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THE EFFECTS OF AFRICAN WALNUT (Tetracarpidium conophorum) CRUDE ETHANOLIC ON THE CARDIOVASCULAR SYSTEM, HEMATOLOGICAL PROFILE AND OXIDATIVE STATUS OF MECURY CHLORIDE TREATED WISTAR RATS

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ABSTRACT

Background: *Tetracarpidium conophorum* commonly referred to as, African walnut has been reported to possess antibacterial activity, anticancer, antidiabetic activities and also boost fertility in male. The influence and mitigating properties of *Tetracarpidium conophorum* on the cardiovascular system and oxidative status of Wistar rats that were administered mercuric chloride (HgCl2) were investigated. **Methods:** A total of forty two rats were divided into seven groups; group A received 1ml of distilled water, group B received tween 80 while group C rats were administered 0.5mg/kg of mercuric chloride. Group D were administered 200mg/kg of *Tetracarpidium conophorum*, group E were administered 200mg/kg *Tetracarpidium conophorum* and o.5mg/kg mercuric chloride, group F were administered 300mg/kg *Tetracarpidium conophorum* and 0.5mg/kg mercuric chloride and group G were treated with 300mg/kg *Tetracarpidium conophorum* only. After 28 days of the experiment, the rats were sacrificed and whole blood (5ml) was collected for hematological analysis. Antioxidants enzymes including, Superoxide (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as, marker of lipid peroxidation (malondialdehyde, MDA) were assayed using standard biochemical procedures. Results and **Discussion:** The heart weight of the group C rats given mercury chloride only demonstrated a significant increase (0.77mg) but declined with a co-administration of 200mg/kg *Tetracarpidium conophorum* (0.68mg) and further decreased with 300mg/kg administration to 0.62mg. The mean aorta weight of the rats administered mercury chloride only (0.08mg) did not show significant change compared to group A (0.09mg) and group B (0.08mg), but the co-administration of mercury chloride with 200mg/kg *Tetracarpidium conophorum* resulted in significant increase in aorta weights for the group E (0.13mg). The co-administration of 200mg/kg and 300mg/kg *Tetracarpidium conophorum* and mercury chloride (group E and F, respectively) led to increases in WBC levels, i.e. 12.50×10-3/µl and 12.80×10-3/µl. The group treated with mercury chloride demonstrated a significant increase in MDA level (7.75 nmole/ml) compared to the control groups; group A (6.71 nmole/ml) and group B (6.33 nmole/ml) but decreased in group E (6.77 nmole/ml). **Conclusion:** Mercuric chloride caused sever damaging effects to both the myocardium and the aorta, but *Tetracarpidium conophorum* was able to ameliorate these effects.

KEYWORDS: African walnut; Mercuric chloride; Myocardium; Aorta; Antioxidant enzymes.

INTRODUCTION

Tetracarpidium conophorum or *Plukenetia conophora* commonly referred to as, African walnut belongs to the family Euphorbiaceae. The other species includes, Olacaceae (Coula edults); the former is mostly found in Nigeria and Cameroon, while the latter if found in Congo, Liberia and Gabon. In Nigeria, it is called Asala or Awusa in Yoruba; Ukpa in Ibo; and Okhue or Okwe in Edo and has been attributed with numerous phyto-medicinal properties. According to Ojobor et al. (2015), *Tetracarpidium conophorum* contains phytochemicals including, alkaloids, glycosides, saponins, flavonoids, tannins, terpenoids, reducing sugars and soluble carbohydrates. They also reported the presence of vitamins A, C, D, E and K. However, Enejiugha (2003) reported the presence of oxalates, phytates, tannins, proteins, fibres, oils, and carbohydrates. *Tetracarpidium conophorum* is one of the very nutritious and medicinal plants in Nigeria which have been underutilized due to paucity of information about its health values or oblivion about its availability. Some of the reported medicinal properties of *Tetracarpidium conophorum* include, antibacterial activity (Ajeiyoba and Fadare, 2006), anticancer (Herbert et al., 1998), and antidiabetic activities (Kaneto et al., 1999). Ojobor et al. (2017) also reported its ability to improve male fertility; increased

epididymal sperm and testosterone concentrations. In addition to their report, histoligical observations included robust seminiferous tubular lumen containing sperm cells and increased production of Leydig cells and Sertoli cells.

Heavy metals have been reported to be highly persistent, bioaccumulate and biomagnify in the food chain, leading to toxicity (Karaboduk et al., 2015; Deepmala et al., 2013). Mercury is a heavy metal that has both natural and artificial sources (Zhang et al. 2013). Mercury can be found in three basic forms, including, elemental, inorganic mercury and organic mercury forms with various toxicological profiles (Oliveria et al. 2012). Inorganic mercury compounds have been reported to induce toxicity in a number of different biological systems, including the reproductive system (Kalender et al. 2013), the central nervous system and urinary system (Patrick 2002). Souza de Assis et al. (2003) also reported that inorganic mercury was capable of producing profound cardiotoxicity. The reported general toxic effect of heavy metals is considered to be a result of the inactivation of enzymes and/or functional proteins by directly binding to them (Tsuji et al. 2002) which may also be partly due to oxidative damage by formation of reactive oxygen species (ROS) (Stohs and Bagchi 1995). Oxidative stress developing with the production of ROS can lead to the development of many pathological changes (Morakinyo et al. 2012). For example, mercury has been attributed to the formation of ROS (Bharathi et al. 2012). Antioxidant enzymes such as SOD, CAT and GPx protect cellular homeostasis from oxidative damage by ROS generated through the reduction of molecular oxygen (Sanz et al. 2002). In this present research, we investigated the influence and mitigating properties of *Tetracarpidium conophorum* on the cardiovascular system and oxidative status of Wistar rats that were administered mercury chloride ($HgCl_2$).

MATERIALS AND METHODS

Experimental rats: The rats used were adult albino male Wistar rats weighing between 100 and 150 g. The rats were acclimatized for 1 week before the administration procedure begun. They were fed standard rat chow and water *ad libitum* (livestock feed). The research ethics committee guideline principles on the handling of animals of the College of Medicine, University of Benin (CMR/REC/2014/57), was adopted and strictly adhered to.

Preparation of *Tetracarpidium conophorum* Crude Extract Stock Solution

A large quantity of fresh parts of the plant were collected from trees from household gardens in Benin City and around the University of Benin, Edo state, Nigeria. The plant was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen number, UBHe0288, was deposited at the Herbarium of Department of Plant Biology and Biotechnology, University of Benin. The properly washed plant samples were pulverized after drying at room temperature (about 25°C) for 4 weeks. The pulverized plant samples were macerated in methanol using jar bottles for 48 h after which they were subjected to filtration using cheese cloth. The obtained extracts were then concentrated *in vacuo* using a rotary evaporator to obtain viscous gels that were air-dried to gel-like solids. The gel-like crude methanolic extracts obtained from the various parts of the plant were reconstituted to obtain a stock solution using distilled–deionized water as solvent. The reconstituted crude extract was stored in small-capped plastic containers in a refrigerator at −4°C until use.

Experimental Protocol

Animals were divided into 7 groups (A to G) with each group containing six rats.
Group A rats served as control and received 1ml of distilled water daily for 28 days.
Group B rats were administered 5mg/kg of tween 80 for 28 days.
Group C rats were administered 0.5mg/kg of mercuric chloride for 28 days.
Group D rats were administered 200mg/kg of *Tetracarpidium conophorum* extract for 28 days.
Group E rats were administered 200mg/kg of *Tetracarpidium conophorum* extract and 0.5mg/kg of mercuric chloride for 28 days.
Group F rats were administered 300mg/kg of *Tetracarpidium conophorum* extract and 0.5mg/kg of mercuric chloride for 28 days.
Group G rats were administered 300mg/kg of *Tetracarpidium conophorum* extract for 28days.

Administration of Extracts

The extracts were administered with the aid of a gavage acting as an orogastric tube. Utmost care was taken not to inflict oral or esophageal injuries on the rats.

Blood Sample Collection for Laboratory Assessment

At the end of the experimental period of 28 days, the rats were killed. Thereafter, whole blood (5ml) was collected into EDTA tubes for hematological analysis. The EDTA whole blood was immediately taken to the University of Benin Teaching Hospital Hematological Department for determining the full hematological profile using the automated blood cell count analyzer.

Biochemical Assays

Superoxide dismutase activity was assayed by method of Misra and Fridovich (1972), catalase activity by Cohen et al. (1970), and glutathione peroxidase by method of Flohe and Gunzler (1984). Marker of lipid peroxidation (malondialdehyde, MDA) was assayed using the method of Vershney and Kale (1990).
Mercuric chloride ($HgCl_2$; 99% purity) were obtained from Sigma Aldrich (Germany).

Histopathology

For histopathological examination, parts of the cardiac tissue obtained from each animal were fixed in Bouin solution. Then the tissue samples were processed using a graded ethanol series, and embedded in paraffin. Paraffin sections were cut into 6-7 µm-thick slices and stained with hematoxylin and eosin (H&E) for histological examination. The sections were examined and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached digital photograph machine (Olympus E-330, Olympus Optical Co. Ltd., Japan).

Statistical Analysis

Values are presented as mean±SEM for five determinations. Data were analyzed with the statistical package for the social sciences (SPSS, version 21.0; IBM Corp., 2012, version 21.0, Armonk, NY, USA), using the Duncan's multiple range analysis of variance (ANOVA) at a confidence interval of 95% (P=0.05).

RESULTS

Table 1: Weights of heart and aorta of rats administered methanolic fruit extract of *Tetracarpidium conophorum*.

Groups	Heart (mg)	Aorta (mg)
Group A	0.57±0.006	0.09±0.002
Group B	0.62±0.006	0.08±0.001
Group C	0.77±0.010	0.08±0.001
Group D	0.73±0.008	0.15±0.003
Group E	0.68±0.010	0.13±0.000
Group F	0.62±0.005	0.05±0.000
Group G	0.53±0.002	0.06±0.002
F value	33.517	82.930
p-value	0.000	0.000

Values are represented as mean±SEM for six determinations. Means with P<0.05 are significantly different at 95% confidence interval using the Duncan's multiple range ANOVA, down the column.

Table 2: Platelets, white blood cells, and differentials of rats administered methanolic fruit extract of *Tetracarpidium conophorum*.

Groups	WBC ($\times10^{-3}$/µl)	Lymphocyte (%)	Monocytes (%)	Granulocytes (%)	Platelets (µl)
Group A	11.75±2.00	49.00±2.00	17.60±0.70	33.40±3.00	886.00±7.00
Group B	7.88±1.00	67.93±12.00	10.78±4.00	21.30±8.00	929.00±12.00
Group C	11.73±0.90	70.90±10.00	10.50±4.00	18.60±7.00	915.67±5.00
Group D	10.80±1.00	87.50±1.00	4.93±0.40	7.58±0.90	884.75±13.00
Group E	12.50±0.50	65.45±1.00	11.25±2.00	23.30±8.00	863.00±2.00
Group F	12.80±3.00	48.33±1.00	12.23±2.00	39.43±1.00	923.00±12.00
Group G	16.36±2.00	51.60±10.00	15.96±3.00	32.44±8.00	1032.20±11.00
F value	24.003	47.289	64.572	59.162	83.359
p-value	0.021	0.003	0.000	0.000	0.000

Values are represented as mean±SEM for six determinations. Means with P<0.05 are significantly different at 95% confidence interval using the Duncan's multiple range ANOVA, down the column.

Table 3: Red blood cell differential of rats administered fruit extract of *Tetracarpidium conophorum*.

Groups	RBC ($\times10^{-3}$/µl)	HGB (g/dl)	HCT	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW	PCT	MPV	PDW
Group A	7.69±0.05	14.70±0.06	44.45±0.70	57.70±1.00	19.05±0.20	33.05±0.30	17.75±0.10	0.78±0.04	8.85±0.30	11.95±0.60
Group B	7.37±0.20	14.40±0.30	44.10±1.00	59.83±0.80	19.53±0.20	32.65±0.50	19.03±0.80	0.70±0.10	7.50±0.30	9.73±0.60
Group C	7.68±0.40	15.37±0.60	46.47±3.00	60.43±0.50	19.97±0.40	33.10±0.90	16.73±0.30	0.66±0.02	7.23±0.50	9.53±0.90
Group D	7.32±0.20	13.88±0.40	41.23±2.00	56.28±1.00	18.90±0.30	33.65±0.90	18.18±0.40	0.64±0.09	7.38±0.40	9.18±0.60
Group E	8.02±0.10	15.25±0.09	47.85±0.03	59.70±1.00	19.00±0.20	31.80±0.20	19.05±0.20	0.69±0.05	8.05±0.30	10.85±0.70
Group F	8.36±0.30	15.90±0.40	48.90±2.00	58.40±0.80	18.97±0.30	32.53±0.40	18.70±0.20	0.63±0.09	6.83±0.20	9.03±0.20
Group G	7.22±0.30	13.68±0.50	39.98±2.00	55.40±2.00	18.94±0.60	34.26±0.40	18.42±0.90	0.72±0.07	7.06±0.20	8.84±0.30
F value	3.289	4.830	12.741	3.251	1.014	1.301	17.728	4.016	5.183	0.170
p-value	0.034	0.042	0.036	0.029	0.146	0.098	0.061	0.038	0.042	0.024

Values are represented as mean±SEM for six determinations. Means with P<0.05 are significantly different at 95% confidence interval using the Duncan's multiple range ANOVA, down the column. WBCs, white blood cells.

Table 4: Antioxidant enzyme activities and level of lipid peroxidation of rats administered fruit extract of *Tetracarpidium conophorum*.

Groups	Malondialdehyde, MDA (nmol./ml of serum)	Glutathione peroxidase, GPx (u/ml of serum)	Superoxide dismutase, SOD (u/ml of serum	Catalase, CAT (u/ml of serum)
Group A	6.71±0.90	86.85±11.00	40.96±8.00	37.22±6.00
Group B	6.33±1.00	113.18±12.00	51.41±12.00	44.54±4.00
Group C	7.75±0.90	82.82±21.00	28.84±6.00	24.34±7.00
Group D	6.77±0.00	84.50±0.00	28.74±0.00	28.50±0.00
Group E	6.77±0.90	81.27±14.00	46.67±10.00	27.59±14.00
Group F	8.03±1.00	98.31±22.00	27.95±5.00	22.92±2.00
Group G	7.83±2.00	94.83±15.00	37.56±5.00	26.41±3.00
F value	11.37	76.29	38.21	44.53
p-value	0.000	0.000	0.000	0.000

Values are represented as mean±SEM for six determinations. Means with P<0.05 are significantly different at 95% confidence interval using the Duncan's multiple range ANOVA, down the column.

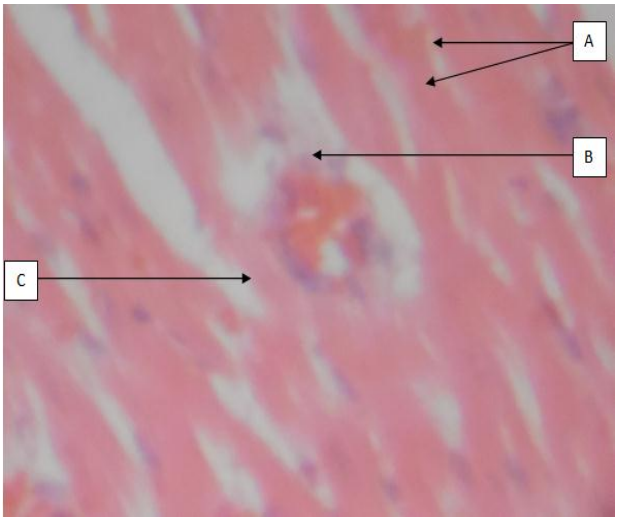

Fig. 1 Control: Rat heart composed of A, bundles of myocardiac fibres, B, coronary artery and C, interstitial space (H&E x 100).

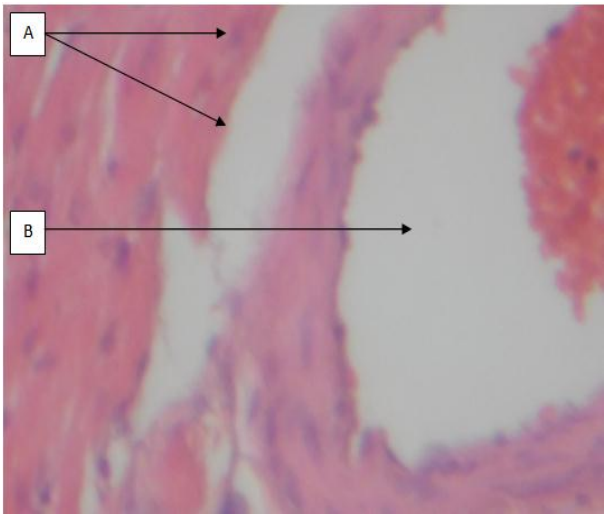

Fig. 3: Rat heart given 200mg/kg extract showing A, normal myocardiac fibre architecture and B, moderate coronary dilatation and congestion (H&E x 100).

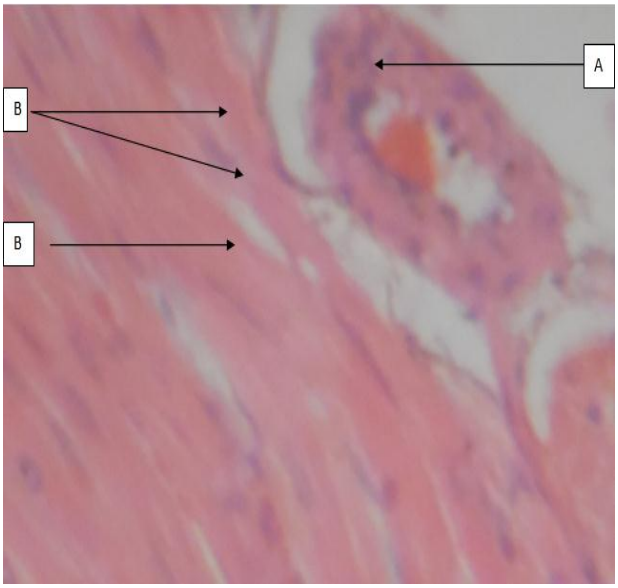

Fig. 2: Rat heart given 5mg/kg Tween 80 only showing A, coronary artery, B, bundles of myocardiac fibres and C, interstitial space (H&E x 100).

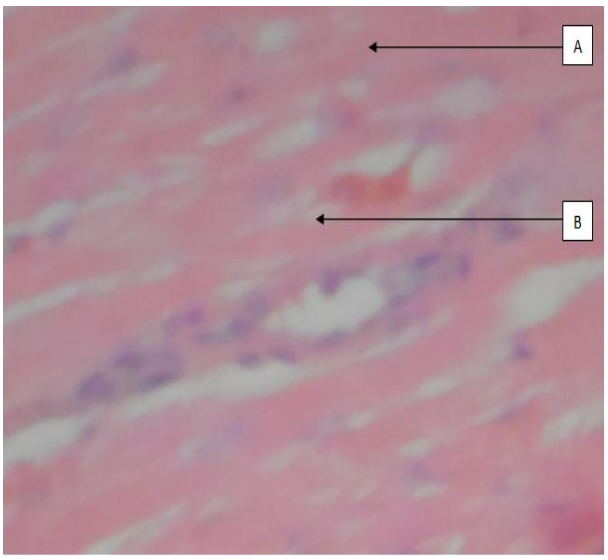

Fig. 4: Rat heart given 300mg/kg extract only showing A, normal myocardiac fibre and B, vascular architecture (H&E x 100).

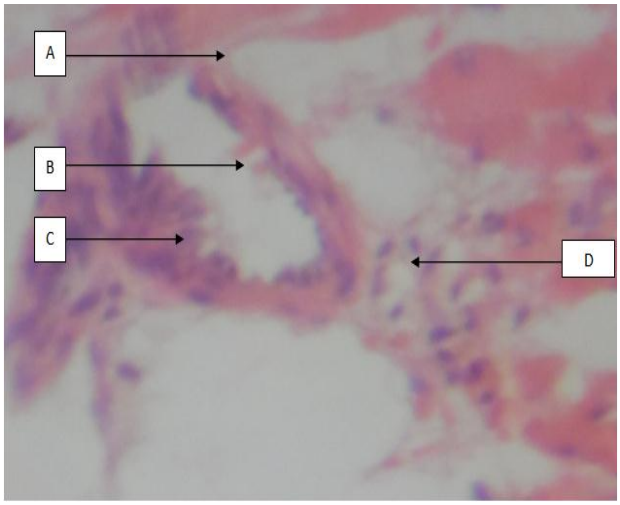

Fig. 5: Rat heart given 0.5mg/kg mercuric chloride only showing A, severe vascular ulceration, B, asymmetric hypertrophy, C, patchy myocardiac degeneration and D, mild myocardiac infiltrates of inflammatory cells (H&E x 100).

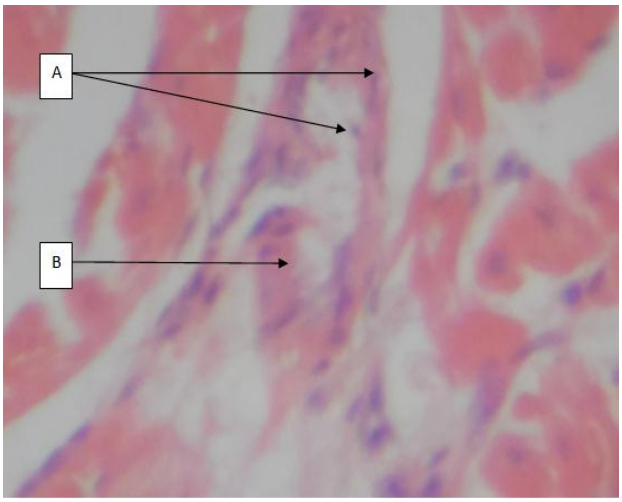

Fig. 6: Same group showing A, severe vascular ulceration and B, moderate myocardiac degeneration (H&E x 100).

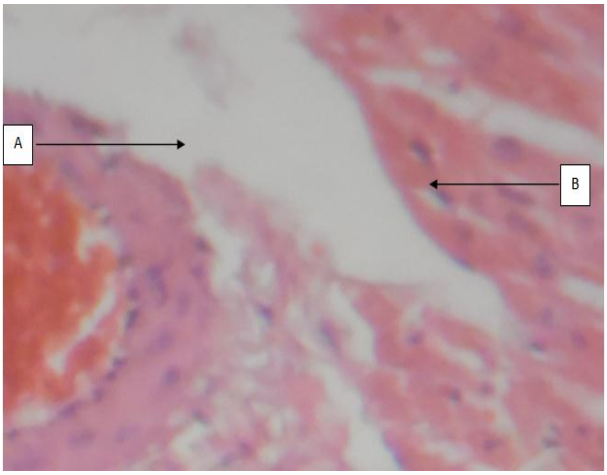

Fig. 7: Rat heart given 0.5mg/kg mercuric chloride plus 200mg/kg extract showing A, normal vascular and B, myocardiac architecture (H&E x 100).

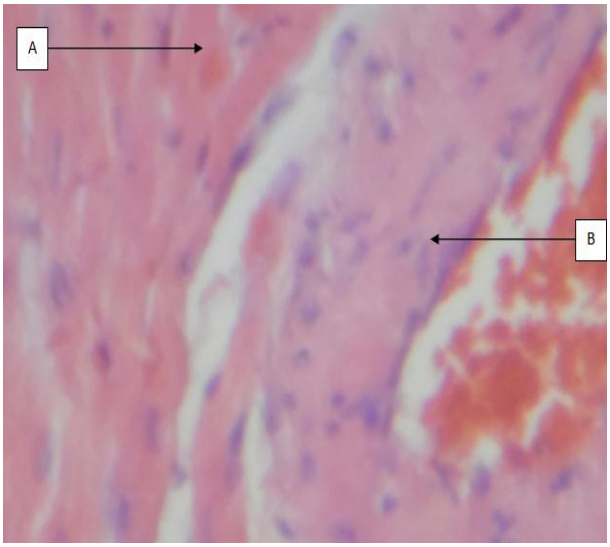

Fig. 8: Rat heart given 0.5mg/kg mercuric chloride plus 300mg/kg extract showing A, normal myocardiac and B, vascular architecture (H&E x 100).

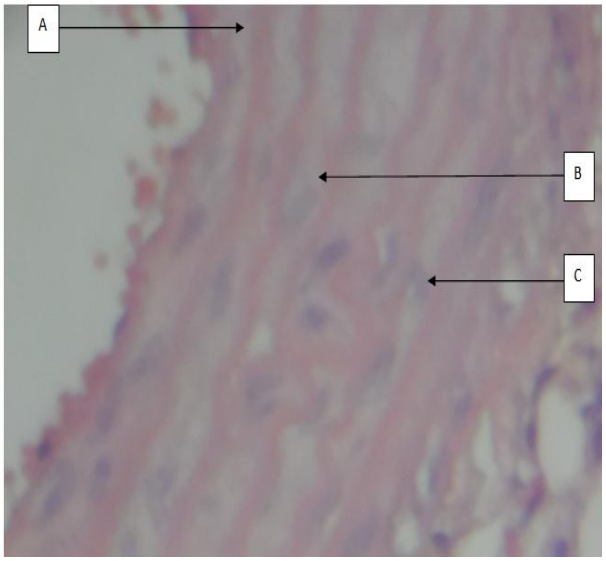

Fig. 9 Control: Rat oarta composed of tunica A, intima, B, media and C, adventitia (H&E x 100).

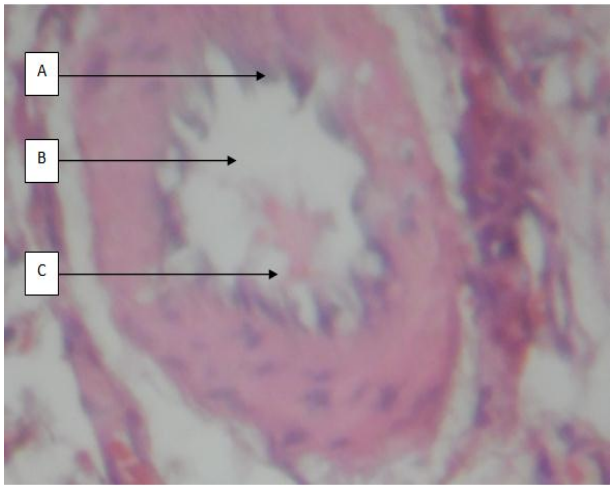

Fig. 10: Same group A, B and C (H&E x 100).

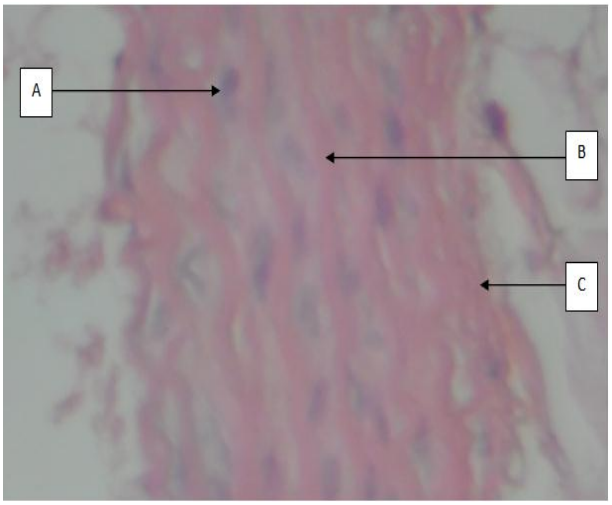

Fig. 11: Rat aorta given 5mg/kg Tween 80 showing A, intima, B, media and C, adventitia (H&E x 100).

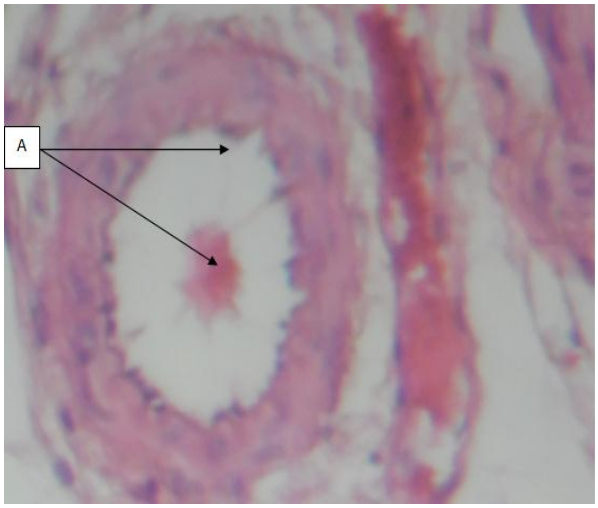

Fig. 14: Same group showing A,normal vascular wall (H&E x 100).

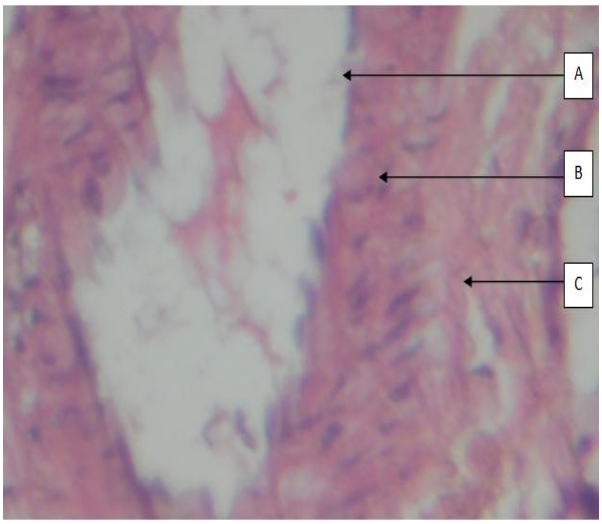

Fig. 12: Same group A, B and C (H&E x 100).

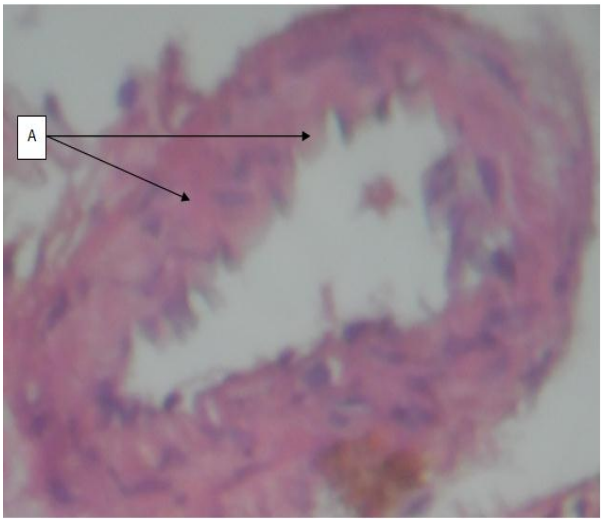

Fig. 15: Rat artery given 300mg/kg extract only showing A, normal vascular architecture (H&E x 100).

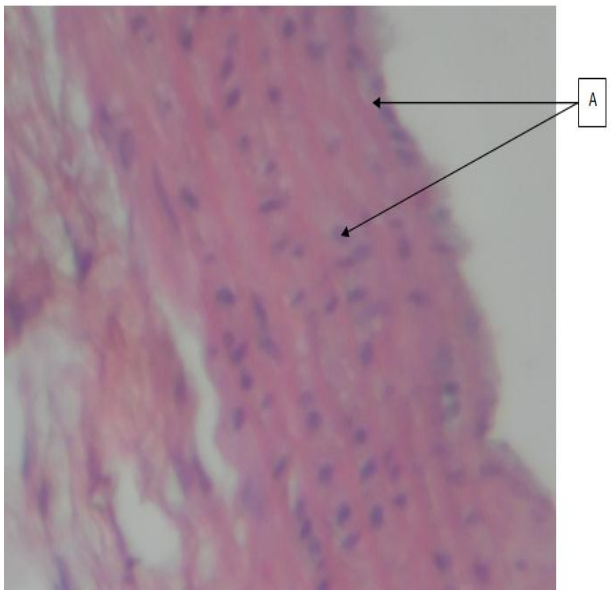

Fig. 13: Rat aorta given 200mg/kg extract only showing A, normal vascular wall architecture (H&E x 100).

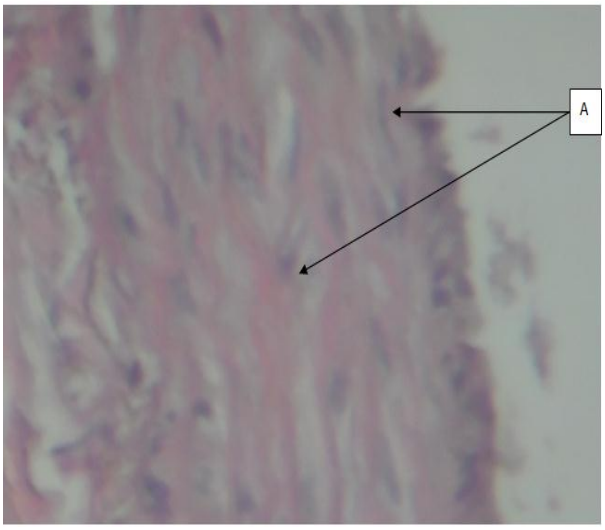

Fig. 16: Same group (aorta) showing A normal vascular wall (H&E x 100).

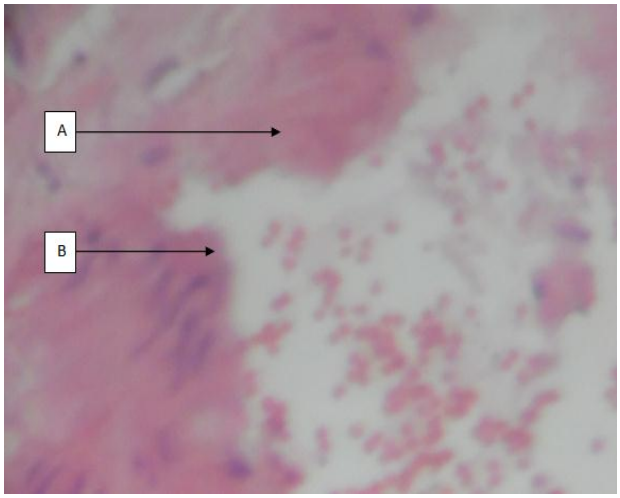

Fig. 17: Rat aorta given 0.5mg/kg mercuric chloride only showing A, severe vascular ulceration and B, mural degeneration (H&E x 100).

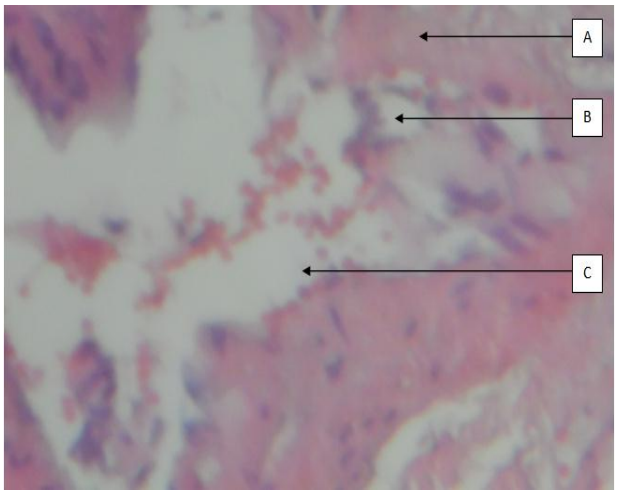

Fig. 18: Same group, rat artery showing A,severe vascular Degeneration, B, ulceration anc C, asymmetric hypertrophy (H&E x 100).

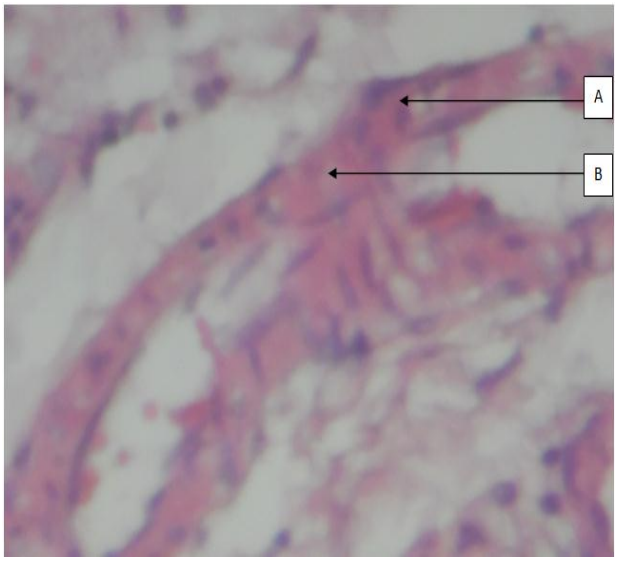

Fig. 19: Same group showing A, severe vascular stenosis and B, obstruction (H&E x 100).

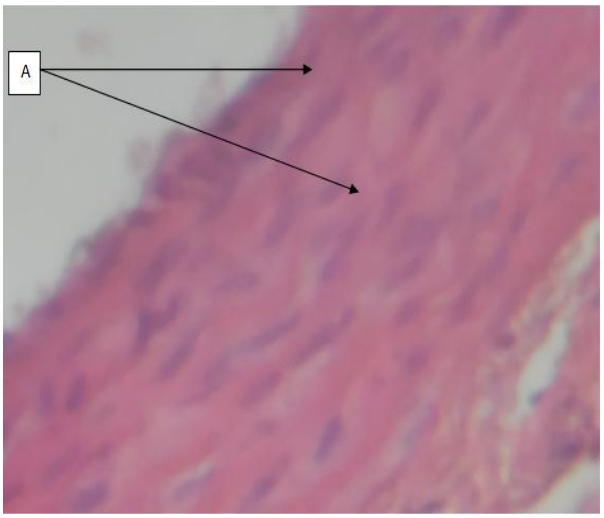

Fig. 20: Rat aorta given 0.5mg/kg mercuric chloride + 200mg/kg extract showing A, normal vascular wall architecture (H&E x 100).

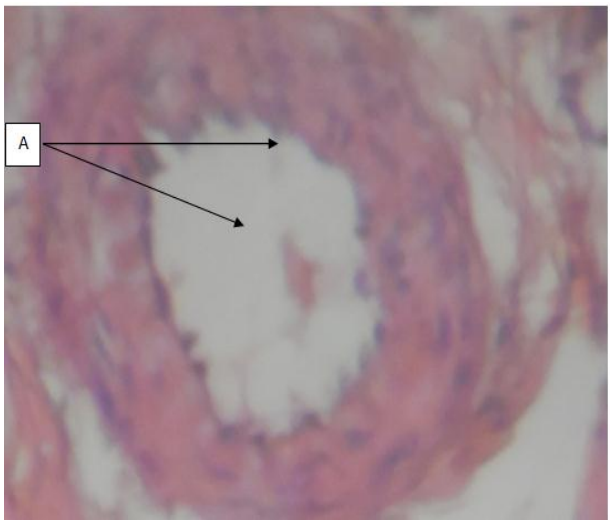

Fig. 21: Same group(artery) showing A, normal vascular wall (H&E x 100).

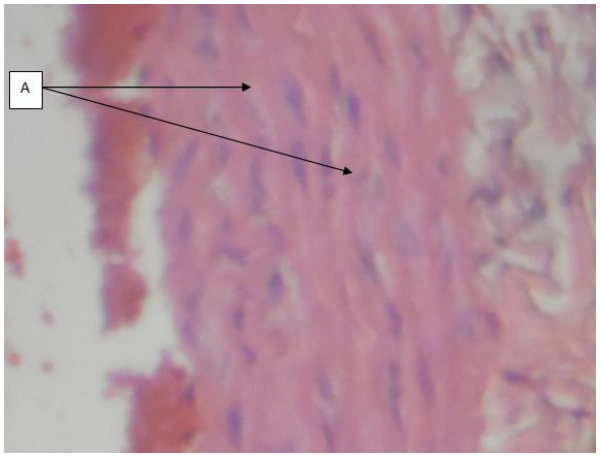

Fig. 22: Rat aorta given 0.5mg/kg mercuric chloride + 300mg/kg extract showing A, normal vascular wall architecture (H&E x 100).

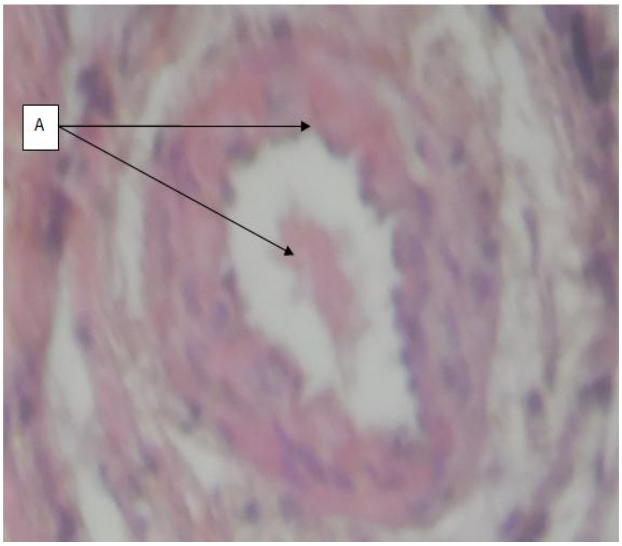

Fig. 23: Same group(artery) showing A,normal vascular wall (H&E x 100).

DISCUSSION

Studies on *Tetracarpidium conophorum* have revealed that it is a rich source of alkaloids, essential vitamins and minerals. Also, the presence of tannins have been reported to be responsible for its anti-inflammatory properties thus buttressing its relevance against asthma, rheumatoid arthritis. The presence of ascorbic acid in high amount also supports its anti-carcinogenic and antioxidant potentials (Ojobor et al., 2015). However, in this present study, rat models of Wistar strain were challenged with high dose mercury chloride (0.5mg/kg), and then treated with crude *Tetracarpidium conophorum* ethanolic extract, i.e., 200mg/kg and 300mg/kg. The ameliorative influence of *Tetracarpidium conophorum* ethanolic extract was assessed by studying the cardiovascular system, hematologic profile and oxidative status of these rats after 28days. The cardiovascular effects of mercury toxicity have not been attentively evaluated until recently. Yoshizawa et al. (2002) reported that mercury exposure was associated with developing cardiovascular disease. Houston (2011) found that mercury toxicity was associated with cardiovascular disorders, including hypertension, coronary heart disease, myocardial infarction, cardiac arrhythmias, reduced heart rate variability and generalized atherosclerosis.

According to table 1, the heart weight of the group C rats given mercury chloride only demonstrated a significant increase (0.77mg). This increase declined with a co-administration of 200mg/kg *Tetracarpidium conophorum* (0.68mg) and further decreased with 300mg/kg administration to 0.62mg. The mean aorta weight of the rats administered mercury chloride only (0.08mg) did not vary significantly with the back controls, i.e., group A (0.09mg) and group B (0.08mg). Strikingly, the co-administration of mercury chloride with 200mg/kg *Tetracarpidium conophorum* resulted in significant increase in aorta weights for the group E (0.13mg) similar with the group administered only 200mg/kg *Tetracarpidium conophorum.* The increased heart weight caused by mercury chloride could be attributed to an increase in heart muscle mass (myocytes) and this was brought to a near control level. Mercury (mercury chloride) has been reported to cause cell membrane damage leading to an imbalance between synthesis and degradation of proteins (Guzzi and La Porta, 2008; Ibrahim, 2015).

Mercury chloride toxicity did not significantly affect the WBC count (table 2; 11.73 $\times 10^{-3}$/µl) compared to the group A (11.75×10^{-3}/µl). However, co-administration of 200mg/kg and 300mg/kg *Tetracarpidium conophorum* and mercury chloride (group E and F, respectively) led to increases in WBC levels, i.e. 12.50×10^{-3}/µl and 12.80×10^{-3}/µl. There was a significant increase in the lymphocyte level of the group C (70.90%) compared to the control group A (49.00%), but on co-administration, groups E and F, the level of lymphocytes was decreased significantly to 65.45% and 48.33% respectively. These observed changes in lymphocytes could be attributed to the immune-challenge of the mercury chloride leading to the activation and mobilization of these immune components, i.e. WBC and lymphocytes towards overcoming the damaging effects of the heavy metal toxicity. These observations are also corroborated by the observations of the monocytes and granulocytes plasma levels. RBC count of the group treated with mercury chloride (7.68 $\times 10^{-3}$/µl) did not vary significantly compared with the group A rats (7.69×10^{-3}/µl), but the group co-administered 200mg/kg and 300mg/kg *Tetracarpidium conophorum* with mercury chloride demonstrated significant increases, 8.02×10^{-3}/µl and 8.36×10^{-3}/µl, respectively, compared to the control groups.

Mercury promotes the generation of ROS such as superoxide and hydrogen peroxides, which induce oxidative stress, resulting in cell injury (Bharathi et al. 2012).

Oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases (Prince et al. 2011).

It has been previously reported that mercuric chloride increased the formation of ROS, which could lead to lipid peroxidation and cause oxidative stress (Durak et al. 2010; Haibo et al. 2011). Lipid peroxidation, a free radical-generating system, has been suggested to be closely related with mercury-induced tissue damage (Tunali-Akbay et al. 2007). MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids (Durak et al. 2010) and increased MDA level is an important indicator of the degree of lipid peroxidation (Tunali-Akbay et al. 2007). Table 4 of this present study corroborates reported observations of mercury toxicity and pro-oxidant properties. The group treated with mercury chloride demonstrated a significant increase in MDA level (7.75 nmole/ml) compared to the control

groups; group A (6.71 nmole/ml) and group B (6.33 nmole/ml). Co-administration of 200mg/kg *Tetracarpidium conophorum* and mercury chloride (group E) decreased (6.77 nmole/ml) while there was a significant increase in plasma MDA level when the concentration of *Tetracarpidium conophorum* ethanolic extract was increased to 300mg/kg.

Mercuric chloride has been reported to significantly inhibit the antioxidant enzyme activities in various rat tissues (Kalender et al. 2013; Aslanturk et al. 2014) which corroborates the observations of this present study. The plasma activities of the antioxidant enzymes significantly decreased for the group administered mercuric chloride indicative of heavy metal toxicity. However, this observed toxicity was ameliorated with the co-administration of ethanolic extract of *Tetracarpidium conophorum.* The enhanced antioxidant capacity and the decreased levels of MDA in antioxidant groups reflected the decreased oxidative damage in the heart tissue. Toxic metals can lead to histopathological changes in the myocardium (Karaboduk et al., 2015). Figures 1 to 23 demonstrate the histological changes of the myocardial tissue, i.e. the heart tissue and aorta, after the various treatments. The group C rats after treatment with 0.5mg/kg mercuric chloride induced severe vascular ulceration, patchy myocardial degeneration, asymmetric vascular hypertrophy and mild vascular infiltrates of inflammatory cells corroborating the hematological observations of the heart tissue. All complications of severe vascular injury and myocardial insult; concomitant administration with mercuric chloride and graded doses of *Tetracarpidium conophorum* ethanolic extract ameliorated the lesions in the coronary arteries and myocardium. For the aorta and its branches, when mercuric chloride was given to the rats, group C, it induced the following lesions: severe vascular ulceration and mural degeneration, as well as, severe vascular stenosis and luminal obstruction.

However, co-administration of mercuric chloride with graded doses of the *Tetracarpidium conophorum* ethanolic extract achieved amelioration of the vascular lesions. Thus, mercuric chloride induced severe vasculitis and myocardial damage in the heart as well as, mural (vascular wall) damage in the aorta and its branches. These lesions were absent when mercuric chloride was co-administered with graded doses of *Tetracarpidium conophorum* ethanolic extract. In addition, the lower dose has a more vaso-dilational properties than the single administration with extract as well as, tween-80 did not cause any injuries to both the heart and the aorta.

CONCLUSION

Mercuric chloride causes sever damaging effects to both the myocardium and the aorta, but *Tetracarpidium conophorum* commonly called African walnut is able to ameliorate these effects.

REFERENCES

1. Aslanturk A, Uzunhisarcikli M, Kalender S, Demir F. Sodium selenite and vitamin E in preventing mercuric chloride induced renal toxicity in rats. Food Chem Toxicol, 2014; 70: 185-190.
2. Bharathi E, Jagadeesan G, Manivasagam T. Influence of s-allyl cysteine against mercuric chloride induced nephrotoxicity in albino rats. J Chem Pharm Res., 2012; 4: 1470-1474.
3. Bharathi E, Jagadeesan G, Manivasagam T. Influence of s-allyl cysteine against mercuric chloride induced nephrotoxicity in albino rats. J Chem Pharm Res., 2012; 4: 1470-1474.
4. Cohen D, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Annals Biochemistry, 1970; 34: 30–38.
5. Deepmala J, Deepak M, Srivastav S, Sangeeta S, Kumar SA, Kumar SS. Protective effect of combined therapy with dithiothreitol, zinc and selenium protects acute mercury induced oxidative injury in rats. J Trace Elem Med Biol., 2013; 27: 249-256.
6. Durak D, Kalender S, Uzun FG, Demir F, Kalender Y. Mercury chloride induced oxidative stres and the protective effect of vitamins C and E in human erythrocytes in vitro. Afr J Biotechnol, 2010; 9: 488-495.
7. Flohé L, Günzler W A. Assays of glutathione peroxidase. Methods Enzymol, 1984; 105: 114-121.
8. Guzzi G, La Porta CA Molecular mechanisms triggered by mercury. Toxicology, 2008; 244: 1-12.
9. Haibo Y, Zhaofa X, Wei L, Yu D, Bin X. The protective role of procyanidins and lycopene against mercuric chloride renal damage in rats. Biomed Environ Sci., 2011; 24: 550-559.
10. Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. J Clin Hypertens, 2011; 13: 621-627.
11. Ibrahim ATA Effects of Mercury Chloride on Oxidative Stress Biomarkers of Some Tissues of the African Catfish Clarias gariepinus (Burchell, 1822). J Veterinar Sci Technol, 2015; 6: 242.
12. Kalender S, Uzun FG, Demir F, Uzunhisarcikli M, Aslanturk A. Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol, 2013; 55: 456-462.
13. Kalender S, Uzun FG, Demir F, Uzunhisarcikli M, Aslanturk A. Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol, 2013; 55: 456-462.
14. Karaboduk, H., Uzunhisarcikli, M. and Kalender, Y. Protective Effects of Sodium Selenite and Vitamin E on Mercuric Chloride-Induced Cardiotoxicity in Male Rats. Brazilian Archives of Biology And Technology, 2015; 58(2): 229-238.
15. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple

assay for superoxide dismutase. J Biol Chem., 1972; 247(10): 3170-3175.
16. Morakinyo AO, Iranloye, BO, Oludare GO, Oyedele OJ, Ayeni OO. Mercury chloride-induced glucose intolerance in rats: Role of oxidative stress. Br J Pharmacol Toxicol, 2012; 3: 7-12.
17. Ojobor Charles Chijioke, Anosike Chioma Assumpta, Ezeanyika Lawrence Uchenna Sunday. Effect of Black Walnut (*Tetracarpidium conophorum*) Leaf Extract on the Reproductive Organ of Male Albino Rats. International Journal of Dual Diagnosis, 2017; 2(3): 22-27.
18. Ojobor, C. C., Anosike, C. A. and Ani, C. C. Studies on the phytochemical and nutritional properties of Tetracarpidium conophorum (Black walnut) seeds. Journal of Global Biosciences, 2015; 14(2): 1366-1372.
19. Oliveira CS, Oliveira VA, Ineu RP, Moraes-Silva L, Pereira ME. Biochemical parameters of pregnant rats and their offspring exposed to different doses of inorganic mercury in drinking water. Food Chem Toxicol, 2012; 50: 2382-2387.
20. Patrick L. Mercury toxicity and antioxidants: Part I: Role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity. Altern Med Rev., 2002; 7: 456-471.
21. Prince PSM, Rajakumar S, Dhanasekar K. Protective effects of vanillic acid on electrocardiogram, lipid peroxidation, antioxidants, proinflammatory markers and histopathology in isoproterenol induced cardiotoxic rats. Eur J Pharmacol, 2011; 668: 233- 240.
22. Sanz N, Diez-Fernandez C, Andres D, Cascales M. Hepatotoxicity and aging: endogenous antioxidant systems in hepatocytes from 2-, 6-, 12-, 18- and 30-month-old rats following a necrogenic dose of thioacetamide. Biochim Biophys Acta., 2002; 1587: 12-20.
23. Souza de Assis GP, Silva CEC, Stefanon I, Vassallo DV. Effects of small concentrations of mercury on the contractile activityof the rat ventricular myocardium. Comp Biochem Physiol., 2003; 134: 375-383.
24. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med., 1995; 18: 321-336.
25. Tsuji N, Hirayanagi N, Okada M, Miyasaka H, Hirata K, Zenk MH, et al. Enhancement of tolerance to heavy metals and oxidative stress in Dunaliella tertiolecta by Zn-induced phytochelatin synthesis. Biochem Bioph Res Co., 2002; 293: 653-659.
26. Tunali-Akbay T, Sener G, Salvarli H, Sehirli O, Yarat A. Protective effects of Ginkgo biloba extract against mercury(II)-induced cardiovascular oxidative damage in rats. Phytother Res., 2007; 21: 26-31.
27. *Varshney* R, *Kale* RK. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol., 1990; 58: 733–743.
28. Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D, et al. Mercury and the risk of coronary heart disease in men. N Engl J Med., 2002; 347: 1755-1760.
29. Zhang J, Lu S, Wang H, Zheng Q. Protective role of Aralia elata polysaccharide on mercury (II)-induced cardiovascular oxidative injury in rats. Int J Biol Macromol, 2013; 59: 301-304.