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High Doses of Anacardium Occidentale Nut Shell Extract Induce Oxidative Damage in Cardiac and Renal Organs of Wistar Rats

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ABSTRACT

Anacardium occidentale (Cashew) *is a* plant reported to show several biological activities. The aim of this study is to examine the effects of methanol extract of Anacardium occidentale nut shell on the antioxidant status and histological features in cardiac and renal tissues of rats. Shells were obtained from the nut, air-dried, pulverized, and subjected to Soxhlet extraction to obtain Anacardium occidentale nut shell extract (AONSE). Forty-five male Wistar rats were divided into nine groups (5 rats each), and given oral gavage of corn oil (Control), and 50, 100, 150, 200, 250, 300, 350 and 400 mg/kg of AONSE, every other day for twenty-eight days. After sacrifice, heart and kidney were removed and divided into two portions each; one portion was homogenized for biochemical assays, while the other was fixed in formalin for histopathology. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST) and Malondialdehyde (MDA) levels in the two organs were spectrophotometrically assayed. Histopathology of the organs was also done. The SOD and catalase were reduced in heart at high doses of AONSE relative to control. The AONSE has no significant effects on GPx, GST and MDA in the two organs, comparable to control. Photomicrographs of heart show, myocardial distortions and fibrosis, while glomerular fluid accumulation and hemorrhagic fibrosis were observed in kidney, at high doses, as against control and low doses of AONSE. This study shows that high doses of *Anacardium occidentale* nut shell extract could induce cytological derangements in heart and kidney of rats, possibly via oxidative mechanism.

KEYWORDS: Anacardium Occidentale Nut Shell

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INTRODUCTION

Cashew (Anacardium occidentale) *is a tree plant* belonging to the order Sapindales, family Anacardiaceae and genus Anacardium. The Anacardiaceae family consists of about 75 genera and 700 species[1]. The plant has been reported to be native to the Central America and North South America, and was introduced to the African Countries, such as Nigeria, Uganda and Gambia, from Brazil by the Cerrados and Restinga[2-3.] Studies by Asogwa et al [4], Orwa et al [5] and Hammed et al[6] have indicated the main producers of Cashew to be Mozambique, Ghana, Cote d'Ivore, Brazil, Vietnam, Guinea Bissau, India, Benin Republic, ,Sri Lanka, Philippine, Nigeria and Tanzania.

Cashew fruit is reported to be rich in water soluble vitamins and minerals (manganese, potassium, zinc, iron, magnesium, selenium, phosphorus and copper) as Documented by the Agricultural Research Service USA[7]. Cashew juice and its pulp have been documented to have a high level of vitamin C[8-9]. However, cashew nut has been reported to contain potential allergens which could not even be decomposed on heating [10]. Other chemical analysis of Cashew fruit have shown the presence of carotenoids, making up to 50% [11] and other compounds including oleic acid, palmitic acid, furfural, lactone, (E) -hexenal, (Z) -hex-3-enol, haxadecanol and 4-hydroxydodecanoic acid [12]. Cashew leaf is rich in essential oils, alkaloids and tannins[13], saponins, cardenolides, flavones, flavonoids, xanthones, catechins, terpenoids, sitosterol, stigmasterol and 3-O-β- D-galactopyranoside [14-15].

Cashew nut shell liquid occurs as a dark brown viscous liquid obtained as a by-product during cashew nut

processing, and it is highly rich in phenolic compounds [16]. It has been reported that Cashew nut shell oil is a mixture of 70% anacardic acid (a Salicylic acid analog, and a strong skin irritant), 18% cardol, and 5% cardanol. The two latter compounds are caustic phenolic substances that readily polymerize, and are useful for making epoxy resins, varnishes, and many high-tech materials that can withstand high temperatures, such as brake linings [5, 17].

A. occidentale plant extracts are reported to have anti-mutagenic and anti-genotoxic [18], antimicrobial, antioxidant, anticancer, and anti-inflammatory[19], and antifungal [20] potentials. Anacardic acid, cardanol and cardol, isolated from cashew nut shell liquid (CNSL) have been reported to have larvicidal and pupicidal effects on *Aedes aegypti* and *Culex quinquefasciatus* at both laboratory and field conditions [21]. Recently, methanol extract of Cashew nut shell was noted to exhibit in-vitro insecticidal effects on the antioxidant and cholinergic enzymes on *Acanthoscelides obstectus* (bean weevils) and *Zonocerus variegatus* (grasshopper) [22]. Cashew nut shell extract was reported to show no significant effects on the body and organ weights of female rats [23]. Furthermore, anacardic acid, a major component of cashew nut shell, has been reported to substantially down-regulate the expression of pro-inflammatory genes responsible for IL-6, COX-2, NF-kB, TNF-α, iNOS, and IL-1β using the RAW 2647 cell line subjected to lipopolysaccharide (LPS) [24]. A study by De Andrade et al [25] against the treatment of Alzheimer's disease, noted that cholinesterase inhibitors derived from cardanol showed both antioxidant and anti-amyloid effects.

The present study investigates *in-vivo* toxicity of the extract of *Anacardium occidentale* nut shell on redox indices and histological features of both cardiac and renal organs of experimental rats.

MATERIALS AND METHODS

Collection and Extraction of *Anacardium occidentale* (Cashew) nut shell

Cashew Nuts were bought from WAZO market, Ogbomosho, Oyo state, Nigeria in May, 2019. The nuts were de-shelled, and the shells were air-dried for three weeks at the room temperature. The shells were then pulverized using electric grinder, and then subjected to Soxhlet extraction (dissolving 25 g of pulverized nut shell in 250ml of methanol). The extract obtained was concentrated using rotary evaporator and then subjected to oven drying at 40^{o}C, to obtain an oily *Anacardium occidentale* nut shell extract (AONSE), which was stored under refrigeration until use.

Experimental Design

Forty-five male Wistar rats of average weight of 149.21g were purchased from LAUTECH animal house, and divided into nine groups with five rats in each group. They were acclimatized for 7 days. Group A (Control) was orally administered with 0.3 ml Corn oil as vehicle, while groups B, C, D, E, F, G, H and I were orally intubated with 0.3 ml of 50, 100, 150, 200, 250, 300, 350 and 400 mg/kg of AONSE, respectively, in corn oil, every other day, and the experiment lasted twenty-eight days.

Sacrifice and Tissue Collection

After twenty-eight days, the experimental rats were fasted overnight and sacrificed by chloroform anesthesia. The heart and kidney were excised and rinsed in 1.15% of KCl solution as washing buffer. Each of heart and kidney was divided into two portions; one portion was fixed using 10% formalin for histology, while the other portion was processed into homogenates for biochemical assays.

Determinations of Total Protein and antioxidant parameters

The total protein levels of the heart and kidney homogenates were determined spectrophotometrically using the Biuret method described by Lowry et al [26]. The superoxide dismutase activities of the organs were estimated according to the method described by Misra and Fridovich [27]. Catalase activities of heart and kidney were estimated according to the method of Aebi [28]. The glutathione peroxidase activities were determined according to the method of Paglia and Valentine [29]. The GST activities of the organs were assayed according to the method of Habig et al [30], while malondialdehyde levels of the organs were determined as described by Ohkawa *et al* [31].

Histopathological examination

Ultra-thin sections of the formalin-fixed heart and kidney were obtained using a microtome knife. The sections were then observed under microscope after staining with hematoxylin and Eosin (H&E) solutions, and the photomicrographs of the organ were taken.

Statistical Analysis

Values were expressed as mean ± SD. Differences in the mean values were estimated statistically by one-way analysis of variance (ANOVA) by using the Statistical Package for Social Sciences (SPSS) software for Windows version 10.0 (USA). Values were considered to be significant at $P < 0.05$.

RESULTS

Table 1 shows the result of the effects of AONSE on the activities of SOD and Catalase in the cardiac and renal tissues of the experimental rats. In the cardiac organ, the activities of both SOD and Catalase were found to be significantly ($p < 0.5$) reduced at high doses (200 – 400 mg/kg) of the extract relative to the control rats. However, in renal tissue, the extract significantly elevated the activities of the two enzymes at certain doses compared with control. In table 2, the activities of both glutathione peroxidase and glutathione-S-transferase were observed to

show no significance ($p > 0.05$) changes in the two organs at all the doses of AONSE applied in the study. Figures 1 and 2 depict the results of AONSE administration on the levels of lipid peroxidation in both heart and kidney of the rats. The AONSE treatment was found to cause no significant ($p > 0.05$) changes in the levels of Malondialdehyde in the two organs.

In figure 3, the photomicrographs of the cardiac organ in groups A1 (Control) and B4 (50 mg/kg of AONSE) showed intact fenestrations or striations in the myocardiocytes. However, the rats in group F2 (250 mg/kg) demonstrated mild distortions in the myocardium. Furthermore, groups F3 (250 mg/kg), I2 (400 mg/kg) and I3 (400 mg/kg), all showed clotted blood, mild fibrosis, with observable large fenestrations in the myocardiocytes. Figure 4 shows the presence of normal glomeruli with normal mesangial cells and capsular spaces, with normal renal tubules and interstitial spaces in groups A (Control) and B4 (50 mg/kg). The rats in group F2 (250 mg/kg) show renal cortex with few sclerotic glomeruli (red arrows) and interstitial spaces with mild vascular congestion and red inflammatory cells. However, groups F3 (250 mg/kg), I2 and I3 (400 mg/kg) show an accumulation of glomeruli with fluid, presence of cast within the tubular lumen (red arrows), congested sclerotic mesangial cells and hemorrhagic fibrosis (red arrows).

Table 1. Effects of *Anacardium occidentale* nut shell extract on superoxide dismutase and catalase activities in heart and kidney of Wistar rats

Treatmnts (mg/kg)	Heart			Kidney
	Superoxide dismutase (U/mg/protein)	Catalase (U/mg/protein)	Superoxide dismutase (U/mg/protein)	Catalase (U/mg/protein)
Control	6.57 ± 1.31	5.43 ± 1.61	2.57 ± 0.12	1.65 ± 0.11
50	5.50 ± 0.99	6.80 ± 1.70	4.22 ± 1.48*	1.97 ± 0.16
100	6.85 ± 0.85	4.01 ± 0.98#	4.01 ± 1.09*	1.82 ± 0.82
150	4.80 ± 0.43#	5.43 ± 1.82	2.80 ± 0.98	2.68 ± 0.12
200	4.17 ± 0.17#	2.46 ± 1.47#	2.00 ±0.36	3.03 ± 0.78*
250	3.33 ± 0.77#	3.59 ± 0.55#	2.35 ± 0.13	5.67 ± 1.52*
300	3.64 ± 0.22#	2.02 ± 1.41#	3.89 ±0.67*	1.44 ± 0.88
350	3.50 ± 0.82#	2.43 ± 1.69#	6.28 ±0.16*	2.34 ± 1.10
400	3.79 ± 0.65#	3.56 ± 1.09#	5.81 ±0.99*	2.19 ± 0.09

Values expressed as mean ± standard deviation
*- significantly higher as compared to control ($P < 0.05$)
- significantly lower compared to control ($P < 0.05$)

Table 2. Effects of *Anacardium occidentale* nut shell extract on glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities in heart and kidney of Wistar rats

Treatments (mg/kg)	Heart			Kidney
	GPx (U/mg/protein)	GST (U/mg/protein)	GPx (U/mg/protein)	GST (U/mg/protein)
Control	242.34 ± 17.60	5.77 ± 1.60	264.50 ± 4.51	6.18 ± 1.87
50	238.65 ± 12.51	6.15 ±1.80	273.02 ± 11.12	6.33 ± 1.54
100	235. 79 ± 22.71	5.69 ± 1.11	256.99 ± 13.04	5.96 ± 2.69
150	240.30 ± 14.13	7.98 ± 0.87*	262.05 ± 19.07	6.30 ± 0.93
200	219.82 ± 12.03	5.82 ± 1.00	267.44 ± 15.68	6.22 ± 1.29
250	187.48 ± 15.59#	6.88 ± 1.90*	240.74 ± 5.69#	5.93 ± 1.21
300	251.65 ± 22.57	5.66 ± 1.16	265.77 ± 11.36	6.36 ± 1.87
350	249.37 ± 9.67	5.85 ± 0.54	265.97 ± 14.59	6.31 ± 1.90
400	242.06 ± 7.36	6.12 ± 1.06	253.40 ± 10.02	6.21 ± 076

Values expressed as mean ± standard deviation
*- significantly higher as compared to control ($P>0.05$)
- significantly lower compared to control ($P<0.05$)

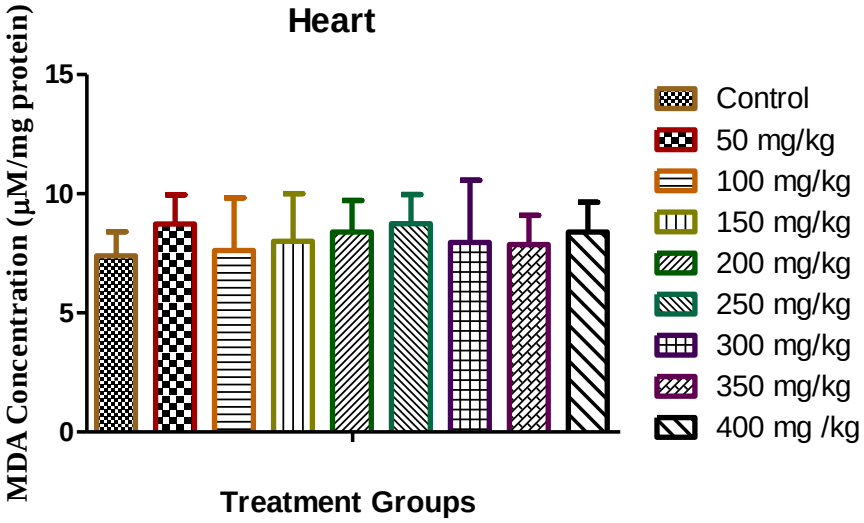

Figure 1. Effects of *Anacardium occidentale* nut shell extract on lipid peroxidation levels in heart of Wistar rats
Values expressed as mean ± standard deviation

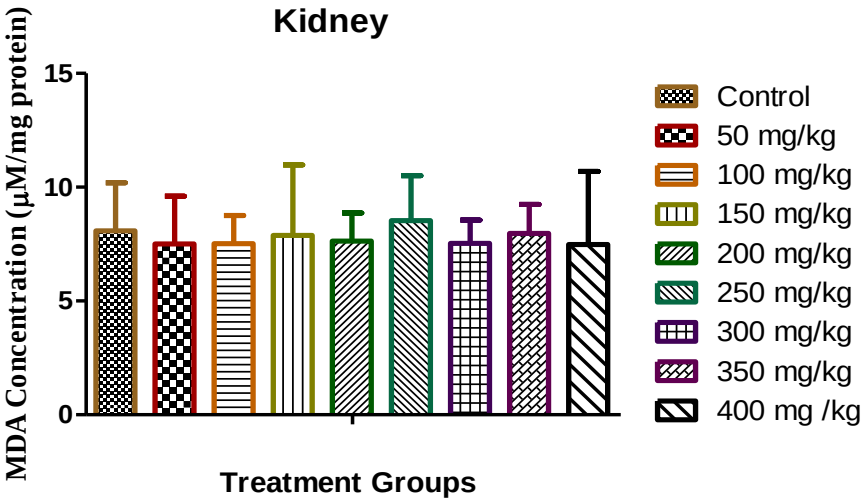

Figure 2. Effects of *Anacardium occidentale* nut shell extract on lipid peroxidation levels in kidney of Wistar rats
Values expressed as mean ± standard deviation

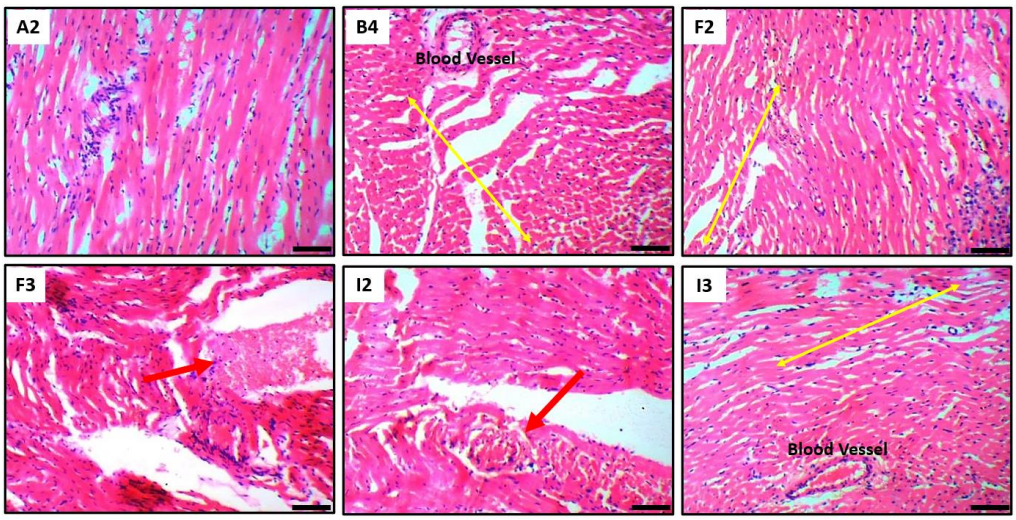

Figure 3. Effects of *Anacardium occidentale* nut shell extract on histology of heart of Wistar rats

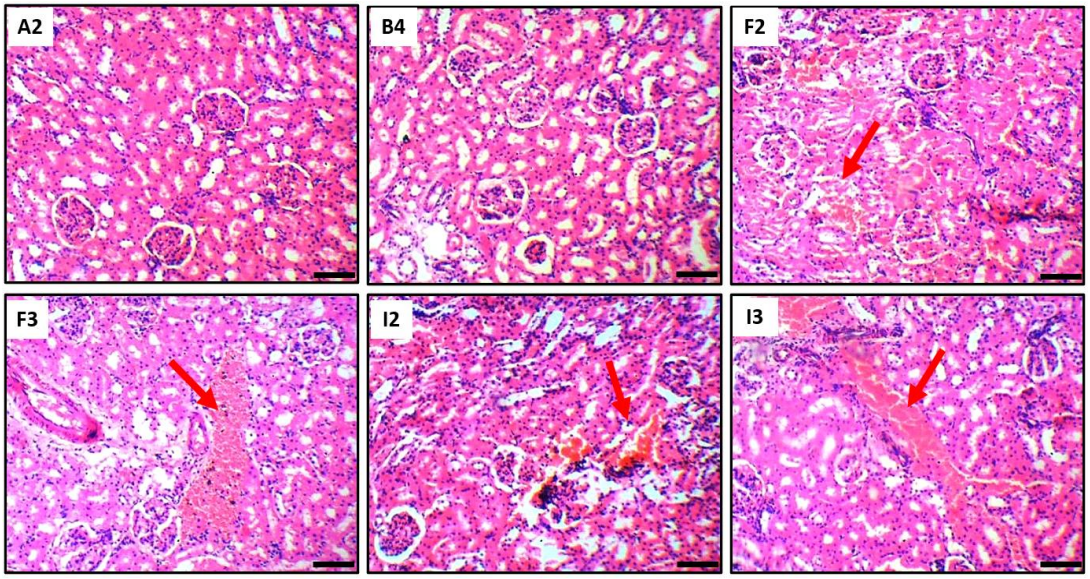

Figure 4. Effects of *Anacardium occidentale* nut shell extract on histology of kidney of Wistar rats

DISCUSSION

Studies have shown that extracts of *A. occidentale* exhibited anti-mutagenic and anti-genotoxic [18], antifungal [20] and antimicrobial, antioxidant, anticancer, and anti-inflammatory [19] potentials. The present study investigated the possible toxic effects of varying doses of *Anacardium occidentale* nut shell extract (AONSE) in cardiac and renal organs of Wistar rats. The study investigated possible induction of oxidative stress and histological derangements in the two organs of the experimental rats.

Oxidative stress occurs when there is an excess level of ROS above the capacity of the antioxidant system, leading to accumulation of reactive oxygen species, oxidation of certain biomolecules and chronic inflammation [32-34]. Oxidative stress also depletes body antioxidant agents and induces ischemic tissue injury [35]. Studies by Oncu et al [36] and Sukprasansap et al [27] revealed that glutathione peroxidase (GPx), glutathione-S-transferase (GST) superoxide dismutase (SOD) and catalase altogether form a protective system against peroxidation of cellular molecules. In the present study, high doses (200-400 mg/kg) of the cashew nut shell extract reduced the SOD and catalase activities in the cardiac tissue, whereas the activities were elevated only at certain doses in the renal tissue. The extract could therefore induce accumulation of superoxide anion and hydrogen peroxide, leading to oxidative stress in the cardiac tissue, whereas such induction may not occur in the kidney. It has been documented that during oxidative stress, signal transduction mechanisms are activated, resulting in cell death and cardiac dysfunction [38-39]. A study by Santos et al [3] revealed that cardiomyocytes have both enzymatic and non-enzymatic defensive pathways against oxidative challenges. These researchers also documented that induction of oxidative stress in heart could be linked to both pathologic and physiologic functions, in regulating the death and survival of cardiomyocytes. Okereke et al [40] reported That cashew nut shell oil caused oxidative stress in kidney and liver of rats due to decrease in the activities of catalase, SOD and GPx, and then concluded that a consumption of the oil could be unsafe in both humans and animals. One of our recent studies noted that high doses of cashew nut shell extract reduced the activity of SOD and catalase in brain and testicular tissues of experimental rats, with substantial derangements in the histological features of the two tissues [41].

Glutathione peroxidase (GPx) catalyzes the detoxification of hydrogen peroxide via reduction of reduced glutathione (GSH), thereby preventing peroxidation in membrane lipids and hemoglobin [36,42]. It was documented by Toussaint[43] that GPx may exhibit greater protection of organs and tissues from oxidative injury than SOD and catalase, since an increased SOD activity causes a high level of hydrogen peroxide. GPx (particularly GPx 1) deficiency induces cardiac hypertrophy and dysfunction [44]. Studies have shown that hydrogen peroxide accumulation could lead to intracellular acidosis, electromechanical dysfunction, with changes in the action potential and contractility of the cardiac organ [45-48]. Decrease in catalase activity causes compromised antioxidant defense, leading to cardiac dysfunction [44, 49]. A reduction in the activity of GPx has been link to generation of ROS and activation of proinflammatory and mitogenic pathways in kidney, culminating in fibrosis and disruption of renal function [50]. However, some recent studies have implicated GPx 2 isozyme in cancer prevention[51-52] and treatment of glioblastomemultiforme [53].

The present study also investigated the effect of the varying doses of AONSE on the activities of glutathione –S-transferase (GST) enzyme in the two organs. The GSTs belong to a family of phase II enzymes in both prokaryotic and eukaryotic organisms. This enzyme family is associated

with detoxification of xenobiotics via conjugation with reduced glutathione [54]. An increased activity of GST has been reported to prevent oxidative stress-induced cardiovascular diseases [55]. We observed that the activities of both GPx and GST were not significantly affected by the AONSE treatment in the two organs. In the same manner, the level of lipid peroxidation (MDA) was not significantly changed in the two organs. Malondialdehyde, a biomarker of lipid peroxidation, interacts with body biomolecules, leading to oxidative stress [56-57], mutagenic development and diseases[58]. A study by Gyurászová [59] showed that a high level of malondialdehyde could be linked to renal dysfunction.

The photomicrographs of cardiac tissue of experimental rats indicate intact fenestrations or striations in the myocardiocytes of control and rats treated with 50 mg/kg of AONSE. The heart of rats treated with 250 mg/kg AONSE exhibited mild myocardiaL distortion, while the rats given 400 mg/kg were observed to show clotted blood, mild fibrosis and large myocardial fenestrations. In a healthy myocardial cell, mitochondrial respiration normally generates reactive oxygen species, which are mopped up via the activity of superoxide dismutase [60]. Studies have demonstrated that antioxidants exert cardiac protection by mediating myocardial ischemia [61-62]. The photomicrographs of the renal tissues show the presence of glomeruli with normal mesangial cells and capsular spaces, with normal renal tubules and interstitial spaces in rats exposed to low doses comparable to controls. However, the rats exposed to high doses of AONSE showed derangements such as few sclerotic glomeruli, mild interstitial vascular congestion, red inflammatory cells, accumulation of glomeruli with fluid, presence of cast within the tubular lumen (red arrows), sclerotic mesangial cells and hemorrhagic fibrosis.

In conclusion, this study has shown that high doses of *Anacardium occidentale* nut shell extract could induce oxidative imbalance in the heart, with cytological derangements in both heart and kidney of experimental rats.

ETHICAL APPROVAL

An approval was obtained from the Ethical Committee of the Ladoke Akintola University of Technology, Ogbomoso, before the conduct of the Study.

REFERENCES

I. Nakasone HY, Paull RE (1998). Tropical Fruits. CAB International, Oxford, UK.

II. Trevisan MTS, B Pfundstein, R Haubner, G Würtele, B Spiegelhalder, H Bartsch, RW Owen (2006). Characterization of alkyl phenols in cashew (Anacardium occidentale) products and assay of their antioxidant capacity.Food and chemical toxicology 44(2), 188-197.

III. Santos G, Silva E, Silva B, Sena K, Lima C. (2011) Influence of gamma radiation on the antimicrobial activity of crude extracts of Anacardium occidentale L., Anacardiaceae, rich in tannins. Rev Bras Farmacogn. 21:444–9.

IV. Asogwa E.U., Anikwe J.C., Ndubuaku T.C.N., Okelana F.A., 2009. Distribution and damage characteristics of an emerging insect pest of cashew, Plocaederusferrugineus L. (Coleoptera: Cerambycidae) in Nigeria: A preliminary report. Afr. J. Biotechnol. 8 (1):053-058.

V. Orwa C., Mutua A., Kindt R., Jamnadass R., Simons A., 2009. Agroforesttree Database: A tree reference and selection guide version 4.0.

VI. Hammed L.A., Lawal B.A., Kolapo K.A., 2011. Growth and nutrient uptake of cashew (Anacardiumoccidentale L.) seedlings as affected by nutsize in the nursery. Afr. J. Agric. Res. 6 (17):3962-

VII. Agricultural Research Service – United States Department of Agriculture. (2015) "Full report (All Nutrients): 12087, Nuts, cashew nuts, database version SR 27"

VIII. Eça K.S., Machado M.T.C., Hubinger MD, Menegalli F.C. (2015). Development of active films from pectin and fruit extracts: light protection, antioxidant capacity, and compounds stability. J Food Sci. 80:C2389-96.

IX. Silva L.M.R., Lima A.C.S., Maia A.G, Sousa P.H.M., Gonzaga M.L.C, Ramos A.M., (2017). Development of mixed nectar of cashew apple, mango and acerola. Int Food Res J. 24: 232-7.

X. McWilliam V., Koplin J., Lodge C., Tang M., Dharmage S., Allen K. (2015). "The prevalence of tree nut allergy: a systemic review". Current Allergy and Asthma Reports. 15 (9): 555.

XI. Abreu VKG, Pereira ALF, Freitas ERD, Trevisan MTS, Costa JMCD (2014). Effect of anacardic acid on oxidative and color stability of spray dried egg yolk. LWT Food Sci. Technol. 55:466-471.

XII. Maia JGS, Andrade EHA, Zoghbi MDGB (2000). Volatile Constituents of the Leaves, Fruits and Flowers of Cashew (Anacardiumoccidentale L.). J. Food Compost. Anal. 13:227-232.

XIII. Ayepola O. and Ishola R (2009). Evaluation of antimicrobial activity of Anacardium occidentale (Linn.). Adv. Med. Dental Sci 3: 1-3.

XIV. Chaves MH, Citó A, Lopes JAD, Costa DAD, Oliveira CAAD, Costa AF, BritoJúnior

FEM (2010). Fenóistotais, atividadeantioxidante e constituintesquímicos de extratos de Anacardiumoccidentale L., Anacardiaceae. Rev. Bras. Farmacogn. 20:106-112.

XV. Doss V.A, Thangavel K.P (2011). Antioxidant and antimicrobial activity using different extracts of Anacardiumoccidentale L. Int. J. Appl. Biol. Pharm. Technol. 2:436-443.

XVI. Garkal D.J. and Bhande R.S. (2021). Review on extraction and isolation of cashew nut shell liquid. International Journal of Innovations in Engineering Research and Technology. 1(1): 1–8.

XVII. Buxton T., Takahashi S., Niwata I., Owusu E.O., Kim C-S., 2017. Isolation and characterization of the insecticidal compounds in Anacardium occidentale (Cashew nut) liquid against the rice weevil, Sitophilusoryzae L. (Coleoptera: Curculionidae). Journal of Entomology and Zoology studies. 5 (2):1241 -1246.

XVIII. Melo-Cavalcante A.A, Dantas S.M, Leite Ade S., Matos L.A, Sousa J.M, Picada J.N, Da Silva J. (2011). In-vivo antigenotoxic and anticlastogenic effects of fresh and processed cashew (Anacardiumoccidentale) apple juices. J. Med. Food 14:792-798.

XIX. Salehi B., Gültekin-Özgüven M.,, Kirkin C.,, Özçelik B, Morais-Braga M.F.B, Carneiro J.N.P., Bezerra C.F., da Silva T.G. et al. (2020). Antioxidant, Antimicrobial, and Anticancer Effects of AnacardiumPlants: An Ethno-pharmacological Perspective. Frontiers in Endocrinology, 11 (295): 1-16.

XX. Jebapritha S.D.S and Karpagam S. (2017). Phytochemical content content and antimicrobial activity of cashew nut shell oil. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 2017; 12 (4): 61-64.

XXI. De Carvalho G.H.F., De Andrade M.A., De Araujo C.N., Santos M.L., De Castro N.A., Chameau S., Monnerat R., Jaime Martins de Santana J.M., Bastos I.M.D. (2019). Larvicidal and pupicidal activities of eco-friendly phenolic lipid products from Anacardium occidentale nutshell against arbovirus vectors. Environmental Science and Pollution Research. 26(6):5514-5523.

XXII. Adeleke G.E., Adedosu1 O.T.., Olayioye A., Olaniyi A.A., Aderoju V.B., Akintaro O.O. (2021). In-vitro Pesticidal effects of Water hyacinth leaf and Cashew nut shell extracts against Acanthoscelides obtectus and Zonocerus variegatus. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 15, (Issue 5 Ser. II): 36-48.

XXIII. Harlita SNHN., Sagi M., Astuti P. 92016). Acute toxicity of cashew nut shell extract (Anacardium occidentale L.) in albino rat (Rattus norvegicus Berkenhout 1769). Pak. J. Biol. Sci. 19(2); 89-94.

XXIV. De Souza, M.Q.; Teotônio, I.M.S.N.; de Almeida, F.C.; Heyn, G.S.; Alves, P.S.; Romeiro, L.A.S.; Pratesi, R.; de Medeiros Nóbrega,Y.K.; Pratesi, C.B. (2018). Molecular evaluation of anti-inflammatory activity of phenolic lipid extracted from cashew nut shell liquid (CNSL). BMC Complement. Altern. Med. 18, 181.

XXV. De Andrade R. G., Souza de Oliveira A., Bartolini M., Naldi M., Liparulo I., Bergamini C., Uliassi E., Wu L., Fraser P.E., Abreu M. et al. (2021). Discovery of sustainable drugs for Alzheimer's disease: Cardanol-derived cholinesterase inhibitors with antioxidant and anti-amyloid properties. RSC Med. Chem. 12:1154–1163.

XXVI. Lowry OH, Rosbrough NJ, Farr AL., et al. (1951). Protein measurement with the Folin- phenol reagent. J. Biol. Chem. 193: 265-275.

XXVII. Misra HP and Fridovch J. (1975). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170-3175.

XXVIII. Aebi H. (1984). Catalase in vitro. In: Packer L. Editor. Methods in Enzymology. Orlando FL: Academic Press. Pp. 121-126.

XXIX. Paglia D.E., Valentine W.N. (1967).Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab Clin. Med. 70: 158-169.

XXX. Habig W, Pabst M, Jakoby W. (1974). Glutathione S-transferase. The first enzymatic step in mercapturic acid formation.J Biol Chem. 249: 7130–7139.

XXXI. Ohkawa H, Ohishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Anal Biochem. 95:351-358.

XXXII. Wadley AJ, Veldhuijzen van Zanten JJ, Aldred S. (2013). The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. Age (Dordr). 35:705–18.

XXXIII. Phaniendra D.B.J., Periyasamy L. (2015). Freeradicals: properties, sources, targets, and their implicationin various diseases. Ind. J. of Clin. Biochem. 30 (1): 11-26.

XXXIV. Bansal A., Simon M.C., 2018. Glutathione metabolism in cancer progression and

treatment resistance. J. Cell Biol. 217:2291- 2298.

XXXV. Mittal M., Siddiqui MR., Tran K., Reddy SP., Malik AB. (2014). Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 20:1126–67.

XXXVI. Oncu M., Gultekin F., Karaoz E., Altuntas T., Delibas N. (2002), Klorprifos Etil tarafindan olusturulan oksidatif hasarin sucan karacigerine etkileri. Turkiye Klinikleri. Journal of Medical Sciences. 22(1): 50-55.

XXXVII. Sukprasansap M., Chanvorachote P., Tencomnao T., 2017. Clestcalyxnervosum var. paniala berry fruit protects neuroxicity against endoplasmic reticulum stress-induced apoptosis. Food Chem. Toxicol. 103: 279-288.

XXXVIII. Fulda S., Gorman A.M., Hori O.. Samali A. (2010). "Cellular stress responses: cell survival and cell death," *International Journal of Cell Biology*, vol. 2010, Article ID 214074, 23 pages, 2010.

XXXIX. Gino A. Kurian,1 Rashmi Rajagopal,1 Srinivasan Vedantham,1 andMohanraj Rajesh (2016). The Role of Oxidative Stress in Myocardial Ischemia and Reperfusion Injury and Remodeling: Revisited**.** Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity. Volume 2016, Article ID 1656450: 1-14

XL. Okereke G., Okezie E., Ude V., Ekweogu CN., Ikpeazu V.O., Ugbogu E.A. (2020). Physicochemical characterristics, acute and sub-acute toxicity of Cashew nut shell oil in Wistar rats. Scientific African. 8: e00391.

XLI. Adeleke G.E., Adedosu O.T., Adeagbo D.P., Oyebamiji A.J., Adegboyega T.E., Babalola K.D., Adegbola P.I., Gbolagade A.M. (2022). Toxicological Profile of *Anacardium occidentale* Nut Shell Extract on Hematologic and Antioxidant Parameters in Brain and Testicular Tissues of Wistar Rats. Int. J. of Sci. and Res. (IJSR). 11 (3): 1533-1540.

XLII. Margis R., Dunand C., Teixeira F.K., Margis-Pinheiro M., 2008. Glutathione peroxidase family - an evolutionary overview. FEBS J. 275: 3959-3970.

XLIII. Toussaint O., Houbion A., Remacle J. (1999). Relationship between the critical level of Oxidative stresses and the glutathione peroxidase activity. Toxicol. 81: 89-101.

XLIV. Arcdenaz N., Yang XP., Cifuentes ME., Haurani MJ., Jackson KW., Liao TD., Carretero OA., Pagano PJ. (2010). Lack of Glutathione peroxidase 1 accelerates cardiac-specific hypertrophy and dysfunction in Angiotensin II hypertension. Hypertension, 55: 116-123.

XLV. Ferdinandy P., Danial H., Ambrus I., Rother RA., Schulz R. (2000). Peroxinitrite is a major contributor to cytokine-induced myocardial contractile failure. Circ. Res. 87: 241-247.

XLVI. Loh SH., Tsai CS., Tsai Y., Chen WH., Hong GJ., Wei j., Cheng TH., Lin CI. (2002). Hydrgen peroxide-induced acidosis and electrochemical inhibition in the disease human ventricular myocardium. Eur. J. Pharmacol. 443: 169-177.

XLVII. Brenner DA., Jain M., Pimentel DR., Wang B., Cnnors L.H., Skinner M., Apstein CS., Liao R. (2004). Human amyloidogenic light chains directly impair cardiomyocytes function through an increase in cellular oxidant stress. Circ. Res. 94: 1008-1010.

XLVIII. Seddon M., Looi Y.H., Shah A.M. (2007). Oxidative stess and redox signaling in cardiac hypertrophy and heart failure. Heart. 93: 903-907.

XLIX. Baumer AT., Flesch M., Wang X., Shen Q., Feuerstein G.Z. Bohm M. (2000). Antioxidative enzymes in human hearts with idiopathic dilated cardiomyopathy. J. Mol. Cell Cardiol. 32: 121-130.

L. Miranda-Diaz A.G., Pazarin-Villasenor L., Yanowsky-Escatell F.G., Andrade-Sierra J. (2016). Oxidative stress in diabetic nephropathy with early chronic kidney disease. Journal Diabetes Research.2016:7047238.

LI. Haung U., Poole E.M., Xiao L., Curtin K., Duggan D., Hsu .L, et al. (2012). Glutathione peroxidase tagSNPs: associations with rectal cancer but not with colon cancer. Genes Chromosomes Cancer. 51:598-605.

LII. Naiki T., Naiki-Ito A., Iida K., EtaniT., Kato H., Suzuki S., et al. (2018). GPX2 promotes development of bladder cancer with squamous cell differentiation through the control of apoptosis. Oncotarget. 9:15847-15859.

LIII. Bangming G., Wenjuan L., Shusheng W. (2021). The clinical significance of GPx 2 in glioblastomamultiforme. Translational Neurosci. 12 (1): 032-039.

LIV. Sheehan D., Meade G., Foley V.M., Dowd C.A., 2001. Review article, Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. 360: 1-16.

LV. Pahwa S., Sharma R., Sigh B. (2018). Role of glutathione-S-transferase on coronary

Artery disease patients with and without type 2 diabetes mellitus. Journal of Clinical and diagnostic Res. 12(8): ZZ01.

LVI. Adeleke G.E., Adaramoye O.A., 2016. Modulatory role of Betulinic acid in *N*-Nitrosodimethylamine- induced hepato-renal toxicity in male rats. Hum and Expert Toxicol. 1-10.

LVII. Ortega A.M.M. and Campos M.R.S., 2019. Bioactive compounds: Health benefits and potential applications. Chapter 5- Medicinal plants and their bioactive metabolites in cancer prevention and treatment. Pp 85-109.

LVIII. Ismail A.F., Moawed F.S., Mohamed M.A., (2015). Protective mechanism of grape seed oil on carbon tetrachloride-induced brain damage in γ -irradiated rats, J. Photochem. Photobiol. B: Biol. 153; 317–332.

LIX. Gyurászová M., Gurecká R., Bábíˇcková J., Tóthová L., (2020). Oxidative stress in the Pathophysiology of kidney disease: Implications for non-invasive monitoring and biomarkers. Oxid. Med. Cell Longev. 2020, 5478708.

LX. Kurian G.A. and Paddikkala J. (2009). "Administration of aqueous extract of Desmodium gangeticum (L) root protects rat heart against ischemic reperfusion injury induced oxidative stress, "Indian Journal of Expt. Biol. 47 (2): 129–135.

LXI. Kurian G.A. and Paddikkala J. (2010). "N-acetylcysteine and magnesium improve biochemical abnormalities associated with myocardial ischaemic reperfusion in South Indian patients undergoing coronary artery bypass grafting: a comparative analysis," Singapore Medical Journal. 51 (5):381–388.

LXII. Giordano F.J. (2005). "Oxygen, oxidative stress, hypoxia, and heart failure." The Journal of Clinical Investigation. 115 (3): 500– 508.