J. Afr. Ass. Physiol. Sci. 7 (1): 1-6, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Minireview

Autonomic Regulation of Cardiovascular Function in Obese Rat versus Obese Human

David Randall[1], Yasser El-Wazir[2]

Departments of Physiology, [1]College of Medicine, University of Kentucky, Lexington, USA and [2]Faculty of Medicine, Suez Canal University. Ismailia, Egypt

Keywords:
Autonomic regulation, obesity, obese rats, obese humans cardiovascular

ABSTRACT

Among the various health problems associated with obesity, cardiac autonomic neuropathy is one of the serious and relatively under-investigated problems. The authors have previously showed, in 2008, that vagal dysfunction is the main underlying factor in cardiac autonomic neuropathy in a rat model of congenital obesity. The aim of this article is to review the published findings in this topic since then and to compare the data in rats with those in human subjects.

INTRODUCTION

Obesity has emerged world-wide as a major public health concern, and Africa is not an exception. It has been estimated that obesity/overweight is increasing by an average of 5% annually in sub-Saharan countries (Ziraba et al., 2009). Epidemiological and experimental studies show that obesity is a causative factor in the development of cardiovascular disease. In this case, the deleterious effects of obesity upon autonomic function are seen, at least in part, as a probable contributor to ultimate cardiovascular dysfunction. Many of the earlier studies that outlined the relationship(s) between obesity and circulatory dysfunction were conducted using animals, especially rats or mice fed calorie rich diets or strains genetically biased to become obese. The results of many of these rodent experiments have only subsequently been followed up with corollary observations in man. Recent human studies reported strong association of waist-hip ratio and low heart rate variability in humans (Yadav et al., 2017). Eleven years ago (2008) our study in Zucker$^{fa/fa}$ obese rats vs. a control strain was among the earlier observations of diminished Parasympathetically-mediated bradycardia during an acute behavioral challenge (i.e., classical aversive conditioning) (El-Wazir, et al., 2008). In the sense of 'translational science' this earlier study is primarily of interest to the degree that the findings are applicable to human obesity. The purpose of the present account is to summarize our earlier work and then to examine the subsequent decade's findings in human studies that are either congruent or inconsistent with our earlier rat findings. As such, it should provide objective validation or refutation of an important aspect – autonomic control - of the use of rats to model human obesity.

*Address for correspondence:
Email ywazir@hotmail.com

Review of our obese rat cardiovascular 'stress response' and its autonomic control

The Zucker obese rats used in our study (El-Wazir et al., 2008) were 9- to 11-weeks of age. The obese animals (n=10) weighed 452 ±45 g (mean ± SD) while the age-matched control rats (n=13) weighed 280 ± 46 g. We found no significant differences in mean arterial blood pressure (mBP_a via indwelling femoral artery catheter) while the rats were unanesthetized and at rest between obese (111.7 ± 5.6 mm Hg) vs. lean (113.1 ± 7.0 mm Hg). Likewise, resting heart rate (HR) was similar in obese (422 ± 22 /min) and lean (413 ± 43 /min.). The major novel finding of the study was that the bradycardia observed in a fully trained rat during a 'stressful stimulus' (a 15 sec. 'conditional stimulus' (CS) tone followed by 0.5 sec. tail shock) was

significantly ($p < 0.05$) smaller in the obese (-17.8 $\pm$21.7 /min) as compared to the lean group (-46.0 $\pm$ 21.5 /min). Fig.1 is a composite analysis (i.e., each recording is an ensemble average across the lean (black) and obese (red) animals). Note particularly the significantly smaller bradycardia during the CS-induced stress in the obese (red) animals (i.e., vs. pre-tone control) ($p < 0.05$). This HR conditional response was essentially eliminated following delivery of atropine (not shown, Fig.1); thus, the HR slowing may be attributed largely to increased cardiac parasympathetic activity. The conditioned increase in mBP_a (not shown, Fig.1) was not significantly different between groups. Finally, the reflex change in HR divided by the change in mBP_a () HR/mBP_a) produced by a bolus iv infusion of phenylephrine, an index of baroreceptor function, was significantly smaller in obese (n=6; -1.36 $\pm$ 0.60) vs. lean (n=5; -2.80 $\pm$ 0.92). Conversely, the reflex tachycardia following iv nitroprusside did not differ between groups.

Our prior work (e.g., Randall 1994) with the conditioning paradigm helps interpret these findings. First, the pattern of changes in SNA relative to pre-tone control consists of an initial 'sudden burst' followed immediately by a momentary decrease which is next replaced during the majority of the CS by a modest increase over control. This patterned, learned cardiovascular response 'evolves' as the animal acquires the association between the tone and shock (El-Wazir, 2005). Second, the accompanying increases in mBP_a are reliably predicted by these changes in SNA (Burgess 1997). If so, our finding that the changes in blood pressure during the CS are similar in the two groups of rats implies that the underlying changes in SNA must be similar between the two groups. Finally, though we have not recorded changes in parasympathetic activity, the bradycardia shown in Figure 1 apparently results from baroreflex activity secondary to the conditional increase in BP (Randall, et al., 1994).

Age-matching of rats and humans

Our present intention is to compare these findings with the closest possible match to subsequent human studies. Perhaps the first issue, therefore, concerns the equivalent human developmental age as compared to 9-11-week old rats: how many rat days are equivalent to one human year? A recent review (Andreollo, et al., 2012) of the relevant literature matching rat development to human development explains that rats become sexually mature at 6 weeks while humans don't experience puberty until between about 12 to 13 years. The 'non-linear' differences in rate of-maturity between the two makes comparison difficult, but over the total lifespan Andreollo and colleagues (2012) suggest that during the 'adult phase' 11.8 rat days is equivalent to 1 human year, while across the entire life-span 13.8 rat days is equivalent to one human year. Using Andreollo's equivalents and accepting 6 weeks / 12 years as sexual maturity in rat / human, we estimate that our animals were roughly equivalent to humans of 23-24 years of age. Given the relative paucity of published human studies we have accepted a somewhat wider human age range for consideration in our comparisons.

Assessments of autonomic function in humans

Autonomic function in humans is often evaluated using heart rate variability (HRV) as assessed either in the time or frequency domain. Moreover, as in our rat study, the physiological response to a challenge can be particularly telling in human tests; challenges to homeostasis have often included standing-up from supine or lying down (e.g., Makary et al., 1999). Parasympathetic influences on heart rate are often evaluated in terms of 'high frequency power' (i.e., power in the HR 'signal' as revealed by Fourier analysis within the frequency range typically, in the human, between 0.15 to 0.40 Hz.) We agree, in fact, since in resting dog – an animal with significant cardiac parasympathetic activity while at rest – selective surgical interruption of the parasympathetic fibers projecting to the SA-node essentially eliminates the high frequency peak (Randall 1991). The low frequency peak (human: 0.04 to 0.15 Hz) is often taken as an approximate index of cardiac sympathetic nervous activity (SNA). In dog, selective SA-nodal parasympathectomy significantly decreases this power, but does not eliminate it; addition of ∃-adrenergic blockade (i.e., administration of propranolol) further, and significantly, decreases this power in the low-frequency range of HRV (Randall 1991). Direct recording of muscle sympathetic nerve activity has also been reported in lean vs. overweight or obese humans (Lambert et al., 2010).

Identification of comparable studies of 'young humans' conducted during the ensuing decade

We identified 5 studies of autonomic function in human obese vs. lean subjects generally within the prescribed age range that were published over the 10 years since our 2008 paper. The average age and weight of Rossi, *et al*.'s (2015) obese individuals (24 men / 20 women) were 20.45 $\pm$ 1.57 years and 102.3 $\pm$ 20.82 kg, respectively, while their 'eutrophic' (24 men/ 24 women) averaged 20.7 $\pm$ 1.39 and 62.89 $\pm$ 10.47 kg.

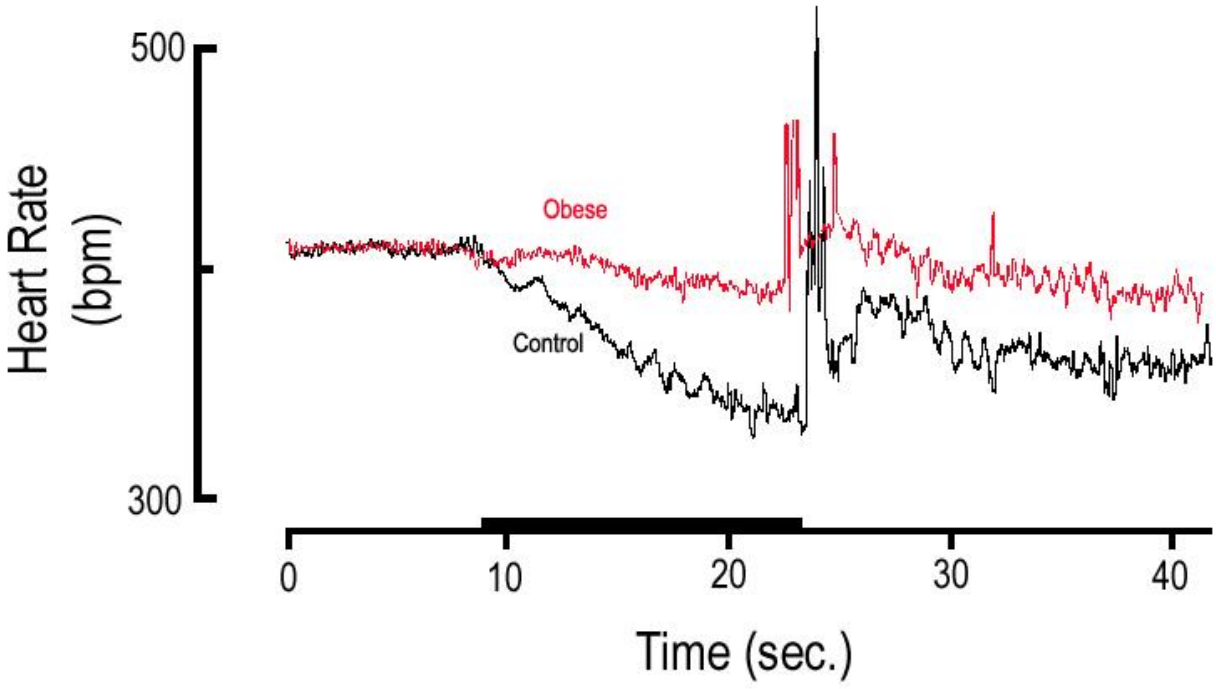

Fig. 1. The rats were un-disturbed while in a comfortable restraining 'sock' for first 9 seconds (i.e., pre-tone control, left portion of time base); the 15 sec. long tone was presented during the dark bar on the X-axis, and the short shock was presented at the end of the dark bar; the irregular fluctuations in the recordings during shock delivery are unreliable because of the rats' flinching during the shock. The heart rate slowing during the stressful stimulus (i.e., 15 sec. tone) was significantly smaller in the obese group vs. the lean animals.

Lee, *et al.* (2014), who included an orthostatic challenge (lower body negative pressure, LBNP) in their work, studied obese individuals who were 27.5 ± 9.3 years of age and 100.8 ± 14.9 Kg. while their non-obese individuals were aged 26.0 ± 7.3 yrs and 66.3 ± 11.9 kg. Muralikrishnan, *et al.* (2013) studied 31 obese people (25.42 ± 7.86 yrs; BMI = 26.84 ± 2.47 kg/m^2 (weight was not provided)) and 31 'normal' (25.38 ± 4.61 years and 21.71 ± 2.99 kg/m^2). Park, *et al.* (2012) reported recordings of muscle sympathetic nerve activity (mSNA) responses to the challenge of the cold pressor test (CPT) and static hand grip (SHG) in 12 overweight subjects (32.3 ± 2.2 yrs; 182.7 ± 7.0 lbs) and in 12 lean individuals (28.9 ± 1.4 yrs (SEM); 138.4 ± 5.0 lbs); although these individuals were older than our estimated rat-human comparison, because the study recorded mSNA and also included a sympatho-excitatory challenge renders it of particular interest. In addition, Indumathy, *et al.* (2015) examined response to standing, deep breathing and isometric handgrip in their obese (29.84 ± 5.88 year; 68.06 ± 7.66 kg) group and their 28.33 ± 7.22 year old controls (54.44 ± 6.40 kg).

Comparisons of effects of obesity on autonomic function in age-matched humans and Zucker rats

We observed no between-group differences in our rats' mBP_a during the pre-tone control. Likewise, Park, *et al.* (2012), Lee, *et al.* (2014) and Muralikrishnan, *et al.* (2013) report similar control mBP_a across their groups, while Indumathy, *et al.* (2015) and Rossi, *et al.* (2015) reported higher arterial pressure in their obese individuals compared to controls. We also reported no difference in pre-tone HR; while Park, *et al's* findings in this regard agree with ours, the overweight or obese subjects for the other studies (Lee, Rossi, Indumathy, Muralikrishnan) had elevated resting HR. HR, in our experience, is quite responsive to the subject's status, emotional and otherwise; we adapted our rats to the sock restraint such that, we believe, their HR was close to 'resting'. In any case, it would be difficult on the basis of control HR alone to draw any firm conclusions on any effects of obesity on autonomic balance.

The HR power spectra from the obese individuals studied by Rossi, *et al.* (2015), as well as Indumathy, *et al.* (2015) had significantly lower HF power than observed in their lean, or control, subjects; such

findings would typically be interpreted to have resulted from decreased parasympathetic control of HR. Absolute LF power in Rossi's study did not differ between groups, but normalized LF power was higher in the obese; Indumathy (2015) reported both a higher total and normalized LF value in his obese subjects. The LF/HF ratio, often regarded as an index of the relative balance of sympathetic and parasympathetic tone, was significantly higher in the obese as compared to the 'eutropic' people in both studies. These investigators conclude that, at rest, obesity was associated with a reduction in parasympathetic and a relative predominance of sympathetic activity. Muralikrishnan, *et al.* (2013) used Poincare Plot analysis to come to the same conclusion. Finally, Park, *et al.* (2012) recorded mSNA via microneurography; baseline bursts/min. in the lean (20.5 $\pm$ 2.2) tended toward fewer per minute than observed in the overweight (27.8 /min.), but the difference fell short of statistical significance ($p = 0.08$).

The study referenced immediately above by Park, *et al.* (2012), is particularly reminiscent of our overall rat work (Randall, et al., 1994) in that they examined changes in mSNA during sympathoexcitatory challenges: the cold pressor test (CPT) and static handgrip exercise at 30% of maximum (SHG 30%). They reported no significant between group differences in the increases (vs. rest) in mBP_a or in HR during the moderate hand grip, though systolic pressure increase in the overweight subjects (+16.6 $\pm$ 1.6 mm Hg) tended ($p = 0.17$) to be blunted compared to the lean (+22.4 $\pm$ 3.5 mm Hg); there was no difference in the magnitude of the increase in mSNA in the overweight (+13.0 $\pm$ 1.6 /min) as compared to the lean (+11.9 $\pm$ 1.3 /min.). Recall that we (El-Wazir et al., 2008) saw no difference (i.e., obese vs. lean rats) in the change in mBP_a during the stress tone which, because changes in arterial pressure 'map' (i.e., are closely related to) changes in SNA (Burgess, 1997), implies that underlying changes in SNA were similar. Conversely, while the changes in pressure and HR were similar between groups during the cold pressor test, the overweight individuals increased mSNA bursts/min more (+18.1 $\pm$ 2.8 /min) than the lean (+10.8 $\pm$ 1.2 /min; $p = 0.03$).

The major focus of Indumathy, *et al's* (2015) study was to investigate the relationship of their indices of sympathovagal imbalance (SVI) to anthropometric indices (e.g., waist or hip circumference), but they also reported comparisons of the "30:15 ratio (ratio of maximum RR interval at 30th beat to minimum RR interval at 15th beat following standing," i.e., orthostatic challenge) and determined the arterial pressure at the first minute and 2nd minute of contraction () DBP_{IHG} or maximum rise in diastolic blood pressure above baseline) during an isometric handgrip test at 30% of maximum voluntary contraction. On the bases of these "classical autonomic function tests" in the obese vs. control group they reported decreased vagal reactivity in their obese subjects during the orthostatic challenge and, during the SHG, they found increased sympathetic reactivity in the obese subjects. As a result of these findings they conclude that differences between the spectral LF:HF ratio in the control (0.69 $\pm$ 0.39; n=43) and obese group (1.41 $\pm$0.73; n=45, $p < 0.001$) are attributable to "concomitant increase in sympathetic activity and reactivity as well as to the decrease in parasympathetic activity and reactivity" (quoted from p. 62). Lee, *et al.* (2014) also reported reduced orthostatic tolerance in obese humans which, on the basis of previously established alterations in autonomic function in humans, "support the contention that autonomic nervous system activity is altered with weight gain and obesity," but they do not offer a more specific analysis.

The subjects in the study by Yadav, *et al.* (2017) were somewhat older (average: 30 – 32 years), and the age range (18 – 75 years) within the normal weight and within the obese individuals was large. Irrespective, we note parenthetically that they report significantly lower HRV HF power in the obese (216 ms^2) as compared to the normal weight (640.5 ms^2; $p = 0.014$); LF power was lower (obese: 248 ms^2; normal weight: 480 ms^2; $p = 0.063$) while LF/HF was higher (obese: 1.2; normal weight: 0.79; $p = 0.045$). In short, they state that variables which reflect the cardiac parasympathetic nerve activity were lower in obese persons than in normal weight persons while the sympathetic marker LF/HF ratio was increased in obese subjects. It may be noteworthy to state that this study was conducted on Asian subjects (Nepal), which may highlight the trans-ethnicity of the obesity-associated cardiac autonomic dysfunction.

Baroreflex function in lean vs. obese individuals

None of the five studies we identified assessed baroreflex function directly in obese vs. lean subjects, so we looked at studies with subjects beyond the optimal age-range and/or published other than within the most recent decade. Using a sequence technique (Randall & Brown, 2014), Skrapari, *et al.* (2007) compared baroreflex sensitivity in obese (BME > 30 kg/m^2) vs. lean (BMI < 25 kg/m^2) women; though aged (42 yrs) somewhat older than our range, the data are worth our noticing. They found a severe reduction in baroreflex sensitivity by the sequence technique in obese (9.18 $\pm$ 3.77 ms/mm Hg), but otherwise healthy,

subjects as opposed to their lean women (19.63 $\pm$ 9.16 ms/mm Hg, $p < 0.001$). These differences were closely associated with the high frequency component (i.e., parasympathetic component) of HRV. Jaju, *et al.* (2016) compared spectral HRV assessments for mental (word conflict test; WCT) and physical (cold pressor test) challenges for large (n=1149) groups of lean (average age: 32.8 yrs; 56.6 kg), overweight (38.0 years; 68.5 kg) and obese (38.1 yrs; 83.4 kg) individuals from 5 large families from an interior province of Oman. Although the average age ranges are near our range, the direct applicability of their findings to ours is limited because of the wide range of the ages of their subjects within any category. Nonetheless, we note that they report no differences in the HR or mBP_a changes (vs. baseline) during WCT or CPT in their autonomic parameters or in their index of baroreflex function.

Interpretations of comparative rat vs. human findings

Stables, *et al* (2013) point out that "it is crucial to be able to determine in what ways the animal models are similar to the human disease, and in what ways they are different" (quoted from p. 75) in their consideration of the applicability of animal (primarily rat) models of diabetes vs. human diabetic cardiac autonomic neuropathy. We attempt such a comparison here of human and rodent (i.e., rat) obesity, where, in our case, the rodent obesity is attributable to a genetically controlled lack of the leptin receptor in the Zucker$^{fa/fa}$ rat: this animal harbors a missense mutation in the leptin receptor gene. Stables, *et al* (2013) point out that such comparison requires a combination of several suggestive tests. In our rat study the primary novel finding depended upon use of a classical aversive conditioning paradigm which is sympatho-excitatory with, thereby, an increase in mBP_a that, in turn, slows HR via activation of the baroreflex with resultant elevated cardiac PNS activity. It is difficult, perhaps impractical, to identify a corresponding human paradigm, which thereby limits our ability to compare strictly our animal with human work. Not unexpectedly, multiple inferences are required.

The human studies are nearly unanimous in positing an overall diminution of parasympathetic nervous activity in obesity, at least as inferred by HRV or other indirect assessments. It is more difficult to convincingly compare findings in these human studies to our major conclusion – decreased parasympathetically mediated bradycardia in the obese rats attributable to differences in baroreflex function – but the findings of Indumathy, *et al's* (2015)"classical autonomic function tests" certainly come close to such a confirmation. Findings are less unanimous as regards differences in sympathetic nervous activity. This is, at least in part, attributable to the weaker linking of changes in LF power (i.e., both sympathetic and parasympathetic activity contribute to LF power) and ambiguity in the LF/HF ratio (i.e., both increases in LF power, or decreases in HF power might contribute to an increased ratio) in assessing 'sympathovagal imbalance' (SVI). Moreover, our conclusions in this regard are inferential as based upon our prior assessment of the relationship between the coupling of changes in SNA and changes in arterial pressure. Nonetheless, our rat findings are generally consistent with a relatively unchanged, or only modest increase in sympathetic arousal with acute challenge (e.g., orthostatic challenge). In sum, it appears from our perusal of the relevant literature that our specific conclusion – diminution of parasympathetic autonomic control of HR early in the development of obesity with only modest, if any, changes in sympathetic activation – is a faithful reflection of the human situation.

What of the underlying mechanism(s) of the lessened bradycardia during stress? Since the bradycardia we observed occurs during an elevation in SNA we believe the HR slowing is secondary to baroreflex activation induced by the arterial pressure increase during the stress. Since the magnitude of the stress-induced pressor response was similar in the obese vs. lean rats, the input to the baroreceptors should have been similar and, at least inferentially, the reflex response of the baroreflex was, therefore, similar. If so, the reduced HR slowing is attributable to diminished parasympathetic control of the SA-node (i.e., rather than diminished sympathetic control). Our finding that the reflex HR slowing to phenylephrine, but not the reflex HR acceleration to nitroprusside, was attenuated is consistent with this interpretation. In sum, our retrospective examination of the age-appropriate human literature published during the last decade indicates that our findings in Zucker rat were in an important and significant sense 'translational' with respect to autonomic function in obese humans as compared to lean individuals.

REFERENCES

Andreollo NA, dos Santos EF, Araδjo MR, Lopes LR. Rat's age versus human's age: what is the relationship? *Arq Bras Cir Dig* 25: 49-51, 2012.

Burgess DE, Hundley JC, Li S, Randall DC, Brown DR. Multifiber renal sympathetic nerve activity recordings predict mean arterial blood pressure in unanesthetized rat. *Am. J. Physiol., 273 (Regulatory Integrative Comp. Physiol.* 36): R851 - R857, 1997.

El-Wazir YM, Li SG, Williams DT, Sprinkle AG, Brown DR and Randall DC. Differential Acquisition of Specific Components of a Classically Conditioned Arterial Blood Pressure Response in Rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., 289: R784 - R788, 2005.

El-Wazir YM, Li S-G, Smith R, Silcox DL, Brown DR Randall DC. Parasympathetic response to acute stress is attenuated I young Zucker obese rats. *Auton Neurosci: Basic Clin* 143: 33-39, 2008.

Indumathy J, Pal GK, Pal P, Ananthanarayanan PH, Parija SC, Balachander J, Dutta TK. Association of sympathovagal imbalance with obesity indices, and abnormal metabolic biomarkers and cardiovascular parameters. *Obes Res Clin Prac* 9: 55-66, 2015.

Jaju D, Al Abri M, Hassan MO, Alvarenga JL, Comuzzie A, Albarwani S, Al Yahysee S, Bayoumi R, Al Hashmi K. Hemodynamic and autonomic reasctivity to mental and physical stress in lean, overweight and obese subjects. *J Obes Overweight* 2(1), 2016 (doi: 10.15744/2455-7633.2.105)

Lambert Elisabeth, Sari Carolina Ika, Dawood Tye, Nguyen Julie, McGrane Mariee, Eikelis Nina, Chopra Reena, Wong Chiew, Chatzivlastou Kanella, Head Geoff, Straznicky Nora, Esler Murray, Schlaich Markus, Lambert Gavin, 2010. Sympathetic Nervous System Activity Is Associated With Obesity-Induced Subclinical Organ Damage in Young Adults. Hypertension 56, 351–358. https://doi.org/10.1161/HYPERTENSIONAHA.110.155663

Lee JF, Harrison ML, Christmas KM, Kim K, Hurr C, Brothers RM. Elevated resting heart rate and reduced orthostatic tolerance in obese humans. *Clin Auton Res* 24: 39-46, 2014.

Makary SM, Selim HA, El-Wazir YMN, Mahmoud A E-A. Physiological assessment of cardiac autonomic reflexes in diabetes. *Suez Canal Univ Med J* 2: 61-72, 1999.

Muralikrishnan K, Balasubramanian K, Ali SMJ, Rao BV. Poincare plot of heart rate variability: an approach towards explaining the cardiovascular autonomic function in obesity. Indian *J Physiol Pharmacol* 57: 31-37, 2013.

Park J, Middlekauff HR, Campese VM. Abnormal sympathetic reactivity to the cold pressor test in overweight humans. Am J Hypertension 25: 1236-1241, 2012 (https: //doi.org/10.1038/ajh. 2012.115).

Randall DC, Brown DR, Raisch RM, Randall WC. SA-nodal parasympathectomy delineates autonomic contributions to heart rate power spectrum. *Am. J. Physiol. 260 (Heart Circ. Physiol*. 29): H985-H988, 1991.

Randall, DC and Brown DR. Closed and open loop mechanisms controlling arterial blood pressure in waking state in human and experimental animal. *Bulletin of the Egyptian Society of Physiol* Sci 34(1): 1-6, 2014.

Rossi RC, Vanderlei LCM, GonHalves ACCR, Vanderlei FM, Bernardo AFB, Yamada KMH, da Silva NT, de Abreu LC. Impact of obesity on autonomic modulation, heart rate and blood pressure in obese young people. *Auton Neurosci: Basic Clin* 193: 138-141, 2015.

Skrapari I, Tentolouris N, Perrea D, Bakoyiannis C, Papazafiropoulou A, Katsilambros N. Baroreflex sensitivity in obesity: relationship with cardiac autonomic nervous system activity. *Obes* 15: 1685 – 1693, 2007.

Stables CL, Glasser RL, Feldman EL. Diabetic cardiac autonomic neuropathy: insights from animal models. *Auton Neurosci: Basic Clin* 177: 74-80, 2013.

Yadav RL, Yadav PK, Yadav LK, Agrawal K, Sah SK, Islam MN. Association between obesity and heart rate variability indices: in intuition toward cardiac autonomic alteration –a risk of CVD. *Diab Met Synd Obes: Targets Therapy* 10: 57- 64, 2017.

Yokoi N, Hosino M, Hidaka S, Yoshida E, Beppu M, Hoshikawa R, Sudo K, Kawada A, Takagi S, Seino S. A novel rat model of type 2 diabetes: the Zucker fatty diametes mellitus ZFDM rat. *J Diab Res* , 2013.

Ziraba AK[1], Fotso JC, Ochako R. Overweight and obesity in urban Africa: A problem of the rich or the poor? BMC Public Health. 15;9:465, 2009.

J. Afr. Ass. Physiol. Sci. 7 (1): 7-16, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Oral administration of acrylamide compromises gastric mucosal integrity in Wistar rats

A.O. Ige*, O.M. Onwuka, I.E. Emediong, A.O. Odetola, B.O. Adele and E.O. Adewoye

Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan, Nigeria

Keywords:
Acrylamide, stomach, gastric mucosa, mucosal integrity, ulcer

ABSTRACT

Background: Acrylamide, a potential toxicant and carcinogen, maybe formed in carbohydrate-rich food cooked at very high temperature. Its effect on gastric mucosa defense is not fully elucidated. Hence, the effect of acrylamide ingestion on gastric mucosal integrity was investigated. **Methods:** Fifty-four (54) Wistar rats (150-200g) were randomly divided into 3 groups; Group I (control) received 0.2mL distilled H_20, Groups II and III received 7.5mg/kg body weight and 15mg/kg body weight acrylamide respectively. Both acrylamide and distilled water were administered orally for 28days. Thereafter, gastric secretion was obtained and analysed for gastric acidity. Gastric antioxidants status (superoxide dismutase (SOD), reduced glutathione, catalase), lipid peroxidation, mucus content, nitric oxide, bicarbonate, prostaglandins-E and gastric mucus content were determined. Blood samples were also collected and evaluated for haematological indices. Histological changes, parietal and mucus cell counts were evaluated on gastric tissues. **Results:** Gastric secretion and acidity increased ($P < 0.05$) in the 15mg/kg acrylamide treated group. Glutathione, SOD, catalase, mucus content, bicarbonate, prostaglandins-E_2, mucous cell count were reduced ($P < 0.05$) while parietal cell count, lipid peroxidation and nitric oxide increased ($P < 0.05$) in both acrylamide treated groups compared to control. White blood cell count in group II was increased compared to control ($P < 0.05$). Acrylamide treated groups displayed gastric epithelial cells with poor architecture, lamina propria, submucosa inflammatory cell infiltration and vascular congestion. **Conclusion:** Acrylamide exposure degenerates gastric mucosal integrity in a dose-dependent manner via reductions in gastric protective factors, which thus predisposes the gastric mucosa to erosions and lesions.

INTRODUCTION

The gastrointestinal barrier has been described as a barrier between the body and a luminal environment that not only contains nutrients, but also is laden with potentially hostile microorganisms and toxins (Allaire *et al.*, 2018). When food is ingested, the gastrointestinal barrier acts as a first line of defence against invasion of foreign pathogens that might have been ingested (Hammer *et al.*, 2015) and disruption of this barrier has been reported to result in severe debilitating disease conditions (Allaire *et al.*, 2018). The gastric mucosa maintains its integrity by a balance between gastro-aggressive (acid and pepsin secretion) and gastro-protective factors (epithelial cells, mucus and bicarbonate concentration, prostaglandins, gastric mucosal blood flow, nitric oxide and antioxidants) (Goel *et al.*, 1985, Abdel-Salam *et al.*, 2001; Goel and Sairam, 2002). These factors constitute a complex system of interacting mediators that contribute to strengthening the gastric mucosa and offer resistance against gastric injury or insults.

Acrylamide is an industrial chemical used in the manufacture of personal care and grooming products, soil conditioners, wastewater treatment, as well as in paper and textile industries (Friedman, 2003; Exon, 2006). High levels of acrylamide have also been detected in tobacco smoke (Pruser and Flynn, 2011). Acrylamide is also a by-product of the cooking process having been reported to be a preparation by-product in

*Address for correspondence:
Email ao.ige@mail1.ui.edu.ng

heat-processed foods high in carbohydrates e.g. snack foods, potato crisps, breads, cereal products, and coffee (Mottram *et al.,* 2002). Acrylamide in diet is formed through the Maillard reaction where reducing sugars (glucose or fructose) react with the amino acid, asparagine. This reaction is responsible for browning food during baking, frying, and roasting of food (Mottram *et al.,* 2002). Therefore, it is likely that the general populace may be exposed to acrylamide through their diets.

Since its discovery in everyday foods (Pellucchi *et al.,* 2011; Virk-Baker *et al.,* 2014), several epidemiological studies have reported its potentially toxic and carcinogenic effects in different organs in the body (Mucci *et al.,* 2003; Hogervorst *et al.,* 2007; Hogervors *et al.,* 2008; Larsson *et al.,* 2009; Virk-Baker *et al.,* 2014). Acrylamide has also been reported to be a potent neurotoxin affecting both central and peripheral nervous systems (Lehning *et al.,* 2002; LoPachin *et al.,* 2002); however, its effect on the gastric intestinal tract has not been fully elucidated. While El-Mehi and El-Sherif, (2015) have reported acrylamide consumption causes mucosal erosions and depletion of the protective surface mucus, the underlying mechanism through which it disrupts the gastric mucosa defense is yet to be fully elucidated.

This study was therefore designed to evaluate the effect of acrylamide consumption on factors that maintain the integrity of the gastric mucosa.

MATERIALS AND METHODS

Animals and grouping

Fifty-four (54) male Wistar rats (150-170g) were housed in standard well-aerated laboratory cages and maintained at room temperature with alternating 12-hour day and night cycles. They were fed on standard rat chow and allowed free access to drinking water *ad libitum.* The animals were randomly divided into 3 groups of 18 rats each.

Treatment protocol

Group 1 - control received distilled water 0.2mLs, groups II and III received 7.5mg/kg body weight and 15mg/kg body weight of acrylamide (Sigma Aldrich, China) (Zenick *et al.,* 1986) respectively. All treatments were given orally for 28days. The Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan approved this experiment. Animals received humane care, and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

Determination of gastric juice acidity and pH

Post-treatment, animals (n = 5) were subjected to surgery under light ether anaesthesia according to Brodie and Knapp (1966). Briefly, under light ketamine anaesthesia (40 mg/kg) the abdomen of each animal was opened through a midline epigastric incision, and the stomach was exposed. The pyloric end was identified and a fine thread was tied round the pylorus, care was taken to avoid inclusion of adjacent blood vessels. The wound was then closed with catgut and the animal returned to its cage where it subsequently regained consciousness. After 4 hours the animal was again anaesthetized, opened up and stomach was removed after clamping the pylorus and the lower end of the oesophagus. 4-hour gastric juice was collected and drained into a graduated test tube and centrifuged at 1400g for 10min (Raji *et al.,* 2011). The supernatant volume and pH were recorded (Saranya and Geetha, 2011) and the total acid content of the gastric juice collected was determined by titrating to pH 7.0 with 0.01N NaOH, using phenolphthalein as indicator.

Determination of ulcer score, gastric oxidative stress, bicarbonate and prostaglandins-E2 levels

Gastric ulcer score was done using a hand lens at X2 magnification as described by Elegbe and Bamgbose (1976) and thereafter the ulcer index and percentage (%) ulcer inhibition was calculated. Stomach tissues (0.5g) from 5 animals in each group were homogenized on ice with ice-cold 0.1 M phosphate buffer (1: 4 w/v, pH 7.4), the homogenates obtained was centrifuged at 2500 rpm for 10 min at 4^0C and the resulting supernatants was frozen at -4°C until use (Saheed *et al.,* 2015). Aliquots of the supernatants were thereafter analysed for catalase (Sinha, 1972), superoxide dismutase (SOD) (Misra and Fridovich, 1972), glutathione (Sedlak and Lindsay, 1968), lipid peroxidation (as malondyaldehyde (MDA) and nitric oxide (Griess reaction as described by Green 1982) levels respectively. The supernatants were also assayed for bicarbonate ion and prostaglandins-E2 level using enzyme-linked Immunosorbent Assay Kits (Bioassay Technology Laboratory, China).

Determination of haematological indices and mucus content in control and acrylamide treated animals

Blood samples were obtained by cardiac puncture after light ketamine anaesthesia (40 mg/kg) from 5 animals in each group into heparinised specimen bottles and analysed for packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelet count, total white blood cell (WBC) count and differential WBC count). Gastric mucous content was

estimated in these same animals using the Alcian blue technique as described by Corne *et al.* (1974).

Parietal, mucous cell counts and Histological evaluation of the gastric mucosa

The stomach samples from animals in each group (n=3) were excised and stored in 10% formalin. Mucous cell count was estimated using the Periodic Acid Schiff (PAS) reaction technique while gastric histopathology and parietal cell count were estimated using Hematoxylin and Eosin-staining techniques as described by Adewoye and Salami (2013).

Statistical analysis

Results are expressed as mean ± SEM and were analysed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. Comparisons between control and experimental groups were carried out and the statistical differences were taken to be significant at $p < 0.05$.

RESULTS

Effect acrylamide on the gastric juice acidity and pH

The pH of gastric effluents in group III (acrylamide 15mg/kg treated)) was significantly reduced ($p<0.05$) compared to group I (3.56 ± 0.28 vs. 4.88 ± 0.29) while group II (acrylamide 7.5mg/kg treated) was not significantly different from control (4.44 ± 0.38) vs. 4.88 ± 0.29). Gastric Acid secretion (mEq/mL/4hours) in groups II (0.28 ± 0.04) and III (0.69 ± 0.12) were significantly increased ($p<0.05$) compared to control (0.07 ± 0.01) (Table 1).

Table 1. Effect of acrylamide on the gastric juice acidity and pH.

Groups	Acidity (pH)	Gastric acid secretion (mEq/mL/4hours)
Group I (Control)	4.88 ± 0.29	0.07 ± 0.01
Group II (Acylamide 7.5mg/kg treated)	4.44 ± 0.38	0.28 ± 0.04*
Group III (Acylamide 15mg/kg treated)	3.56 ± 0.28#	0.69 ± 0.12#

* Indicates significant differences between group II and control, # indicates significant differences between group III and control.

Gastric oxidative stress and bicarbonate level in control and acrylamide treated animals

Gastric antioxidants (superoxide dismutase (SOD), reduced glutathione and catalase were significantly reduced ($p<0.05$) in groups II (acrylamide 7.5mg/kg treated) and III (acrylamide 15mg/kg treated) compared to control. Gastric MDA (µmol/g) in groups III (0.234 ± 0.035) and II (0.059 ± 0.006) were significantly increased ($p<0.05$) compared to control (0.0177±0.002). Gastric bicarbonate (mmol/l) was significantly reduced ($p<0.05$) in groups III (4.05 ± 0.18 vs 7.66 ± 0.55) and II (4.85 ± 0.23 vs 7.66 ± 0.55) compared to control (Table 2).

Table 2. Effect of acrylamide on antioxidant status enzymes activities and lipid peroxidation parameter

Groups	SOD (µmol/g protein)	CAT (µmol/g protein)	GSH (µg/g)	HCO_3^- (mmol/L)	MDA (µmol/g)
Group I (Control)	6.18±0.89	28.54±1.89*	10.87±0.76	7.66±0.55	0.0177±0.02
Group II (Acrylamide 7.5mg/kg treated)	2.08±0.53*	19.14±1.86*	6.41±0.83*	4.85±0.23*	0.059±0.006*
Group III (Acrylamide 15mg/kg treated)	1.08±0.24#	15.18±1.67#	5.00±0.18#	4.05±0.18#	0.233±0.035#

* Indicates significant differences between group II and control, # indicates significant differences between group III and control.

Gastric ulcer score, index and inhibition in control and acrylamide treated animals

Gastric ulcer score was significantly increased ($p<0.05$) in group III (15mg/kg acrylamide treated) compared to control (group I) while values in group II (7.5mg/kg acrylamide treated) were not different from controls (Table 3). Ulcer index and percentage inhibition in group III was 0.49 and -88.46%, in group II it was 0.30 and -15.39% while in control it was 0.26 and 0% respectively (Table 3).

Table 3. Effect of acrylamide on ulcer score (units), ulcer index and ulcer inhibition

Groups	Ulcer score (units)	Ulcer index	% Ulcer inhibition
Control	5.1 ± 0.70	0.26	–
7.5mg/kg of acrylamide	6.0 ± 2.41	0.30	- 15.39
15mg/kg of acrylamide	9.8 ± 1.91[#]	0.49[#]	- 88.46

[#] Indicates significant differences between group III and I.

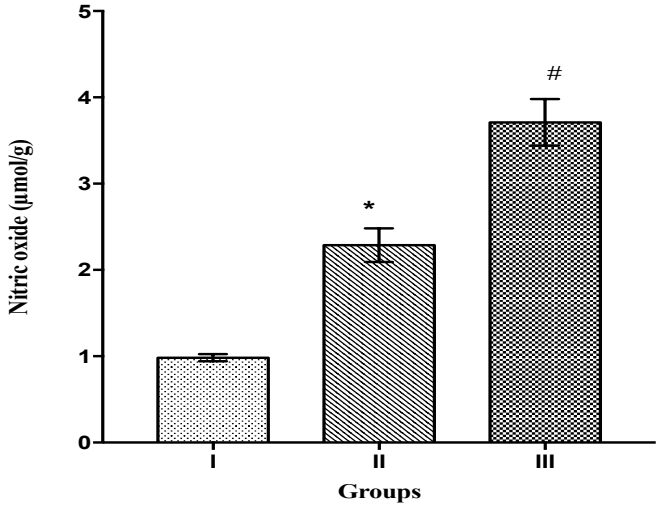

Fig. 1. Effect of acrylamide on gastric nitric oxide concentration. *Indicates significant differences between group II and control, [#] indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

Gastric nitric oxide and prostaglandins E_2 levels in control and acrylamide treated animals

Gastric nitric oxide (µmol/g) was significantly increased in groups III (3.71 ± 0.27) and II (2.29 ± 0.20) compared to group I (0.98 ± 0.04) (Fig 1). Prostaglandins-E_2 (ng/mL) values in group III (2.59 ± 0.07) were significantly reduced while that in group II (2.71 ± 0.08) was not significantly different to group I (2.89 ± 0.07) (Fig. 2).

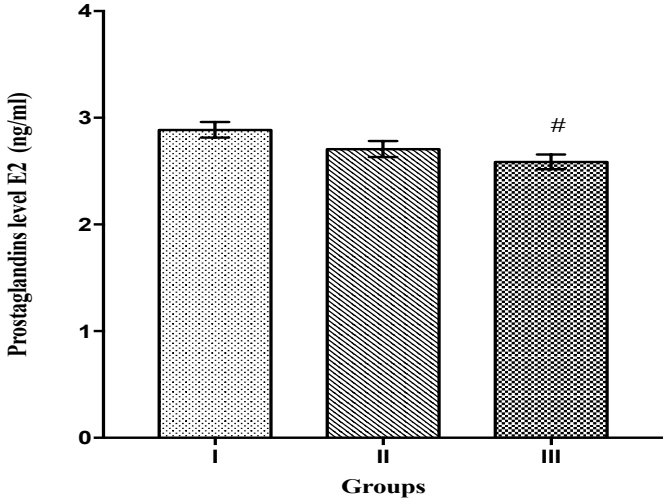

Fig. 2. Effect of acrylamide on gastric prostaglandin E2 level. [#]Indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

Gastric mucus concentration, parietal and mucous cell counts in control and acrylamide treated animals

Gastric mucus concentration (µg/g) was significantly reduced in both experimental groups compared to control (Fig. 3). Parietal cell count (cells /field) was significantly decreased ($p<0.05$) in group II (acrylamide 7.5mg/kg treated) and increased in group III (acrylamide 15mg/kg treated) compared to group I. Mucous cell count (cells /field) in groups II (486.0 ± 102.2) and III (361.7 ± 30.6) were significantly decreased compared to group I (814.7 ± 19.5) (Table 4).

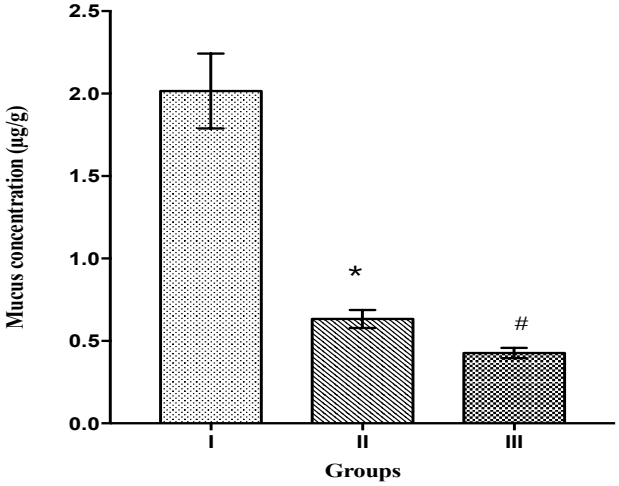

Fig. 3. Effect of acrylamide on gastric mucus concentration. *Indicates significant differences between group II and control, [#]indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

Table 4. Effect of acrylamide on parietal and mucus cell counts

Groups	Parietal cell count cells/field)	Mucus cell count (cell/field)
Group I	419.3±7.84	814.7±19.5
Group II	385.7±5.24*	486.0±102.2*
Group III	492.3±8.29#	361.7±30.6#

* Indicates significant differences between group II and I, # indicates significant differences between group III and I.

Haematological indices in control and acrylamide treated animals

Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell counts (RBC) and platelet counts in all treatment groups were not significantly different from group I (Table 5). However, total white blood cell count (WBC) in group II (acrylamide 7.5mg/kg treated) ($42.0 \pm 3.51 \times 10^5$) was significantly increased ($p<0.05$) compared to group I ($32.6 \pm 4.24 \times 10^5$). Group III (acrylamide 15mg/kg treated) WBC counts were not significantly different from group I (Table 5). Monocytes and eosinophils in the experimental groups were not significantly different from control (group I) values. Lymphocyte values were significantly increased in group II (acrylamide 7.5mg/kg treated) (72.2±1.28) but decreased in group III (acrylamide 15mg/kg treated) (62.4±3.08) compared to control (67.6±1.03). Neutrophil count was significantly decreased in group II (low dose - 7.5mg/kg of acrylamide) (20.0±4.34) but increased in group III (acrylamide 15mg/kg treated) (35.4±3.20) compared to control (29.6±0.81) (Table 5).

Table 5: Effect of acrylamide on haematological indices

Haematological indices	Group I	Group II	Group III
PCV (%)	38.6±0.75	40.6±1.03	34.6±0.68
Hb (g/dL)	12.8±0.23	13.46±0.41	11.28±0.30
RBC count (10^{12}/L)	6.31±0.07	6.69±0.20	5.52±0.19
WBC count (10^9/L)	3.26±0.42	4.20±0.35*	3.36±5.85
Platelets (mm^3/L	15.84±1.96	9.86±0.78*	13.5±1.09
Neutrophil	29.6±0.81	20.0±4.34*	35.4±3.20
Lymphocyte (%)	67.6±1.03	72.2±1.28	62.4±3.31
Monocytes	1.8±0.37	1.8±0.37	1.4±0.25
Eosinophil	1.2±0.58	1.8±0.58	1.2±0.58

* Indicates significant differences between group II and I.

Histopathology of the gastric mucosa

The gastric mucosa of the control group (group I) had normal architecture, well preserved mucosa epithelial cells layer (white arrow) and the mucosa layer showed no infiltration of the gastric glands and lamina propria (slender arrow). The submucosal (blue arrow) and circular muscle (red arrow) layers were normal and were not infiltrated by inflammatory cells. Group II animals (Acrylamide 7.5mg/kg treated) had gastric mucosa with poor architecture, poorly preserved mucosa epithelial cell layer (white arrow) and mild infiltration of the lamina propria. The submucosal layer in this group had inflammatory cell infiltration, however the circular muscle layer appears normal. Group III (Acrylamide 15mg/kg treated) showed mucosa layer with eroded epithelial cells (white arrow), infiltrated lamina propria. The submucosal layers in this group appear moderately infiltrated by inflammatory cells (blue arrow) while the circular muscle layer appeared normal. Mild vascular congestion was also observed (Fig. 4 A-C).

DISCUSSION

Acrylamide has been described as a toxicant and an irritant (Zamani *et al.*, 2017). The discovery that it may be produced when cooking, frying, toasting and baking high carbohydrate foods has increased investigations into its potential biologic effects. These investigations have reported the neurotoxicity, reproductive toxicity and immune-toxicity of acrylamide consumption (Zamani *et al.*, 2017. In this study the effects of acrylamide on gastric mucosal integrity was evaluated at two doses, 7.5mg/kg and 15mg/kg, which have been reported to be equivalent to 1/20 and 1/10 of LD_{50} for acrylamide (LD_{50} 150 mg/ kg) respectively (Zenick *et al.*, 1986). The significantly increased acidity and secretion of gastric juice especially in the high dose (15mg//kg acrylamide) compared to control (Table 1) suggests a predisposition of the treated animals to gastric ulceration as excess acidity of gastric juice has been reported to favour aggressive factors that predispose to gastric ulceration (Wormsley, 1974). Furthermore, parietal cells, which are responsible for acid secretion (Pavelka and Roth, 2010; Ige *et al.*, 2016), had increased counts in the high dose group compared to control (Table 4) suggesting a likely increase in gastric acidity and secretion in this group. This may thus be responsible for the significantly increased ulcer score and index seen in the acrylamide-exposed groups compared to control (Table 3).

Gastric antioxidants, an essential component of the gastrointestinal defence system that scavenge free radicals, have been reported to play an integral role in

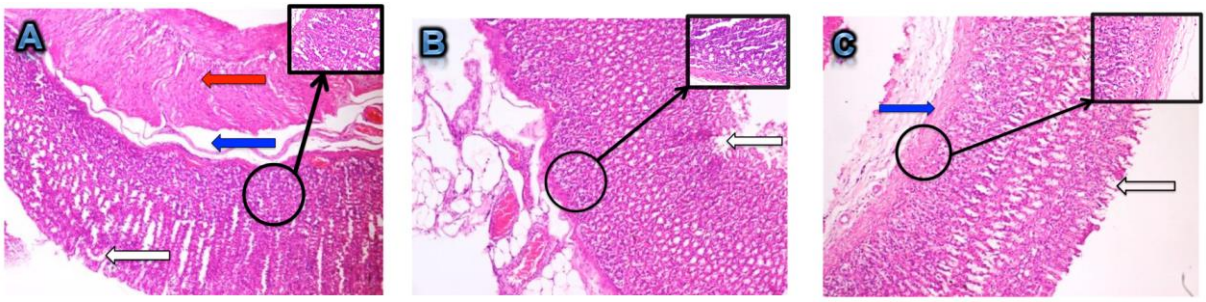

Fig. 4. (A-C) Photomicrograph of stomach samples in control and experimental groups at low magnification (x100) and high magnification (x400) Group 1 (Control) displayed normal architecture of gastric mucosa, with well-preserved mucosa epithelial cells layer (white arrow), the mucosa layer showed no infiltration of the gastric glands and lamina propria. The submucosal layers appeared normal and were not infiltrated by inflammatory cells (blue arrow), the circular muscle layer (red arrow) appears normal. Group 2 (Acrylamide 7.5mg/kg treated) exhibited poor architecture, the mucosa epithelial cells layer was poorly preserved (white arrow), and the mucosa layer displayed mild infiltration of the lamina propria and the gastric gland. The submucosal layers appear mildly infiltrated by inflammatory cells; the circular muscle layer appears normal. Group 3 (Acrylamide 15mg/kg treated) exhibited mucosa layer with eroded epithelial cells (white arrow), the mucosa layer shows mild infiltration of the lamina propria. The submucosal layers appeared moderately infiltrated by inflammatory cells (blue arrow), the circular muscle layer appeared normal. There is also was mild vascular congestion (Fig. 4, A-C).

the formation of gastric lesions (Hassan *et al.*, 1998). This study also shows depletion of gastric antioxidants and significant increase in gastric lipid peroxidation compared to control (Table 2) suggesting a decline in the antioxidant capacity and increased oxidative stress in the gastric mucosa of the acrylamide exposed animals. Mucus, secreted by mucus cells, and bicarbonate ions secreted by gastric and duodenal epithelial cells constitute an integral component of the gastrointestinal barrier against erosion and invasion (Engle *et al.*, 1995). The mucus produced reduces the shear stresses on the epithelium and contributes to barrier function through various mechanisms, which include binding to bacteria thus preventing epithelial colonization and retarding diffusion of agents that can damage the epithelial surface e.g. acid secretion. Bicarbonate ion, on the other hand, serves to maintain a neutral pH along the epithelial plasma membrane, despite the highly acidic conditions existing in the gastric lumen (Engle *et al.*, 1995). This study shows a dose dependent and significant decrease in gastric bicarbonate concentration (Table 2), mucous content (Fig. 3) and mucous cell count (Table 4) compared to control which suggests an impairment in the ability of the gastric mucosa of the acrylamide treated animals to sustain its barrier function and prevent trans-epithelial migration of bacteria and antigens. It is thus likely that increased exposure to acrylamide enhances gastro-aggressive and suppresses gastro-protective factors that may predispose the stomach to gastric ulceration and lesions.

Inflammation within the gastrointestinal tract has been reported to result in the activation of inducible nitric oxide synthase (iNOS) leading to an increase in nitric oxide (NO) production that results in increased production of reactive oxygen radicals and oxidative stress (Muscara and Wallance, 1999; Lanas, 2008). An increase in NO was seen in the acrylamide-exposed groups compared to control (Fig. 1) and suggests a likely inflammatory mediated pathway for acrylamide-induced disruption of the gastric mucosa. Furthermore, prostaglandins whose gastro-cytoprotective effects are exerted by their ability to stimulate mucosal mucus and bicarbonate secretion, increase mucosal blood flow and partially limit back diffusion of acid into the epithelium (Wallance, 2008) was reduced in the high dose acrylamide group compared to control (Fig. 2) thus suggesting an impairment of prostaglandin enabled gastro-protection and increased susceptibility of the gastric barrier to damage.

Haematological and serum biochemical indices are important tools in evaluating the health status of an individual (Ige *et al.*, 2015). This study shows no significant difference in red cell indices (red blood cell count, packed cell volume and haemoglobin levels) across the groups (Table 5) which is consistent with Rawi *et al.*, (2012) who reported no change in haemoglobin, erythrocyte count and haematocrit levels in immature male rats and a decrease of these same indices in immature female following acrylamide (15mg/kg) treatment. This thus suggests the question of a likelihood of a gender effect regarding acrylamide

toxicity and will form a subject for subsequent research in our laboratory. However, elevations in total white blood cell counts accompanied by reductions in neutrophil count were observed in the acrylamide treated, especially the low dose group, compared to control (Table 5). This suggests stimulation of the immune system arising from acrylamide exposure. Interestingly the high dose acrylamide group showed elevations in neutrophil counts and reduction in lymphocyte count compared to control, which suggests nutritional impairment and immune suppression in this group (Gonda *et al.,* 2017). Neutrophils are of particular importance to gastrointestinal integrity as diverse insults to the gastric mucosa, including infectious processes, ischemia and damaging chemicals have been reported to promote infiltration of the gastric mucosa by neutrophils (Gayle *et al.,* 2000). This study shows neutrophil infiltration of the gastric mucosa in groups 2(Fig 4B) and 3 (Fig 4C), which again suggest gastric tissue damage in the acrylamide-exposed groups. Furthermore, histological analysis in the different groups are consistent with the result of biochemical assays carried out and the report of El-Mehi and El-Sherif, (2015) who stated that acrylamide effects on the gastric mucosa include mucosal erosions, depletion of the protective surface mucus and inflammatory infiltration of the mucosal layer.

In conclusion, it may be inferred from this study that increased dietary acrylamide exposure, compromises the integrity of the gastric mucosal barrier by increasing the activity of gastro aggressive factors (decreased gastric acid pH and mucous cell count, increased gastric acid secretion, gastric lipid peroxidation, nitric oxide production, parietal cell and neutrophil counts respectively) and suppressing gastro protective factors (decreased gastric mucus, prostaglandins, antioxidants, bicarbonate ion. Hence, excessive browning while frying or toasting should be avoided as this causes acrylamide formation and accumulation in food, which may result to gastric mucosal damage or exacerbate already formed gastric ulcers. peroxidation in the serum of the high salt diet groups suggests an increase in oxidative stress in rats in these groups. This finding is consistent with other studies that report increasing oxidative stress effect of a high salt diet (Huang *et al.*, 2016; Tian *et a.l*, 2007). ROS activities in other organ systems, such as the heart, nervous system, and kidneys, have also been implicated in the pathophysiology of hypertension (Imaizumi *et al.*, 2016; Huang *et al.*, 2016; 2017). In particular, increased renal O_2^- production is associated with NO bio-inactivation, which influences afferent arteriolar tone, tubuloglomerular feedback responses, and sodium reabsorption, which are important in long-term BP regulation (Wilcox, 2002). High salt diet has been reported to affect both cardiac and renal functions negatively (Huang *et al.*, 2016; 2017). It could be that the negative impact of a high salt diet on the heart and kidney is mediated through its ROS generating effect. Orchidectomy attenuated the increase in lipid peroxidation, as observed in the orchidectomised and high salt diet group, while testosterone replacement following orchidectomy increased the lipid peroxidation level almost back to what is obtained in the sham groups. This result implicates testosterone in the oxidative stress promoting effect of a high salt diet. This current result is consistent with that obtained from the cardiac and renal weight indices experiment. The significant increase in the cardiac and renal weight indices of the high salt fed rats could be due to the increased oxidative stress observed in the serum of rat from this group because increased ROS generation has been implicated in cardiac and renal hypertrophy (Huang *et al.*, 2016; 2017). Likewise, the finding that orchidectomy reduced the cardiac and renal hypertrophic effect of a high salt diet is also consistent with the finding that orchidectomy attenuated the ROS generating effect of a high salt diet in this study.

Superoxide dismutase (SOD) is one of the most important antioxidant enzymes in the body (Berry *et al.*, 2001). Maintaining a balance between ROS generation and antioxidant system in the body is necessary in preventing oxidative stress and its consequential negative effect. The serum level of SOD was measured as an indicator of the antioxidant system in the body. The decrease in the level of SOD from serum of rats fed a high salt diet suggests the depletion of this important endogenous antioxidant system in this group of rats. This finding is consistent with the elevated level of lipid peroxidation in the serum of the rats fed a high salt diet. It implies that the available SOD is used up in quenching the generated ROS by a high salt diet as suggested by the elevated level of lipid peroxidation. This is consistent with the data on lipid peroxidation discussed above. An increase in the level of lipid peroxidation in the body indicates an increase in ROS generation e.g. O_2^- which is a substrate for SOD. SOD reacts with O_2^- converting it to hydrogen peroxide (H_2O_2) and H_2O_2 in another step reaction is converted to water and molecular oxygen (Berry *et al.*, 2001). The significant increase in SOD level of the orchidectomy plus high salt diet group when compared with sham plus high salt diet group suggests orchidectomy counteracted ROS generating effect of high salt diet therefore, reducing the usage of antioxidant system (SOD) or it increased the production

of SOD in the body as a way of preventing oxidative stress. The implication of testosterone in increased oxidative stress in animals fed a high salt diet is reinforced by elevation of lipid peroxidation and concomitant reduction in SOD level in testosterone replacement groups. These findings are indicative of testosterone – dependence of oxidative stress elevating effect of a high salt diet in the rat.

The result of the present experiment indicates that high salt diet decreased the total serum bilirubin levels. Bilirubin is not merely an end product of haem degradation but a potent endogenous antioxidant which can be destroyed by ROS (Stocker *et al.*, 1987; Vitek, 2017). Bilirubin usually acts by inhibition of NADPH oxidase (Lanone *et al.*, 2005) and of protein kinase C activity (Sano *et al.*, 1985; Amit *et al.*, 1993). The reduction in the serum bilirubin level in the high salt diet group could be as result of an increase in the ROS level in these groups of rats. An increase in the ROS level will consequently lead to a decrease in the level of antioxidant system such as bilirubin, as the latter is used to mop up the excess ROS. Some studies have reported a relationship between serum bilirubin and oxidative stress-mediated diseases, including coronary artery disease (Endler *et al.*, 2003; Novotny *et al.*, 2003), angiotensin II-mediated hypertension (Pflueger *et al.*, 2005), and renal ischemia-reperfusion injury (Adin *et al.*, 2005; Kirkby *et al.*, 2006). High salt diet generates ROS that consume bilirubin, this possibly might be the reason for the reduced serum level of bilirubin observed in rats fed a high salt diet in this study. Reduction in the serum bilirubin level observed in rats fed a high salt diet was attenuated by orchidectomy, while testosterone replacement re-established it. This finding implicates testosterone in the antioxidant activities of bilirubin. Although it is not imminently clear how testosterone reduces concentration of serum bilirubin, but sex disparity in the haem oxygenase activity, which is the rate-limiting enzyme to produce bilirubin has been reported. For instance, Toth *et al.*, (2003) reported that trauma and haemorrhage doubled the hepatic HO-1 expression, in female rats compared with male rats. Likewise Chin *et al.*, (2009) reported that subjects with higher bilirubin level showed a lower incidence of hypertension than did the subjects with lower bilirubin level, especially in females. Novotny and Vitek, (2003) reported that in humans, mildly increased serum bilirubin levels is a decreased risk for the development of coronary artery disease and atherosclerosis. Likewise, in hyperbilirubinaemic Gunn rats infused with angiotensin II, the rise in systolic blood pressure was markedly blunted, and oxidative stress was attenuated when compared with control (Pflueger *et al.*, 2005). The finding of the present study agrees with the above reports. In this study, an observation worthy of note is the lower blood pressure parameters in groups with higher serum level of bilirubin. Blood pressure reducing effect of orchidectomy is consistent with its serum bilirubin elevating effect in rats fed a high salt diet. Therefore, increasing serum bilirubin and SOD levels, either by promoting their production or preventing excess ROS generation which could have reduce the bilirubin and SOD bioavailability could be one of the mechanisms by which orchidectomy prevents or attenuates blood pressure elevation in rats fed a high salt diet. On the other hand, blood pressure elevating effect of testosterone could be partly mediated by decreased serum bilirubin and SOD, which increases oxidative stress and consequently promotes endothelial dysfunction.

In conclusion, testosterone potentiates the cardiac and renal hypertrophic as well as oxidative stress effect of a high salt diet, and these mechanisms appear to underlie the sexual differences in response to salt stress.

REFERENCES

Abdel-Salam OM, Czimmer J, Debreceni A, Szolcsányi J. and Mózsik G (2001). Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. An overview, *J Physiol* 95(1-6): 105-27.

Adewoye EO, and Salami AT (2013). Anti-ulcerogenic mechanism of magnesium in indomethacin induced gastric ulcer in rats. *Niger. J. Physiol. Sci.* 28: 193–199.

Allaire Joannie M, Crowley SM, Law HT, Chang S-Y, Ko H-J and Vallance BA (2018). The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends immunol* 39(9): 677-696

Brodie DA and Knapp PG (1966). The mechanism of the inhibition of gastric secretion produced by esophageal ligation in the pylorus-ligated rat. *Gastroenterology*. 50 (6): 787-95.

Pelucchi C, La Vecchia C, Bosetti C, Boyle P, and Boffetta P (2011). Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. *Ann. Oncol* 22: 1487–1499, doi:10.1093/annonc/mdq610

Corne SJ, Morrissey SM and Woods RJ (1974). Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J Physiol*., 242(2): 116-117.

El-Mehi AE and El-Sherif NM (2015). Influence of acrylamide on the gastric mucosa of adult albino rats

and the possible protective role of rosemary. *Tissue Cell.* 47(3): 273-83. doi: 10.1016/j.tice.2015.03.005.

Elegbe RA and Bambgose SA (1976). Protective dietary factors in experimental ulceration study of some Nigerian cereals and tubers. *Prostgrad. Med. Journ.* 52: 258-63

Engle E, Guth PH, Nishizaki Y, and Kaunitz, JD (1995). Barrier function of gastric mucus gel. *Am J Physiol* 269:G994-999.

Exon JH (2006). A review of the toxicology of acrylamide. *J. Toxicol. Environ. Health B Crit. Rev.* 9: 397-412.

Friedman, M (2003). Chemistry, biochemistry and safety of acrylamide. A review: *J. Agricult. Food Chem.*, 51:4504-26

Gayle JM, Blikslager AT and Jones SL (2000). Role of neutrophils in intestinal mucosal injury. *J Am Vet Med Assoc* 217:498-500.

Goel RK, Chakraharty A, and Sanyan AK (1985). The effect of biological variables on the antiulcerogenic effect of vegetable plantain banana. *Planta. med*. 2:82–88.

Goel RK and Sairam K (2002). Anti-ulcer drugs from indigenous sources with emphasis on Musa sapientum, Tamrabhasma, Asparagus racemosus and Zingiber officinale, *Indian J. Pharmacol.* 34: 100–110.

Gonda K, Shibata M, Sato Y, Washio M, Takeshita H, Shigeta H, Ogura M, Oka S, and Sakuramoto S. 2017. Elevated neutrophil-to-lymphocyte ratio is associated with nutritional impairment, immune suppression, resistance to S-1 plus cisplatin, and poor prognosis in patients with stage IV gastric cancer. *Mol Clin Oncol.* 7(6): 1073-1078.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, and Tannenbaum SR (1982). Analysis of nitrate, nitrite and [15N] nitrate in biological fluids, *Anal. Biochem.* 126 (1): 131–138.

Hammer AM, Morris NL, Earley ZM and Choudhry, MA (2015). The First Line of Defense: The Effects of Alcohol on Post-Burn Intestinal Barrier, Immune Cells, and Microbiome. *Alcohol Res*. 37(2): 209-22.

Hassan A, Martin E, and Puig-Parellada P (1998). Role of antioxidants in gastric mucosal damage induced by indomethacin in rats, Methods Find. *Exp. Clin. Pharmacol.* 20 (10) 849–854.

Hogervorst, JG, Schouten LJ, Konings EJ, Goldbohm RA, and van den Brandt PA (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev*.16 (11):2304–2313.

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, and van den Brandt PA (2008). Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *Am J Clin Nutr.* 87(5): 1428–1438.

Ige AO, Adewoye EO, Okwundu NC, Alade OE, and Onuobia PC (2016). Oral magnesium reduces gastric mucosa susceptibility to injury in experimental diabetes mellitus. *Pathophysiology*, 23(2): 87-93.

Ige AO, Adeyomoye OI, Adewoye EO (2015). Effect of oral Magnesium treatment on Haematological, Biochemical profile and Liver Glycogen content in Male Wistar rats. *Archives of Basic and Applied Medicine*, 3(2): 65 – 70

Lanas A (2008). Role of nitric oxide in the gastrointestinal tract. *Arthritis Res Ther*. 10 Suppl 2(Suppl 2): S4.

Larsson SC, Akesson A, and Wolk A (2009). Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. *Cancer Epidemiol Biomarkers Prev.* 18(6): 1939– 1941.

Lehning EJ, Balaban CD, Ross JF and LoPachin R M (2002). Acrylamide neuropathy, II Spatiotemporal characteristics of nerve cell damage in rat brainstem and spinal cord. *NeuroToxicology* 23: 415-429.

LoPachin RM, Foss JF, and Lehning EJ (2002). Nerve Terminals as the Primary Site of Acrylamdie Action: A Hypothesis. *NeuroToxicology*, 23: 43-59.

Misra HP and Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.*, 247(10): 3170–3175.

Mottram DS, Wedzicha BL and Dodson AT (2002). Acrylamide is formed in the Maillard reaction. *Nature* 419(6906): 448-9.

Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K (2003). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *Br J Cancer*. 13, 88(1):84–89.

Muscara MN and Wallace JL (1999). Nitric oxide V. Therapeutic potential of nitric oxide donors and inhibitors. *Am J Physiol* 276: G1313-1316.

Pavelka M., and Roth, J. (2010). Parietal Cells of Stomach: Secretion of Acid. In: Functional Ultrastructure. Springer, Vienna.

Pruser KN and Flynn NE (2011). Acrylamide in health and disease. Front., Biosci., (Scholar edition), 3: 41-51

Raji Y., Oyeyemi W. A., Shittu, S. T., and Bolarinwa A. F., (2011). Gastro-protective effect of methanol extract of Ficus asperifolia bark on indomethacin-

induced gastric ulcer in rats, *Nig J Physiol Sci*. 26(1): 43–8.

Rawi Sayed M, Marie Mohamed-Assem S., Fahmy Sohair R and El-Abied Salma A (2012). Hazardous effects of acrylamide on immature male and female rats. *Afr J Pharm Pharmaco* 6(18), pp. 1367-1386.

Saheed S, Taofeeq G, Taofik SO, Oladipipo AE, Olarewaju SA, Ismaila NO and Abdulazeez B (2015). Indomethacin-induced Gastric Ulceration in Rats: Protective Roles of Spondias mombin and Ficus exasperate. *Toxicol.Rep.* 2: 261 – 267

Saranya, P. and Geetha, A. (2011). A biochemical study on the gastroprotective effect of hydroalcoholic extract of Andrographis paniculata in rats. Indian, J Pharmacol. 43(4):402–408. doi: 10.4103/0253-7613.83110

Sedlak J, and Lindsay RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal.Biochem*, 25,192–205.

Sinha AK (1972). Colorimetric assay of catalase, *Anal., Biochem.*, 47: 389-394

Virk-Baker MK, Nagy TR, Barnes S, and Groopman J (2014). Dietary Acrylamide and Human Cancer: A Systematic Review of Literature. *Nutr Cancer*. 66(5): 774–790 doi:10.1080/01635581.2014.916323.

Wallace JL (2008). Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn't the Stomach Digest Itself? *Physiol Rev* 88: 1547–1565, doi:10.1152/physrev.00004.2008.

Wormsley KG (1974). The pathophysiology of duodenal ulcer. *Gut* 15:59-81.

Zamani, E., Shokrzadeh, M., Fallah, M. and Shaki, F. (2017). A review of acrylamide toxicity and its mechanism. mazums-pbr. 3 (1): 1-7

Zenick H, Hope E and Smith MK (1986). Reproductive toxicity associated with acrylamide treatment in male and female rats. J. Toxicol. Environ. Health, 17: 457.

J. Afr. Ass. Physiol. Sci.7 (1): 17-22, July, 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

The relationship between physical activity level and obesity among medical students at International University of Africa, Sudan

N.A. Mohammed and H.S. Ahmed*

Department of Physiology-Faculty of Medicine –International University of Africa, Khartoum, Sudan

Keywords:
Obesity, physical activity, medical students, Sudanese.

ABSTRACT

Background: Obesity is a leading preventable cause of death worldwide, with increasing rates in adults and children . Several studies had investigated the relationship between physical activity and obesity but this relationship is still controversial and few attempts were made to study this relationship among medical students especially in Sudan. Therefore, the aim of this study was to determine the relationship between physical activity level and obesity among the medical students of the International University of Africa. **Methods**: This was cross section descriptive facility- based study conducted among 200 medical students at International University of Africa (IUA) selected by stratified random sampling. Data were collected by self-administered questionnaire which included sociodemographic data. Anthropometric measurements were done for each participant. Physical activity level was determined by the short form of the international physical activity questionnaire (SF-IPAQ). Data were analyzed using SPSS version 23 program. Descriptive data were presented as means and standard deviations of means or percentages. Correlation analysis and Chi-Square test were used to assess associations/ differences between different variables. $P<0.05$ was considered statistically significant **Results:** The mean age of participants was 21.76±2.48 years. The prevalence of obesity among students was 6.5% and most of the students had either low or moderate physical activity level (24.5% and 48.5% respectively). Male students had higher level of physical activity than female students ($P<0.001$). There was no significant relationship between physical activity level and obesity in both male and female students. **Conclusion:** The study revealed insignificant relationship between obesity and physical activity level.

INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health (WHO, 2015). Obesity is a leading preventable cause of death worldwide, with increasing rates in adults and children (WHO, 2015). In 1997 the WHO formally recognized obesity as a global epidemic (WHO, 2015) and in 2013, the American Medical Association classified obesity as a disease (Andrew Pollack, 2013). In 2014, 600 million adults (13%) and 42 million children under the age of five were obese (WHO, 2015). Kuwait, Bahrain, Saudi Arabia and United Arab Emirates are in the list of top ten countries worldwide in term of obesity (Ono and Guthold, 2005). Recent surveys found that in Kuwait, 48% of females and 36% of males were obese, whereas 77% of females and 74% of males were overweight (World Health Organization (WHO), 2009). In Saudi Arabia 44% of the female population and 28% of the male population were found to be obese. However, 71% of women and 66% of men were reported to be overweight (World Health Organization (WHO), 2009). A study conducted in Sudan revealed that the combined prevalence of obesity and overweight is 37.50% (18.75% in each case) among the included subjects (Ahmed et al., 2011). Sedentary lifestyle plays a significant role in obesity. The level of physical activity is reduced in developing countries and sedentary behaviors have risen (Musaiger et al., 2016), and there has been a large shift towards less physically demanding work (World Health Organization, 2004). Currently at least 30% of the world's population gets insufficient exercise, this is primarily due to increasing

*Address for correspondence:
Email: humeda2004@hotmail.com

use of mechanized transportation and a greater prevalence of labor-saving technology in the home (World Health Organization, 2004), which may contribute to the occurrence of obesity and other chronic diseases (Musaiger et al., 2016). Prospective observational population studies in adults, from the last 20 year, with physical activity measured at baseline were few, and had given inconsistent results with regard to the effect of physical activity on body weight change and development of obesity (Andersen et al., 2000). Several studies reported a negative association between physical activity and weight gain, i.e. that lower physical activity predicts higher subsequent weight gain (Schmitz et al., 2000; Sherwood et al., 2000; Wagner et al., 2001; Wenche and Holmen, 2004). On the other hand some studies did not find an association (Rainwater et al., 2000; Ball, 2002) and one study reported a reverse association, suggesting that higher baseline levels of BMI predict future low levels of physical activity (Petersen et al., 2004).These discrepancies in results may be due to variations in sample size, study designs, publication bias and presence of confounding factors (Wareham et al., 2005). It has been suggested that physical activity at baseline is not related to weight gain, but that the converse is true, as a higher BMI at baseline is related to an increased risk of later physical inactivity (Petersen et al., 2004). This raises important questions about reverse causality. However, it is difficult to determine the direction of causality for this type of data because of the marked difference in the measurement precision of physical activity and obesity (Wareham et al., 2005). Longitudinal studies that use more accurate measurement of both activity and weight change might be able to more accurately estimate the true relationship between changes in these measures (Wareham et al., 2005).However, ultimately there remains a 'chicken and egg' argument, which may not be resolvable using observational data (Wareham et al., 2005).

Since medical student are the future doctors and role models in our community. They will have heavy stressful work in future and they need to be healthy and to keep themselves away from risk factors of diseases like obesity. Doctors' behavior doesn't just impact them and their families it had an impact on all community (Yousif et al., 2019). Although several studies had been conducted worldwide to determine the relationship between obesity and physical activity among medical students, to the best of our knowledge minor attempts were made to investigate this relationship among Sudanese medical students. Therefore, the aim of this study was to determine the relationship between physical activity level and obesity among the medical students of the International University of Africa in Khartoum, Sudan.

METHODS

This was observational descriptive cross-sectional facility-based study conducted among medical students at the International University of Africa. Any student with chronic diseases like chronic renal failure, liver failure, tuberculosis or endocrine disorders was excluded from the study. Two hundred students, selected by systematic random sampling, were included in the study. Sociodemographic data were collected by self -administered questionnaire (age, gender). Weight was measured using normal weighing scale with subject taking off shoes and recorded to the nearest 0.5 Kg. Height was measured using a measuring tape, with the individual standing straight next to the wall, with the heels, buttocks, shoulders and occiput touching the wall without shoes and recorded to the nearest 0.1 cm. Body Mass Index was calculated from the anthropometric data collected using the following equation:

BMI = weight (Kg)/Height2 (m2) (WHO, 2015)

Physical activity level was assessed using the short form international physical activity questionnaire (IPAQ) which assesses the individuals physical activity in the past 7 days as part of their everyday lives, which includes the following domains like walking, moderate activity which requires moderate physical effort like carrying light loads and double tennis and vigorous activity which requires hard physical effort like heavy lifting, digging and aerobics; and all these activities should have taken at least 10 minutes at a time. Scores were given for each domain which was converted into MET- minutes/week. The following values were used for the analysis of IPAQ data: Walking = 3.3 METs, Moderate PA = 4.0 METs and Vigorous PA = 8.0 METs (Research, 2004). Finally, the candidates were categorized into low, moderate and high physical activity groups based on their MET values (Brisbois et al., 2012).

For further analysis the participants were classified into male and female students (96 and 104 respectively). Data were analyzed using SPSS version 23. Descriptive data were presented as means and standard deviations of means or percentages. Correlation analysis was used for assessing associations between BMI and physical activity level. Chi-square test with 95% confidence level was used to determine the relationship between the gender and physical activity and between gender and obesity. $P<0.05$ was considered statistically significant.

Ethical consideration:
The study was approved by the ethical committee at the international university of Africa and written consent was obtained from each participant.

RESULTS

The mean age of students was 21.76 years ±2.48. Fifty two percent (104) of the study participants were females while 48% (96) were males. The mean weight of the students was 65.17 kg ±15.5. Their mean height was 1.69 m±0.09. The mean body mass index (BMI) was 22.48±4.42 **(**Table 1)

Table 1: Anthropometric measurements of students (N=200)

	Weight (Kg)	Height (m)	BMI (Kg/m2)
Mean	65.17	1.69	22.48
SD	15.51	0.09	4.43

According to the WHO classification of obesity, more than half of male and female medical students were normal (61.5% and 56.7 respectively), while 11.5% of males and 18.3% of females were overweight were and only 9.4 % of males and3.8% of females were obese. Chi-square test revealed no significant difference in BMI classification between male and female students (P value = 0.22) **(Fig. 1).** Our study shows that 24.5% of student had low physical activity level, 48.5% had moderate and 27 % had high physical activity level. When the analysis was applied to each sex separately, 39.6% of male students had high level of physical activity than female students (39.6% and 15.4% respectively) while 42.7% of male and 53.8 % of females had moderate level. Female students had lower level of activity than males (30.8% and 17.7% respectively). Chi-square test revealed significant difference in physical activity level between male and female students (Chi-square value = 15.85, P value <0.001) (Fig. 2). Pearson correlation test shows insignificant relationships between physical activity and BMI in both male and female students (Pearson correlation = 0.05, P value =0.33 for males and Pearson correlation = 0.25, P value= 0.16 for females). (Figs. 3A and B).

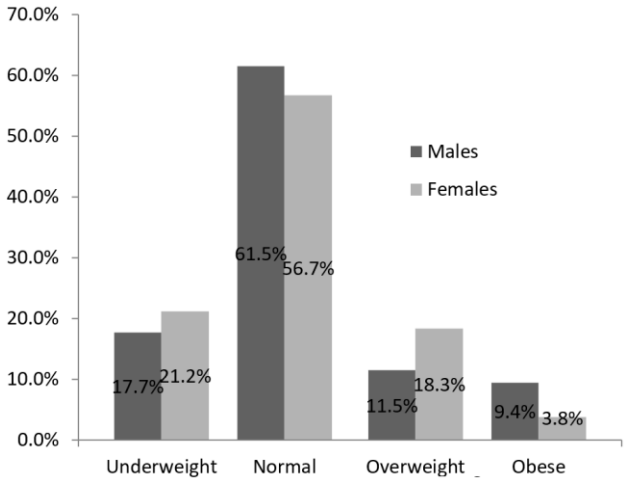

Fig. 1. Association between gender and Body mass Index classification Chi-square value = 4.38 P-value =0.22

The prevalence of obesity among medical students, observed in this study, is in consistence with various studies conducted nationally and worldwide. In this study 6.5% of the undergraduate medical students were obese, which is less than the prevalence among medical students at Ribat university 9.2% (Abdalla and Mohamed, 2008) . However, it is similar to Ajman study in which the prevalence was 6.9% (Ahmed et al., 2015).

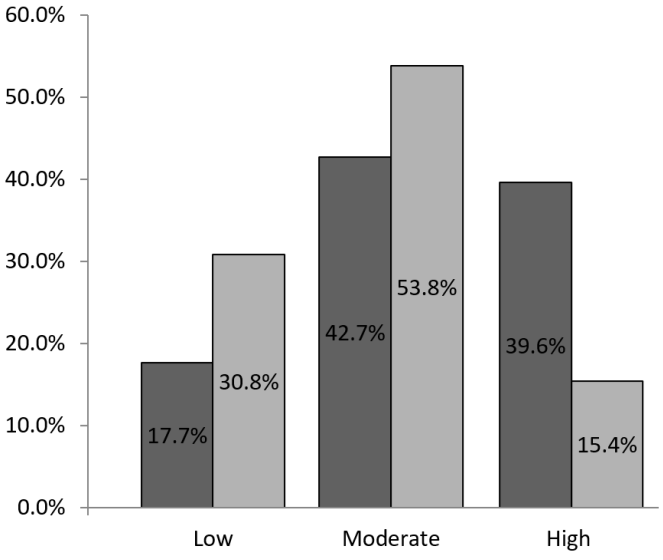

Fig. 2. Association between gender and Physical activity (Chi-square value = 15.85, P-value of association test < 0.001)

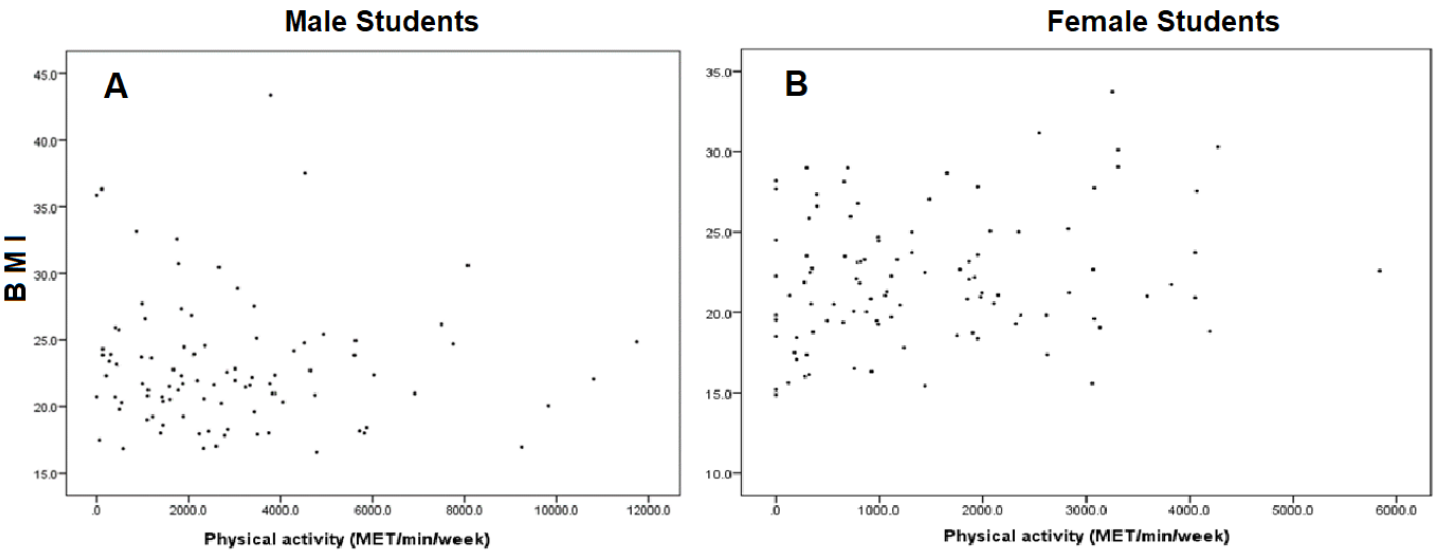

Fig. 3. The relationship between physical activity and BMI across gender (A: Male students, B: Female students)

medical students at Ribat university 9.2% (Abdalla and Mohamed, 2008) . However, it is similar to Ajman study in which the prevalence was 6.9% (Ahmed et al., 2015) . . An Indian study conducted at Azeezia medical college showed a prevalence of 6.3%(Napolean and Stephen, 2014) , another closely related result are the Indian Kanchipuram 8.6%(Kokila Selvaraj, 2013) and west Bengal 9%(M. Basu, K. Sarkar, B. Shahbabu, S. Ray, G. Barik, 2016) studies performed on medical students of both districts, slightly lower figures 4.3% were seen in the study of Crete Greece(Bertsias et al., 2003), the Malaysian study by Boo N Y et al. 2010 (3.3%)(Boo N Y, Chia G J Q, Wong L C, Chew R M, Chong W, 2010) and the AIMST University in Malaysia which showed a prevalence of 5.2%(Gopalakrishnan et al., 2012).

The impact of exercise intensity on change in body composition is uncertain (Ekelund et al., 2007). Most of the students in this study are minimally active 48.5% and highly active 27% followed with least figures by inactive individuals with 24.5% this was in consistence with the Napolean Reny et al. 2014 study which found that 40.2% of students were doing exercise of which 15% did daily exercise and 25% did regular exercise while the rest do exercise occasionally; 59.8% of candidates were physically inactive (Napolean and Stephen, 2014). In the Wattanapisit A et al. 2016 study, less than half of participants (49.5%) were physically active (Wattanapisit et al., 2016) , in line with this is the Basu et al. 2016 results which concluded that only 34% of the study population had habit of regular exercise(M. Basu, K. Sarkar, B. Shahbabu, S. Ray, G. Barik, 2016). This was not the case in the sandheep sugathan et al. 2014 study were overweight and obese students did engage in exercise for more than 60 minutes duration per week, but was not meeting the required physical activity level (Sugathan and Bagh, 2014). The present study showed significant high physical activity levels among male compared to female students. This finding in in agreement with previous studies (Rao et al., 2012; Al-Asousi and El-Sabban, 2016; Wattanapisit et al., 2016) However, other studies either disclosed insignificant difference (Bakr, Ismail and Mahaba, 2002; Alkahtani and Awad, 2016)or females predominance(Bergier et al., 2012)

In the present study, no significant association was found between the physical activity level and obesity in both sexes which is supported by the studies of Napolean Reny et al. 2014 (Napolean and Stephen, 2014), Boo et al. 2010 (Boo et al 2010); Wattanapisit et al. (2016) as well as the Kanchipuram study which also revealed that physical activity and obesity were statistically insignificantly related; although, most participants follow a sedentary lifestyle e(Kokila Selvaraj, 2013). In the Swedish prospective case-control study by U Ekelund et al. 2007 Physical activity and Physical Activity Energy Expenditure are only weakly related to gain in Body Weight and Fat Mass, therefore among obese individuals change in activity level was not related to change in BW and FM (Ekelund et al., 2007) which was also in line with our study. But in some of the studies conducted on medical students, physical inactivity was significantly associated with obesity; this is the case in Basu et al. 2016(M. Basu, K. Sarkar, B. Shahbabu, S. Ray, G. Barik, 2016) and sandheep sugathan et al. 2014 (Sugathan and Bagh, 2014) results.

Conclusion: In this study, most of medical students had

low to moderate physical activity levels and male students had higher activity level than females. Male students had more physical activity level than females. There was no significant relationship between obesity and physical activity level. Further large-scale cohort or interventional studies are needed to reinvestigate this relationship.

ACKNOWLEDGMENT

The authors would like to thank department of physiology at International University of Africa for their great help and support. Also, many thanks to the medical students who willingly participated in this study.

REFERENCES

Abdalla, S. M. and Mohamed, E. Y. (2008) 'Obesity Among Medical Students of The National Ribat University , Khartoum 2008', (10), pp. 16–19.

Ahmed, Aisha M. B; Ahmed, Hoyam, A; Mohammed, Tagwa, E; Alawad, Samia, S. (2011) 'Prevalence of obesity and overweight among the female students in basic school levels in Omdurman- Sudan', Sudan Medical Laboratory Journal, 1(2), pp. 20–25.

Ahmed, E. A. et al. (2015) 'Obesity Among University Students: A cross-Sectional Study in Ajman, UAE', Gulf Medical Journal, 4, pp. 14–23.

Al-Asousi, M. and El-Sabban, F. (2016) 'Physical Activity among Preclinical Medical Students at The University of Malaya, Malaysia', Journal of Nutritional Health & Food Science, 4(2), pp. 1–8.

Alkahtani, S. and Awad, N. (2016) 'Comparing the Physical Activity Patterns of Male and Female Students in the Preparatory Year in Saudi Arabia', Journal of Obesity & Weight Loss Therapy, 6(2). doi: 10.4172/2165-7904.1000308.

Andersen, L. B. et al. (2000) 'All-cause mortality associated with physical activity during leisure time, work, sports, and cycling to work.', Archives of internal medicine, 160(11), pp. 1621–8. doi: 10.1001/archinte.160.11.1621.

Andrew Pollack (no date) 'AMA recognizes obesity as a disease', NewYork Times.

Bakr, E. M., Ismail, N. A. and Mahaba, H. M. (2002) 'Impact of life style on the nutritional status of medical students at Ain Shams University.', The Journal of the Egyptian Public Health Association, 77(1–2), pp. 29–49. Available at: https://www.scopus.com/inward/record.uri?eid=2-s2.0-33847021241&partnerID=40&md5=38915ad453ad2b5f72912c7a0ec31dc5.

Ball K, B. W. & C. D. (2002) 'Who does not gain In, weight? Prevalence and predictors of weight maintenance young women', International Journal of Obesity and Metabolic Related Disorders, 26, pp. 1570–1578.

Bergier, J. et al. (2012) 'Physical activity of Polish adolescents and young adults according to IPAQ: A population-based study', Annals of Agricultural and Environmental Medicine, 19(1), pp. 109–115.

Bertsias, G. et al. (2003) 'Overweight and obesity in relation to cardiovascular disease risk factors among medical students in Crete, Greece', BMC Public Health, 3(1), p. 3. doi: 10.1186/1471-2458-3-3.

Boo N Y, Chia G J Q, Wong L C, Chew R M, Chong W, L. R. C. N. (2010) 'The prevalence of obesity among clinical students in a Malaysian medical school The prevalence of obesity among clinical students in a Malaysian medical school', Singapore Med J, 51(2), pp. 126–132.

Brisbois, T. D., Farmer, A. P. and Mccargar, L. J. (2012) 'Obesity Diagnostic and Prevention Early markers of adult obesity : a review', International Association for the Study of Obesity, 13(April), pp. 347–367. doi: 10.1111/j.1467-789X.2011.00965.x.

Ekelund, U. et al. (2007) 'Does physical activity equally predict gain in fat mass among obese and nonobese young adults?', International journal of obesity (2005), 31(1), pp. 65–71. doi: 10.1038/sj.ijo.0803361.

Gopalakrishnan, S. et al. (2012) 'Prevalence of overweight/obesity among the medical students, Malaysia.', The Medical journal of Malaysia, 67(4), pp. 442–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23082463.

Kokila Selvaraj, P. S. (2013) 'A Study on the Prevalence of Overweight and Obesity among Medical Students of Kanchipuram District Introduction ':, National Journal of Research in Community Medicine, 2(2), pp. 140–144.

M. Basu, K. Sarkar, B. Shahbabu, S. Ray, G. Barik, S. C. and R. N. M. (2016) 'pattern and determinants of overweight and obesity among medical students of kalkota', International Journal of Pharmaceutical Sciences and Research, 7(1), pp. 377–386. doi: 10.13040/IJPSR.0975-8232.7(1).377-86.

Musaiger, A. O., Al-Khalifa, F. and Al-Mannai, M. (2016) 'Obesity, unhealthy dietary habits and sedentary behaviors among university students in Sudan: growing risks for chronic diseases in a poor country', Environmental Health and Preventive Medicine, 21(4), pp. 224–230. doi: 10.1007/s12199-016-0515-5.

Napolean, R. and Stephen, J. (2014) 'Prevalence of Overweight and Obesity Among Students of a Medical College in South India ':, Indian Journal of Clinical Practice, 25(4), pp. 333–337.

Petersen, L., Schnohr, P. and Sørensen, T. I. a (2004) 'Longitudinal study of the long-term relation between physical activity and obesity in adults.', International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity, 28(1), pp. 105–12. doi: 10.1038/sj.ijo.0802548.

Rainwater DL, Mitchell BD, Comuzzie AG, VandeBerg JL, S. and Changes, M. & M. J. (2000) 'Association among 5-year in weight, physical activity, and cardiovascular disease risk factors in Mexican Americans', American journal of epidemiology, 152(974–982).

Rao, C. R. et al. (2012) 'Practice of physical activity among future doctors: A cross sectional analysis', International Journal of Preventive Medicine, 3(5), pp. 365–369. doi: 10.1017/CBO9781107415324.004.

Research, I. C. (2004) Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) - Short Form ,.

Schmitz KH, Jacobs DR Jr, Leon AS, S. P. & S. B. and Ten (2000) 'Physical activity and body weight: associations over Development, years in the CARDIA study. Coronary Artery Risk And, in Young Adults.', International Journal of Obesity Related Metabolic Disorders, 24, pp. 1475–1487.

Sherwood NE, Jeffery RW, French SA, H. P. & M. D. (2000) 'Predictors of weight gain in the Pound of Metabolic study.', International Journal of Obesity and Related Disorders, 24, p. 395–403.

Sugathan, S. and Bagh, D. S. (2014) 'Prevalence and Correlates of Overweight and Obesity among Medical Students in Ipoh, Malaysia', Academic Medical Journal of India, II(1), pp. 22–24.

T. Ono, R. Guthold, and K. S. (2005) WHO Global Comparable Estimates. Available at: https://apps.who.int/infobase/Comparisons%0A.aspx. Wagner A, Simon C, Ducimetiere P, Montaye M, B. V and And, Y. J. et al. (2001) 'Leisure-time physical activity With, regular walking or cycling to work are associated PRIME, adiposity and 5 y weight gain in middle-aged men: the Metabolic, Study', International Journal of Obesity and Related Disorders, 25, pp. 940–948.

Wareham, N. J., van Sluijs, E. M. F. and Ekelund, U. (2005) 'Physical activity and obesity prevention: a review of the current evidence', Proceedings of the Nutrition Society, 64(2), pp. 229–247. doi: 10.1079/PNS2005423.

Wattanapisit, A. et al. (2016) 'Physical activity among medical students in Southern Thailand: a mixed methods study', BMJ Open, 6(9), p. e013479. doi: 10.1136/bmjopen-2016-013479.

Wenche DB, Holmen J, K. O. & M. K. (2004) 'Leisure time physical activity and change in body mass Health, an 11-year follow-up study of 9357 normal weight healthy women 20–49 years old.', Journal of Women's Health, (13), pp. 55–62.

WHO (2015) Obesity and overweight fact sheet No.311, January, 2015

World Health Organisation (WHO) (2009) World Health Statistics.

World Health Organization (2004) 'Promoting Mental Health: Concepts, Emerging Evidence, Practice: Summary Report. Geneva, World Health Organization. Retrieved', Http://Www.Who.Int/Mental_Health/Evidence/En/Promoting_Mhh.Pdf, p. 68. doi: 10.1111/j.1466-7657.2004.00268.x.

Yousif, M. M., Kaddam, L. A. and Ahmed, H. S. (2019) 'Correlation between physical activity , eating behavior and obesity among Sudanese medical students Sudan'. BMC Nutrition, 5(6), pp. 1–8.

J. Afr. Ass. Physiol. Sci. 7 (1): 23-29, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Modulatory role of lauric acid supplement on lipid peroxidation and some antioxidant enzymes activity in high fat diet, streptozotocin-induced type 2 diabetic male wistar rats.

E. A. Alex[1*], Y. Tanko[1], K. Muhammed[1], A.B. Dubo[1], F.M. Yahuza[1], D.C. Ejiogu[1], N.D Aisha[1], M. Olubiyi[5], P.O. Ikeogu[4], O.O. Ayegbusi[3]

[1]Departments of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria, [2]College of Medicine, Kaduna State University, Kaduna State, [3]Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital Zaria, Kaduna State, Nigeria, [4]Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University Zaria, Kaduna State and [5]Kogi State University, Ayangba, Nigeria

Keywords:
Lipid peroxidation; antioxidant enzymes; lauric acid; metformin; type 2 diabetes

ABSTRACT

Background: Diabetes mellitus is a major health challenge in the world and is diagnosed by the presence of sustained hyperglycemia (high glucose levels in the blood). Oxidative stress is known to be actively involved in the onset and progression of diabetes and its complications. Antioxidants have important roles in biological systems by scavenging free radicals which may result in oxidative damage of biological molecules such as lipids, proteins and DNA. **Aim:** This study was designed to evaluate the effect of lauric acid on lipid peroxidation (serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase and Catalase) activities in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats. **Study Design:** Thirty-five apparently healthy male wistar rats aged 6-8 weeks, weighing between 70-90 g were assigned into seven groups of five animals each (n=5) and administered graded doses of lauric acid supplement after validation of diabetes for a period of twenty-one (21) days. **Methodology:** Group 1: (Normal control), Group 2: (Diabetic control untreated), Groups 3: (Normoglycemic) received 125 mg/kg Lauric acid, Group 4, 5 and 6 were administered 125, 250 and 500 mg/kg body weight of lauric acid, Group 7: (Standard control) received metformin 100 mg/kg. At the end of twenty-one (21) days, rats were anaesthetized using ketamine and xylazine at 75 and 25 (mg/kg). Blood samples were taken from all treated groups for evaluation of serum MDA, SOD and CAT level. **Results:** Serum malondialdehyde (MDA) levels were significantly ($P < 0.05$) reduced (1.32±0.04, 1.40±0.04 and 1.42 ± 0.06 nmol/l) respectively when compared with the diabetic control (untreated) group with a value of (2.25±0.10 nmol/l), while there was up-regulated activities of serum endogenous antioxidant enzymes: SOD (1.97±0.08, 2.02±0.16, 1.98±0.12 IU/L), CAT (44.5±0.64, 43.2±0.85, 43.7±0.85 IU/L) relative to diabetic control (untreated) (1.35±0.02 and 34.0±0.91 IU/L) respectively. **Conclusion:** In conclusion, lauric acid decreases lipid peroxidation while increasing serum antioxidant activity in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats after 21 days oral administration.

INTRODUCTION

Diabetes mellitus is a term that describes a metabolic syndrome of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and proteins metabolisms resulting from defects in insulin secretion, insulin action or both (WHO, 2016). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels (Cheungpasitporn *et al.*, 2016; Kalteniece *et al.*, 2018; Punthakee *et al.*, 2018). Diabetes represents one of the world's four major non

*Address for correspondence:
Email **:** jenoch22@yahoo.com

communicable disease that are of great public health concern in the healthcare system. It accounts for about 1.6 million deaths each year globally (WHO, 2017). In 2015 in Nigeria alone, 1.56 million cases were reported including 105,091 deaths (IDF, 2015) and about 95% of all cases reported were attributed to Type-2 diabetes mellitus (T2DM), (IDF, 2015; WHO, 2016). In spite of advancements recorded to date on basic and clinical investigations into diabetes, a properly effective final remedy does not exist.

Oxidative stress contributes significantly to the patho-physiology of several diseases which include diabetes. It is believed that in the onset and progression of late diabetic complication, free radicals play a major role due to their ability to cause alteration in enzymatic systems, lipid peroxidation, impaired glutathione metabolism and DNA damage (Ayepola, 2014; Ullah, *et al.,* 2016). Lauric acid (LA) or a dodecanoic acid is saturated fatty acids with a 12 carbon atom chain thus falling into the group of medium chain fatty acids (C6-C12) (Anzaku *et al.,* 2017). LA is the primary fatty acid of coconut oil which is present at approximately 45–53 % and its metabolic and physiological properties account for many of the medicinal and healing properties attributed to coconut oil. It is found in a wide variety of fruits, seeds (Silva *et al.,* 2015) and as a human milk component (Silberstein *et al.,* 2013).

Numerous methods for modeling diabetes have been developed. Among these techniques, the construction of a diabetic model with streptozotocin (STZ) combined with high-fat diet has been widely used. STZ, a monofunctional nitrosourea derivative, was first isolated from Streptomyces achromogene. It is also an alkylating agent and can lead to cell death. When STZ acts on β-cells, it may lead to beta cell death, insulin release decreases, and increase in blood glucose in the body. In addition, a high-fat diet can lead to obesity and insulin resistance. Therefore, diabetes induced by a high-fat diet combined with STZ can better mimic the pathogenesis of human type 2 diabetes and can be used widely in studies on hypoglycemic activity (Chen *et al.,* 2018).

This study was designed to evaluate the effect of oral administration of lauric acid on lipid peroxidation (using serum malondialdehyde concentration) and some antioxidant enzymes (superoxide dismutase and Catalase) activities in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats.

MATERIALS AND METHODS

Experimental Site

This study was carried out in the Physiology Laboratory of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Kaduna state Nigeria. Zaria is located between latitudes 110 and 3′ N, and between 70 and 42′ E, at an altitude of 670 m above the sea level and 664 km away from the sea, in the Northern Guinea Savanna zone (Marthin, 2006)

Chemicals and Reagents

Lauric acid (white crystalline powder with CAS No-143-07-7, malaysia), Streptozotocin (Bristol sigma, Lagos), Metformin, Chloroform, (Zayo sigma company, Jos, Nigeria) Normal saline (0.9% w/v), Methylated spirit, Tween 80, Citrate buffer, fructose solution (Kem Light Laboratories PVT Ltd, India), Simas Margarine (PT Salim Ivomas Pratama Tbk, Indonesia), Groundnut meal, and ground nut oil were purchased in Samaru-Zaria market, Kaduna Nigeria. All chemicals were commercially obtained and were of analytical grade.

Experimental animals

Thirty-five (35) wistar rats aged 6-8weeks weighing 70-100 g were used for this study. The wistar rats were purchased from Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in plastic cages with bedding material (saw dust), in the animal house of department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, and were provided with food (pelletized growers mash) and water *ad libitum* for a period of two (2) weeks to acclimatize before the study began.

Preparation of High Fat Diet

Composition of the high fat diet was 25% margarine, 25% ground nut meal, 15% ground nut oil with 35% of the animal feed which was a modification of the composition described by Tanko *et al.,* (2016) and Okoduwa *et al.,* (2017).

Induction and Confirmation of Hyperglycaemia

Insulin resistance was induced by feeding the animals with high fat diet (25% margarine which is made up of (fat 99.9%, Emulsifiant E471, E322, E306, Beta Carotene C175130, Vitamin D 30,000 IU/kg), 25% ground nut meal, 15% ground nut oil with 35% of the animal feed (composed of crude protein 13%, fat 8%, crude fibre 15%, calcium 0.90%, available phosphorus 0.35%, methionine 0.37%, lysine 0.70%, metabolizable energy 2600kcal/kg) orally for a period of eight weeks along with 10% fructose solution as drinking water. On the 8th week, a single intraperitoneal dose (30mg/kg) of streptozotocin (STZ) dissolved in 0.1M fresh cold citrate buffer pH 4.5 was injected into overnight fasted

rats. The rats were provided 5% glucose solution as drinking water in the first 24 hrs after STZ induction (Srinivasan *et al*., 2005; Zhang *et al*., 2008; Wilson and Islam, 2012; Xiang *et al.*, 2018) as modified. After three (3) days, the blood glucose level of the rats were measured, and after two weeks, it was measured again and only animals with fasting blood glucose level greater than 11.1 mmol/L were considered diabetic (Xu *et al.*, 2017).

Experimental Design

Animal grouping

Thirty-five (35) wistar rats were divided into seven (7) groups containing 5 wistar rats each (n=5) as follows:

Group 1: Normoglycemic rats administered 1 ml/kg Distilled water via intragastric intubation once daily for three weeks

Group 2: Diabetic rats administered 1 ml/kg Tween 80 via intragastric intubation once daily for three weeks

Group 3: Normoglycemic rats administered 125 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 4: Diabetic rats administered 125 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 5: Diabetic rats administered 250 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 6: Diabetic rats administered 500 mg/kg of Lauric Acid via intragastric intubation once daily for three weeks

Group 7: Diabetic rats administered 100 mg/kg Metformin via intragastric intubation once daily for three weeks (Tikoo *et al.*, 2016)

Estimation of Biomarkers of Oxidative Stress and Lipid Peroxidation

Catalase (CAT) activity:

Catalase (CAT) activity was measured using the method described by Aebi (1984). Exactly 10µl of serum was added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of $0.041mM^{-1}\text{-}cm^{-1}$ was used to calculate the Catalase activity. Catalase Conc.= Absorbance/E. Catalase Activity = Catalase Con./Protein Conc. (mg/ml)

Superoxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity was determined by the method described by Fridovich (1989).

Principle:

The ability of superoxide dismutase (SOD) to inhibit auto-oxidation of adrenaline at pH 10.2 forms the basis of this assay.

Procedure:

An aliquant mixture of 0.2ml of the diluted micro some was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of freshly prepared 0.3mM Adrenaline (0.01g of adrenaline dissolved in 17ml of distilled water) and The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm

Calculations:

Increase in absorbance per minute = $(A_2 - A_1)/2.5$

% Inhibition = 100 - { (Incr. in absorbance for sample/Incr. in absorbance of blank) x 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

Estimation of lipid peroxidation (malondialdehyde):

Lipid peroxidation was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS). A principal component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. The assay for malondialdehyde was estimated colorimetrically by measuring malondialdehyde (MDA) by the method of Albro *et al.* (1986) and Das *et al.* (1990). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37%, 0.25N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank.

Lauric Acid Preparation and Administration

Lauric acid, due to its low solubility in water, was suspended in 1ml/kg of tween 80, and administered orally.

Ethical Approval

The rats were handled in accordance with the principles guiding the use and handling of experimental animals Ahmadu Bello University Zaria, Nigeria.

Statistical Analysis

Data collected was analyzed by one-way analysis of variance, (ANOVA), followed by *Tukey's post-hoc* test

which was used to compare the level of significance between the controls and treatment groups. Results were expressed as mean ± SEM. Statistical package for social science (SPSS) version 22.0 was used for the analysis and Values of $p < 0.05$ was considered significant.

RESULTS

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Malondialdehyde (MDA) level in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats.

Fig. 1 Shows, a significant increase (P < 0.05) in malondialdehyde (MDA) levels in the diabetic control (untreated) group (2.25±0.10 nmol/l) when compared with that of normal control (normal saline) (1.37±0.06 nmol/l) and a significant decrease (P < 0.05) in malondialdehyde level in groups treated with lauric acid at doses of 125 mg/kg (1.32±0.04 nmol/l), 250 mg/kg (1.40±0.04 nmol/l) and 500 mg/kg (1.42 ± 0.06 nmol/l) respectively when compared with the diabetic control (untreated) group with a value of (2.25±0.10 nmol/l)

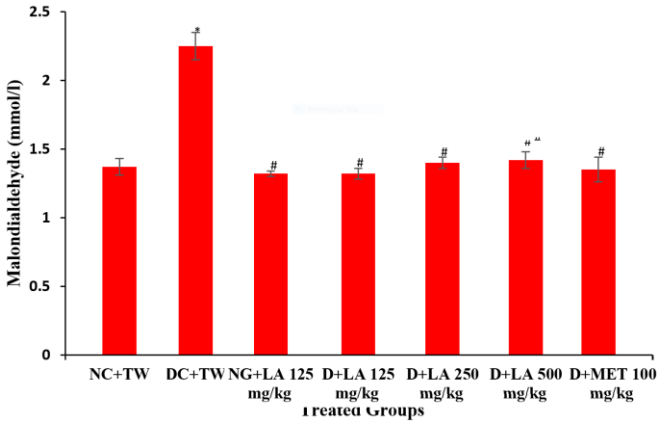

Fig 1. Se.rum Malondialdehyde (MDA) level in HFD/STZ-Induced Type 2 Diabetes mellitus in Male Wistar Rats Treated with Different Doses of Lauric acid for 21days. *= Means with this superscript are significant p< 0.05 compared to normal control #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated). DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 8.

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Superoxide Dismutase (SOD) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats.

The results (Fig. 2) show a significant (P < 0.05) increase in serum superoxide dismutase (SOD) activity in groups treated with lauric acid 125 mg/kg (1.97±0.08 IU/L), 250 mg/kg (2.02±0.16 IU/L) and 500 mg/kg (1.98±0.12 IU/L) as compared with diabetic control (untreated) (1.35±0.02). The result also shows significant increase (P < 0.05) in serum superoxide dismutase (SOD) activity in groups treated with lauric acid 125 mg/kg (1.97±0.08 IU/L), 250 mg/kg (2.02±0.16 IU/L) and 500 mg/kg (1.98±0.12 IU/L) as compared with standard control (metformin treated group) (1.80±0.07). However, only group treated with lauric acid 250 mg/kg (2.02±0.16 IU/L) shows significant increase (P < 0.05) when compared with normal control (1.97±0.08 IU/L)

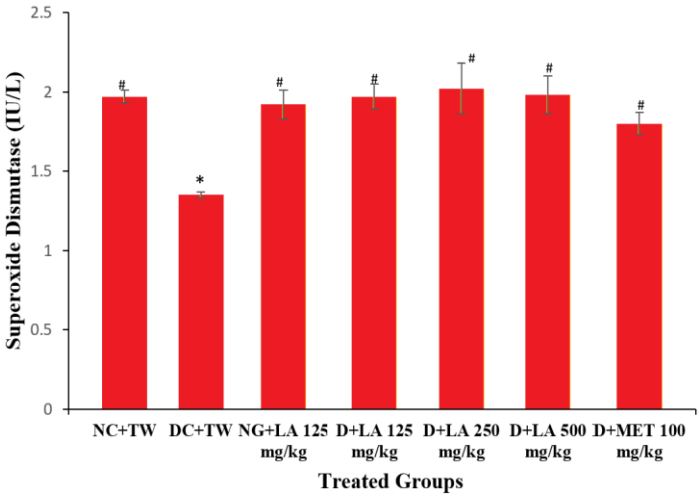

Fig. 2. Serum Superoxide Dismutase (SOD) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats Treated with Lauric acid and Metformin for 21days. *= Means with this superscript are significant p< 0.05 compared to normal control; #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated); DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 80

Effect of Three Weeks Oral Administration of Lauric Acid on Serum Catalase (CAT) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rat

The results in Fig. 3 show a significant (P < 0.05) increase in serum catalase (CAT) activity in groups treated with lauric acid 125 mg/kg (44.5±0.64 IU/L), 250 mg/kg (43.2±0.85 IU/L) and 500 mg/kg (43.7±0.85 IU/L) as compared with diabetic control (untreated) (34.0±0.91). The result also shows significant increase (P < 0.05) in serum catalase (CAT) activity in groups treated with lauric acid 125 mg/kg (44.5±0.64 IU/L), 250 mg/kg (43.2±0.85 IU/L) and 500 mg/kg (43.7±0.85 IU/L) as compared with standard control (metformin treated group) (40.7±0.47 IU/L).

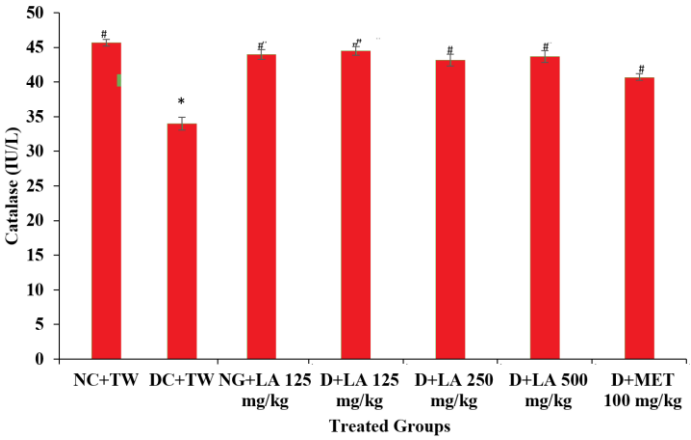

Fig. 3. Showing Serum Catalase (CAT) Activity in HFD/STZ-Induced Type 2 Diabetes Mellitus in Male Wistar Rats Treated with Lauric acid and Metformin for 21days. ; *= Means with this superscript are significant p< 0.05 compared to normal control; #= Means with this superscript are significant p< 0.05 compared to diabetic control (untreated); DC= Diabetic control, NC= Normal (Non-diabetic) control, NG= Normoglycemic, D = Diabetic, LA = Lauric acid, MET = Metformin, TW= Tween 80

DISCUSSION

Oxidative stress is a product of an imbalance between reactive oxygen species (ROS) such as superoxide anion (O_2^-) and the antioxidant defense systems such as superoxide dismutase (SOD). Antioxidants enzymes involved in the elimination of ROS include SOD, CAT and GSH, respectively. Antioxidant enzymes are a critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. They form the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress and act cooperatively at different sites in the metabolic pathway of free radicals (Cheng and Kong, 2011).

The present study showed a significant decrease in the activity of measured antioxidants enzymes (SOD and CAT) in diabetic control (untreated) rats when compared with normal control while there was a significant increase in the level of malondialdehyde (MDA) when compared with the normal control. There was also a significant increase in the activity of measured antioxidants enzymes (SOD and CAT) in diabetic rats treated with graded doses of lauric acid (125, 250, 500 mg/kg respectively) when compared with diabetic control untreated while there was a significant decrease in the level of malondialdehyde (MDA) when compared with diabetic control untreated. There was a significant increase in activity of antioxidant enzymes (SOD and CAT) and a significant decrease in MDA levels in diabetic rats treated with graded doses of lauric acid (125, 250, 500 mg/kg respectively) when compared with standard control (metformin) however only group that received 250 mg/kg lauric acid has a significant increase in SOD activity when compared with normal control. There was no significant difference between normoglycemic group that received 125 mg/kg lauric acid when compared with normal control (1 ml/kg distilled water).

This finding is consistent with the reports of various researchers (Cheng and Kong, 2011; Singh *et al.*, 2012; Cheng *et al.*, 2013 Iranloye *et al.*, 2013; Akinnuga *et al.*, 2016). This indicates a decrease in the antioxidant defense system and an increase in lipid peroxidation. Hyperglycemia is a main cause of increase production of ROS by generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins (Al-Faris *et al.*, 2010) which could lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in biological system. The SOD, a superoxide radical scavenging enzyme is considered the first line of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O_2^- radical to H_2O_2 and O_2^- thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. As a result, reduction in SOD activity in diabetic animals observed in the present study may be as a results of an increased formation of O_2^- radical which overwhelms the enzymes ability to counteract its effect and hence may reflects the cause of tissue injury (Singh *et al.*, 2012).

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide formed from the dismutation of superoxide anion by SOD to water (H_2O) and oxygen (O_2) which protects the tissues from highly reactive hydroxyl radicals (Onyeka *et al.*, 2012); however, treatment with graded doses (125, 250 and 500 mg/kg) of LA significantly increased the activities of the antioxidant enzymes (SOD and CAT) and also significantly reduced lipid peroxidation as seen in the increased levels of MDA in the treatment groups when compared with the diabetic control (untreated) group. SOD and CAT must have been up regulated by LA and this may indicate a pronounced antioxidant effect of the treatment given to the group. Since oxidative stress contributes significantly to the pathophysiology of diabetes and its complications substances that suppress oxidative stress might be therapeutically beneficial.

CONCLUSION

Lauric acid possesses potent antioxidant activity and also decrease lipid peroxidation by decreasing serum malondialdehyde concentration in HFD/STZ-induced type 2 diabetes mellitus in male wistar rat treated for twentyone days.

REFERENCES

Aebi, H. (1984). Catalase *in vitro. Methods in Enzymology*, 105: 121– 126.

Akinnuga, A. M., Jeje, S. O., Bamidele, O., Omu, I. E., Onun, T. B., Sunday, V. E. and Odu, O. P. (2016). Virgin Coconut Oil (Vco) Supplemented Diet Ameliorates Blood Glucose, Renal Tissue And Antioxidant Enzymes In Diabetic Rats. *Journal of International Research in Medical and Pharmaceutical Sciences.* 9(2): 49-53.

Albro, P. W., Corbett, J. T. and Schroeder, J. L. (1986). Application of the Thiobarbituric Acid Assay to the Measurement of Lipid Per- oxidation Products in Microsomes. *Journal of Biochemical Biophysical Methods*. 13:185-194

Al-Faris, N.A., Al-Sawadi, A.D. and Alokail, M.S., (2010). Effect of Samh seeds supplementation (*Mesembryanthemum forsskalei* Hochst) on liver enzymes and lipid profiles of streptozotocin (STZ)-induced diabetic Wistar rats. *Saudi Journal of Biological Sciences*, 17(1): 23-28.

Anzaku, A.A., Akyala, J.I., Juliet, A. and Obianuju, E.C. (2017). Antibacterial Activity of Lauric Acid on Some Selected Clinical Isolates. *Annala of Clinical Laboratory Research*; 5:2. doi:10.21767/2386-5180.1000170

Ayepola, O.R., Brooks, N.L. and Oguntibeju, O.O. (2014). Kolaviron Improved Resistance to Oxidative Stress and Inflammation in the Blood (Erythrocyte, Serum, and Plasma) of Streptozotocin-Induced Diabetic Rats. *Scientific World Journal*. 2014: 921080. doi: 10.1155/2014/921080

Chen, X., Tan, F., Yi, R., Mu, J., Zhao, X. and Yang Z. (2018). Effects of Lactobacillus on Mice with Diabetes Induced by High-Fat Diet with Streptozotocin (STZ). *Applied Sciences;* 8(8): 1249 DOI: 10.3390/app8081249

Cheng, D. and Kong, H. (2011). The effect of lycium barbarum polysaccharide on alcohol induced oxidative stress in rats. *Molecules*, 3(16): 2542–2550.

Cheng, D., Liang, B.and Yunhui Li, Y. (2013). Antihyperglycemic Effect of *Ginkgo biloba* Extract in Streptozotocin-Induced Diabetes in Rats. *BioMedical Research International, 2013:162724:* 7 pages.

Cheungpasitporn, W., Thongprayoon, C., Vijayvargiya, P., Anthanont, P. and Erickson, S.B. (2016). The risk for new-onset diabetes mellitus after kidney transplantation in patients with autosomal dominant polycystic kidney disease: A systematic review and meta-analysis. *Canadian Journal of Diabetes*, 40(6): 521–528.

Das, B.S., Thurnham. D.I., Patnaik, J.K., Das, D.B., Statpathy, R. and Bose, T.K. (1990). Increased plasma lipid peroxidation in riboflavin-deficient, malaria infected children. *American Journal of Clinical Nutrition.* 51:859-63

Fridovich, I. (1989). Superoxide dismutases. An adaptation to aparamagnetic gas. *Journal of Biological Chemistry*. 264:7761-7764

Iranloye, B., Oludare, G. and Olubiyi. M. (2013). Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. *Journal of Diabetes Mellitus*, 3(4), 221-226.

Kalteniece, A., Ferdousi, M., Azmi, S., Marshall, A., Soran, H. and Malik, R.A. (2018). Keratocyte Density Is Reduced and Related to Corneal Nerve Damage in Diabetic Neuropathy. *Investigative Ophthalmology and Visual Science*, 59. 3584–3590.

Marthins MI. Zaria and its regions. Department of Geography Ahmadu Bello University, Zaria. 2006;41.

Okoduwa, S.I.R., Umar, I.A., James, D.B. and Inuwa, H.M (2017). Appropriate Insulin Level in Selecting Fortified Diet-Fed, Streptozotocin-Treated Rat Model of Type 2 Diabetes for Anti-Diabetic Studies. *PLoS ONE* 12(1): e0170971. doi:10.1371/journal.pone.0170971

Onyeka, C.A., Aligwekwe, A.U., Nwakanma, A.A., Bakare, A.A. and Ofoego U.C. (2012). Effects of Ethanolic Root Bark Extract of *Chrysophyllum albidum* on Serum Superoxide Dismutase, Catalase and Malondialdehyde in Rat. *International Journal of Pharmaceutical Sciences and Research,* 3(3): 347-351

Punthakee, Z., Ronald, G. and Pamela K. (2018). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Canadian Journal of Diabetes.* 42 (2018) S10–S15

Silberstein, T., Burg, A., Blumenfeld, J., Sheizaf, B., Tzur, T. and Saphier, O. (2013). Saturated fatty acid composition of human milk in Israel: a comparison between Jewish and Bedouin women. *Israel Medical Association Journals*, 15:156–159

Silva, R.B., Silva-Júnior, E.V., Rodrigues, L.C., Andrade, L.H., Silva, S.D… and Harand, W. (2015). A comparative study of nutritional composition and potential use of some

underutilized tropical fruits of Arecaceae. *Annals of the Brazilian Academy of Sciences*. doi:10.1590/0001-3765201520140166

Singh, K., Bal, B.S., Chopra, S., Singh, S. and Malhotra, N. (2012). Ameliorative Effect of Lycopene on Lipid Peroxidation and Certain Antioxidant Enzymes in Diabetic Patients. *Journal of Diabetes and Metabolism*, 3(6): 202

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C.L. and Ramarao, P. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacological Research*, 52(3):13-20

Tanko, Y., Olaleye, T.S., Mohammed, K.A., Jimoh, A., Dewu, M.A., Dallatu, M.K., Yerima, M. and Mohammed, A. (2016). Effects of L-Ascorbic acid and α-tocopherol and co-administration on serum lipid profile and liver enzymes on high fat diet-induced insulin resistance in wistar rats. *Nature and Science*, 13(6): 62-68.

Tikoo, K., Sharma, E., Amara, V.R., Pamulapati, H. and Dhawale, V.S. (2016). Metformin improves /metabolic memory in high fat diet-induced renal dysfunction. *Journal of biological chemistry*, 291:21848-21856

Ullah, A., Khan, A., and Khan, I. (2016), Diabetes mellitus and oxidative stress—A concise review, *Saudi Pharmaceutical Journal* 24(5): 547–553.

Wilson, R.D. and Islam, S. (2012). Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *Pharmacological Report*,

64 (1): 129-139.

World Health Organisation (2016). Global Report on Diabetes; World Health Organisation: Geneva, Switzerland.

Xiang, C., Da-Wei, Q., Shu, J., Er-Xin, S., Zhen-Hua, Z. and Jin-Ao, D. (2018). Scutellariae Radix and Coptidis Rhizoma Improve Glucose and Lipid Metabolism in T2DM Rats via Regulation of the Metabolic Profiling and MAPK/PI3K/Akt Signaling Pathway. *International Journal of Molecular Science*, 19(11), 3634.

Xu, G.K, Qin, X.Y., Wang, G.K., Xie, G.Y., Li, X.S., Sun, C.Y. Qin, M.J. (2017). Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of *Bombax ceiba* leaves inhigh-fat-diet- and streptozotocin-induced Type 2 diabetic rats Chinese *Chinese Journal of Natural Medicines*, 15(3): 168-177

Zhang, M., Lv, X.Y., Li, J., Xu, Z.G. and Chen, L. (2008). The characterization of high-fat and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Experimental Diabetes Research*, 2008:704045

J. Afr. Ass. Physiol. Sci. 7 (1): 30-37, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Protective effect of co-administration of vitamins C and E on reserpine-induced oxidative stress in mice

T. Danboyi[1], J. Abdullazeez, [2]A.W. Alhassan[2] and E. Hassan-Danboyi[2]

Departments of Human Physiology, [1]Kaduna State University, Kaduna, Nigeria and [2]Ahmadu Bello University, Zaria, Nigeria

Keywords:
Co-administration, neuroprotective, oxidative, reserpine-induced, stress

ABSTRACT

Background: Several studies have shown potential benefits of antioxidants in the treatment of Parkinson's disease (PD) but none have combined vitamins C and E targeting the oxidative stress (OS). **Aim**: To evaluate the neuroprotective effect of co-administration of vitamins C + E or single vitamin, on parameters of reserpine-induced OS in mice. **Methods**: Twenty-five mice were randomly assigned into 5 groups.: Group I received only distilled water (control); other groups received reserpine 0.1 mg/kg intraperitoneally on alternate days. In addition, Group III received vitamin E 200 mg/kg/day orally; group IV, had vitamin C 250 mg/kg/day orally and group V, had both vitamins orally. All drugs were given concurrently for 28 days. The mice were humanely acrificed and brain homogenate made to assess for biomarkers of OS. Data were expressed as mean ± SEM and values at $p < 0.05$ were considered significant. **Results**: The significant increase in malondialdehyde concentrations observed in the Res group (42.2±0.28 Umol/L) compared to control (37.54±1.27 Umol/L), was ameliorated in all the vitamin-treated groups with significance in the Res+Vit C group (35.0±1.69 Umol/L) compared to the Res group (p=0.002). Superoxide dismutase (SOD) activity increased significantly (p=0.003) across the vitamin-treated groups (24.9±2.11 Umol/mg, 24.0±1.78 Umol/mg and 22.4±1.50 Umol/mg in the Res+Vit E, Res+Vit C and co-administered groups respectively) compared to control (14.3±1.65 Umol/mg), with non-significant increase in the Res group (20.6±1.42 Umol/mg); catalase activity increased significantly in the Res+Vit C (28.0±3.70 Umol/mg) and co-administered (30.2±2.22 Umol/mg) groups compared to controls (14.3±1.65 Umol/mg) and Res (20.6±1.42 Umol/mg) groups (p=0.000), with non-significant increase in the Res+Vit E group (17.6±0.68 Umol/mg). The highest GSH level was seen in the Res group (45.2±2.65 Umol/mgpr) and the lowest level seen in the Res+Vit E group (38.58 ± 1.78 Umol/mgpr) with no significant difference across all the groups (p=0.104). **Conclusion**: The co-administration of vitamins C and E fails to confer significant superior neuroprotection against reserpine-induced OS compared to single vitamin administration.

INTRODUCTION

Parkinson's disease (PD), first described by James Parkinson (1817), is the most common movement disorder affecting about 1.5% of the world's population over 65 years of age (~10million) (Blesa and Przedborski, 2014). This prevalence increases with advancing age to about 5% in those above 85 years (Farn, 2003) and the prevalence is projected to double by 2040 due to increasing life expectancy with higher aging population and increasing industrialization (Dorsey *et al.*, 2018). It is the second most common neurodegenerative disease (NDD) (Tanner and Aston, 2000; Lee *et al*. 2009). Neurological disorders are now noted to be the leading cause of disability worldwide, among which, PD is the fastest growing (Dorsey and Bloem, 2018).

Several conventional (Schapira, 2005; Clark, 2007; Davie, 2008; Weinreb *et al*., 2010; Thomas, 2017) and other treatment strategies such as nicotine (Barreto *et al*., 2015), non-steroidal anti-inflammatory drugs, NSAIDs (Wahner *et al*., 2007; Gagne *et al*., 2010), docosahexanoic acid (Ozsoy *et al*., 2011), caffeine

*Address for correspondence:
Email timothy.danboyi@kasu.edu.ng

(Joghataie *et al*., 2004; Saaksjarvi *et al*., 2008) etc. have been explored in the treatment of PD but some of these findings for example nicotine, are controversial (Ferrea and Winterer, 2009). Even the gold standard in PD treatment, l-dopa and carbidopa (a peripherial decarboxylase inhibitor), is associated with dyskinesia and end-of-dose "wearing-off" (Fahn, 1999) and it has been suggested that l-dopa might be neurotoxic, hastening the progression of PD via oxidative stress (Cracium *et al.,* 2016). Although the motor symptoms initially respond well to pharmacologic therapies, none has been shown to slow down or halt the disease progression (Elmer and Hauser, 2013). Hence, the need to explore newer neuroprotective agents that can target the main pathophysiology of the disease with the aim of preventing further damage or neuronal loss, halting the progression, ameliorating symptoms and initiating repair or healing processes.

Given the well-established central role of oxidative stress (OS) in the pathogenesis of PD (Jenner, 2003), the focus in PD therapy is now shifting to antioxidants such as vitamins E and C, which might protect against oxidative damage by neutralising free radicals. Both vitamins C and E had been shown to have neuroprotective effects as individual antioxidants in some studies (Roghani and Behzadi, 2001; Miyake *et al*., 2011; Harrison, 2012) but the co-administration of both vitamins was shown to be of greater efficacy against oxidative stress and nephrotoxicity in rodents (Khadkhodaee *et al*., 2008), with more potent antidiabetic effects (Tanko *et al*., 2014), although, in another study, this was shown to be otherwise (Mohammed *et al*., 2015). The combined administration of the two vitamins has been shown to potentiate the effect of each other on reserpine-induced oral dyskinesia in rodents (Faria *et al*., 2005), also suggesting that the combination of both vitamins provide a complete antioxidant defence (Mahadik *et al*., 2001).

Vitamin C plays an important role in recycling of vitamin E (Kohen and Nyska, 2002; Harrison, 2012), a process that results in the formation of vitamin C (semi-ascorbyl) radical (Powers and Jackson, 2008; Pillai and Yao, 2015). Therefore, a combination of the two vitamins, due to their synergistic interaction (Baptista-Ortega and Ruiz-Feria, 2010; Yarube and Ayo, 2011; Dawud *et al*, 2014; Bursac-Metrovic *et al*., 2016), may enhance their antioxidant benefit. The aim of this study is to evaluate the effects of co-administration of vitamin C (*l*-ascorbic acid) and vitamin E (α-tocopherol) on reserpine-induced oxidative stress and parkinsonism in mice.

METHODS

Vitamins C and E and reserpine were of analytical grade and purchased from MedChem Express, U.S.A. The reserpine was dissolved in 0.5% glacial acetic acid, vitamin E (alpha-tocopherol acetate) in dimethyl sulfoxide and vitamin C (*l*-ascorbic acid) in distilled water.

Ethical Consideration

Ethical clearance/approval was sought from the Ahmadu Bello University Ethical Committee on Research Animals Use and Care. The study was conducted in accordance with the guidelines of the A.B.U Animals Use and Care Policy.

Experimental Animals

A total of 25 mice, 8-12 weeks old and weighing 20-30g were housed in plastic cages under standard environmental conditions with free access to commercial grower mash feed and water.

Induction of Parkinsonism

Parkinsonism was induced by alternate day's administration of 0.1mg/kg reserpine injection *i.p* over 4weeks (Sarmento-Silva *et al.,* 2014).

Animal Grouping

The mice were randomly assigned into five (5) groups of 5 animals each:

Group I (n=5): were fed with normal diet *ad libitum* plus normal saline (1ml/kg) throughout the study period. This group served as normal control.

Group II (n=5): received reserpine (0.1mg/kg) *i.p* on alternate days (Sarmento-Silva *et al*., 2014) for 4 weeks. They served as positive control.

Group III (n=5): were pre-treated with vitamin C (250mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4 weeks.

Group IV (n=5): were pre-treated with vitamin E (200mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4 weeks.

Group V (n=5): were pre-treated with vitamin C (250mg/kg) *i.p* and vitamin E (200mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4weeks.

Homogenate Preparation

The mice were humanely sacrificed by anaesthetizing them with i.p ketamine (10 mg/kg) and i.p diazepam (2 mg/kg). As soon as the effect of anaesthesia is evident, the brain was carefully harvested and a homogenate was prepared as described by Freitas *et al.* (2005), using phosphate buffer solution. The homogenate was

used for analysis of biomarkers for OS (according to the manufacturer's manual/guidelines).

Assay for Biomarkers of Oxidative Stress

Protocol for assay of superoxide dismutase (SOD) activity

Activity of SOD was carried out according to the method described by Misra and Fridovich (1972). The assay is based on the principle of superoxide dismutase (SOD) inhibition of auto-oxidation of adrenaline at pH of 10.2. 0.2 ml of diluted homogenate (0.1ml of homogenate plus 0.9ml of distilled water i.e. dilution factor of 1:10) was mixed with 2.5 ml of 0.05 mM carbonate buffer (14.3 g of Na_2CO_3 and 4.2 g $NAHCO_3$ plus 1,000 ml distilled water at pH of 10.2) and 0.3 ml of 0.3mM adrenaline (0.01 g of adrenaline plus 17 ml of distilled water). The absorbance was measured over 60s at 450 nm. The % inhibition was calculated as:

$$\%\ Inhibition\ of\ adrenaline\ oxidation = \frac{Increase\ in\ substrate\ absorbance/min}{Increase\ in\ absorbance\ of\ blank/min} X\ 100$$

Hence, the concentration of the SOD activity (in Umol/mg) is the amount of SOD required to elicit 50% inhibition of adrenaline auto-oxidation per minute.

Protocol for assay of reduced glutathione (GSH) activity

Reduced glutathione (GSH) activity was carried out according to the method described by Ellman (1959) as modified by Rajagopalan *et al.* (2004). It is based on the reaction of 5,5'-dithiobis nitro benzoic acid (DNTB) with GSH. To 150 uL of the homogenate 1.5 ml of 10% trichloroacetic acid (10g of TCA dissolved in distilled water up to 100 ml in a volumetric flask) was added and centrifuged at 1500 g for 5 minutes. 1ml of the supernatant was mixed with 0.5 ml of Ellman's reagent (19.8g of DNTB in 100ml of 0.1% sodium).

Protocol for assay of catalase (CAT) activity

Catalase activity was measured according to the method described by Aebi (1984). The principle is based on the decrease in absorbance of the sample induced by the catalytic decomposition of H_2O_2 to H_2O and O_2 in the presence of catalase. This enzymatic decomposition of H_2O_2 is a first order reaction, the rate of which is directly proportional to the amount of H_2O_2 present in the homogenate. 2.8 ml of the homogenate was added to 0.1 ml of 30mM H_2O_2 in a test tube and the rate of decomposition was measured at 240 nm for 5minutes using the spectrophotometer. Catalase activity was calculated using the molar extinction coefficient (E) of $0.041 mM^{-1}cm^{-1}$ as:

Catalase concentration = absorbance / E

Protocol for determination of MDA concentrations

The level of thiobarbituric-acid (TBA) reactive substance (TBARS), as an index of lipid peroxidation was determined by quantitative measurement of MDA concentration based on the method described by Okhawa *et al.* (1979), as modified by Atawodi *et al.* (2011). The principle is based on the reaction of 14% trichloroacetic acid (TCA) and 0.67% TBA solution both of which were added to about 100 µL of the supernatant, forming a TBARS, an adduct that absorbs strongly at 532 nm. The supernatant was deproteinized by addition of the TCA and the mixture was heated in a water bath at 80°C for 30minutes before cooling on ice then centrifuged at 2000 g for 10 minutes. The product of lipid peroxidation, MDA is expected to be released by the deproteinization and react with the TBA forming the coloured product, MDA-TBA (or TBARS), absorbance of which was measured at 532 nm with a UV spectrophotometer. The concentrations of TBARS were calculated as the absorbance/molar extinction coefficient of malondialdehyde: 1.56×105 mol/L/cm. All TBARS concentrations are expressed in µmol/g tissue protein.

Statistical Analysis

Data were expressed as mean ± SEM and analysed using one-way analysis of variance (ANOVA), followed *Tukey's* post-hoc test. Values at $p<0.05$ were considered statistically significant using SPSS version 25.0.

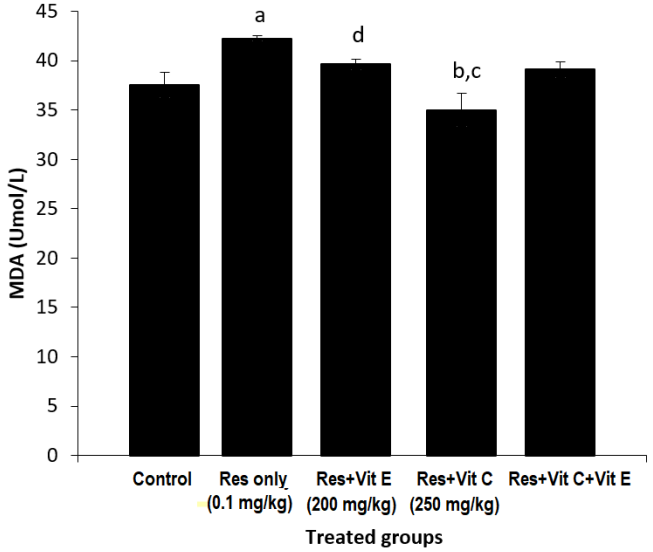

Fig. 1. MDA concentration by group: Res= Reserpine, Vit= vitamin. $^{a}p < 0.05$ indicates statistically significant difference compared to control, $^{b}p < 0.05$ compared to Res group, $^{c}p < 0.05$ compared to Res + Vit E, and $^{d}p < 0.05$ compared to Res + Vit C group (One-way ANOVA followed by Tukey's *post-hoc* test).

Level of SOD activity

There was a significant increase in the level of SOD activity across the vitamin-treated groups (24.0±1.78 Umol/mg by vitamin C, 24.9±2.11 Umol/mg by vitamin E and 22.4±1.5 Umol/mg by both vitamins). F = 5.660, p = 0.003.

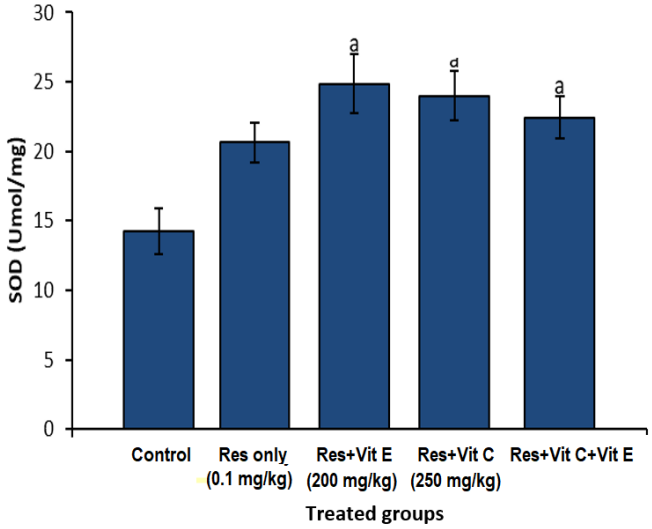

Fig. 2. Level of SOD activity by group: Res= Reserpine, Vit= vitamin. $^{a}p < 0.05$ indicates statistically significant difference compared to the control (One-way ANOVA followed by Tukey's *post-hoc* test).

Level of CAT activity

There was significant increase in catalase activity in the Res+Vit C (28.0±3.70 Umol/mg) and co-administered (30.2±2.22 Umol/mg) groups compared to both control (14.3±1.65 Umol/mg) and Res (20.6±1.42 Umol/mg) groups, with non-significant increase in the Res+Vit E group (17.6±0.68 Umol/mg). F = 2.214, p = 0.000.

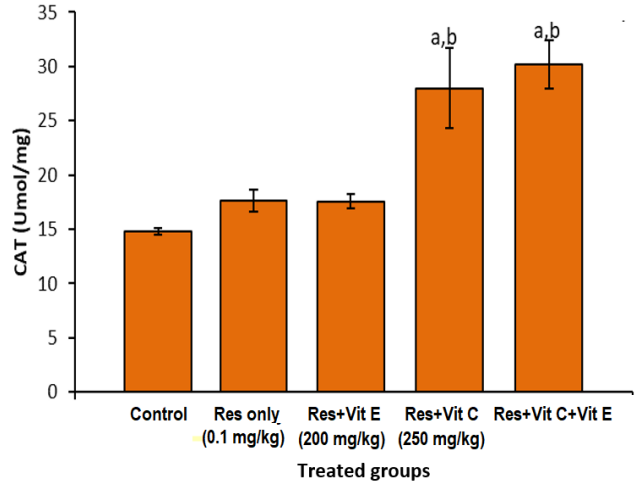

Fig. 3. Level of CAT activity by group: Res = Reserpine, Vit = vitamin. Superscripts $^{a}p < 0.05$ indicates statistically significant difference compared to control and $^{b}p < 0.05$ compared to Res group (One-way ANOVA followed by Tukey's *post-hoc* test).

GSH level

There was no significant difference in the GSH level across the groups, the highest level seen in the Res group (45.18 ± 2.65 Umol/mgpr) and the lowest level seen in the Res + Vit E group (38.58 ± 1.78 Umol/mgpr). F = 2.214, p = 0.104.

Table 1: Reduced glutathione levels by groups

Groups	Reduced Glutathione (GSH) (Umol/mgpr)
Control	39.72±1.45
Res only (0.1 mg/kg)	45.18±2.65
Res + Vit E (200 mg/kg)	38.58±1.78
Res + Vit C (250 mg/kg)	43.08±1.67
Res + Vit C + Vit E	41.82±0.74

Res= Reserpine, Vit= vitamin. No statistically significant difference (One-way ANOVA followed by Tukey's *post-hoc* test).

DISCUSSION

Oxidative stress (OS) can be defined as an imbalance between the levels of ROS produced and the ability of the biological system to neutralize them, creating a state of possible cellular damage (Dias *et al.*, 2013). It can also be defined as the presence of ROS in excess of the available antioxidant buffering capacity (Czerska *et al.*, 2015). These ROS may damage DNA, proteins, lipids, proteins and carbohydrates changing the organism's structure and functions (Czerska *et al.*, 2015). There is normally a balance between production of ROS and the antioxidant defence against them and any disturbance of such balance is capable of causing OS. Within the cell where oxygen metabolism takes place, ROS are normally and constantly formed as part of normal physiological processes with the majority of endogenous ROS generated by the mETC during the production of ATP (Eckert *et al.*, 2003). The antioxidant defence consist of enzymatic and non-enzymatic antioxidants.

The mechanism involved in toxin-induced PD in well-established animal models such as MPTP, 6-OHDA, rotenone and paraquat is associated with OS (Dias *et al.*, 2013). Although, apart from PD, OS had also been linked to other NDD such as Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) despite having distinct pathological and clinical features (Lin and Beal, 2006), supporting the role of oxidative stress in neurodegeneration (Anderson, 2004).

Markers of lipid peroxidation such as HNE and MDA are increased in the SN of PD patients, while PUFAs

are decreased (Montine *et al*., 2004). Our result showed a significant evidence of lipid peroxidation (figure 1), characterized by a significant increase in MDA concentration in the reserpine only treated group compared to control. This was however, significantly ameliorated by vitamins C and E as single administrations. This is in line with the findings of Nayak *et al.,* (2018), which showed neuroprotective effect of vitamin E against lipid peroxidation at different regions of rat brain. Surprisingly, vitamin C conferred the greater significant protection against lipid peroxidation, not being a membrane lipid-soluble antioxidant itself. This finding on the co-administered group is unexpected, as vitamin C to regenerate vitamin E in a synergistic action to produce even much greater significant protection against lipid peroxidation. This might be due to pro-oxidant activity of vitamin C at certain dosages, depending on the presence of transition metal ions such as Fe^{3+} (Paolini *et al.,* 1999; Halliway *et al.*, 1999).

The increase in SOD activity seen in the reserpine-only treated group, could be a compensatory response to the OS resulting from the excessive production of free oxygen radicals. This is a first line of antioxidant defence against OS, scavenging O_2^{2-} in both cytosol and mitochondrial inter-membrane space (Rodriguez *et al.,* 2011). However, the activity of SOD in the vitamin groups was even significantly higher, probably due to augmentation of the endogenous antioxidant enzyme by the vitamins. Again, the co-administration did not show more efficacy probably due to the pro-oxidant effect of vitamin C. This finding is consistent with the work of Serra *et al.* (2001), but contrary to other more recent studies (Sunday *et al*., 2014; Cracium *et al.,* 2016).

The significant increase in CAT activity across vitamin groups, with the greatest significance observed in the group given both vitamins is probably due to their synergistic effect against OS (Faria *et al*., 2005; Khadkhodaee *et al*., 2008; Baptista-Ortega and Ruiz-Feria, 2010; Yarube and Ayo, 2011; Dawud *et al*, 2014; Bursac-Metrovic *et al*., 2016). CAT is part of the second line antioxidant defence, scavenging for H_2O_2 generated by SOD activity intracellularly (Pillay and Yao, 2015). Hence, increase in SOD activity will lead to increase in H_2O_2 generation, leading to a compensatory increase in CAT activity.

The mild increase in GSH level, although not statistically significant, may be due to a compensatory response to increase generation of free radicals, which is contrary to the work of Sian *et al.* (1994), that showed decrease in GSH level in the SNc of PD brains. Although expected, there was no appreciable increase in the GSH level in the groups treated with vitamins. This may be due to an overwhelming level of OS and it's in line with the findings of Nayak *et al.* (2018), who also found no significant alteration in GSH level in rat brain exposed to aluminium and ethanol toxicity, following vitamin E supplementation for 4 weeks. Since GPx catalyses a reaction in which GSH detoxifies H_20_2 to water, the GSH level is expected to increase when there is decrease in the GPx activity, as seen in the present study. This is also in line with the findings of Sunday *et al.* (2014), who found decrease in GPx as a reciprocal to SOD activity, although other studies showed no significant alteration (Cracium *et al.,* 2016) in the GPX activity.

All the above findings showed a significant evidence of OS induced by reserpine in agreement with several other studies (Sanghavi *et al*., 2010; Fernandez *et al*., 2012; Eftimov *et al*., 2014; Hsiang-Chien *et al*., 2015; Dwivedi and Tomar, 2016). However, the administration of the antioxidants (vitamins C and E) was significantly protective against the OS, with the co-administration of the two antioxidants more potent than single vitamin administration. These findings of the present study on biomarkers of OS is contrary to other studies who showed generalised decrease in the SOD, CAT, GPx activities and GSH level (Napolitano *et al.,* 2011; Koppula *et al*., 2012).

The beneficial effect of antioxidants and antioxidant enzymes is seen in scavenging of free radicals generated during OS and neuroprotection These antioxidant systems prevent the generation and actions of ROS and provide a potential mechanism for ameliorating OS. Vitamin E, being a chain-breaking antioxidant within the lipid membrane (Kamal-Eldin and Appelqvist, 1996), protected against lipid peroxidation to a very significant extent as seen in this study and, it was itself regenerated by the vitamin C (Ambali *et al*, 2010; Harrison, 2012), which is a powerful water-soluble antioxidant within the cytoplasm and outside the membrane, and may help to boost the endogenous antioxidant levels. The vitamin E in turn, probably played a role in the biosynthesis of more endogenous vitamin C (Machlin and Gabriel, 1980) to boost the antioxidant neuroprotective effect.

CONCLUSION

From this study, co-administration of vitamins C and E was only partially neuroprotective against reserpine-induced OS in mice. Although, no significant neuroprotection was observed over the single vitamin administration, this finding depicts a great potential in slowing down the progression of NDDs like PD. Introducing such neuroprotective therapy, which are readily available and affordable, into the standard

treatment of PD will be highly beneficial. More studies are however needed, to explore these vitamins C and E at different dosages.

AKNOWLEDGEMENT

No external source of funding obtained. We appreciate the contributions of Mr Emmanuel Solomon Nachamada to the success of this study.

REFERENCES

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105: 121-126.

Ambali, S. F., Ayo, J. O., Ojo, S. A. and Esievo, K. A. (2010). Vitamin E protects wistar rats from chlorpyrifos-induced increase in erythrocyte osmotic fragility. *Food Chemistry and Toxicology*, 48(12): 3477-3480.

Andersen, J. K. (2004). Oxidative stress in neurodegeneration: Cause or consequence? *Nature's Medicine*, 10: 18–25.

Atawodi, S. E., Adekunle, O. O. and Bala, I. (2011). Antioxidant, organ protective and ameliorative properties of methanol extract of A*nogeissus leiocarpus* stem bark against carbon tetrachloride-induced liver injury. *International Journal of Pharmacological Sciences and Research*, 2(6): 1443-1448.

Baptista-Ortega, J. and Ruiz-Feria, C. A. (2010). L-Arginine and antioxidant Vitamins E and C improve the cardiovascular performance of broiler chickens grown under chronic hypobaric hypoxia. *Poulty Science*, 89 (10): 2141-2146.

Barreto, G. E., Larkov, A. and Moran, E. V. (2015). Beneficial effect of nicotine, cotinine and its metabolites as potential agents for Parkinson's disease. *Frontiers in Aging Neuroscience*, 6(340). doi: 10.3389/fnagi.2014.00340.

Blesa, J. and Przedborski, S. (2014). Parkinson's Disease: animal models and dopaminergic cell vulnerability. *Frontiers in Neuroanatomy*. doi: 10.3389/fnana.2014.00155.

Bursac-Metrovic, M., Milovanovic, D. R., Mitic, R., Jovanovic, D., Sovrlic, M., Vasiljevic, P., Tomovic, J. and Manojlovic, N. (2016). Effects of L-ascorbic acid and alpha-tocopherol on biochemical parameters of swimming-induced oxidative stress in serum of guinea pigs. *African Journal of Traditional, Complementary and Alternative Medicines*, 13(4): 29-33.

Clark, C. E. (2007). Parkinson's disease. *British Medical Journal*, 335(7617): 441-445.

Cracium, E. C., Dronca, E. and Leach, N. V. (2016). Antioxidant enzyme activity in subjects with Parkinson's disease under L-DOPA therapy. *Human and Veterinary Medicine Bioflux*, 8(2): 124-127.

Czerska, M., Mikolajewska, K., Zielinski, M., Gromadzinska, J. and Wasowicz, W. (2015). Today's oxidative stress markers. *Medycyna Pracy*, 66(3): 393-405.

Davie, C. A. (2008). A review of Parkinson's disease. *British Medical Bulletin*, 86: 109-127.

Dawud, F. A., Mabrouk, M. A. and Umar, I. A. (2014). Effects of vitamins C and E on aspirin-induced gastric mucosal damage and oxidative stress. *Journal of Biological Sciences*. 6(1): 36-41.

Dias, V., Junn, E. and Mouradian, M. M. (2013). The role of oxidative stress in Parkinson's disease. *Journal of Parkinson's Disease,* 3(4): 461-491.

Dorsey, E. R. and Bloem, B. R. (2018). The Parkinson pandemic – a call to action. *Journal of American Medical Association - Neurology*, 75(1): 9-10.

Dorsey, E. R., Sherer, T., Okun, M. S. and Bloem, B. R. (2018). The emerging evidence of the Parkinson pandemic. *Journal of Parkinson's Disease*, 8: 3-8.

Dwivedi, G. and Tomar, V. (2016). Protective effects of ipomoea aquatic against reserpine-induced oxidative stress in brain using mice. *International Journal of Basic and Clinical Pharmacology,* 5(5): 2123-2129.

Eckert, A., Keil, U., Marques, C. A., Bonert, A., Frey, C., Schussel, K. and Muller, W.E. (2003). Mitochondrial dysfunction, apoptotic cell death and Alzheimer's disease. *Biochemical Pharmacology*, 66: 1627-1634.

Eftimov, M., Dobreva, C., Velkova, D. and Valcheva-Kuzmanova, S. (2014). Effect of *Aronia melanocarpa* fruit juice on reserpine-induced hypokinesia and oxidative stress in rats. *Scripta Scientifica Pharmaceutica*, 1(2): 29-34.

Ellman, G. L. (1959). Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics*, 82: 70-77.

Fahn, S. (1999). Parkinson disease, the effect of levodopa and the ELLDOPA trial (earlier vs later L-dopa). *Archives of Neurology*, 56: 529–535.

Fahn, S. (2003). Description of Parkinson's disease as a clinical syndrome. *Annals of New York Academy of Science*, 991: 1–14.

Faria, R. R., Abilo, V. C., Grassi, C., Chinen, C. C., Negrao, L. T. R., deCastro, J. P. M. V., Fukushiro, D. F., Rodrigues, M. S. D., Gomes, P. H. Z., Registro, S., de Carvalho, R. C., DAlmeida, V., Silva, H. R., Ribeiro, R. A. and Frussa-Filho, R. (2005). Beneficial effects of vitamin c and vitamin e on reserpine-induced oral dyskinesia in rats: critical role of striatal catalase activity. *Neuropharmacology*, 48(7): 993-1001.

Fernandez, V. S., Santos, J. R., Leao, A. H., Medeiros, A. M., Melo, T. G., Izidio, G. S., Cabral, A., Ribeiro, R. A., Abilio, V. C., Ribeiro, A. M. and Silva, R. H. (2012). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. *Behavioural Brain Research*, 231(1): 154-163.

Ferrea, S. and Winterer, G. (2009). Neuroprotective and neurotoxic effects of nicotine. *Pharmacopsychiatry*, 42(6): 255-265.

Freitas, R. M., Vasconcelos, S. M. M., Souza, F. C. F., Viana, G. S. B. and Fonteles, M. M. F. (2005). Oxidative stress in the hippocampus after pilocarpine-induced status epilepticus in wistar rats. The FEBS Journal, 272(6): 1307-1312.

Gagne, J.J., Power, M.C. (2010). Anti-inflammatory drugs and risk of Parkinson's disease. *Neurology*, 74(12): 995-1002.

Harrison, F. E. (2012). A critical review of vitamin C for the prevention of age-related cognitive decline and Alzheimer's disease. *Journal of Alzheimer's Disease*, 29(4): 711-726.

Hsiang-Chien, T., Mao-Hsien, W., Hung-Sheng, S., Yi, C., and Kuo-Chi, C. (2015). Epigallocatechin-3-gallate prevents the reserpine-induced impairment in short-term social memory in rats. *Behavioral Pharmacology*, 26(8): 741-747.

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neuroloogy*, 53(3): 26-36

Joghataie, M. T., Roghani, M., Negahdar, E. and Hashemi, L. (2004). Protective effect of caffeine against neurodegeneration in a model of Parkinson's disease in rat: behavioral and histochemical evidence. *Parkinsonism and Related Disorders*, 10(8): 465-468.

Kamal-Eldin, A. and Appelqvist, L. A. (1996). The Chemistry and Antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31:671-701.

Khadkhodaee, M., Khaster, H., Faghihi, M., Ghaznavi, R. and Zahmatkesh, M. (2008). Effects of co-supplementation of vitamins E and C on gentamycin-induced nephrotoxicity in rat. *Experimental Physiology*, 90(4), doi: 10.1113/expphysiol.2004.029728.

Kohen, R. and Nyska, A. (2002). Oxidation of biological systems: oxidative stress phenomenon, antioxidants, redox reaction and methods for their quantification. *Toxicologic Pathology*, 30(6): 620-650.

Koppula, S., Kumar, H., More, S. V., Kim, B. W., Kim, I. S. and Choi, D. K. (2012). Recent advances of the neuroprotective potentials of antioxidants in experimental models of Parkinson's disease. *International Journal of Molecular Sciences*, 13. doi:10.3390/ijms130810608

Lees, J., Hardy, J. and Revesz, T. (2009). Parkinson's disease. *The Lancet*, 373 (9680): 2055–2066.

Lin, M. T. and Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, 443:787–795

Machlin, L.J. and Gabriel, E. (1980). Interactions of vitamin E with vitamin C, vitamin B12 and zinc. *Annals of the New York Academy of Science*, 355(1): 98- doi: 10.1111/j.1749-6632.1980tb21330.x

Mahadik, S. P., Evans, D. and Lal, h. (2001). Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. *Progress in Neuropsychopharmacology, Biology and Psychiatry*, 25: 463-493.

Misra, H. P. and Fridivich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*, 247(10): 3170-3175.

Miyake, Y., Fukushima, W., Tanaka, K., Sasaki, S., Kiyohara, C., Tsuboi, Y., Yamada, T., Oeda, T., Miki, T., Kawamura, N., Sakae, N., Fukuyama, H., Hirota, Y., Nagai, M. and Fukuoka K. (2011). Dietary intake of antioxidant vitamins and risk of Parkinson's disease: a case-control study in Japan. *European Journal of Neurology*, 18(1): 106-113.

Mohamed, S., Jawis, N., Abass, S. and Krasilshchikov, O. (2015). Effects of dietary vitamins C and E supplementation on hepatic and renal function on young weightlifters. *Periodisation, British Journal of Sports Medicine*, 2(2): 24-29.

Montine, K. S., Quinn, J. F., Zhang, J., Fessel, J. P., Roberts, L. J., Morrow, J. D. and Montine, T. J. (2004). Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chemistry and Physics of Lipids*, 128: 117–124.

Napolitano, A., Manini, P. and D'Ischia, M. (2011). Oxidation chemistry of catecholamines and neuronal degeneration: an update. *Current Medical Chemistry*, 18(12): 1832–1845.

Nayak, P., Sharma, S. B. and Chowdary, N. V. S. (2018). Alpha-tocopherol supplementation restricts aluminium- and ethnol-induced oxidative damage in rat brain but fails to protect against neurobehavioral damage. *Journal of Dietary Supplements*, 10.1080/19390211.2018.1451940.

Okhawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry, 95(2): 351-358.

Ozsoy, O., Seval-Celik, Y., Hacioglu, G., Yargicoglu, P., Demir, R., Agar, A. and Aslan, M. (2011). The influence and the mechanism of docosahexanoic acid on a mouse model of Parkinson's disease. *Journal of Neurochemistry International,* 59(5): 664-670.

Pillai, A. and Yao, J. K. (2015). Antioxidant interventions in neuropsychiatric disorders. In: Dietrich-Muszalska, A., Chauhan, V. and Grignon, S. (eds.). *Studies on Neuropsychaitric Disorders: Oxidative Stress in Applied Basic Research and Clinical Practice*. New York, NY: Humana Press, pp 505-520. doi: 10.1007/978-1-4939-0440-2_25.

Powers, S. K. and Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiologic Review*, 88(4): 1243-1276.

Rajagopalan, R., Kode, A., Penumatha, S. V., kallikat, N. R. and Venugopal, P.M. (2004). Comparative of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacology and Pharmaceutical Sciences*, 83: 2747-2752.

Rodriguez, R. A., Wuwial, E., Perez, V. I., Lambert, A. J., Edrey, Y. H., Lewis, K. N., Grimes, K., Lindsey, M. L., Brand, M. D. and Buffenstein, R. (2011). Walking the oxidative stress tightrope: a perspective from the naked mole-rat, the longest living rodent. *Current Pharmaceutical Design*, 17: 2290-2307.

Roghani, M. and Behzadi, G. (2001). Neuroprotective effect of vitamin E on the early model of Parkinson's disease in rat: behavioral and histochemical evidence. *Brain Research,* 891(1): 211-217.

Saaksjarvi, K., Knekt, P., Rissanen, H., Laaksonen, M. A., Reunanen, A. and Mannisto, S. (2008). Prospective study of coffee consumption and risk of Parkinson's disease. *European Journal of Clinical Nutrition*, 62(7): 908-915.

Sanghavi, C. R., Barhate, S. A., Mahajan, S. M., Mohan, M. and Kasture, S. B. (2010). Korean ginseng extract attenuates reserpine-induced orofacial dyskinesia and improves cognitive dysfunction in rats. 704-715. doi: https://doi.org/10.1080/14786410802583031 .

Sarmento-Silva, J. A., Lima, R. H., Cabral, A., Meurer, Y., Ribeiro, A. M. and Silva, R. H. (2014). Alpha-Tocopherol Counteracts Cognitive and Motor Deficits Induced by Repeated Treatment with Reserpine. Biochemistry and Pharmacology (Los Angeles) 3: 153.doi:10.4172/2167-0501.1000153

Schapira, A. H. V. (2005). Present and future drug treatment for Parkinson's disease. *Journal of Neurol. Neurosurg. and Psychiatry,* 76: 1472-1478.

Serra, J. A., Dominguez, R. O., de Lustig, E. S., Guareschi, E. M., Famulari, A. L., Bartolome, E. L., Marschoff, E. R. (2001). Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. *Journal of Neurologic Transmission*, 108(10): 1135-1348

Sian, J., Dexter, D. T., Less, A. J., Daniel, S., Jenner, R. and Marsden, C. D. (1994). Glutathione-related enzymes in brain in Parkinson's disease. *Annals of Neurology,* 36: 356-361.

Sunday, O. P., Adekunle, M. F., Temitope, O. T., Richard, A. A., Samuel, A. A., Olufunminyi, A. J. and Elizabeth, O. O. (2014). Alteration in antioxidants level and lipid peroxidation of patients with neurodegenerative diseases {Alzheimer's disease and Parkinson disease}. *International Journal of Nutrition, Pharmacology and Neurological Diseases*, 4(3): 146-152.

Tanko, I., Benjamin, N., Yahuza, F., Jimoh, A., Mohammed, K.A., Muhammad, A., Yerima, M. And Mohammed, A. (2014). Ameliorative effect of l-ascorbic acid and α- tocopherol co-administration on blood glucose levels and some haematological indices on high fed fat diet-induced diabetes on wistar rats. *Nigerian Journal of Scientific Research, 13(2): 26-31.*

Tanner, C. M. and Aston, D. A. (2000). Epidemiology of Parkinson's disease and akinetic syndromes. *Current Opinions in Neurology*, 13: 427–430.

Thomas, L. (2017). Parkinson's Disease Tremor. Retrieved on 13th March, 2018 from https://www.news-medical.net/health/Parkinsone28099s-disease-tremor.aspx

Wahner, A. D., Bronstein, J. M., Bordelon, Y. M. and Ritz, B. (2007). Non-steroidal anti-inflammatory drugs may protect against Parkinson's disease. *Neurology*, 69(19): 1836-1842.

Weinreb, O., Amib, T., Bar-Am, O. and Youdim, M. (2010). Rasagiline: a novel antiparkinsonian monoamine oxidase-B inhibitor with neuroprotective activity. *Progress in Neurobiology*, 92:330-344.

Yarube, I. U. and Ayo, J. O. (2011). Modulatory role of antioxidant vitamins C and E on erythrocyte osmotic fragility induced by chronic sodium nitrate administration in rats. *Bayero Journal of Pure and Applied Sciences*, 4 (1): 115-120

J. Afr. Ass. Physiol. Sci. 7 (1): 38-43, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Evaluation of Milk Yield and Some Lactogenic Hormones in Lactating Wistar Rats after treatment with Ascorbic acid and α-Tocopherol

A.E. Ogweje, I.G. Bako and M.B. Akor-Dewu

Department of Human Physiology, Faculty of Basic medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Nigeria

Keywords:
Milk yield, Vitamin C Vitamin E, Prolactin, Oxytocin, Pup and Dams.

ABSTRACT

Background: Milk synthesis and ejection is essential for breastfeeding and is influenced by nutritional and non-nutritional factors. Vitamin C (L-ascorbic acid) is an essential nutrient for humans and certain animal species. Vitamin E (α-Tocopherol) is a form of vitamin E that is preferentially absorbed. This study was designed to assess milk yield, serum prolactin and oxytocin hormone in lactating Wistar rats following ascorbic acid and α-Tocopherol supplementation. **Methods**: At parturition, the animals were randomly divided into five groups thus: Group I: (Normal control) was given commercial feed and distilled water, orally (1 ml/kg), Group II: metoclopramide (5 mg/kg), Group III: 100 mg/kg of Vitamin E. Group IV: 100 mg/kg of Vitamin C and Group V was treated with the co-administration of vitamin C and E 100 mg/kg each. Administration was carried out orally from day 3 to day 13 of lactation at 06:00 hours daily. The animals were euthanized on day 14 using ketamine and diazepam at 75 mg/kg and 25 mg/kg given intraperitoneally and thereafter sacrificed. Milk yield18 hours after gavage as well as serum levels of prolactin and oxytocin were evaluated. **Result**: Statistical analysis was carried out using SPSS version 20 with the aid of one-way analysis of variance (ANOVA) and tukey's post-hoc test. Values of $p \leq 0.05$ were considered significant. There was a statistically significant ($p < 0.05$) increase in milk yield in groups IV and V when compared to control: 3.72±0.37, 3.60±0.33 vs 2.28±0.08 respectively, and also a significant decrease ($p < 0.05$) in group V compared to metoclopramide-treated group; 9.32±0.57 vs 11.48±0.72. Serum oxytocin level was significantly increased ($p < 0.05$) in group IV compared to the control group 13.32±1.16 vs 9.46±0.81and a significant decrease in group V compared to group IV 8.80±0.95 vs 13.32±1.16 was observed. **Conclusion**: This study has shown that Vitamin C possesses more lactogenic activity which increased more milk yield and serum oxytocin level in comparison to vitamin E and metoclopramide.

INTRODUCTION

Antioxidants are man-made or natural substances that may prevent or delay some type of cell damage (Yadav *et al*., 2016). Under normal physiological conditions, antioxidants and oxidants are in a dynamic equilibrium with antioxidants scavenging ROS generated in the body. Vitamins C (ascorbic acid) and E (α-tocopherol) are the main natural antioxidants occurring in biological system. Vitamin C as an extracellular fluid antioxidant reduces excessive ROS generation. Vitamin E is a chain-breaking antioxidant, which exerts its antioxidant effects mainly on cell membrane. it also regenerates the activity of tocopherol by reducing the tocopheroxyl radicals (Abdel-Khalek *et al*., 2008). Antioxidants are widely used as dietary supplements and numerous studies suggest that supplements of vitamin C and/or vitamin E may contribute to lowering the risk of specific chronic diseases and enhancing the antioxidant activities of breast milk during lactation. Vitamin C is a powerful dietary antioxidant which influences iron absorption and helps fight cell-damaging free radicals. Natural sources of vitamin C

*Address for correspondence:
Email **:** abigailogweje@gmail.com

are fruits and vegetables and they include oranges, grapes, strawberries, red bell peppers, tomatoes, cabbage, spinach green peas just to mention a few while Vitamin E can be found in various foods and oils for instance nuts, seeds, green leafy vegetables and fortified cereals (Moser and Chun, 2016), (Rizvi *et al*., 2016).

Milk production is dependent on the state of the maternal wellbeing. It has been reported that milk yield can be affected by stressors enhancing ROS production through down regulation of milk synthesis leading to an increase in dopamine and adrenaline levels which in turn inhibit hormones of lactation (Hassioutou, and Geddes, 2012). Insufficient milk let down/supply (hypogalactia) is still being observed as a major and frequently-cited problems associated with lactation leading to early discontinuation of breastfeeding (Odom *et al*., 2013).

Pregnancy period through lactation has been proposed as one of the central sources of oxidative stress as it is associated with increase in energy metabolism beginning with cost of fetal development, milk production, and energy expenditure from high maternal maintenance and physical activities (Ziomkiewick *et al*., 2016).

Galactogogues are substances or medications used in assisting initiation and maintenance or augmentation of milk production (Yahuza *et al*., 2016). Some of this galactogogues include dopamine antagonists such as Metoclopramide, domperidone, antipsychotics, sulpiride, chlorpromazine; hormone synthetic analogues such as oxytocin, thyroxine and medroxyprogesterone are also included in the synthetic galactogogues list (Zuppa *et al*., 2010). This study was designed to assess milk yield in lactating Wistar rats following treatment with ascorbic acid and α-tocopherol.

METHODS

Experimental Protocol

A total of 30 female Wistar rats and 15 male Wistar rats, weighing 150-250g were purchased from the experimental animal house of the Department of Human Physiology, Ahmadu Bello University Zaria. Female Wistar rats were randomly assigned into groups of two each (n=2), and then mated alongside one male counterparts in the ratio 2:1 in a stainless steel metal cage. The rats were fed with commercial feed and tap water *ad libitum* and were provided with approximately 12hrdark/light cycle. Ethical approval was obtained from the Ethical Committee of Ahmadu Bello University, Zaria on animal handling, consistent with standard animal welfare guideline. At parturition, weight of dams and pups were recorded and the number of pups per dam was culled to 4(Yahuza *et al*., 2016). Lactating Wistar rats were randomly grouped into five groups of six animals each (n=6).

Experimental Design

The animals were grouped as shown in Table 1. Administration of agents was carried out orally for a period of ten (10) days starting from day 3 to day 13 of lactation (Bako *et al*., 2015).

Table 1. Experimental groups used in the study

Groups	Treatment
Group I	1 ml/kg normal saline
Group II	5 mg/kg metoclopramide (Jiangsu Pengyao Pharmaceutical Inc. China)
Group III	100 mg/kg Vitamin E (Michelle laboratories LTD)
Group IV	100 mg/kg Vitamin C (Michelle laboratories LTD)
Group V	100mg/kg co-administration of vitamin C and E

Evaluation of milk yield in lactating Wistar rats

Milk yield and body weight of dams and weight of pups were measured each day with an electronic balance (Salter). Milk yield was estimated 18 hours after gavage indirectly from the relationship between weight gain of pups pre and post suckling (Sampson and Jansen, 1984; Ann and Linzell, 2003). Following administration of agents at 06:00 pm, the pups were weighed every day during the study period at 07:00 am the next day and recorded as (W_1) and then isolated from their dams for a period of four (4) hours (Samson and Jensen, 1984). At 11:00 am, the pups were weighed (W_2) and re-united with their dams and allowed to feed for 1 h. At 12:00 am, they were weighed again (W_3). Milk yield 18 hours after gavage was estimated as $W_3 - W_2$ with a correction for weight loss due to metabolic processes in the pups as $(W_2 - W_1)/4$ (Ouedraogo *et al*., 2004; Bako *et al*., 2013).

Milk synthesis and ejection 18 hours after gavage = $W_3 - W_2$

Where W_3 = Post suckling weight of pups (12:00 pm)

W_2 = Pre-suckling weight of pups (11:00 am)

*Correction for weight loss due to metabolic processes was calculated as follows:

Weight loss correction 18 hours after gavage = $W_3 - W_2/4$

Where W_2 = pre-suckling weight pups, W_1 = pre-isolation weight of pups (taken four hours before W_2 at 7:00 am (Morag, 1970).

Prolactin Analysis

Blood samples were obtained via cardiac puncture into specimen bottles and allowed to clot and separated by centrifugation at 2,000 × g for 10 minutes using Centrifuge Hettich (Universal) and the sera obtained and used for biochemical assays. The analysis was conducted at the Department of Human Anatomy, Ahmadu Bello University, Zaria. Prolactin analysis was carried out using the specie-specific Prolactin rat ELISA kit which was designed for the quantitative evaluation of rat prolactin according to the manufacturer's manual. The microplate was first coated with monoclonal antibody prolactin. The sera sample containing the antigen was pipetted into an antibody-coated microplate and allowed for two (2) hours during which the prolactin antigen in the sample binds to the antibodies fixed on the inner surface of the wells. Non-reactive components were removed by washing steps. Afterwards a second polyclonal horseradish peroxidase-labelled antibody was added, a sandwich complex which consisted of two antibodies and the rat prolactin was formed during 1 hour of incubation. The excess enzyme conjugate was washed out and a chromogenic substrate, tetra-methyl benzidine (TMB) was added to the wells and was allowed to incubate for 30 minutes, during which the substrate was converted to a coloured end product (blue) by the fixed enzyme. The enzyme reaction was inhibited by adding hydrochloric acid as the stop solution. Absorbance was measured using a microplate reader at 450 nm. A standard curve was obtained by plotting the concentration of standard versus the absorbance from which the prolactin concentration was gotten. The lowest detectable level of prolactin with this test was 0.8 ng/mL (Bako *et al*., 2015).

Oxytocin Analysis

Oxytocin analysis was carried out using the oxytocin rat ELISA kit according to the manufacturer's manual. The oxytocin specie-specific enzyme-linked immunosorbent assay (ELISA) kit in microplate was designed for the quantitative evaluation of rat oxytocin. The analysis was conducted at the Department of Human Anatomy, Ahmadu Bello University, Zaria. Oxytocin analysis was carried out using the specie-specific Oxytocin rat ELISA kit which was designed for the quantitative evaluation of rat Oxytocin according to the manufacturer's manual. The microplate is pre coated Oxytocin. The sera sample which containing the antigen was pipetted into an antibody coated microplate, and allowed for two (2) hours, during the reaction, oxytocin in the sample or standard competed with a fixed amount of oxytocin on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to oxytocin. Excess conjugate and unbound sample or standard were washed from the plate, and HRP-Streptavidin (SABC) was added to each microplate well and incubated. Then TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm.

A standard curve was obtained by plotting the concentration of standard versus the absorbance from which the oxytocin concentration was gotten. The lowest detectable level of oxytocin with this test was 6.3 pg/mL.

Data analysis

All data were expressed as Mean ± Standard Error of Mean. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Results were considered significant at $p \leq 0.05$. Statistical Package for Social Sciences (SPSS) version 20 was used.

RESULTS

Milk Yield

The result of milk yield in lactating Wistar rats treated with ascorbic acid and α-tocopherol showed a statistically significant ($p < 0.05$) increase in group IV (ascorbic acid (vitamin C-treated group) and also group V (co-administration of both vitamin C and E) when compared to control; 3.72±0.37 and 3.60±0.33 versus 2.28±0.08 respectively (Fig. I below).

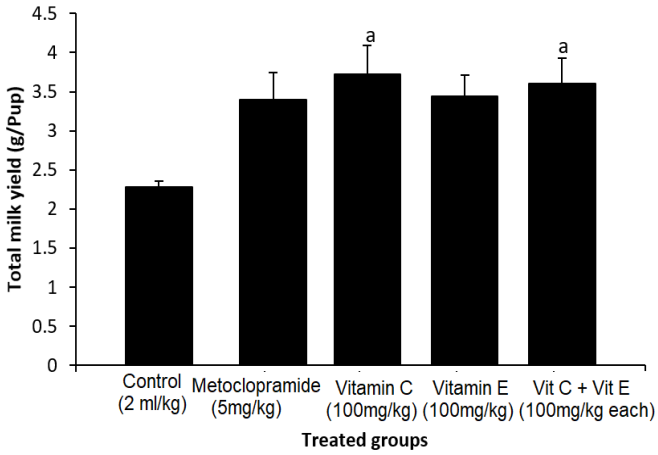

Fig. 1. Mean milk yield in lactating Wistar rats following supplementation with vitamins C, E and their co-administration. Superscripts a indicate statistical significance at $p < 0.05$ compared to control

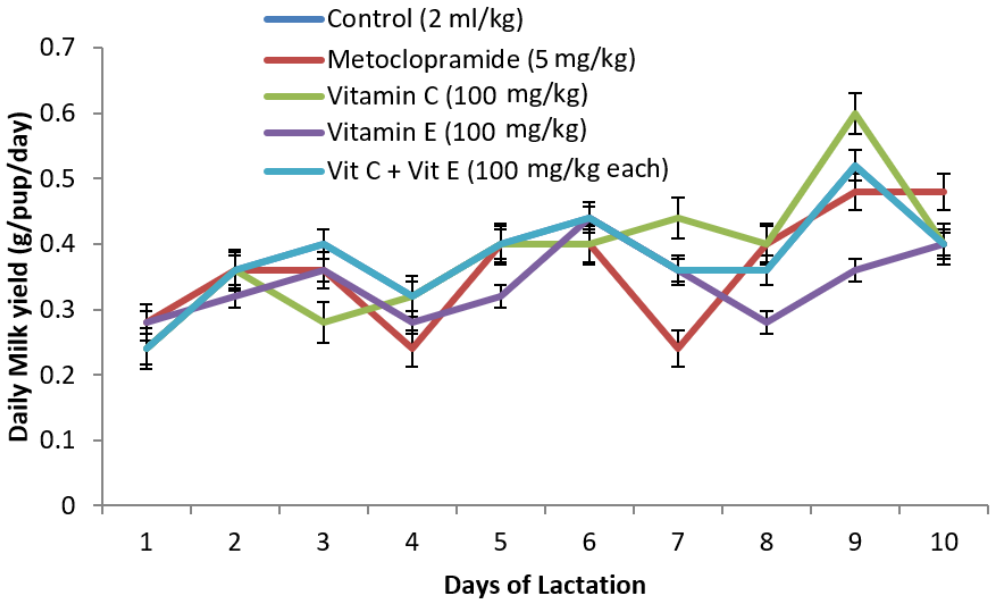

Fig 2. Daily milk yield 18h after gavage in lactating Wistar rats treated with ascorbic acid, α-tocopherol, vitamins C and E combined and metoclopramide are statistically significant at p< 0.05.

There was a significant increase in milk yield (p< 0.05) from day 3 to day 4 in the vitamin C and E combined treated group, and also a significant increase in the vitamin C-treated group from day 7 to day 9 when compared to the Control group. The highest milk yield recorded was on day 9 of lactation with its lowest being day 7 in group II (metoclopramide-treated group). Although there was an increase in the milk yield in metoclopramide-treated group and α-tocopherol (vitamin E) treated group from day 7 to day 10 it, was however not statistically significant.

Serum Prolactin

There was a statistically significant increase (p< 0.05) in group II (metoclopramide treated group) compared to control; 11.48±0.72 versus 9.12±0.29, and also a significant decrease (p< 0.05) in group V (vitamin C and E co-administration group) compared to metoclopramide treated group; 9.32±0.57 versus 11.48±0.72 figure III.

Serum oxytocin

Serum oxytocin level in lactating Wistar rats in the experiment was significantly higher (p< 0.05) in group IV (vitamin C-treated group) 13.32±1.16 compared to the control group (9.46±0.81). A significant decrease was also observed in group V (combination treated group) 8.80±0.95 compared to vitamin C-treated group (13.32±1.16). Although there was an increase in the vitamin E- treated group, it was not statistically significant.

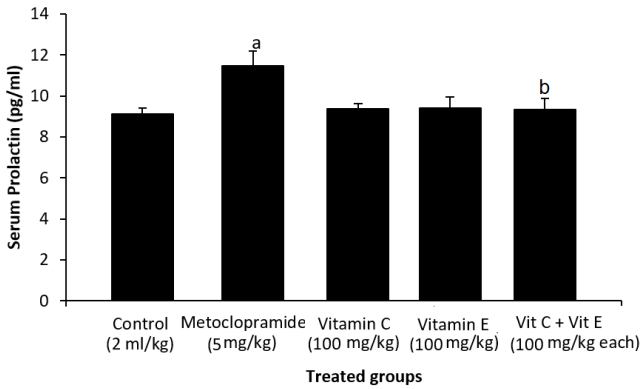

Fig. 3. Effect of ascorbic acid and α-tocopherol on serum prolactin level in lactating Wistar rats. Different superscripts a,b indicate statistical significance at p< 0.05 compared to control and metoclopramide respectively.

DISCUSSION

The increase in the milk yield in the vitamin C and their combination could be attributed to the increase in the serum oxytocin level. Reports from studies have shown that water soluble vitamins increase milk yield as they serve as cofactors for the enzymes responsible for the synthesis of amino acids involved in milk production. Vitamin C as a water-soluble vitamin act as a cofactor in a number of reactions and among these is its role as a cofactor to peptidyl-gylcine alpha-amidating monooxygenase (PAM) enzyme in enhancing the

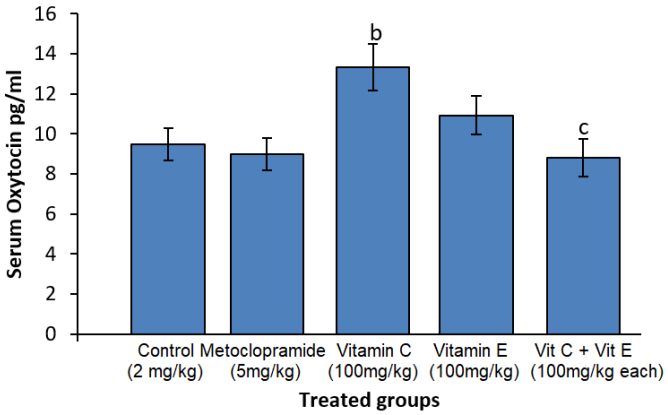

Fig. 4. Effect of ascorbic acid and α-tocopherol on serum oxytocin level in lactating Wistar rats. Different superscripts a,b,c indicate statistical significance at p< 0.05 compared to control, metoclopramide and vitamin c-treated group respectively.

synthesis of oxytocin hormone (Epipper *et al*., 1994). Increase synthesis of oxytocin enhances the release of oxytocin upon stimulation of the nipple during sucking. The increase in milk yield in the combination group could be due to a synergistic effect of vitamin C in addition to the vitamin E, the high milk yield in the vitamin E group even though not significant can be attributed to the increase in oxytocin. Literatures have shown that most traditional galactogogues like *Hibiscus sabdariffa* (Okasha *et al*., 2008) exhibit their lactogenic activity through stimulation of prolactin production, however in this study antioxidants (vitamin C and E) increased milk yield without stimulating prolactin hormone compared to the standard drug metoclopramide which caused a significant increase in serum prolactin hormone level. It is therefore apparent that vitamin C and E do not have any effect on prolactin hormone, this agrees with reports of Simelane *et al*., (2012); Yahuza *et al*., (2016) and is suggestive that most galactogogues with antioxidant properties stimulate milk production and milk yield without stimulating prolactin hormone and therefore have less effect on this lactation hormone. Metoclopramide as a standard drug has been known for its ability to increase prolactin level through its antidopaminergic effect on the dopaminergic cells leading to a subsequent suppression of dopamine release resulting in increased prolactin release (Bako *et al.*, 2013). Prolactin is known for its numerous roles with its major effect seen on the mammary gland ranging from development of the mammary gland to milk synthesis and maintenance of milk secretion (Freeman *et al*., 2006). After parturition, prolactin induces lactation by stimulating the synthesis of milk in the epithelial cells and also causes proliferation of secretory cells (Yahuza *et al*., 2016). Oxytocin aside its other roles is known for its role in milk ejection brought about by neuroendocrine reflexes. In this study vitamin C could have increased oxytocin level by stimulating oxytocin release while inhibiting prolactin release causing oxytocin to have more effect on the myoepithelial cells of the mammary gland, enhancing more milk yield through the milk ejection reflex. This effect on oxytocin level could be responsible for the increase in the milk yield observed in the vitamin C-treated group in this study. The effect of these supplements agrees with Abdel khalek *et al* (2008) who observed a better performance in lactating doe rabbits following treatment with supplements of vitamins C. This is suggestive that vitamin C promotes milk letdown via an inhibitory mechanism on the dopaminergic cells along the hypothalamo-hypophyseal axis (Esmaeilpour-Bezenjani, and Abbasnejad, 2013) thereby inducing an increase in oxytocin synthesis in the lactotrophs cells on the adenophysis. This occurs through inhibition of D_2 receptors on dopaminergic cells resulting in potassium (K^+) channel opening which in turn increases its intracellular concentration while reducing calcium Ca^{2+} entry and its intracellular concentration. As a result of decreased calcium concentration within these cells, there is a corresponding decrease in dopamine release (Kauppila *et al*., 2001; Gupta and Gupta 2005). Vitamin C could also have increased oxytocin level by enhancing activities of peptidylglycine alpha amino amidating mono oxygenase (PAM) enzyme during oxytocin synthesis leading to an increase in the serum oxytocin level (Epipper *et al*., 1994). These supplements could have also elicited the observed activity through activation of phospholipase C (PLC) and protein kinase C (PKC) thereby increasing Ca^{2+} intracellular concentrations and mobilizations from the endoplasmic reticulum within the lactotrophic cells thereby increasing formation of vesicular oxytocin and subsequent release (Rizvi *et al*., 2015). Suckling triggers milk ejection reflex through neuroendocrine reflexes which causes release of oxytocin from the hypothalamo hypophyseal axis leading to milk ejection (Yahuza *et al*., 2016). Vitamin C from this study with higher oxytocin level has been found to increase milk yield/milk production in lactating Wistar rats through the milk ejection mechanism however the receptors to which vitamin C act upon to enhance oxytocin secretion remains unknown.

CONCLUSION

The study has shown that Vitamin C supplementation in lactating Wistar rats improved milk yield by enhancing milk ejection due to its effect on serum

oxytocin hormone better than vitamin E and the co-administration of vitamin C and E.

REFERENCES

Abdel-Khalek, A.M., Selim, N.A., El-Medany, S.A. and Nada, S.A. (2008). Response of doe rabbits to dietary antioxidant vitamins E and C during pregnancy and lactation. In: Xiccato, G.,Trocino, A. and Lukefahr, S.D. (eds). *Proceedings of the 9th World Rabbit Congress*, Verona Italy, Pp 519-5623.

Allen, H.L. (2005). Multiple micronutrients in pregnancy and lactation: an overview. The *American Journal of Clinical Nutrient*, 81(5): 1206-1212.

Ann, H. and Linzell, J.L. (2003). A simple technique for measuring the rate of milk secretion in the rat. Comparative Biochemistry and physiology part A: Physiology, 43(2): 259-70.

Bako, I.G., Mabrouk, M.A., Abubakar, M.S. and Mohammed, A. (2013). Lactogenic study of the ethyl-acetate fraction of Hibiscus sabdariffalinn seed on pituitary prolactin level of lactating albino rats. *International Journal of Applied Research in Natural Products*, 6(2) : 30-37

Bako, I.G., Maje, M.I., Emmanuel, S.N. and Muhammad, U.A. (2015). Lactogenic study of the crude extract of Nigella sativa seed on serum prolactin level of lactating albino rats. *Nigerian Journal of Scientific Research*; 14(2): 85-89.

Epipper, B.A., Milgram, S.L., Husten, J.E., Yun, H.Y and Mains R.E. (1993). Peptidylglycine α-amidating monooxygenase: A much functional protein with catalytic processing and routing domains. *Protein Science*; 2:489-497.

Esmaeilpour-Bezenjani, K.H. and Abbasnejad, M. (2013). Effect of administration of ascorbic acid and dopamine D_2 receptor agonist in the hippocampal CAI area on spatial learning and memory in adult male rats. *Iranian journal of vetinary research shiraz university*; 14 (2): 126-132.

Freeman, M.E., Bela, K., Leran, T.A and Nagy, G. (2006). Prolactin structure, function and regulation of secretion. *Physiological Reviews*, 80: 1523-1631.

Gupta, A.P. and Gupta, P.K. (2005). Metoclopramide as a lactogogue *Clinical Pediatrics* 24(5): 269–272.

Hassioutou, F. and Geddes, D. (2012). Anatomy of the human mammary gland: current status of knowledge. *Clinical Anatomy*, 26(1) : 29-48.

Kauppila, A., Kivinen, S. and Ylikorkala, O. (2001). A dose response relation between improved lactation and Metoclopramide. *The Lancet*, 317(8231): 175–177

Moser, M.A and Chun, O. (2016). Vitamin C and Heart Health: A Review Based on Findings from Epidemiologic Studies. *International Journal of Molecular Science*, 17(8): 1328

Morag, M. (1970). Estimation of milk yield in the rat, *Laboratory Animal*, 4(2): 259-72.

Odom, E.C., Li, R. and Scanlon, K.S. (2013). Reasons for earlier than desired cessation of breastfeeding. Paediatrics, 131: e726-e732.

Okasha, M.A.M., Abubakar, M.S., Bako, I.G. (2008). Study of *hibiscus sabdariffa* linn seed extract on serum prolactin level on lactating female Albino rats. *European Journal of Scientific Research* 4: 575-583.

Ouedraogo, Z.L., Heide, D.V., Beek, E.M.V., Swarts, H.J.M., Mattheij, J.A.M. and Sawadogo, L. (2004). Effect of aqueous Acacia niloticasppadansonii on milk production and prolactin release in the rat. *Journal of Endocrinology*, 182: 257- 266.

Rizvi, S., Raza, S.T., Ahmed, F., Ahmad, A., Abbas, S. and Muahdi, F. (2016). The Role of Vitamin E in Human Health and Some Diseases. *Sultan Qaboos University Medical Journal*, 14(2): 157–165.

Sampson, D.A. and Jansen, G.R. (1984). Measurement of milk yield in lactating rat from pup weight and weight gain. *Journal of Pediatry, Gastroenterology and Nutrition*, 3: 613–617.

Simelane M.B.C., Lawal, O.A., Djarova, T.G., Musabayane, C.T., Singh, M and Opoku, A.R. (2012). Lactogenic activity of rats stimulated by *Gunnera Perpensa* L (Gunneraceae) from south Africa. African journal of tradition, complementary and alternative medicine. 9 (4): 561-573.

Yadav, A., Kumari, R., Yadav, A., Mishra, J.P., and Prabha, S. (2016). Antioxidants and its functions in human body. A review research. Research in environment and life science; 9: 1328-1331.

Yahuza, F.M., Bako, I.G. and Kawu, M.U. (2016). Study of lactogenic activity of resveratrol in female lactating Albinom wistar rats.

Zuppa,A., Sindico, A., Orchi, C. (2010). Safety and efficacy of galactogogues: substances that induce, maintain, and increase breast milk production. *Journal of Pharmacy and Pharmaceutical Sciences* 13(2).

Ziomkiewick, A., Sancilio, A., Galbarczyk, A., Kilmex, M., Jasienska, G. and Bribiesces, R.G. (2016). Evidence for the cost of reproduction in humans: High life time productive effort is associated with greater oxidative stress in post-menopausal women. *Plos One*, 11(1): e0145753.

J. Afr. Ass. Physiol. Sci. 7 (1): 44-52, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Effect of salt loading and gender influences on plasma atrial natriuretic peptide levels and cardiovascular parameters in normotensive and hypertensive Nigerians

[1*]A.O. Abidoye, [2]S.I. Jaja and [2]O.A. Sofola

Departments of Physiology, [1]Lagos State University College of Medicine, Ikeja, Lagos and [2]College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria.

Keywords:
Atrial natriuretic peptide, blood pressure, salt loading, gender, hypertension.

ABSTRACT

Background: Prevalence of hypertension is higher among Sub-Saharan Africans than whites and there is a sex difference in the prevalence. Though, salt retention has been implicated in pathogenesis of hypertension, the basis is not completely known. Atrial natriuretic peptide (ANP) might play a role. **Method:** 43 apparently healthy normotensive male and female Nigerians and 37 aged-matched hypertensive counterparts were orally administered 11.6g of dietary salt each per day for 5 days. Their plasma ANP levels, heart rate (HR), systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MABP) were determined before and after salt loading. **Results:** Normotensive and hypertensive male and female subjects had similar basal ANP levels. However, salt loading significantly raised the ANP concentrations ($p = 0.0001$) in normotensive subjects but not significantly in hypertensive counterparts. ANP levels rose significantly in the normotensive males ($p = 0.0024$) and females ($p = 0.0002$) but not significantly in the hypertensive counterparts. Besides, salt significantly decreased HR in normotensive ($p = 0.0095$) and hypertensive ($p = 0.0397$) subjects but increased SBP ($p < 0.01$) and MABP ($p< 0.01$) in both study groups and DBP ($p = 0.0014$) in hypertensive group only. SBP, DBP and MABP were all significantly elevated ($p < 0.05$) in hypertensive males and females but not significantly in normotensive females. **Conclusion**: Although, basal ANP levels were similar in the study normotensive and hypertensive subjects, other findings in this study suggest that ANP as well as female gender could ameliorate increased blood pressure response to salt loading especially in a normotensive state

INTRODUCTION

Prevalence of hypertension in Africa is estimated at 46% in adult individuals aged 25 years and above with higher prevalence in males than females (WHO, 2013a; Akinlua *et al*., 2015). Hypertension affects more than one billion people, globally (Mills *et al*., 2016) and is responsible for about 9 million deaths, annually, worldwide (WHO, 2013b). It is the single most important risk factor for stroke and coronary artery disease (Garg, 2014). The current levels (9 – 12 g/day) of salt consumption in most countries, have exceeded daily recommended levels (<5 g/day) (Samogan *et a*l., 2014; Dötsch-Klerk *et al*., 2015). Salt retention has been implicated in the pathogenesis of hypertension (He and MacGregor, 2007; Hauck *et al*., 2012). Although, most people exhibit an increase in blood pressure on salt loading (Weinberger, 1996), the quantity of salt required to induce a hypertensive state, varies (Richardson *et al*., 2013). The basis for this variation is still a subject of intense research.

The mechanisms by which salt raises blood pressure are complex, multi-factorial and incompletely understood (Hauck *et al*., 2012). Some of the mechanisms include a genetic defect in salt transport, increased sympathetic activity, pathological condition of the kidney, adrenal cortex or pituitary gland (Strazzulo *et al*., 2001; Brooks *et al*., 2005; Blaustein *et al*., 2010). Other mechanisms such as reduced production of vasodilators like nitric oxide, dopamine and kallikrein have also been

*Address for correspondence:
Email: oladele.abidoye@lasucom.edu.ng
Tel: +234 803 502 0338

implicated in the pathogenesis of hypertension (Fujwara *et al.,* 2000). The role of atrial natriuretic peptide in the development of salt-induced hypertension has not been fully investigated. There is paucity of data regarding the effect of salt loading on plasma atrial natriuretic peptide concentrations in normotensive and hypertensive Nigerians. Previous measurements of plasma atrial natriuretic peptide levels, assessed among individuals with essential hypertension in European and North American population, have provided conflicting results. Some investigators have reported low ANP concentrations in hypertension (Macheret *et al.,* 2012) while others have documented high amounts (Irzmanski *et al*., 2007).

Atrial natriuretic peptide (ANP) is an endocrine hormone that regulates salt and water balance as well as blood pressure by promoting renal loss of sodium and water (Song *et al*., 2015). It is produced mainly in the cardiac atria and released into the circulation in response to volume expansion and increased atrial distension (Klar *et al.,* 2007; Wang *et al*., 2012). It has been suggested that Sub-Saharan Africans have lower plasma atrial natriuretic levels than their white counterparts (Guptal *et al*., 2015). The higher prevalence of hypertension reported in Sub-Saharan Africans might be due to abnormal ANP concentrations. In mice, ANP deficiency caused salt-sensitive hypertension and cardiac hypertrophy (Song *et al.,* 2015). It is not clear if sex difference exists on plasma ANP levels among Africans particularly in a hypertensive condition when high salt diet is consumed; hence, the study was designed to assess plasma ANP levels in a Nigerian population as well as to determine the effects of salt loading and gender influences on plasma ANP levels and blood pressure in normotensive and hypertensive Nigerian volunteers.

METHODS

Forty-three (43) apparently healthy normotensive and thirty-seven (37) age-matched newly diagnosed hypertensive subjects who were yet to be on antihypertensive medications, participated in the study. Ethical approval with Ref No: CM/HREC/10/16/101; dated February 16, 2017 was obtained from Health Research Ethic Committee of College of Medicine, University of Lagos. The volunteers were briefed about the study and duly signed informed consent forms were obtained.

Inclusion Criteria:

The normotensive subjects had their blood pressure below140/90 mmHg. They were not on any antihypertensive medication, not suffering from chronic kidney, cardiovascular or cerebrovascular disease. They did not have any abnormal ECG findings such as left ventricular hypertrophy, ischaemic heart disease, myocardial infarction, atrial fibrillation and they were not diabetic.

The hypertensive volunteers had sustained systolic blood ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or both (Franklin, 2004). The subjects were not on any antihypertensive drug, not diabetic or on any hypoglycaemic medication and they were not suffering from any complication arising from their hypertensive condition.

Exclusion Criteria:

The normotensive volunteers who were diabetic, smokers or had history of chronic consumption of alcohol were excluded from the study. Hypertensive volunteers that had severe hypertension (BP ≥ 180/110 mmHg) or history of cardiovascular disease or chronic kidney disease as evident by plasma $K^+ \geq 5.5$ mmol/l and or creatinine ≥ 150 µmol/l, were also excluded from the study. Pregnant women were not allowed to participate in the study for medical and ethical reasons.

Experimental procedures

Anthropometric data of the subjects were determined. Age was recorded in years and body weight was measured with the aid of weighing scale and recorded in kilogrammes. Height in metres was measured using stadiometer and the body mass index (BMI) was calculated by dividing the body weight (kg) by square of the height (m) (Monterio*et al*., 2012).

Measurements of Cardiovascular Parameters of the Subjects

The cardiovascular parameters were determined before and after salt loading in the study groups of subjects

Determination of Heart Rate

Heart rate (beats/minute) was determined using an electrocardiograph machine. The procedure was briefly explained. Subjects were allowed to rest for10 minutes in sitting position. The placement of electrodes was done in conformity with the American Heart Association recommendations (Kligfield *et al*., 2007). The heart rate was determined from the R-R intervals in Lead II of the electrocardiogram, using formula: 25/R –R x 60 (beats/min) (Kligfield *et al*., 2007).

Determination of Blood Pressure

Blood pressure was determined by auscultatory method using Accoson mercury sphygmomanometer (Accoson, United Kingdom, 2014), as per the described instructions of American Heart Association (Beevers *et*

al., 2001). Subjects were allowed to rest for 10 minutes in sitting position. Appropriate cuff size was wrapped on the right arm with the midline of cuff over the brachial arterial pulsation and inflated rapidly while palpating radial pulse. Reading at which pulse disappeared was noted and pressure was further elevated 20 – 30mmHg above this value. Then the cuff was slowly deflated while listening to the Korotkoff's sounds using a stethoscope placed on brachial arterial pulsation. Systolic blood pressure and diastolic blood pressure were recorded to the nearest 2mmHg as the first appearance and disappearance of the Korotkoff's sounds, respectively. The blood pressure was taken thrice and the average determined and recorded.

Determination of Mean Arterial Blood Pressure

Mean arterial blood pressure was determined from the sum of diastolic blood pressure and one-third of pulse pressure (Zheng *et al*., 2008). The pulse pressure is the difference between systolic and diastolic blood pressure.

Measurements of Laboratory Parameters

The laboratory parameters measured in the subjects were fasting blood sugar, plasma creatinine, sodium, potassium and ANP levels as well as the urine sodium and potassium concentrations and urine volumes.

Protocol for Venous Blood Collection

The subjects fasted overnight and reported at 9a.m in the laboratory for their blood collection. They were briefed about the procedure. Venous blood was withdrawn from the antecubital vein under aseptic condition and emptied into appropriately labeled blood sample bottles. Lithium heparin bottles were used for blood creatinine, sodium and potassium estimations while chilled EDTA bottles were used for ANP estimation. The withdrawn blood samples were spinned immediately at 2000 x g at 4°C for 10 minutes. The supernatants were stored at – 40°C until analyses were carried out.

Fasting blood sugar was measured using Accu-check glucometer (Roche Diabetes Care Inc; USA, 2008). Plasma creatinine was determined using COBAS C 111 machine (Roche Diagnostics, USA, 2012). Plasma and urinary sodium and potassium concentrations were measured before and after salt loading, using ion selective electrode (ISE 6000) machine (SFRI, France, 2011). The 24- hour urine volumes were also measured before and after salt loading, using a measuring cylinder. Plasma ANP concentrations were determined before and after salt loading, using Human Atrial Natriuretic Peptide Eliza Kits (Sunlong Biotech, China, 2016). The ANP levels were analyzed as described by the manufacturer's instructions.

Acute Salt Loading in the Study Subjects

Having measured and recorded the baseline cardiovascular and laboratory parameters, each of the subjects in the study group ingested 200mmol of sodium (11.6 g of dietary salt) per day in two divided doses for 5 days (Tzemos *et al*., 2008; Elias *et al*., 2014). The subjects were followed up during the course of salt administration and compliance with the salt ingestion was assessed by determining their 24-hour urine sodium excretions before and after the salt loading. They reported in the laboratory on the 6th day for measurements of their cardiovascular and laboratory parameters, following the 5-day period of salt ingestion.

Data Analysis

Data analysis was carried out with the help of GraphPad Statistical software, Version 5 for Windows (GraphPad Software, San Diego, California, USA). Data was expressed as mean ± standard deviation. Paired student's t- test was employed to analyze data within the group and unpaired t- test for data between the study groups. Statistical significance was accepted at $p < 0.05$ level.

RESULTS

Baseline Characteristics of the Subjects in the Study Groups

The baseline characteristics of the participants in the study groups are shown in Table 1.

Although, the mean body weight and BMI of subjects in the hypertensive group were significantly higher than those of normotensive counterparts, there was no significant difference in their mean ages. The fasting blood sugar and creatinine levels were also observed to be similarr in the study groups (Table 1).

Table 1: The Baseline Characteristics of the Study Groups of Subjects

Parameters	Normotensive Group (n = 43)	Hypertensive Group (n = 37)	p Value
Age (yr)	43.09 ± 8.14	46.43 ± 6.73	0.0513
Body weight (kg)	67.60 ±10.62	77.00 ±14.53	0.0012
Height (m)	1.64 ± 0.07	1.65 ± 0.07	0.9019
BMI (kg/m^2)	25.10 ± 3.92	28.48 ± 4.60	0.0007
FBS (mg/dl)	81.86 ±10.12	83.78 ± 9.40	0.3838
Creatinine(µmol/l)	69.00 ± 18.54	71.65 ± 17.94	0.5208

Values are means ± SD as analyzed by student's t-test. n = number of subjects; SD = standard deviation; BMI = body mass index; FBS = fasting blood sugar NS=not significant

Effect of Salt Loading on the Plasma ANP Levels in the Study Groups

The basal plasma ANP levels observed in the normotensive and hypertensive subjects were similar (Table 2). However, salt loading significantly increased

plasma ANP levels (p = 0.0001) in the normotensive group but had no significant effect on ANP concentrations in the hypertensive group. In addition, when comparing the mean changes in the plasma ANP concentrations observed in the study groups after salt loading, the mean change in the normotensive group was significantly higher (p = 0.0137) than that of the hypertensive group (Table 2).

Influence of Gender on Plasma ANP Levels

The plasma ANP levels before and after salt loading in male and female normotensive and hypertensive volunteers are also shown in Table 2. In the normotensive group, basal ANP concentrations between males and females were similar. Also, in the hypertensive group, the basal ANP levels observed in males and females were not significantly different. The basal ANP concentrations seen in the normotensive males and hypertensive females or normotensive females and hypertensive males were not significantly different (Table 2). However, after salt loading in these subjects, plasma ANP levels rose significantly in both normotensive males (p = 0.0024) and females (p < 0.0002) but there were no significant increases in the hypertensive male and female counterparts (Table 2).

The mean ANP concentration after salt loading in the normotensive females was observed to be significantly higher (p = 0.0263) than that of the hypertensive female counterparts but those of the normotensive and hypertensive males were not significantly different. In addition, no significant difference was seen in the mean changes in the normotensive males and females. The mean ANP changes in the hypertensive male and female subjects were also similar (Table 2).

Effect of Salt Loading on Cardiovascular Parameters Measured in the Normotensive and Hypertensive Subjects

The mean values of heart rates, systolic and diastolic blood pressure as well as the mean arterial blood pressure measured before and after salt loading in the study groups are shown in Tables 3.

Heart Rates:

There was no significant difference between the mean values of the heart rates observed in the normotensive and hypertensive subjects before salt loading (Tables 3). However, after salt loading, the heart rates fell significantly in both normotensive (p = 0.0095) and hypertensive (p = 0.0397) groups (Table 3). There was no significant difference in the mean heart rate changes, observed in these study groups.

Systolic Blood Pressure:

Hypertensive subjects had significantly higher basal SBP (p = 0.0001) than normotensive subjects. Salt loading significantly elevated SBP in the normotensive (p = 0.0023) and hypertensive (0.0037) volunteers (Tables 3). When comparing the mean changes in SBP observed in the study groups after the salt ingestion, they were not significantly different (Table 3).

Diastolic blood pressure:

Hypertensive volunteers also had significantly higher basal DBP (p = 0.0001) than normotensive counterparts (Tables 3). Salt loading increased DBP significantly (p = 0.0014) in hypertensive subjects but not significantly in the normotensive counterparts. The mean changes in DBP in the study groups though, higher in the hypertensive subjects, were not significantly different.

Mean arterial blood pressure (MABP):

Basal MABP was significantly higher (p = 0.0001) in the hypertensive subjects than normotensive volunteers. Salt loading significantly elevated mean MABP in both normotensive (p = 0.0025) and hypertensive (p =0.0014) subjects and the mean changes in MABP after salt loading, were not significantly different.

Table 2: Plasma ANP Levels Measured before and after Salt Loading in Normotensive and Hypertensive Groups of Subjects

Subjects m (f)	Normotensive 20 (23) ANP (pg/ml)	Hypertensive 16 (21) ANP (pg/ml)	p Value
Before Salt loading			
Males	10.16 ± 1.17(n =22)	10.75 ± 2.13 (n = 16)	0.2932
Females	10.99 ± 2.70(n – 21)	10.33 ± 1.61 (n – 21)	0.3383
Combined (M & F)	10.60 ± 2.15 (n – 43)	10.52 ± 1.84 (n = 37)	0.8440
After Salt Loading			
Males	11.24 ± 1.76**	11.23 ± 0.38	0.9783
Females	12.26 ± 2.76***	10.68 ± 1.70	0.0286
Combined (M & F)	11.78 ± 2.38***	10.92 ± 1.63	0.0649
Δ (Changes after Salt)			
Males	1.05 ± 1.42	0.48 ± 1.37	0.2348
Females	1.26 ± 1.43	0.35 ± 1.17	0.0263
Combined (M & F)	1.16 ± 1.42	0.40 ± 1.25	0.0137

Values are expressed in mean ± SD as analyzed by student's t-test.**:** n = number of subjects; SD = standard deviation M & F = combined male and female subjects in the group; Δ = changes in ANP levels after salt loading m (f) = males (females). **p = 0.0024 in ANP levels observed in the normotensive males before and after salt loading' ***p = 0.0002 in ANP levels observed in normotensive females before and after salt loading; ***p = 0.0001 in ANP levels observed before and after salt loading in normotensive group (M&F combined)

Influences of Gender on the Cardiovascular Parameters Measured in the Normotensive and Hypertensive Subjects

Influences of gender on the cardiovascular parameters measured in the study normotensive and hypertensive subjects are shown in Table 4. The basal heart rates in

the normotensive male and female subjects were not significantly different but hypertensive females had a significantly higher basal heart rate (p = 0.0161) than their counterpart males. After salt loading, heart rates significantly fell in normotensive (p = 0.0236) and hypertensive (p =0.0171) females but not significantly in normotensive and hypertensive males (Table 4).

In addition, normotensive male and female subjects had similar basal systolic blood pressure values and there was no significant difference in the basal systolic blood pressure observed in the hypertensive male and female counterparts (Table 4). However, after salt loading, systolic blood pressure was significantly elevated (p = 0.0008) in the normotensive males but not significantly in the normotensive females. In the hypertensive group, on the other hand, the systolic blood pressure was significantly increased in both males (p = 0.0490) and females (p = 0404).

Furthermore, normotensive males had a significantly higher basal diastolic blood pressure (p = 0.0375) than normotensive females. In the same vein, hypertensive males had a significantly higher basal diastolic blood pressure (p = 0.0113) than their females. However, salt loading significantly increased diastolic blood pressure in both hypertensive males (p = 0.0310) and females (0.0208) but not significantly in the normotensive male and female counterparts (Table 4).

The basal mean arterial blood pressure values observed in normotensive males and females were not significantly different. Also, in the hypertensive male and female subjects, the basal MABP values were similar. However, after salt loading, the mean arterial blood pressure values were significantly raised in the hypertensive males (p = 0.0147) and females (p = 0.0353) but no significantly in the normotensive male and female counterparts (Table 4)

DISCUSSION

The subjects in the study groups were aged- matched. This was ensured so as to eliminate age factor that has been reported to be associated with high blood pressure (Rockwood and Howlett, 2011). The participants were selected primarily based on their health status. They were not suffering from any end organ damage such as left ventricular hypertrophy, ischaemic heart disease, congestive heart failure, chronic kidney disease or cerebrovascular disease; as any of these disease conditions, has been reported to be associated with abnormally high plasma ANP concentrations (Minamino and Nishikimi, 2013; Volpe *et al*., 2016; Ogawa *et al*., 2015). In addition, none of the subjects was diabetic, as abnormally low ANP concentrations have been documented to be observed in diabetes mellitus patients (Wang *et al*., 2007; Magnusson *et al.*, 2012).

The basal plasma ANP levels in the study population were similar. This finding disagrees with earlier studies carried out among Caucasians that hypertensive individuals have higher basal ANP levels than normotensive counterparts (Irzmanski *et al*., 2007; Hu *et al.*, 2015). However, when compared the basal ANP levels observed in the study population with those reported in western population (Castro and Gombein, 1994), the levels observed in the Nigerian population were found to be lower. Abnormally low plasma ANP concentrations have been implicated in the pathogenesis of salt induced hypertension (Song *et al.*, 2015). This finding agrees with what Gupta and his co-workers (2015) reported that African Americans have lower plasma ANP concentrations when compared with Caucasians.

Table 3: Cardiovascular Parameters Measured before and after Salt Loading in Normotensive and hypertensive Groups of Subjects

Cardiovascular Parameters Measured	Normotensive Group (n = 43)			Hypertensive Group (n = 37)		
	Before Salt Loading	After Salt Loading	p -Value	Before Salt Loading	After Salt Loading	p Value
HR(beats/min)	73.00 ± 7.29	69.77 ±8.54	0.0095	76.11 ± 8.67	73.51 ±10.66	0.0397
SBP(mmHg)	116.60 ± 10.09	122.00 ± 12.43	0.0023	136.4*** ± 9.00	142.80 ± 13.10	0.0037
DBP (mmHg)	80.21 ± 7.24	82.81 ± 10.01	0.0642	97.00*** ± 5.91	101.9 ± 9.44	0.0014
MABP(mmHg)	92.35 ± 7.50	96.40 ±10.02	0.0025	110.50*** ± 5.55	115.50 ± 8.37	0.0014

Values are expressed in mean ± SD, as analyzed by student's t-test. MABP = mean arterial blood pressure; n = number of subjects in the group; SBP = systolic blood pressure; DBP = diastolic blood pressure SD = standard deviation; HR = heart rate, ***P = 0.0001 in SBP values between normotensive and hypertensive groups of subjects before salt loading. ***P = 0.0001 in DBP values between normotensive and hypertensive groups of subjects before salt loading. ***P = 0.0001 in MABP values between normotensive and hypertensive groups of subjects before salt loading.

Salt loading increased plasma ANP concentrations significantly in the normotensive subjects but the hypertensive counterparts demonstrated a blunted ANP response to the salt challenge. Furthermore, the normotensive volunteers had higher mean change in the plasma ANP levels than hypertensive counterparts. The slight increase in the plasma ANP levels, seen in the hypertensive subjects, might be due to impaired response of stretch receptors to increased blood volume, caused by salt loading in these individuals. Stretch-

induced ANP release is said to be suppressed by an endogenous angiotensin II (Oh *et al*., 2011) which has since been documented to be raised in hypertension (Catt *et al.*, 1971).

Table 4: Cardiovascular Parameters Measured before and after Salt Loading in the Normotensive and Hypertensive Male and Female Subjects

Cardiovascular Parameters Measured	Normotensive Group					
	Male Subjects (n = 20)			Female Subjects (n = 23)		
	Before Salt	After Salt	p Value	Before Salt	After Salt	p Value
HR (mmHg)	71.85 ± 6.49	69.70 ± 8.53	0.2052	74.00 ± 7.92	69.83 ± 8.73	0.0236
SBP (mmHg)	117.20 ± 8.52	125.00 ± 9.83	0.0008	116.10 ± 11.46	119.40 ±14.01	0.2117
DBP (mmHg)	82.65* ± 6.04	86.95 ± 7.98	0.5047	78.09 ± 7.64	79.22 ±10.35	0.5324
MABP (mmHg)	94.17 ± 6.15	99.63 ± 7.81	0.0086	90.77 ± 8.31	93.58 ±11.01	0.1112
Cardiovascular Parameters Measured	**Hypertensive Group**					
	Male Subjects (n = 16)			Female Subjects (n = 21)		
	Before Salt	After Salt	p Value	Before Salt	After Salt	p Value
HR (beats/min)	71.81 ± 5.49	71.69 ± 6.72	0.9355	79.38* ±10.94	74.90 ±12.88	0.0171
SBP (mmHg)	134.80 ± 7.48	141.90 ± 13.18	0.0490	137.70 ± 9.99	143.50 ±13.33	0.0404
DBP (mmHg)	99.75* ± 6.73	104.20 ± 8.70	0.0310	94.90 ± 4.27	100.20 ± 9.82	0.0208
MABP (mmHg)	111.40 ± 6.01	116.70 ± 9.29	0.0147	109.70 ± 5.20	114.60 ± 9.55	0.0353

Values are expressed in mean ± SD as analyzed by student's t-test; MABP = mean arterial blood pressure, n = number of subjects; SBP = systolic blood pressure; DBP = diastolic blood pressure HR = heart rate; SD = standard deviation; before salt = before salt loading; after salt = after alt loading * p = 0.0161 in basal HR values observed values between hypertensive males and females, * p = 0.0113 in basal DBP values observed values between hypertensive males and females. * p = 0.0375 in basal DBP values between normotensive males and females

Regarding influences of gender on plasma ANP concentrations in the normotensive and hypertensive subjects in this study, the basal ANP levels were similar in males and female subjects of the study groups. However, after salt ingestion what was observed in the study subjects was quite interesting as males and females in the normotensive group demonstrated significant increases in their ANP levels but salt loading did not significantly elevate ANP levels in their hypertensive male and female counterparts. The blunted ANP response demonstrated by these hypertensive individuals, might be an underlying cause for poor salt handling that has been reported to be seen in a hypertensive state (O'Shaughnessy and Karet; 2004). The higher ANP response observed in the normotensive females than males might be due to oestrogen effect as it has been documented that oestrogen induces ANP release from the heart via oestrogen receptor (Vishwarkama *et al*., 2016).

On the cardiovascular parameters measured in the study population, basal heart rates were similar in the study groups. However, basal systolic, diastolic and mean arterial blood pressure were observed to be significantly higher in the hypertensive subjects than normotensive counterparts. These were not quite surprising as hypertension is a chronic disease condition, characterized with abnormal elevations in systolic and diastolic blood (Michael *et al.*, 2007).

In this study, salt loading significantly increased systolic and mean arterial blood pressure as well as causing significant decreases in the heart rates in both normotensive and hypertensive groups of subjects. The magnitude of these heart rate decreases though found to be higher in the normotensive than hypertensive subjects, the decreases were necessitated by a reflex response to increased blood pressure demonstrated by the subjects (McNeely *et al*., 2008; Messerli, 2013).

The expansion in blood volumes that led to increase in blood pressure caused stretch receptors (baroreceptors) in the carotid sinus and aortic arch to be stimulated, leading to increased vagal discharge to the heart from nucleus ambiguus and dorsal motor nucleus of the vagus in the medulla oblongata (Wang *et al*., 2001; Messerli, 2013). Hence, the observed decreases in heart rates in the normotensive and hypertensive subjects.

Salt loading significantly elevated systolic and mean arterial blood pressure in both study groups but diastolic blood pressure was not significantly raised in the normotensive group. Although, impaired baroreflex function has been documented in hypertension (Heusser *et al.*, 2005), the differential response in diastolic blood pressure that was seen in the normotensive subjects following salt loading, might also be due to a significant increase in their ANP concentrations. ANP is a vasodilator that reduces peripheral resistance leading to a decrease in blood pressure (Chopra *et al.*, 2013; Song *et al*., 2015; Ogawa *et al*., 2015). It is also inhibitory to sympathetic nervous activity (Nakagawa *et al.*, 2015).

On the effect of gender on the measured cardiovascular parameters on salt loading, female gender played a protective role on cardiovascular function especially in a normotensive condition as evident by insignificant increases observed in heart rate, systolic, diastolic and mean arterial blood pressure seen in the normotensive females after salt ingestion but these were not the case in

the hypertensive female counterparts is as all these parameters (SBP, DBP and MABP) were significantly elevated in them. This implies that oestrogen though is reported to play a protective role in cardiovascular function in premenopausal women (Lorga *et al*., 2017), this role seems to be impaired in a hypertensive state.

CONCLUSION

In the study subjects, basal ANP levels were similar in normotensive and uncomplicated hypertensive states. However, salt loading significantly elevated ANP concentrations in normotensive subjects with greater ANP response in females than males but not significantly increased in hypertensive volunteers. Findings in this study suggest that ANP as well as female gender could ameliorate increased blood pressure response to salt loading primarily in normotensive individuals.

REFERENCES

Akinlua J.T,, Meakin R, Umar A.M., Freemantle N. (2015). Current prevalence pattern of hypertension in Nigeria: A systematic review. *PLoS ONE*; 10(10): 1 - 18.

Beevers G., Gregory Y.H., and O'Brein E. (2001). Blood pressure measurement: Part II conventional sphygnomanometry: technique of auscultatory blood pressure measurement. *BMJ*; 322: 1043-1047.

Blaustein M.P., Hamlyn J.M. (2010). Signaling mechanisms that link salt retention to hypertension: Endogenous ouabain, the Na^+ pump, the Na^+/Ca^{2+} exchanger and TRPC proteins. *Bio chemical Biophys Acta;* 1802(12): 1219 – 1229.

Brooks V.L., Haywood J.R., Johnson A.K. (2005). Translation of salt retention to central activation of sympathetic nervous system in hypertension. *Clin Exp Pharmacol Physiol*; 32(5-6):426 – 432.

Castro L.C., Hobel CJ., Gombein J. (1994). Plasma levels of atrial natriuretic peptide in normal and hypertensive pregnancies: a meta-analysis. *Am J Obstet Gynaeco*; 17 (16): 1642 -1651.

Catt K.J., Zinnat P.Z., Cam M.D., Crab E., Best J.B., Coghlan J.P. (1971). Angiotensin II blood levels in human hypertension. DOI:https://doi.org/10.1016/S0140-6736(71)81085-3

Chopra S., Cherian D., Verghese P., Jacob J.J. (2013). Physiology and clinical significance of natriuretic hormones. *Ind J Endocrinol and Metab*; 17(1): 83 – 90.

Dötsch-Klerk M., Goossens W.P., Meijer G.W, van het Hof K.H. *(2015).* Reducing salt in food; setting product-specific criteria aiming at a salt of 5 g per day. *Eur J Clin Nutri*; 69(7): 799 – 804.

Elias SO, Sofola OA and Jaja SI. (2014). Vascular reactivity and salt sensitivity in normotensive and hypertensive Nigerians. *J. Afr. Ass. Physiol. Sci*; 2 (2): 95-103.

Franklin SS. (2004). Systolic blood pressure: it is time to take control. *American Journal of hypertension*; 17 (S3): 49S -54S.

Fujwara N., Osanai T., Kamada T., Katoh T., Takarashi K., Okumura K. (2000). Study on the relationship betweenplasma nitrite and nitrate level and sal sensitivity in human hypertension. *Circulation*; 101(8):856- 861.

Garg R. (2014). What health problems are associated with hypertension? *Indian Journal of Clinical Practice*; 25(7): 683 – 684.

Gupta, D.K., Claggett B., Wells Q., Chen S., Li M., Maruthur N., Selvin E., *et al.* (2015). Racial differences in circulating natriuretic peptide Levels: The Atherosclerosis Risk in Communities Study." *J Ame Heart Assoc*; 4 (5): 1- 8.

Hauck C., Frishman W.H. (2012). Systemic hypertension: The roles of salt, vascular Na^+/K^+ ATPase and the endogenous glycosides, Ouabain and marinobufagenin. *Cardiol Rev*; 20(3): 130–138.

He F.J., MacGregor G.A. (2007). Salt, blood pressure and cardiovascular disease. *Curr Opin Cardiol* 22(4):298 – 305.

HU W., Zhou P., Zhang X., Xu G., Wang W. (2015). Plasma concentrations of adrenomedullin and natriuretic peptides in patients with essential hypertension. *Exp Ther Med*; 9(5) : 1901 -1908.

Irzmanski R., Barylski M., Banach M., Piechota M., Kowalski J., Cierniewski C., Pawlicki L. (2007). The concentration of atrial and brain natriuretic peptide in patients with idiopathic hypertension. *Med Sci Monit*; 13(10):CR449-56.

Klar A., Haver E., Lichtstein D., Lichstein D., Hurvitz H., Foan-Shauli T.. (2009). Atrial natriuretic peptide in young and elderly children with mild gastroenteritis. *Gastroenterol Res Pract*; 2009: 1 – 4.

Kligfield P. (2007). Recommendations for standardization and interpretation of electrocardiogram. *J Am Coll of Cardiol*; 49(10):1109-27.

Kohno M, Yasunari K., Toshifunmi M., *et al*. (1987). Circulating atrial natriuretic polypeptide in essential hypertension. *Am Heart J*; 113(5);1160 – 1163.

Lorga A., Cunningham C.M., Moazeni S., Ruffenach G., Umar S., Eghbali M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biol Sex Differ*; 8: 33. doi: 10.1186/s13293-017-0152-8

Macheret, D., Costello-Boerrigter L.C., Boerrigter G., McKie P., Bellavia D., Mangiafico S., Ikeda Y, Bailey K., Scott C.G., Sandberg S., Chen S.H.H., Malatino L., Redfield M.M, Rodeheffer R., Burnett J, JR., Cataliotti A.. (2012). Human hypertension is characterized by a lack of activation of the antihypertensive cardiac hormones ANP and BNP. *J Am Coll Cardiol*; 60(16): 558 – 1565.

Magnusson I, Jujic A, Hedblad B., Engstrom G., Perrson M., Struck J., Morgenthaler NG., Newton-Cheh C., Wang T. J., Melander O. (2012). Low plasma level of atrial natriuretic peptide predicts development of diabetes: the prospective malmo diet and cancer study. *J Clin. Endocrinol. Metab*; 97(2): 635 – 645.

McNeely D., Windham B.G., Anderson D.E. (2008). Dietary sodium effects on heart rate variability in salt-sensitivity of blood pressure. *Psycholophysiology*; 45(3): 405 – 411.

Messerli F.H. (2013). Cardiovascular disease in the elderly, second edition; page 133.

Michael S.K., Surks H.K., Wang Y., Zhu Y., Blanton R., Jamnogiit M., Aronovitz M., Baur W., Ohtani K., et al. (2007). High blood pressure arising from a defect in the vascular function. Proc Natl AcadSci; 105 (18): 6702 – 6707.

Mills K.T., Bundy J.D., Kelly TN., Reed J.E., Keamey P.M., Reynoids K., Chen J., He J. (2016). Global disparities of hypertension prevalence and control: A systematic analysis of population-based studies from 90 countries. *Circulation;* 134(6): 441 – 450.

Minamino N., Nishikimi T. (2013). Natriuretic peptides. *Handbook of Biologically Active Peptides*, 2nd Edition; 1415 – 1422.

Monteiro G., Chathoth V.M., Kishan K. (2012). Cardiac autononomic responses during a cold pressor test in normal and overweight adults. *International Journal of Biomedical and Advance Research*; 3(6): 514 – 516.

Nakagawa H., Nakada Y., Saito Y. (2015). Natriuretic peptides regulate sympathetic nervous activity independent of mineralocorticoid receptor. *BMC Pharmacol Toxicol*. 2015; 16(Suppl 1): A71 doi: 10.1186/2050-6511-16-S1-A71

Ogawa N., Komura H., Kuwasako K., Kitamura K., Kato J. *(2015).* Plasma levels of natriuretic peptides and development of chronic kidney disease. *BMC Nephrology*; 16:171.

Oh Y., Gao S., Amin S., Kim J.H., Park E.H., Kim S.H., (2011). Endogenous angiotensin II suppresses stretch-induced ANP secretion via AT1 receptor pathway. *Peptides* 32(2) 374 -381.

O'Shaughnessy K.M., Karet F.E. (2004). Salt handling and hypertension. *J Clin Invest*; 113(8):1075–1081.

Richardson S.I., Freeman B.I., Ellison DH., Rodriguez C.J. (2013). Salt sensitivity: a review with a focus on non-Hispanic blacks and Hispanics. *J Am Soc of Hypertens*; 7(2): 170–179.

Rockwood M.R.H., Howlett S.E. (2011). Blood pressure in relation to age and frailty. Can Geriatr J; 14(1): 2 - 7

Sarmogan R and Worsley A. (2014). Current levels of salt knowledge: A review of the literature. *Nutrients;* 6(12): 5534 – 5559.

Song W, Sang H and WU Q. (2015). Atria natriuretic peptide in cardiovascular biology and disease (NPPA). *Gene;* 569 (1): 1 – 6.

Strazzulo P., Barbato A., Vuotto P., Galletti F. (2001). Relationship between salt sensitivity of blood pressure and sympathetic nervous system activity: A short review of evidence. *Clin and Expe Hypertens* 23(1-2): 25- 33.

Talartschick T, Eisenbauer T and Schrader J. (1990). Low atrial natriuretic peptide plasma concentrations in 100 patients with essential hypertension. Am J Hypertens; 3(2): 45 – 47.

Tzemos N, Lim PO, Wong S, *et al*. (2008). Adverse cardiovascular effects on acute salt loading in young normotensive individuals. *Hypertension*; 51(6):1525 – 1530.

Vishwakarma V.K., Gupta J.K., Qureshi S., Agrawal V.(2016). Role of atrial natriuretic peptide in various conditions. *Int. J Pharma Bio Sci; 7 (3): 20 – 27.*

Volpe M,. Carnovail M and Mastromarino V. (2016). The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis of treatment. *Clin Sci*; 130 (2):57 – 77.

Wang J., Neff R.A., Imaten M., Venkatesan P. (2001). Synaptic and neurotransmitter activation of cardiac vagal neurons in the nucleus ambiguous. *Ann N Y Acad Sci*; 940(1):237 – 246.

Wang T.J., Larson MG., Keyes M.J., Levy D., Benjamin E.J., Vasan R.S. (2007). Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. *Circulation* 115(11): 1345–1353.

Wang W., Shen J., Cui Y., Jiang J., Chen S., Peng J.,Wu Q. (2012). Impaired sodium excretion and salt-sensitive hypertension in corin-deficient mice. *Kidney Inter;* 82(1):26 – 33.

Weinberger M.H. (1996). Salt-sensitivity of blood pressure in humans. *Hypertension*; 27(3 pt2): 481-490.

World Health Organization (2013a). Global brief on hypertension: Word Health Day. Available at: www.who.int/cardiovascular_diseases/publications/global_brief_hypertension/en

World Health Organization Bulletin. (2013b). Nigerians wake up to high blood pressure. 91:242-243. doi: http://dx.doi.org/10.2471/BLT.13.020413

Zheng L., Sun Z., Li J., Zhang R., Zhang X., Liu S., Li J., Xu C., Hu D., Sun Y. *(2008).* Pulse pressure and mean arterial pressure in relation to ischaemic stroke among patients with uncontrolled hypertension in rural areas of China. *Stroke*; 39(7): 1932 – 1937.

J. Afr. Ass. Physiol. Sci. 7 (1): 53-58, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Co-infection of *Plasmodium falciparum* and HIV among pregnant women in Edo State, Nigeria

F.O. Akinbo[1], T.A. Olowookere[1], M.O. Oriakhi[2]

[1]Department of Medical Laboratory Science, University of Benin, Benin City, Nigeria and [2]Department of Obstetrics and Gynaecology, Central Hospital, Benin City, Nigeria

Keywords:
Edo State, HIV, pregnancy, P. falciparum Co-infection

ABSTRACT

Background: *P. falciparum* and HIV diseases affect the poorest group of a population that are made vulnerable by the lack of access to quality education, information and health facilities, all of which are characteristic of sub-Saharan Africa. This study was conducted to determine the co-infection of *P. falciparum* and HIV among pregnant women in Edo State, Nigeria. **Methods:** A total of 459 HIV infected pregnant women attending antenatal clinics at the Central Hospital Benin City, were enrolled. The age of participants ranged from 20 – 48 years. Blood specimens were collected from participants and analysed for HIV and *P. falciparum* detection, full blood count and $CD4^+$ T cells count estimation. Chi squared (X^2) was used for frequency data whereas odd ratio (OR) was analysed for each potential risk factor. **Results:** An overall prevalence of 27.2% of *P. falciparum* infection among HIV infected pregnant women in Edo State, Nigeria was observed. HIV infected pregnant women that are 20-29 years age group, those single, primary school leavers, traders, first trimester, primiparous, use of insecticide-treated bed nets, rainy season and anaemia significantly affected the prevalence of *P. falciparum* infection (P<0.0.0001). **Conclusion:** The administration of intermittent preventive treatment (IPT) as early as possible during pregnancy, use of insecticide-treated bed nets and effective and prompt malaria management are advocated.

INTRODUCTION

Malaria and HIV infections are among the most important public health problems worldwide (UNAIDS, 2012). Malaria is prevalent in resource-poor settings, particularly occasioned by poverty, caused by inadequate sewage treatment, poor hygiene and substandard housing (Gallup and Sachs, 2001). Malaria remains one of the challenging infections affecting the lives of many HIV infected pregnant women in sub-Saharan Africa (WHO, 2004). It is estimated that about 24 million pregnant women are affected by *P. falciparum* yearly, majority of which are from sub-Saharan Africa and about 1 million are co-infection with HIV (Steketee *et al.*, 2001; WHO, 2004). HIV infection constitutes the leading cause of death among women of reproductive age and as such is considered an important maternal problem worldwide (Tang and Nour, 2010). HIV infected pregnant women are at increased risk of maternal anaemia, adverse pregnancy outcomes including low birth weights, stillbirths, infant morbidity and high mortality (Braddick *et al*., 1990; Robbins *et al*., 2007; Naniche *et al*., 2009; Zaba *et al*., 2013).

It has been reported that HIV infected pregnant women have an increased susceptibility to malaria (van Eijk *et al*., 2003). The co-infection of malaria and HIV infection have been reported to cause more than 4 million deaths annually (WHO, 2003). HIV and malaria epidemics overlap in sub-Saharan Africa (Meshnick *et al*., 2006). *P. falciparum* and HIV diseases affect the poorest group of a population that are made vulnerable by the lack of access to quality education, information and health facilities, all of which are characteristics of sub-Saharan Africa (Kwenti, 2018). These two diseases interactions are particularly significant in pregnant women as they form one of the most vulnerable population groups in sub-Saharan Africa (Uneke and Ogbonna, 2009). The interactions between the two diseases are bidirectional

*Address for correspondence:
Email: fredrick.akinbo@uniben.edu
Tel: +2348033796874

with each disease exacerbating the other (Gonzalez *et al*., 2012; Gonzalez and Naniche, 2015).

In Nigeria, both diseases are still serious life-threatening problems among pregnant women. *P. falciparum* and HIV are among the leading causes of morbidity in pregnancy where modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and the neonates (Corbett *et al*., 2002). Without a timely intervention, it is estimated that approximately 25 – 45 % of the HIV infected women will transmit infection to their children (Dabis and Ekpini, 2002). Information is lacking on the co-infection of *P. falciparum* and HIV among pregnant women in Edo State, Nigeria. As such pregnant women and their foetuses are exposed to the risk of malaria and HIV infections as both diseases overlap in Edo State. Against this background, this study was conducted to determine the co-infection of *P. falciparum* and HIV among pregnant women in Edo State, Nigeria.

METHODS

Study area

The study was conducted at the Central Hospital, Benin City, Edo State, a secondary referral health institution and a centre for HIV/AIDS management.

Study population

This study was conducted between November 2018 and June 2019 at the Central Hospital, Benin City, Edo State. A total of 459 HIV infected pregnant women attending antenatal clinics at the Central Hospital, Benin City were enrolled in this study. HIV infected pregnant women on HAART attending antenatal care clinics, those that were 20 years and above and those that consented to participate in the study were enrolled. Pregnant women that were less than 20 years and those with life-threatening medical and obstetrical conditions and those not on HAART were excluded from this study. The agents used in the HAART regimen for HIV infected patients consist of zidovudine, lamividine and nevirapine. A well- structured questionnaire bothering on biodata and sociodemographic characteristics was administered to each participant. Informed consent was obtained from study participants prior to specimen collection. The protocol for this study was approved by the Ethics and Research Committee of the Ministry of Health, Benin City, Edo State.

Collection of specimens

About 5 ml of venous blood was obtained from each participant and dispensed into ethylene diamine tetra acetic acid containers.

About 5 ml of venous blood was obtained from each participant and dispensed into ethylene diamine tetra acetic acid containers.

Processing of specimens

Pregnant women were screened for HIV using the Determine HIV 1/2 rapid immunoassay test strip. Positive cases were confirmed using the trinity Unigold HIV 1/2 kit (CDC, 2014).

Thick and thin blood films were made from each blood specimen, allowed to air-dry and stained in 1:10 dilution of Giemsa stain for 30 min. The stained blood films were rinsed in buffer solution and allowed to air dry. The stained thick films were examined for malaria parasites detection whereas the thin blood film was used for speciation by light microscopy. A total of 200 high power fields per blood film were examined (Cheesbrough, 2000).

The blood samples were analysed for full blood count using an auto-analyser Sysmex Kx-21 (Sysmex Corporation, Kobe, Japan). Anaemia was determined using haemoglobin concentration <11g/dl for pregnant women (Beutler and Waalen, 2006).

$CD4^+$ T cells counts were determined using the flow cytometry (Partec, Gmbh, Germany). Briefly, 20µL of monoclonal antibodies were added and incubated in the dark for 15 min at room temperature after which 800µL of buffer was added. The tube containing the mixture was plugged to the flow cytometer for counting and the value of $CD4^+$T cells obtained from a programmed monitor connected to the flow cytometer.

Data analysis

The data obtained were analysed using Chi squared (X^2) test in comparing the frequency data while the odd ratios (OR) were calculated for potential risk factors at 0.05 significance level. The statistical package used in the data analyses was INSTAT® (GraphPad software Inc, La Jolla, CA, USA).

RESULTS

Out of the 459 HIV infected pregnant women attending antenatal clinics at the Central Hospital, Benin City, 125 (27.2%) had *P. falciparum* infection. Age significantly affected the prevalence of *P. falciparum* infection among HIV infected pregnant women (P=0.0183) with the 20-29 years age group having the highest prevalence (31.1%). Marital status strongly affected *P. falciparum* infection among HIV infected pregnant women (P=0.0050) where single HIV infected pregnant women had the highest prevalence (44.3%). HIV infected pregnant women who are primary school leavers presented with the highest prevalence of *P. falciparum* (49.3%) followed by those with tertiary education

(20.7%) and the least was those with secondary education (17.6%). In addition, educational status significantly affected the prevalence of *P. falciparum* infection among HIV infected pregnant women. Traders were significantly more prone to *P. falciparum* infection among HIV pregnant women (P<0.0001). HIV infected pregnant women in their first trimester were more likely to be infected with *P. falciparum* (P<0.0001). Primiparous HIV infected pregnant women were more prone to malaria infection (P<0.0001) with a 1 to 4-fold risk of acquisition. Participants that used insecticide treated bed nets had significantly lower prevalence of *P. falciparum* infection (P=0.0021). Seasonal variation significantly affected the prevalence of *P. falciparum* infection among HIV infected pregnant women (P<0.0001) with the rainy season recording higher prevalence (45.5%) than the dry season (14.4%) (Table 1).

Table 1: Relationship between demographic characteristics and co-infection of *P. falciparum* and HIV among pregnant women

Characteristic	No. Tested	No. infected (%)	OR	95% CI	P value
Age (year)					
20-29	305	95(31.1)			0.0183
30-39.	124	22(17.7)			
40 & Above	30	8(26.6)			
Marital status					
Single	70	31(44.3)			0.0050
Married	351	83(23.6)			
Divorced.	23	6(26.1)			
Widowed.	15	5(33.3)			
Educational status					
Primary	146	69(47.3)			<0.0001
Secondary	284	50(17.6)			
Tertiary	29	6(20.7)			
Occupational status					
Trader	84	40(47.6)			<0.0001
Civil servant	91	9(9.9)			
Artisan	192	59(30.7)			
Business woman	92	17(18.5)			
Gestational age					
First trimester	138	56(40.6.)			<0.0001
Second trimester	192	49(25.5)			
Third trimester	129	20(15.5)			
Parity					
Primiparous	204	79(38.7)			<0.0001
Multiparous	255	46(18.0)			
Preventive measures					
Insecticide	67	23(34.3)			0.0021
Insecticide treated net.	189	5(18.5)			
Window netting	203	67(33.0)			
Seasonal variation					
Rainy season	189	86(45.5)	4.945	3.172, 7.710	<0.0001
Dry season	270	39(14.4)			

OR=Odd ratio; CI=Confidence interval; * *P*<0.05

There was a strong association between anaemia and *P. falciparum* infection among HIV infected pregnant women (OR=4.738; 95% CI= 2.851, 7.875; P<0.0001). $CD4^+$ T cells less than 200cells/μL did not strongly associate with the prevalence of *P. falciparum* infection among HIV infected pregnant women (P=0.9081) (Table 2).

DISCUSSION

Human immunodeficiency virus and malaria infections represent the most important health problems in sub-Saharan Africa, where these infections overlap and co-infection is rampant (Abu-Raddad *et al*., 2006). Co-infection with malaria and HIV presents specific complications for pregnant women and foetal development. The interactions between these two diseases during pregnancy are complex (Guyatt and Snow, 2004) thus, putting the life of the pregnant women and their unborn babies at risk of medical and poor obstetrical conditions and threatening antiretroviral and antimalarial treatments effectiveness. To our knowledge, this is the first study on the co-infection of *P. falciparum* and HIV among pregnant women in Edo State, Nigeria.

An overall prevalence of 27.2% of *P. falciparum* infection was observed among HIV infected pregnant women in Edo State. The prevalence of 27.2% reported in our study is lower than the 33.4% observed by Houmsou *et al*. (2014) in Benue State, Central Nigeria, the 47.7% reported by Sanyaolu *et al*. (2013) in Lagos, South West Nigeria and 49.83% recorded by Johnbull *et al*. (2014) in Enugu, South East Nigeria. In areas with stable malaria transmission such as our study area, HIV has been reported to increase the risk of malaria infection and clinical malaria in adults, especially those with advanced immunosuppression (Berg *et al*., 2014). The difference in our work and that of other studies may be attributed to geographical locations and the efforts of the MTCT unit at caring for HIV-infected pregnant women and ensuring compliance in taking their medications.

Table 2: Effect of anaemia and $CD4^+$ T cell counts on the co-infection of *P. falciparum* and HIV in pregnant Women; **P*<0.05

Parameter	No. Tested	No. infected (%)	OR	95% CI	P value
Anaemia					
<11g/dl	269	103(38.3).	4.738.	2.851, 7.875	<0.0001
>11g/dl	90	22(11.6)			
CD4 Count					
<200 cells/μL.	131	35(26.7)	0.9641.	106, 1.522	0.9081
>200	328.	27.4(27.4)			

Age has been reported as a co-factor in disease progression, and the immunity to malaria and HIV infection has been observed to be age-dependent (Schwartz *et al*., 2001). In this study, age significantly affected the prevalence of *P. falciparum* infection

among HIV infected pregnant women (P=0.0183) with the 20-29 years age group having the highest prevalence (31.1%). This finding is in contrast to that observed by Houmsou *et al*. (2014) that did not find an association between malarial infection in HIV infected pregnant women in Benue State, Nigeria.

Being single strongly associated with *P. falciparum* infection among HIV infected pregnant women (P=0.0050). This finding is at variance with the report of Houmsou *et al*. (2014) that observed increased malaria infection among divorcees. The reason for the difference may be due to the fact that singles are more likely to engage in several occupations that may expose them to mosquito bites.

In this study, educational status significantly impacted on the prevalence of *P. falciparum* infection (P<0.0001) where those with primary school education presented with the highest prevalence (47.3%).

Traders were observed to be more likely to acquire *P. falciparum* infection among HIV infected pregnant women (P<0.0001). This is because they are exposed to the bite of mosquitoes as they carry out their activities mostly around breeding mosquito sites. This may explain the reason for this finding.

HIV infected pregnant women who are in their first trimesters were found to be at risk in acquiring *P. falciparum* (P<0.0001) when compared with other trimesters which imply that most of the women enrolled in our study are at risk of adverse pregnancy outcomes. This finding is in tandem with the previous study of Houmsou *et al.* (2014) that observed that HIV infected pregnant women in their first trimester are more likely to be infected with *P. falciparum*. It has been observed that pregnant women in their first trimester are unlikely yet to be administered sulphadoxine-pyrimethamine used as the intermittent preventive treatment (IPT) that are usually given only to women at their second and third trimesters (Houmsou *et al.*, 2014).

First pregnancy is believed to be the most critical as women develop pregnancy-specific immunity against placental parasites over successive pregnancies resulting from repeated exposure (Flateau *et al*., 2011). Albeit, available information suggest that women who are infected with HIV have the same low level of immunity to malaria in subsequent pregnancies as they do in their first pregnancy and are twice as susceptible to clinical malaria, which increases the risk of adverse outcomes (Brenthinger *et al*., 2006; Flateau *et al*., 2011). In our study, primiparous HIV infected pregnant women are at 1 to 4 -fold increased risk of acquiring *P. falciparum* infection when compared with their multiparous counterparts (OR=2.871; 95% CI= 1.876, 4.396; P<0.0001).

Generally, the use of insecticide treated bed net is a recognized effective way of preventing malaria infection (Akinbo *et al.,* 2014). HIV infected pregnant women that used insecticide treated bed nets had significantly the least prevalence of *P. falciparum* infection (18.5%; P=0.0021) compared with other preventive measures. The World Health Organization recommends a three-pronged approach for malaria control during pregnancy in sub-Saharan Africa: intermittent preventive treatment (IPT), insecticide treated bed nets and effective case management of malaria illness (WHO, 2012). Thus, HIV infected pregnant women be issued and encouraged to use insecticide treated bed nets in order to reduce the adverse pregnancy outcomes occasioned by *P. falciparum* infection.

Parasite transmission and development are regarded to be influenced by climatic conditions particularly within the temperature range of 25-30^{o}C (Egbendewe-Mondzozo *et al*., 2011). There is a direct influence of temperature and rainfall on the number and productivity of breeding sites, ultimately the vector density (Afrane *et al*., 2012). This study observed *P. falciparum* infection peaked during rainy season among HIV infected pregnant women (P<0.0001). Rainy season has been reported to provide ecological changes favouring the breeding of the mosquito vectors which enhance the transmission of malaria (Erhabor *et al.,* 2014).

HIV infected pregnant women with malaria had a 2 to 7-fold higher risk of developing anaemia (OR= 4.738; 95% CI= 2.851, 7.875; P<0.0001). This observation agrees with previous reports of Johnbull *et al*. (2014) and Houmsou *et al*. (2014). The cause of anaemia in pregnancy is multifactorial and may include haemodilution infections particularly HIV and malaria, inadequate erythropoiesis (Agan *et al*., 2010). Both HIV and malaria are known causes of maternal anaemia (Moses et al., 1998; Guyatt and Snow, 2001). In addition, the presence of zidovudine among the HAART regimen used by our subjects and antibodies to HAART agents have been reported to be associated with anaemia (Moyle, 2002). The risk of anaemia secondary to malaria and HIV infection could be reduced through prompt, effective treatment and follow up during pregnancy in order to increase the chances of delivering a healthy infant. Adequate information about nutritional diet could also play a big role in reducing adverse pregnancy outcomes necessitated by anaemia.

It has been reported that independently, HIV and malaria interact with the host immune system to bring about complex activation of immune cells, which causes dysfunctional levels of cytokine and antibody production (Hochman and Kim, 2009). Furthermore, $CD4^{+}$ T cells play a major part in the development and

maintenance of antimalaria immunity, but HIV interferes with immunity (Troye-Blomberg and Berzins, 2008) by widespread lymphoid necrosis throughout the lymph nodes, spleen, and gut mucosae, hyperactivation of $CD4^+$ and $CD\ 8^+$ effector cells to secrete cytokines, HIV induced downregulation of $CD4^+$ T cells, decreases in $CD8^+$ T-cells counts, and upregulation of parasitemia, consequently leading to fatal malaria and a rapid progression to AIDS (Ryan-Payseur *et al.*, 2011). Surprisingly, $CD4^+$ T-cells count did not strongly associate with co-infection of malaria and HIV among pregnant women in this study (OR= 0.9641; 95% CI = 0.6106, 1.522; P=0.9081). The low malarial infection observed in those with $CD4^+$ T cell counts greater than 200 cells/μL could be the reconstitution of their immune system due to highly active antiretroviral therapy (HAART) or recently infected pregnant women that still have strong immune system. This may explain the reason for our finding.

CONCLUSION

This study reveals a prevalence of 27.2% of *P. falciparum* infection among HIV infected pregnant women in Edo State, Nigeria. HIV infected pregnant women that are 20-29 years age group, those single, primary school leavers, traders, first trimester, primiparous, those that use insecticide treated bed nets and rainy season significantly affected the prevalence of *P. falciparum* infection in this study (P<0.0001). In addition, anaemia strongly associated with *P. falciparum* infection among HIV infected pregnant women (P<0.0001). The administration of intermittent preventive treatment as early as possible during pregnancy, use of insecticide-treated bed nets and effective and prompt malaria management are advocated.

ACKNOWLEDGEMENTS

Authors acknowledge the participants used in this study for their cooperation and the Hospital Management Board for allowing us to use their laboratories.

REFERENCES

Abu-Raddad LJ, Patnaik P, Kublin JG. (2006) Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science*. 314(5805): 1603-1606.

Afrane YA, Lawson BW, Brenya R, Kruppa T, Yan G. (2012). The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. *Parasit Vectors*. 5: 233.

Agan TU, Ekabua JE, Iklaki CU, Oyo-Ita A, Ibanga I. (2010). Prevalence of asymptomatic malaria parasitaemia. *Asian pacific journal of tropical medicine*. 2:1–5.

Akinbo FO, Osanyinbi BI, Omoregie R, Ande ABA (2014). Asymptomatic Malaria among pregnant women in Edo State. *Journal of Medicine and Biomedical Research*. 13(1): 61-69.

Braddick MR, Kreiss JK, Embree JB, Datta P, Ndinya-Achola JO, Pamba H, Maitha G, Roberts PL, Quinn TC, Holmes KK. (1990) Impact of maternal HIV infection on obstetrical and early neonatal outcome. *AIDS*. 4(10): 1001-1005.

Berg A, Patel S, Aukrust P, David C, Gonca M, Berg ES, Dalen I, Langeland N. (2014) Increased severity and mortality in adult co-infected with malaria and HIV in Maputo, Mozambique: a prospective cross-sectional study. *PloS One*. 9(2): e88257.

Beutler E, Waalen J. (2006) The definition of anemia: What is the lower limit of normal of the blood hemoglobin concentration? Blood. 107: 1747–1750.

Brentlinger PE, Behrens CB, Micek MA. (2006) Challenges in the concurrent management of malaria and HIV in pregnancy in sub-Saharan Africa. *Lancet Infect Dis.* 6(2): 100-111.

CDC (2014) Laboratory testing for the diagnosis of HIV infection: Updated Recommendations.

Cheesbrough M. (2000) Medical Laboratory Manual for Countries Part 2. Cambridge University Press. Cambridge. UK. 2nd edition. 2000.

Corbett EL, Steketee RW, ter Kuile FO, Latif AS, Kamali A, Hayes RJ. (2002) HIV-1/AIDS and the control of other infectious diseases in Africa. *Lancet*. 359: 2177-2187.

Dabis F, Ekpini ER. (2002) HIV-1/AIDS and maternal and child health in Africa. *Lancet*. 359: 2097-2104.

Egbendewe-Mondzozo A, Musumba M, McCarl BA, Wu X. (2011) Climate change and vector-borne diseases: an economic impact analysis of malaria in Africa. Intern J Environ Res Public Health. 8: 913-930.

Erhabor O, Mohammed HJ, Ahmed HM, Ezimah ACU. (2014) Effect of Plasmodium Parasitaemia on some Haematological Parameters in Children Living in Sokoto, North Western, Nigeria. *Intern J Clin Med Res.* 1(22): 57-64.

Flateau C, Le Loup G, Pialoux G. (2011) Consequences of HIV infection on malaria and therapeutic implications: a systemic review. *Lancet Infect Dis*. 11(7): 541-546.

Gallup JK, Sachs JD (2001) The economic burden of malaria. *Am J Trop Med Hyg*. 64(1): 85-96.

Gonzalez R, Ataide R, Naniche D, Menendez C, Mayor A. (2012) HIV and malaria interactions:where do we stand? *Expert Rev Anti Infect Ther*. 10(2): 152-165.

Gonzalez R, Naniche D. (2015) HIV and malaria interaction in the pregnant women. Springer Sci. doi 10.1007/978-1-4614-8757-967-1.

Hochman S, Kim K. (2009) The impact of HIV and malaria coinfection: what is known and suggested venues for further study. Interdiscip Perspect *Infect Dis.* 2009: 617-954.

Guyatt HL, Snow RW. (2001) The epidemiology and burden of *Plasmodium falciparum* -related anaemia among pregnant women in sub-Saharan Africa. *Am J Trop Med Hyg.* 64: 36-44.

Guyatt HL, Snow RW. (2004) Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev.* 17(4): 760-769.

Houmsou RS, Wama BE, Elkanah SO, Garba LC, Hile TD, Bingbeng JB, Kela SL, Amuta EU (2014) Malarial infection in HIV infected pregnant women attending a rural antenatal clinic in Nigeria. *Adv Epidemiol*. doi.org/10.1155/2014/6994213.

Johnbull OS, Uche AP, Kesiena AJ, Francis FA, Oyemocho A, Obianwu IM, Akabueze J. (2014). Prevalence and risk factors of malaria in HIV-infected pregnant women on antiretroviral therapy in Enugu, South East Nigeria. *J AIDs Clin Res*. 5: 321-326.

Kwenti TE. (2018) Malaria and HIV coinfection in sub-Saharan Africa:prevalence, impact, and treatment strategies. *Res Rep Trop Med*. 9: 123-136.

Meshnick SR, Nwapasa V, Rogerson SJ (2006). Protecting pregnant women from malaria in areas of high HIV infection prevalence. *J Infect Dis*. 194: 273-275.

Moses A, Nelson J, Bagby GC Jr. (1998) The influence of human immunodeficiency virus-1on hematopoiesis. *Blood.* 91: 1479-1495.

Moyle G. (2002) Anaemia in persons with HIV infection: Prognostic marker and contributor to morbidity. *AIDS Rev.* 4: 13-20.

Naniche D, Bardaji A, Lahuerta M, Berenguera A, Mandomando I, Sanz S, Aponte JJ, Sigauque B, Alonso PL, Menendez C. (2009) Impact of maternal human immunodeficiency virus infection on birth outcomes and infant survival in rural Mozambique. *Am J Trop Med Hyg.* 80(5): 870-876.

Rollins NC, Coovadia HM, Bland RM, Coutsoudis A, Bennish ML, Patel D, Newell ML (2007) Pregnancy outcomes in HIV-infected and uninfected women in rural and urban South Africa. *J Acquir Immune Defic Syndr*. 44(3): 321-328.

Ryan-Payseur B, Ali Z, Huang D, Chen CY, Yan L, Wang RC, Collins WE, Wang Y, Chen ZW (2011). Virus infection stages and distinct TH1 or Th17/Th22 T-cell responses in malaria/SHIV coinfection correlate with different outcomes of disease. *J Infect Dis*. 204(9): 1450-1462.

Sanyaolu AO, Fagbenro-Beyioku AF, Oyibo WA, Badaru OS, Onyeabor OS, Nnameka CI. (2013) Malaria and HIV co-infection and their effect on haemoglobin levels from three healthcare institutions in Lagos, South West Nigeria. *Afr Health Sci.* 13(2): 295-300.

Schwartz E, Sadetzki S, Murad H, Raveh D. (2001) Age as a risk factor for severe *Plasmodium falciparum* malaria in non-immune patients. *Clin Infect Dis.* 33: 1774-1777.

Steketee RW, Nahlen BL, Parise ME, Menendez C. (2001) The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg*. 64: 28-35.

Tang J, Nour NM (2010) HIV and pregnancy in resource-poor settings. *Rev Obstet Gynae.* 3(2): 66-71.

Troye-Blomberg M, Berzins K. (2008) Immune interactions in malaria co-infections with other endemic infectious diseases: implications for the development of improved disease interventions. *Microbes Infect.* 10(9): 948-952.

UNAIDS. (2012) Global report: UNAIDS report on the global AIDS epidemic. 2012. Geneva. WHO UNAIDS/JC2417E.

Uneke CJ, Ogbonna A. (2009) Malaria and HIV co-infection in pregnancy in sub-Saharan Africa: Impact of treatment using antimalarial and antiretroviral agents. *Trans R Soc Trop Med Hyg.* 109(8): 761-767.

Van Eijk AM, Ayisi JG, ter Kuile FO, Misore AO, Otieno JA, Rosen DH, Kager PA, Steketee RW, Nahlen BL. (2003) HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. AIDS. 17(4): 595-603.

WHO. (2003) The African Malaria Report 2003. Geneva. 2003; World Health Organization.

WHO. (2004) Malaria and HIV/AIDS interactions and their implications for public health policy. Report of a technical consultation. 23-25 June 2004. Geneva. 2004; World Health Organization.

WHO. (2012) Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine. Updated WHO Policy Recommendations. WHO; 2012. Geneva.

Zaba B, Calvert C, Martson M, Isingo R, Nakiyingi-Miiro, J, Lutalo T, Crampin A, Robertson L, Herbst K, Newell ML, Todd J, Byass P, Boerma T, Ronsmans C. (2013) Effect of HIV infection on pregnancy-related mortality in sub-Saharan Africa: secondary analyses of pooled community-based data from the network for Analyzing Longitudinal Population-based HIV/AIDS data on Africa. *Lancet.* 20381(9879): 1763-1771.

J. Afr. Ass. Physiol. Sci. 7 (1): 59-63, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Antidiabetic and hypolipidaemic potentials of ethanol fruit pulp extract of *Persea americana* (avocado pear) in rats

I.O. Umoh*[1], O.O. Samuel[1], T.B. Kureh[2] and K.G. Davies[2]

[1]Department of Human Physiology, Faculty of Medicine, St. Francis University College of Health and Allied Sciences (A constituent college of St. Augustine University of Tanzania), Ifakara, Tanzania and [2]Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo, P.M.B 1017, Uyo, Nigeria.

Keywords:
Diabetes mellitus, Blood glucose level, serum lipid, phytochemical constituents

ABSTRACT

Background: Development of new therapies capable of improving glycaemia and lipid profiles in diabetes management without side effects, reduction in efficacy and toxicity has been of great scientific interest. *P. americana* seed has been reported to have diverse applications in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin treatment and beautification however there is dearth of information regarding it use in treatment of diabetes and hypolipidemic effect following its use for other purposes. **Aim**: Therefore, this study was undertaken to investigate the antidiabetic and hypolipidaemic potentials of *P. americana* ethanol fruit pulp extract. **Methods:** Phytochemical screening for classes of secondary plant metabolites was done using standard methods. 250 and 500mg/kg of *P. americana* ethanol fruit pulp extracts were administered to alloxan- induced diabetic rats orally twice daily for 3 weeks. Glycemic levels were checked every 3 days and serum lipid profile assay was carried out at the end of the treatment period. **Results:** Phytochemical screening of the extract revealed presence of various classes of phytochemicals such as saponins, tannins, alkaloids and steroids**.** Both doses of the extract significantly reduced blood glucose levels when compared to the control group. The higher dose (500mg/kg) significantly decreased total cholesterol, triglycerides and low-density lipoproteins compared with the control. There was also a marginal increase in HDL-Cholesterol. **Conclusion:** *P. americana* ethanol fruit pulp extract reduces hyperglycaemia and hyperlipidaemia associated with type I diabetes mellitus.

INTRODUCTION

Persea americana fruit tree originated in South Central Mexico (Royal Botanic Gardens, Kew and Missouri Botanical Garden 2010; Chen *et al*., 2008). It is classified as a member of the flowering plant family *Lauraceae*, the fruit of the plant also called avocado pear or alligator pear, is botanically a large berry containing a single large seed known as a "pit" or a "stone" (Morton, 1987; Storey, 1973). The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and of smooth, almost creamy texture (Morton, 1987). *P. americana* leaves have been reported to have anti-inflammatory and analgesic activities (Adeyemi *et al*., 2002). The seed of *P. americana* has diverse applications in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin diseases and beautification (Pamplora and Roger, 1999). Antioxidant activity due to the phenolic content of seeds of avocado pear was found to be greater than 70% (Song and Barlow, 2004). Avocados are one of the few fruits that give “good” fats because it contains lipids such as phytosterols, ß-sitosterol, campesterol, and stigmasterol as well as monounsaturated fatty acids mainly oleic acid and it also reduces the risk of cardiovascular disease (Olagunju *et al*., 2017).

Diabetes mellitus is a complex metabolic disorder that mainly occurs due to defects in either insulin secretion, insulin action, or both and is characterized by high blood sugar (glucose) levels **(**Kooti *et al*., 2016**)**. The disorder can also lead to serious complications affecting human health with long-term effects that causes micro and macro vascular problems (Mohana *et*

*Address for correspondence:
Email iumoh@sfuchas.ac.tz
Tel: +255622351221

al., 2012). The World Health Organization reports suggests that the prevalence of diabetes in adults worldwide would increase to 300 million in years 2025, making the disease one of the main threats to human health in the 21st century and also the fifth leading cause of deaths in most developed countries (Kazi, 2014).

Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) involves ß-cell destruction with little or no endogenous insulin secretory capacity and is triggered by autoimmune idiopathic factors (Bastaki, 2005). A major feature of Type 2 diabetes mellitus is insulin resistance or deficiency, which can cause hyperglycemia (Laakso, 2001). High prevalence, variable pathogenesis, progressive process, and complications of diabetes all highlight the urgent need for effective treatments such as insulin therapy, pharmacotherapy, and diet therapy **(**Kooti *et al*., 2016**)**.

Despite the significant progress made in the treatment of diabetes, treatment outcomes are are still far from perfect due to drug resistance (reduction of efficacy), side effects, and toxicity (Hui *et al.,* 2005). However, the use of medicinal plants is now being recommended because most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides which often have anti-diabetic effects (Michael *et al*., 2005; Kooti *et al*., 2015). The aim of this study was to investigate the antidiabetic and hypolipidaemic potentials of ethanol fruit pulp extract of *P. americana* on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of Fruits pulp extract

Ripe fruits of *P. americana* were obtained from Enugu, and were authenticated in Botanical unit, of the Department of Biological Sciences, Madonna University Nigeria. The fruits were thoroughly washed and the pericarp (peel) removed alongside the seeds from the mesocarp (pulp). The fruit pulps (Mesocarp) were cut into smaller pieces and air dried for 4 days at ambient temperature and thereafter grounded into powdered form using mortar and pestle. The powdered sample was extracted with ethanol using Soxhlet apparatus, concentrated to dryness in a water bath and preserved at 4ºC until required for use (Redfern *et al*., 2014). Weighed samples of the extract (1g in 10 ml distilled water) were then used to prepare the stock solution (100 mg/ml).

The brand of metformin (METFORMINA® 500 mg) used in this study was Manufactured by S Kant Healthcare Ltd. India. The Alloxan monohydrate (Sigma St. Louis, M.O., USA) solution was prepared by dissolving 1.25g of Alloxan monohydrate in 25ml of distilled water.

Experimental Animals and Treatment Protocol

Twenty-five male albino rats (160-200g) used for this experiment were obtained from the University Animal house and kept in standard rat cages, fed with pelletized commercial feed and tap water *ad libitum* followed by 1 week of habituation before the commencement of the research. Animal studies were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1999). The animals were then assigned into five groups of five animals each as shown below:

Group 1 – Normal Control (2 mg/kg of distilled water p.o., twice daily)

Group 2 – Diabetic Control

Group 3 – Diabetic + *P. americana* (250 mg/kg p.o., twice daily)

Group 4 – Diabetic + *P. americana* (500 mg/kg p.o., twice daily)

Group 5 – Diabetic + Metformin (500 mg/kg p.o., once daily)

Group's 2 – 5 animals were fasted overnight then diabetes was induced by a single intraperitoneal (IP) injection of freshly prepared 150 mg/kg of alloxan monohydrate solution (Yanarday and Colac, 1998). Animals were considered diabetic if the blood glucose values were ≥ 200mg/dl 48 hours after alloxan injection. Blood glucose levels were checked using a glucometer (Bioland glucometer, Germany).

Oral administration was done for 21 consecutive days with aid of a rubber cannula attached to a calibrated syringe. Blood glucose levels were checked every three days by 6am during the treatment period of 21 days by prickling the tail vein of the animals. The animals were fasted overnight on the 21st day, rendered unconscious under chloroform fumes, sacrificed and blood withdrawn via cardiac puncture was collected in plain-capped sample bottles. Blood samples collected were centrifuged at 3000rpm for 10 minutes. Then serum was separated gently and stored in labeled plain sample bottles at -20º C until required for the lipid profile assay.

Lipid Profile Analysis

Serum total cholesterol, triglyceride and high-density lipoprotein (HDL) were determined using Randox kits produced by Human Diagnostic-Germany (Schettler and Nussel, 1975; Trinder, 1981; Trinder and Webster, 1984). Serum very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated using the Friedewald's method (Friedwald, 1972).

Phytochemical Analysis of P. americana ethanol pulp Extract:
Using standard methods, the extract was screened for bioactive ingredients. Saponins and oxalates determined by the method of Kokate, (1997). Alkaloid, tannins and Phenols were determined by method of Trease and Evans (1989). Phytates, steroids and cardiac glycosides were determined by method of Harbone, (1973).

Statistical Analysis:
Data was analysed using one-way ANOVA and difference between groups compared using Least Significance Difference (LSD). Data are expressed as mean ± standard error of mean and values of $P<0.05$ were considered significant. SPSS version 15.0 was used for this analysis.

RESULTS

Effect of treatment on blood glucose levels of diabetic rats
Results in Table 1 shows the mean blood glucose concentrations of the control groups (normal, diabetic controls), the two doses of the *P. americana* ethanol fruit pulp extract and the standard drug (metformin).

Table 1: Effect of oral 21-day doses of *P. americana* ethanol fruit pulp extract on blood glucose levels of alloxan-induced diabetic rats.

Groups	Mean Blood glucose levels (mmol/l)	
	Initial	Final
Normal Control (2 mg/kg of distilled water p.o. twice daily)	6.70 ± 0.8	7.5 ± 0.6
Diabetic Control	16.65 ± 5.0*	29.3 ± 4.0*
Diabetic + *P. americana* (250 mg/kg)	20.10 ± 7.0*	14.4 ± 2.0bc
Diabetic + *P. americana* (500 mg/kg)	20.40 ± 5.0*	9.4 ± 1.0b
Diabetic + metformin (500 mg/kg)	25.20 ± 1.0*	6.7 ± 0.0b

Values are expressed in mean ± SEM, n = 4; * = $P<0.05$ indicates a significant difference compared with normal control; b = $P<0.05$ indicates a significant difference compared with diabetic control; c = $P<0.05$ indicates a significant difference compared with standard drug, metformin. Initial = 0 week after induction of diabetes and before commencement of treatment. Final = mean values obtained after 21 days of treatment.

Both doses of *P. Americana* ethanol fruit pulp extract (250 mg/kg and 500 mg/kg) significantly decreased ($P<0.05$) blood glucose levels in the diabetic treatment groups 3 (14.4 ± 2.0 mmol/l) and 4 (9.4 ± 1.0 mmol/l) respectively when compared to the diabetic control group (29.3 ± 4.0 mmol/l).
Also, in the alloxan-induced diabetic group treated with metformin (6.7 ± 0.0 mmol/l), a significant decrease ($p<0.05$) was observed in the blood glucose concentrations when compared to the diabetic control group (29.3±4.0 mmol/l).

Effect of treatment on serum lipid profiles of diabetic rats
Results obtained for serum lipid profiles showed significant ($P<0.05$) increases in total cholesterol, Triglycerides and low-density lipoproteins of the diabetic control group when compared to the normal control group and the Metformin group 4 (Table 2).
P. americana (500mg/kg) diabetic treatment group showed significant ($P<0.05$) decreases in TC, TG and LDL, when compared with the diabetic control. HDL-C also showed marginal increase in *P. americana* (500mg/kg) diabetic treatment group. (Table 2).

Table 2: Effect of oral 21-day doses of *P. americana* ethanol fruit pulp extract on serum lipid profiles of alloxan-induced diabetic rats.

Groups	Total cholesterol (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal control (2 mg/kg of distilled water p.o. twice daily)	4.2 ± 0.1	1.47 ± 1.1	1.9 ± 1.5	0.70± 0.1
Diabetic Control	11.3± 1.1*c	6.8± 0.4*c	1.4 ± 0.1	12.1 ± 0.4*c
Diabetic + *P. americana* (250 mg/kg)	6.4 ± 0.0bc	3.9 ± 0.4bc	2.1 ± 0.1	8.04 ± 0.1bc
Diabetic + *P. americana* (500 mg/kg)	2.5 ± 0.0b	1.9 ± 0.1b	2.8 ± 0.5	1.93 ± 0.4b
Diabetic + *Metformin* (500 mg/kg)	1.8 ± 0.0b	0.7 ± 0.3b	3.1 ± 0.2*b	0.63 ± 0.1b

TG; Triglycerides, HDL; high-density lipoproteins, LDL; low-density lipoproteins. Values are expressed in mean ± SEM, n = 4; * = $P<0.05$ indicates a significant difference compared with normal control; b = $P<0.05$ indicates a significant difference compared with diabetic control
c = $P<0.05$ indicates a significant difference compared with standard drug, metformin.

Phytochemical screening of ethanol pulp extract of Persea americana
The phytochemical analysis of the ethanolic pulp extract of *P. americana* revealed the presence of various concentrations of phytochemicals as shown in Table 3.

Table 3: Phytochemical constituent of ethanol pulp extract of *P. americana*

Constituent	Inference
Alkaloids	+
Saponins	+
Tannin	+
Phytate	+
Phenol	+
Cardiac-glycosides	+
Oxalate	+
Steroids	+

Key: + = present.

DISCUSSION

Uncontrolled diabetes can lead to serious micro and macro vascular problems on the long term (Mohana *et al*., 2012). In addition it causes many chronic complications including blindness, heart disease and renal failure (Mamun-or-Rashid *et al*., 2014).

Results obtained from phytochemical screening of the *P. americana* ethanol pulp extract showed it contains saponins, alkaloids, steroid, phytate, phenol, oxalate, tannins and glycosides, suggesting that they contribute to the therapeutic efficacy/anti-diabetic properties of the plant extract.

A significant change occurs in the structure and metabolism of lipid in diabetes leading to lipid peroxidation associated with hyperlipidaemia (Kooti *et al*., 2016). This finding was also observed in our study as the increased blood sugar was accompanied by alterations the lipid profiles of the untreated diabetic animals.

The study showed that daily oral administration of doses of ethanol fruit pulp extract of *P. americana* significantly reduced the blood glucose levels of the alloxan- induced diabetic rats close to normal values. These results are in line with findings by Alhassan *et al*. (2012) who reported that consumption of the aqueous seed extract of *P. americana* exerts significant hypoglycaemic effects on alloxan induced diabetic rats. The significant anti-diabetic activity of the ethanol fruit pulp extract of *P. americana* is due to the presence of hypoglycemic agents such as saponins, tannins, alkaloids and steroids which contain insulin stimulatory substances such as insulin receptors substrate (IRS), pro-hormone convertase, glycogen synthase, the beta-3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor and peroxisome proliferators (Broadhust, 1997).

Results of this study also showed the ability of *P. americana* ethanol fruit pulp extract to significantly reduce TC, TG, LDL while marginally (non-significantly) increasing HDL levels in diabetic treated animals. HDL has the ability to promote efflux of cholesterol from cells, which may minimize the accumulation of foam cells in the artery wall thereby preventing the development of atherosclerosis (Olagunju *et al*., 2017).

This research also supports findings by Olagunju *et al*. (2017) who reported that cardiovascular disease marker on TC/HDL ratio of male albino rats fed with aqueous and ethanol extracts of *P. americana* fell within the low risk acceptable range and boosting the "good cholesterol" (HDL) which is good for cardiovascular health. Therefore, the significant hypolipidaemic activity of *P. americana* ethanol fruit pulp extract in this study can be attributed to the presence of phytochemicals such as alkaloids, phenols, saponins and sterols in the extract (Bopanna *et al*., 1997; Katsumata, *et al*., 1999).

In conclusion, the administration of *P. americana* ethanol fruit pulp extract produced significant reduction of blood glucose levels and lipid related dysfunction in alloxan-induced diabetic rats. Thus, in the light of these studies, further pharmacological investigations are needed to determine the chemical compositions of the extract and their exact mechanisms of action in the management of diabetes.

REFERENCES

Adeyemi O.O., Okpo S.O. and Ogunti OO. (2002). Analgesic and anti-inflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia*; 73: 375–380.

Alhassan A.J., Sule M.S., Atiku M.K., Wudil A.M., Abubakar H. and Mohammed S.A. (2012). Effects of aqueous avocado pear (*Persea americana*) seed extract on alloxan induced diabetes rats. *Greener Journal of Medical Sciences*; 2(1):005-11.

Bastaki S. (2005). Review of Diabetes mellitus and its treatment. *International journal of Diabetes and Metabolism*; (13): 111-4.

Bopanna K.N., Bhagy A., lakshmi N., Kanna J., Balaraman R. and Rathod S.P. (1997). Antidiabetic and Antihyperlipidemic effects of neem seed and kernel powder on Alloxan-induced Diabetic rats. *Phamacol. Res*; 46(3):251-55.

Chen H., Morrell P.L., Ashworth V.E., De La Cruz M. and Clegg M.T. (2008). Tracing the geographic origins of major avocado cultivars. *Journal of Heredity*; 100(1):56-65.

Friedwald W.T. (1972). Methods for the determination of LDL Cholesterol. *Clin Chem*; 18: 499-502.

Harbone J.B. (1973). Phytochemical methods. *A guide to modern techniques of plant analysis*, Springer Science & Business Media, pp: 1-13.

Hui H., Zhao X. and Perfetti R. (2005). Structure and function studies of glucagon-like peptide-1 (GLP1): the designing of a novel pharmacological agent for the treatment of diabetes. *Diabetes Metab Res Rev*; 21: 313-31.

Katsumata K.Y., Katsumata T.O. and Katsumata K. (1999). Potentiating Effects of combined usage of three sulphonylurea Drugs on the occurrence of Alloxan-induced Diabetes in rats. *Horm. Metab. Res*; 25:125-26.

Kazi S. (2014). Use of traditional plants in diabetes mellitus. *Int J Pharm*; 4(4):283-9.

Kooti W., Moradi M., Akbari S.A., Sharafi-Ahvazi N., Asadi Samani M. and Ashtary-Larky D. (2015). Therapeutic and pharmacological potential of *Foeniculum vulgare* Mill: A review. *J HerbMed Pharmacol*; 4: 1-9.

Kooti W., Farokhipour M., Asadzadeh Z., Ashtary-Larky D. and Asadi-Samani M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*; 8(1):1832.

Kokate K.C. (1997). Practical pharmacognosy, 4th ed Delhi: Vallabh Prakashan; p.218.

Laakso M. (2001). Insulin resistance and its impact on the approach to therapy of type 2 diabetes. *International journal of clinical practice. Supplement*;(121):8-12.

Mamun-or-Rashid A., Hossain M.S., Naim Hassan B., Kumar Dash M., Sapon A. and Sen M.K. (2014). A review on medicinal plants with antidiabetic activity. *Journal of Pharmacognosy and Phytochemistry*; 3(4): 149-59.

Morton J.F. (1987) Fruits of warm climates. Creative Resource Systems, Inc., Winterville, NC and Center for New Crops & Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN. pp. 91–102.

Michael P.K., Asim A.B. and Robert S.B. (2005). The Utility of Oral Diabetes Medications in Type 2 Diabetes of the Young. *Curr Diab Rev*; 1: 83-92.

Mohana L., Sandhya R. and Kiran U. (2012). A review on diabetes milletus and the herbal plants used for its treatment. *Asian Journal of Pharmaceutical & Clinical Research*; 5(4): 15-21.

NRC. (1999). Guide for the care and use of laboratory animals. Publication of the National Institute of Health, Bethesda, MD.

Olagunju H.T., Oruambo I.F., Oyelowo H.O. and Obediah G.A. (2017). Effects of Some Selected Solvent Extracts of Avocado Pear (Persea americana) on Cholesterol/HDL Ratio in Albino Rats. *Biochem Anal Biochem*; 6: 342.

Royal Botanic Gardens, Kew and Missouri Botanical Garden (2010). "*Persea americana* Mill. The Plant List, Version 1" (http://www.theplantlist.org/tpl/record/kew-2529835).

Redfern J., Kinninmonth M., Burdass D. and Verran J. (2014). Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of microbiology & biology education*. 15(1):45.

Schettler G. and Nussel E. (1975). Massnahmen Zur Prevention der Artherosklerose. *Arb Med Soz Med Prav Med* ; 10: 25.

Storey W.B. (1973). "What kind of fruit is the avocado. *California Avocado Society 1973–74 Yearbook*; 57: 70–1.

Song Y. and Barlow P.J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem*; 88(3):411–17.

Trease G.E. and Evans W.C. (1989). Pharmacognosy.11th ed. London: Brailliar Tiridel and Macmillian Publishers.

Trinder P. (1981). Oxidase determination of plasma cholesterol as cholest-4-en-3-one using isooctane extraction. *Ann Clin Bioche* 18: 64-70.

Trinder P and Webster D. (1984). Determination of HDL-cholesterol using 2,4,6-tribromo-3hydrobenzoic acid with a commercial CHOD-PAP reagent. *Ann Clin Biochem*; 21: 430-33.

Yanarday R. and Colac H. (1998). Effect of chard (*Beta vulgaris L. varcicla*) on blood glucose level in normal and alloxan-induced diabetic rabbit. *J. Ethnopham*; 4: 309-311.

J. Afr. Ass. Physiol. Sci. 7 (1): 64-69, July 2019

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Effect of aqueous extract of *Spondias mombin* leaves on Nitric Oxide level and some liver biomarkers in dietary palm oil supplement-fed Wistar rats.

E. E. Nkanu[1], S.O. Jeje[2], and G.U. Otu[1]

Departments of Physiology, [1]Cross River University of Technology, Calabar, Nigeria and [2]School of Health and Health Technology, Federal University of Technology, Akure

Keywords:
Spondias mombin, Thermooxidized Oil, Nitric Oxide, Liver Biomakers

ABSTRACT

Background: Consumption of thermally oxidized palm oil over a period of time may lead to altered biochemical indices. Exposure to extract of *Spondias mombin* leaves has been reported to improve biochemical indices in rats. This study was designed to examine the effects of aqueous extract of *Spondias mombin* (SPM) leaves on nitric oxide level and some liver biomarkers in dietary oil supplemented Wistar rats. **Methods:** Thirty-six male Wistar rats weighing between 180-200 g, were used. The animals were randomly divided into six groups containing six rats each. Group 1 (control) received normal rat chow. Group 2 received normal rat chow+ SPM (300 mg/kg body weight orally), Group 3 was fed on feed mixed with fresh palm oil (FPO) (15% w/w), Group 4 was fed on feed mixed with FPO (15% w/w)+SPM (300mg/kg body weight orally), Group 5 was fed on feed mixed with thermooxidised palm oil (TPO) (15% w/w), Group 6 received feed mixed with TPO(15% w/w)+SPM (300 mg/kg body weight orally) for five weeks. **Results:** Result obtained shows a significant ($p<0.05$) increase in NO level in *Spondias mombin* (SPM) group when compared with the control. Serum glucose was significantly ($p<0.05$) high in FPO and TPO. However, SPM, FPO+SPM and TPO+SPM serum glucose levels were decreased by 24.07%, 10.93% and 39.28% respectively when compared with their untreated groups. Total protein and globulin concentration were increased significantly ($p<0.05$) in SPM, FPO+SPM and TPO+SPM. Albumin level was raised in FPO and FPO+SPM compared to control. Total Cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL-c) and Very low density lipoprotein (VLDL) were significantly ($p<0.05$) elevated in TPO but Significantly ($p<0.05$) decreased upon treatment with SPM. **Conclusion**: We conclude that aqueous *Spondias mombin* leave extract promote liver protein synthesis and increases nitric oxide level in normal condition but not in consumption of thermally oxidized oil.

INTRODUCTION

Palm oil is one of the most widely consumed vegetable oil in the world. This is attributable not only to its use as food supplement, but its value in terms of nutritional content (Edem 2002). Palm oil has been reported to contain both saturated and unsaturated fats assumed to be in a ratio of 1:1 (Cottrel, 1991; Edem 2002).

Despite the presence of saturated fatty acids up to 50%, it does not promote atherosclerotic plague formation and thrombosis in the vessels. Some identified components available in fresh palm oil include vitamin C and Vitamin E such as tocopherols, tocotrienols and β-carotenes. The major carotenoids in palm oil are α- and β-carotene (Edem, 2002, Adam *et al.*, 2008). These constituents act as antioxidants that have the potentials to protect against lipid peroxidation/oxidative stress, protein oxidation as well as blood pressure reduction. Subjecting palm oil to repeated heating leads to thermoxidation and production of free radicals that are cytotoxic and may lead to decreased membrane fluidity and loss of enzyme and receptor activity (Leong et al., 2008). In quest for therapeutic approaches to contribute to handling of the developing cardiovascular and other prevalent diseases, the use of folk medicine has been sort. *Spondias mombin,* commonly known as hog plum

*Address for correspondence:
Email: etahnkanu4@gmail.com
Tel: +2347030299991

in English, 'akika'('Iyeye') in Yoruba, ijikara in Igbo, tsardarmaser in Hausa and nsukakara in Efik, is a fructiferous tree in the family *Anacardiaceous* (Gill, 1992). It is mostly found in rain forests and coastal areas, attaining a height of 15-22 meters (Ayoka et al., 2008). *Spondias mombin* leaves have been reported to contain some bioactive substance such as tannins, saponins, flavonoids, phenolics and anthraquinone glycosides antioxidant vitamins; alpha-tocopherol and ascorbic acid (Abo et al., 1999; Maduka et al., 2014). These medicinal potential attributes of the plant extracts may makes it efficacious and therapeutic in protecting against hepatic toxicity and altered plasma protein following consumption of thermally oxidized palm oil.

METHODS

Experimental design

Thirty-six male Wistar rats weighing between 150-200 g were used in this study and they were randomly divided into six groups containing six rats each. The animals were housed in plastic cages and kept under standard laboratory condition. Group 1 (Control) received normal rat chow. Group 2 received normal rat chow+ SPM (300 mg/kg body weight orally), Group 3 was fed on feed mixed with fresh palm oil (FPO) (15% w/w), Group 4 was fed on feed mixed with FPO (15% w/w)+SPM (300 mg/kg body weight orally), Group 5 was fed on feed mixed with thermooxidised palm oil (TPO) (15% w/w), Group 6 received feed mixed with TPO (15% w/w)+SPM (300 mg/kg body weight orally) for five weeks. All groups had free access to water and feed *ad libitum*. Ethical approval for the study was obtained from the Faculty animal research ethical committee of the Cross River University of Technology, Calabar (CRT/ARC/16/021),

Preparation of leaf extract of Spondias mombin.

The modified method of extraction according to Eno and Itam (1998) was used. Fresh leaves of Spondias mombin were collected and washed free of dirts. The leaves were dried on laboratory table for 5 days, after which they were grounded to powder. A quantity of the ground sample (20g) was weighed and Soxhlet extraction was done with 300 ml distilled water at 100 ^{0}C for 12 hours. The liquid extract obtained was slowly evaporated to dryness in vacuo. The total yield was 8.0 g. The extract was stored till further use.

Reparation of Oil diets

Palm oil used for this study was purchased from Okuku market, in Yala Local Government, Cross River State, Nigeria and used fresh or heated five times, according to the modified method of Owu, et al. (1998). To obtain the five times heated palm oil, 1 kg of sliced yams with 2.5 L of palm oil in a stainless was heated. Oil was allowed to cool for five hours and then the heating process was repeated with a fresh batch of yams. This heating process was repeated four times to obtain the five times heated oil. At the course of heating, no oil was topped to make up for loses. The diet was formulated by mixing 15% (w/w) of fresh or heated oils respectively with the feed.

Measurement of biochemical parameters

Blood samples were collected orbital sinus into EDTA sample bottle, centrifuge at 4000 rpm for 10 minutes to prepare the plasma used for biochemical assays and Fasting Blood Glucose (FBS) was determined via tail puncture after overnight fast by using Accu-Check ActiveGglucometer (Roche Diagnostics Germany).

Total cholesterol level in blood was determined as described by Siedel *et al.,* (1983). Triglyceride was determined by the method of Sullevian *et al.,* (1985). The equation of Friedwald *et al.,* 1972 (LDL =TC-HDL+ TG/2.23) was used to estimate LDL-c level

Serum total protein and albumin levels were estimated using the method of Lowry, (1951 and Doumas *et al.,* (1971) respectively

Determination of NO

Plasma nitric oxide levels were determined by the presence of nitrite metabolites according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Samples of 50 µL each were put into a microtiter plate and then 50 µL of modified Griess reagent added (Sigma-Aldrich, St. Louis, MO, USA). The nitrite concentration was then measured after 15 minutes of incubation at 540 nm using an Emax ELISA microplate reader. The procedures for NO determination were carried out in a dark environment. The nitrite concentrations were quantified with standard curve generated using known concentrations of sodium nitrite (Sigma-Aldrich, St. Louis, MO, USA).

Statistical Analysis

All results were presented as mean ± SEM. One-way analysis of variance (ANOVA) was done using Graph pad prism version 5 statistical software. Bonferroni's multiple comparison test was used for pair wise comparison and differences were considered significant at $P<0.05$.

RESULTS

Nitric Oxide Level, Serum Blood Glucose Level and Lipid Profile in Rats Fed on Dietary Oils

The results obtained showed that Nitric oxide level was significantly ($p<0.05$) reduced in the TPO group. Co-

TABLE 1: Effects of aqueous extract of Spondias mombin leaves on blood lipid profile

Variables (mmol/L	Control Mean ± SEM	SPM Mean ± SEM	FPO Mean ± SEM	FPO+SPM Mean ± SEM	TPO Mean ± SEM	TPO+SPM Mean ± SEM
Total Cholesterol	0.75±0.009	0.71±0.01	0.70±0.02*	0.63±0.009$^{*\#}$	0.85±0.007$^{*\#}$	0.70±0.006^{*a}
Triglyceride	0.43±0.024	0.42±0.008	0.41±0.009	0.40±0.019	0.58±0.016$^{*\#}$	0.44±0.009^{a}
HDL-Cholesterol	0.22±0.003	0.18±0.003*	0.20±0.004$^{*\#}$	0.19±0.003*	0.16±0.003$^{*\#}$	0.21±0.002$^{\#a}$
VLDL-cholesterol	0.22±0.009	0.18±0.009*	0.21±0.004$^{\#}$	0.22±0.007$^{\#}$	0.28±0.007*	0.20±0.006^{a}

SPM- *Spondias mombin* group, FPO (Fresh Palm Oil Group) TPO Thermoxidized Palm Oil Group) *,#, a P<0.05-significantly different from the control, SPM , and TPO respectively

treatment with SPM does not significantly improve the level of Nitric oxide. *Spondias mombin* only group had a significant (p<0.05) increase in nitric oxide level when compared with the control, TPO and TPO+SPM groups. Nitric oxide level was not significantly different in FPO and FPO+SPM (FPOT) when compared with the control (Figure 2).

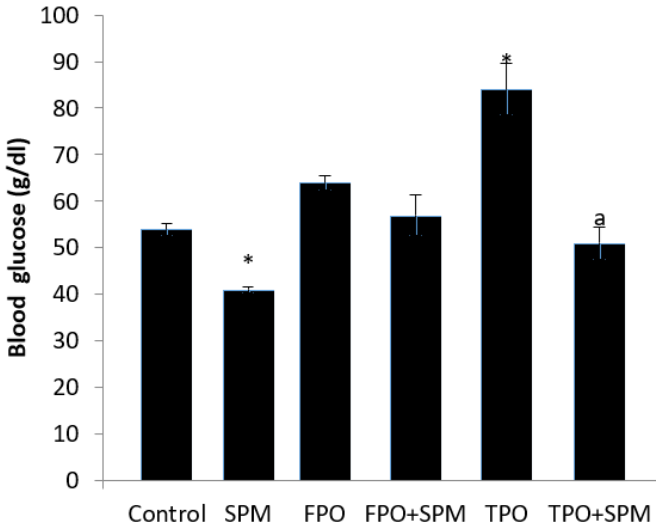

Fig.1: Effects of aqueous extract of *Spondias mombin* leaves on serum blood glucose. SPM- *Spondias mombin* group, FPO (Fresh Palm Oil Group) TPO Thermooxididsed Palm Oil Group) ***, #,**a P<0.05-significantly different from the control, SPM , and TPO respectively

Blood glucose level was significantly (p<0.05) raised in TPO group. Treatment with SPM extract significantly (p<0.05) reduce the blood glucose level in both SPM and TPO+SPM groups (Figure 1). The results of lipid profile are also presented in table 1. Serum TC, TG, LDL-c and VLDL were significantly (p<0.05) elevated in TPO compared to all other groups. Treatment with the extract significantly (p<0.05) reduced the lipid profile level in the TPO.

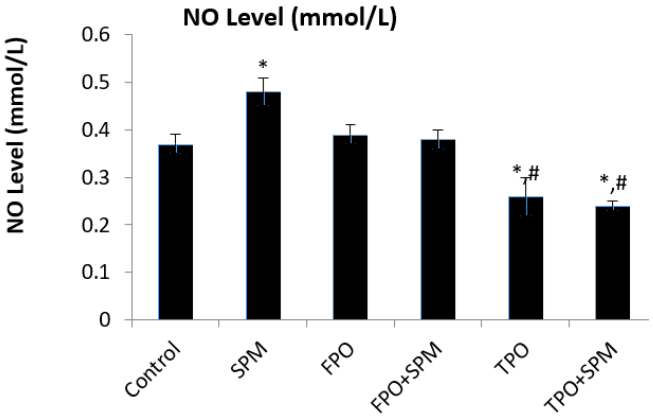

Fig. 2: Effects of aqueous extract of *Spondias mombin* leaves on NO. SPM- *Spondias mombin* group, FPO- (Fresh Palm Oil Group) TPO- (Thermooxididsed Palm Oil Group) *,#,a P<0.05-significantly different from the control, SPM , and TPO respectively

.

Total Protein, Albumin and Globulin in Rats Fed on Dietary Oils

In Figure 3, total protein level in all the treated groups were significantly (p<0.05) different from the Cntrol. Co-treatment of TPO with extract significantly (p<0.05) increased the total protein level compared to TPO only group. Globulin concentration in SPM group and TPO +SPM groups were significantly (p<0.05) raised compared to all other groups. Albumin concentration in the TPO+SPM was increased by 8.1% compared to TPO only group. There was no significant difference between FPO and FPO+SPM compared to control.

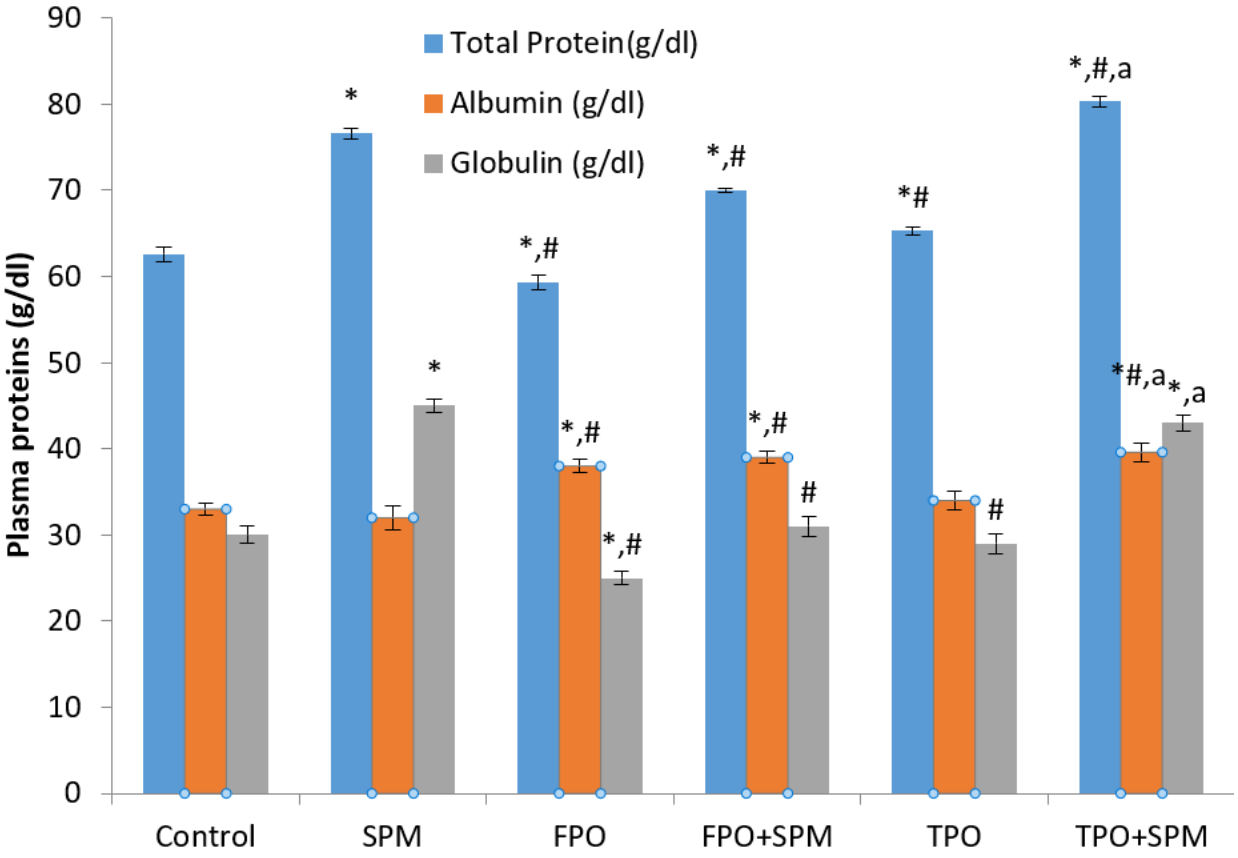

Fig. 3: Effect of aqueous extract of *Spondias mombin* leaves on total protein and liver biomarkers level in Wister rats fed on dietary oils. SPM- *Spondias mombin* group, FPO- (Fresh Palm Oil Group) TPO- (Thermoxidized Palm Oil Group) *,#,a P<0.05-significantly different from the control, SPM , and TPO respectively.

DISCUSSION

Palm oil is commonly used for cooking either in its fresh state or fried. Depending on the frequency of frying, it loses its unsaturation status. The altered nature of these oils affects the body's physiologic function (Osim *et al.*, 1996; Owu *et al.*, 1998). The aim of this study therefore was to investigate the effect of aqueous extract of *Spondias mombin* leaves NO level and some liver biomarkers in oil diet supplemented Wistar rats. In our study, thermally oxidized oil remarkably reduced nitric oxide level. This result is in accordance with earlier reported works by Jaarin *et al. (*2011). Generally, repeated heating of oils at high temperatures predisposes to fatty acid oxidation and release of free radicals including peroxides, and superoxide anions. These products of oxidation down regulates the synthesis of nitric oxide (Carr & Frei 2000; Gao & Lee, 2001; Hayashi et al, 2004; *Jaarin et al.*, 2011) thereby limiting its physiologic function. That nitric oxide level depreciated in this study is therefore not surprising. Even though nitric oxide concentration was significantly increased in the rats that received *Spondias mombin* extracts only, it was observed that treatment of the thermally oxidized group (TPOT) with the same extract had no significant effect. The probable and possible reason for this observed inactivity is that the presence of the free radicals may be counteracting the effect of the extract on the site of synthesis of the cell signaling molecule or acting to inhibit the nitric oxide producing cells. According to Adams *et al.* (2007), heated palm oil reduced the vitamin E content of palm oil. Vitamin E is an important dietary free radical scavenger.

Data presented in this study also showed that prolonged consumption of thermally oxidized palm oil significantly raised serum blood glucose concentration. Similarly, the lipid profile status in TPO was challenged. The total cholesterol (TC), triglyceride (TG), Low density lipoprotein (LDL-c) and VLDL were significantly increased while the HDL-c often referred to 'good' cholesterol was reduced. This result is in line with earlier observations reported by Osim *et al.*, (1996)

and Owu *et al.,* (1998). This may suggest the presence of lipid metabolism disorder that could result in systemic diseases such as high blood pressure.

Interestingly, treatment with *Spondias mombin* leave extract reasonably decreased serum glucose concentration in FPO+SPM and TPO+SPM as well as the lipid profile in TPO +SPM. This hypoglycemic and hypolipidemic effect of the leave extract may be linked to its medicinal attributes occasioned by the rich phytochemical constituents and the reported free radical scavenging property (Maduka et al., 2014). In the liver, overproduction of reactive oxygen species may result in oxidative stress. This affects hepatic functions. Our study has shown that total protein, albumin, globulin, concentrations were significantly decreased in TPO. The recorded significant decrease in total protein and albumin of the groups fed on thermally oxidized palm oil diets shows that this form of oil may alter protein synthesis in the liver. Similar result had earlier been reported by Ayodeji et *al*., (2015). Treatment with extract recorded a tremendous increase in these parameters, suggesting ameliorating and protective property. Research has shown that bilirubin and albumin are pivotal in detoxification and antioxidant protection by scavenging superoxide, peroxides and hypochlorous acids (Frei *et al.,* 1988; Wu *et al.,* 1994). Hypoalbuminemia is associated with liver disorder (Ayodeji et *al*., (2015). With the increase in these liver biomarkers recorded in this study following treatment with *Spondias mombin* leave extract, there is a strong indication that the available phytochemical constituents may be promoting liver hepatocyte function.

In conclusion, aqueous extracts of *Spondias mombin* leaves promote liver protein synthesis, hypoglycemic, and hypolipidemic activity but does not influence NO level in thermally oxidized oil consumption.

ACKNOWLEDGEMENT

The authors acknowledged with thanks the technical assistance received from laboratory technologist of the Department of Physiology, Cross River University of Technology

REFERENCES

Abo, K.A., Ogunleye, J.O. and Asindi, J.S. (1999). Antimicrobial potential of *Spondias mombin Croton zambesicus* and *Zygotritoniacrocea*. *Phytother Res*. 13: 494497. 2.

Adam, S.K., Sulaiman, I.N., Umar, N.A., Mokhtar, N., Mohammed, N., Jaarin, K.(2008).Effect of repeatedly heated palm oil on serum lipid profile, lipid peroxidation and homocysteine levels in postmenopausal rat model. McGill *J Med* 11(2): 145-151.

Adam, S.K., Sulaiman, N.A., Mat Top, A.G., Joarin, K, (2007).Heating reduces vitamin E content in palm and soy oils. *Malaysia Journal of Biochemistry and Molecular Biology*. 15; 76-79.

Ayodeji, O. F.,Ganiyu, O., Adedayo, O.A., Oluwatoyin, V. O.(2015). Consumption of thermally oxidized palm oil diets alters biochemical indices in rats. *Beni-suef University Journal of Basic and Applied Sciences*. 4 (2): 150-156

Ayoka, A.O, Akomolafe, R.O, Akinsomisoye, O.S. and Ukponmwan, O.E. (2008). Medic inaland Economic Value of *Spondias mombin*. *Afri J Biomedic Res. 11; 129 – 136. 5.*

Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. Free Radic Biol Med. 2000;28: 1806–14. 10.1016/S0891-5849(00)00225-2

Cottrell, R.C (1991). Introduction: nutritional aspects of palm oil. *Am J Clinical Nutri* 53: 989s-1009s

Edem,D.O.(2002).Palmoil:biochemical physiological,nutrtitional haematological and toxicological aspects; a review. Plant Food and Human Nutrition.57 (3-4): 319-341.

Eno, A.E. and Itam E.H. (1998): Stimulation of Cholinoreceptors in the rat uterus by a crude extract from *Elaecophorbia drupilera* leaves. *Pharmacol*. 36(2): 97-102

Frei B., Stocker R., Ames B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* 85, 9748975210.1073/pnas.85.24.9748

Friedwald, W.T., Levy, R.J., Friedricken, D.S.,(1972). Estimation of HDL-c in the plasma without the use of preparative ultracentrifuge.*Clinical Chemistry*.18: 449 -502

Gao YJ, Lee RMKW. Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A_2 production. Br J Pharmacol. 2001;134:1639–46.

Gill L.S. (1992). Ethnomedicinal uses of plantsin Nigeria (UNIBEN press), Nigeria, pp. 222-223

Hayashi Y, Sawa Y, Nishimura M, Fukuyama N, Ichikawa H, Ohtake S, et al. Peroxynitrite, a product between nitric oxide and superoxide anion, plays a cytotoxic role in the development of post-bypass systemic inflammatory response. Eur J Cardiothorac Surg. 2004;26:276–80

Jaarin, Kamsiah,S-Nik,Aziz.,S-Tan.,Siew., I-syen., Zahir (2011). Changes in serum lipid profile and malondialdehyde following consumption of fresh

or heated red oil. *Medical Journal of Islamic Academy of Science*.14 :(2), 79-86

Leong, X.F., Aishah, A., Nor Aini, U., Das, S., Jaarin, K. (2008). Heated palm oil causes rise in blood pressure and cardiac changes in heart muscle in experimental rats. *Archives of Medical Research*. 39(6567-572

Lowry, O.H., Rosebrough, N.J., Farr,A.L., Randall,R.J., (1951). Protein measurement with the Folin Phenol Reagent. Journal of Biological Chemistry 193; 269-275.

Maduka, H.C.C, Okpogba, A.N, Ugwu, C.E, Dike, C.C., Ogueche, P.N., Onwuzurike, D.T and Ibe, D.C (2014). Phytochemical, antioxidant and microbial inhibitoryeffects of Spondias mombin leaf and stembark extracts. *J Pharm Biol Sci*. 9(2): 14-17. 27.

Osim, E.E., Owu, D.U., Etta, K.M.(1996). Arterial pressure and lipid profile in rats following chronic ingestion of palm oil diets. *African Journal of Medicine and Medical Science*. 25:335-340.

Owu, D.U., Osim, E.E., Ebong,P.E.(1998). Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta tropics* 69: 65-73.

Siedel, J. Hagele, E.O. Ziegenhorn, J., Wahlefield, A.W.(1983). Reagent for the enzymatic determination of serum cholesterol with improved lipolytic efficiency. *Clinical Chemistry*.20:1075.

Sullivian D.R. Kruijswijk Z, West, C.E, Kohlmeier M, Katan M.B. (1985): Determination of serum Triglycerides by an accurate enzymatic method not affected by free glycerol. *Clin. Chem*.31:1227-12

Wu T. W., Fung K. P., Yang C. C. (1994). Unconjugated bilirubin inhibits the oxidation of human low density lipoprotein better than Trolox. *Life Sci.* 54, 477–48110.1016/0024- 3205(94)90140-146