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Toxicity Assessment of Cashew Nut Shell Methanol Extract on Hematology and Redox Status in Lungs and Liver of Wistar Rats

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ABSTRACT

This study examines the effects of Cashew nut shell methanol extract (CNSME) on hematology, antioxidant parameters and histopathology in lungs and liver of rats. Forty-five male Wistar rats were used for the study, being divided into nine groups - A, B, C, D, E, F, G, H and I (five rats per group). The groups were orally intubated with corn oil (Control), 50, 100, 150, 200, 250, 300, 350 and 400 mg/kg of CNSME, respectively, every other day. After twenty-eight days, the rats were sacrificed under chloroform anesthesia. Blood was collected into EDTA bottles for determination of red blood cell (RBC) and mean corpuscular hemoglobin (MCH). Lungs and liver were excised and divided into two portions each. One portion was fixed in 10% formalin for histology, while the other was processed into homogenates for spectrophotometric assays of Superoxide dismutase (SOD) and catalase activities. The CNSME slightly reduced RBC, and increased MCH levels relative to controls. Both SOD and catalase were increased in lungs and liver, and reduced in liver and lungs, respectively by CNSME against controls. Rats given 250 and 400 mg/kg of CNSME showed degenerated pulmonary parenchyma in lungs, whereas liver showed heamoragic congestion in central venules, pyknotic hepatocytes and fibrosis. These lesions were not observed in controls and rats given 50 mg/kg of CNSME. In conclusion, high doses of cashew nut shell methanol extract could induce cytological damage in lungs and liver of rats via redox disruption, without any adversely effect on the red blood cell groups.

KEYWORDS: Cashew nut shell extract, blood indices, redox imbalance, organ degeneration

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INTRODUCTION

Anacardium occidentale (Cashew) is a tropical nut tree plant crop which belongs to the Anacardiaceae family and Anacardium genus[1]. This crop has been a great source of food, income, industrial raw materials and foreign exchange in many countries of Africa, Asia and Latin America [2]. This tree crop has been reported to originate from the Northeastern part of Brazil, and has been spread to many tropical nations including Indonesia, India, Thailand, Vietnam, Tanzania [3]. The emergence of cashew tree in African Countries including the Gambia, Nigeria and Uganda has also been documented [4, 5]. The countries which are main producers of Cashew have well documented by Orwa et al. [6], Asogwa et al. [7] and Hammed et al.[8]

A document by the US Agricultural Research Service [9] showed that the juice of A. occidentale contains many minerals such as manganese, potassium, zinc, iron, magnesium, selenium, phosphorus and copper, while the reports of Eca et al.[10] and Silva et al.[11] revealed the high level of Vitamin C. The presence of carotenoids[12], palmitic acid, oleic acid, lactone, furfural, haxadecanol, 4-hydroxydodecanoic acid (E) -hexenal, (Z) -hex-3-enol [13] in the juice, have also been reported. Recent studies by Adeleke et al.[14] using High-performance Liquid Chromatography (HPLC) revealed the presence of catechin, rutin and quercetin as flavonoids, while Gas Chromatography-Flame ionization detection (GC-FID) quantified the contents of anacardic acid (39%), cardol (18%), lutein (10%), beta-sitosterol (9%) and cardanol (8%) in methanol extract of cashew nut shell.

A. occidentale leaves are reported to possess strong ferric reducing property, as well as the scavenging potential against DPPH radical, Nitric Oxide and free radical [15 – 17]. Due

to the presence of the several chemical compounds, extracts of *A. occidentale* plants have been documented to exhibit activities against genotoxic, mutagenic [18], microbial [3, 19], oxidative, tumor[20], inflammatory [21] and fungal [22] processes. In addition, the ability of anacardic acid (6-nonadecyl salicylic acid), a major constituent of cashew nut shell [14], has been shown to inhibit inflammation by suppressing the genes encoding TNF-α, IL-1β, NF-kB, iNOS and COX-2 [23, 24]. A study carried out by Santos et al. [25] noted the pharmaceutical application of the by-products of cashew nut as anti-inflammatory and anti-arthritis agents.

Toxicological profiling of cashew nut shell extract has been documented by several researchers. However, cashew nut shell extract has been reported to show possible damage in some insect pests via induction of oxidative and cholinergic stresses, and lowering carboxylesterase, an enzyme responsible for resistance to pesticides [26, 27]. Pathological effects of cashew nut shell oil occur at high doses, biochemical markers of liver (aspartate transaminase, alanine transaminase and bilirubin) and kidney (urea and creatinine) functions have been reported to be elevated, as acute responses, in experimental rats [28]. Cashew nut shell extract significantly reduced Total WBCs count, while platelets and granulocytes were increased compared with controls, in a recent study carried out by Adeleke et al [27].

In this study, we investigated the in - vivo effects of methanol extract of Cashew nut shell on the hematology, as well as antioxidant indices and histology of lungs and liver of experimental Wistar 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 cashew nut shell methanol extract (CNSME), which was refrigerate at 4^{0}C prior to use.

Experimental Design

This study used forty-five male Wistar rats (Average weight = 149.21g) bought from Animal house of the Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The rats were divided into nine groups (5 rats per group). They were acclimatized for one week. 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 administered (every other day) with 0.3 ml of 50, 100, 150, 200, 250, 300, 350 and 400 mg/kg of CNSME in corn oil, as vehicle, respectively.

Sacrifice and Tissue Collection

After twenty-eight days, the experimental rats were fasted overnight and sacrificed by chloroform anesthesia. Blood samples were collected into EDTA bottle by cardiac puncture, for hematology. The lungs and liver were excised and rinsed in washing buffer (1.15% of Potassium chloride solution). Each of lung and liver tissues was divided into two portions; one portion was fixed using 10% formalin for histology, while the other portion was processed into homogenates for biochemical indices.

Determination of blood parameters

Red blood cell, hemoglobin, hematocrit, Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) counts of the rats were estimated using Azotta hematological auto-analyzer (China) and mean values calculated according to Ugochukwu et al. [29].

Determinations of Biochemical parameters

The total protein levels of the lungs and liver homogenates were determined spectrophotometrically using the Biuret method described by Lowry et al [30]. The superoxide dismutase activities of the organs were estimated according to the method described by Misra and Fridovich [31]. Catalase activities of the two organs were estimated according to by Aebi [32]. The glutathione peroxidase activities were estimated by the method of Paglia and Valentine [33]. The glutathione-S- transferase activities of the organs were assayed according to the method of Habig et al [34], while malondialdehyde levels of the organs were determined as described by Ohkawa et al [35].

Histopathological examination

Ultra-thin sections of the formalin-fixed lungs and liver were obtained using a microtome knife. The sections were then observed under microscope after staining with hematoxyline and Eosin (H&E) solutions, and the photomicrographs of the organs 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

Hematology

Table 1 depicts that the CNSME caused a slight decrease in the RBC count of the experimental rats across the treatment groups relative to control rats. However, the levels of both MCH and MCHC were significant elevated, while the levels of both HGB and HCT were not significantly affected by the doses of the extract compared with the control rats.

Antioxidant Indices

Table 2 shows that SOD was increased, while catalase was reduced in lungs. However, in Liver, SOD was reduced, while catalase was elevated compared with controls. Table 3 reveals the effects of CNSME on the activities of GST and GPx. The results show that the extract exerted no significant effects on the activities of the two enzymes. In figure 1, the levels of Malondialdehyde (MDA) in both lungs and liver were not significantly affected by the treatment with the extract.

Histopathology of lungs and liver

Figure 2 shows the representative photomicrographs of the lungs of rats administered with CNSME. The Controls (Group A2) and rats treated with 50 mg/kg (Group B4) showed no observable morphological derangements, indicated by well-outlined alveoli and bronchi, with distinct layering of pulmonary cells. However, groups F2 and F3 (250 mg/kg) and I2 and I3 (400 mg/kg of CNSME) showed loss of pulmonary parenchyma, not comparable to controls and rats with lower doses. Figure 3 depicts the photomicrographs of liver of the experimental rats treated with varying doses of CNSME, in which the controls (Group A2) and rats treated with 50 mg/kg (Group B4) showed hepatocytes with normal central nenules (without congestion), sinusoids and no infiltration. The livers of rats in group F2 (250 mg/kg) showed central venules with mild congestion (indicated with red arrows), portal tract with moderate infiltration, dilated portal triad and sinusoids with mild infiltration. However, the livers of rats in groups F3 (250 mg/kg), I2 (400 mg/kg) and I3 (400 mg/kg) showed distorted venules with heamoragic congestion (indicated with red arrows), pyknotic and scanty hepatocytes and infiltrated sinusoids with presence of fibrosis across the plate.

Table 1: Effects of Cashew nut shell methanol extract (CNSME) on some hematological parameters

Treatment Groups (mg/kg)	RBC (L $X10^3$(mm^3)	HGB (g/dL)	HCT (%)	MCH (pg)	MCHC (g/dL)
A (Control)	$7.81x10^3$ ±$1.57x10^3$	15.80 ± 3.20	55.53 ± 9.80	20.61 ± 4.11	31.3 ± 8.11
B (50)	$6.7x10^3$ ±$1.22x10^3$	16.91 ±3.80	55.05 ± 3.55	26.11± 4.72*	35.8 ±1.20*
C (100)	$6.38x10^3$ ± $2.16x10^3$	15.63 ± 2.49	52.83 ± 8.57	25.54 ±5.36*	37.13 ±1.44*
D (150)	$6.6x10^3$ ± $1.18x10^3$	16.48 ±4.61	45.56 ±6.51	26.88 ± 3.40*	36.23 ±5.99*
E (200)	$6.57x10^3$ ± $2.03x10^3$	16.18 ±1.95	45.78 ±2.02	24.94 ± 2.00*	37.04 ±2.65*
F (250)	$6.64x10^3$ ± $1.73x10^3$	15.84 ±1.21	55.25±2.06	22.58 ±1.14	35.83 ±1.69*
G (300)	$6.57x10^3$ ± $2.91x10^3$	15.12 ±2.82	55.02 ±2.74	24.30 ± 0.62*	37.22 ± 0.82*
H (350)	$5.98x10^3$ ±$1.41x10^3$	14.44 ±4.31	54.24±9.41	25.58 ± 2.25*	35.56 ±3.72*
I (400)	$6.25x10^3$ ± $2.7x10^3$	14.76 ±1.50	42.63 ±4.92#	25.79 ± 0.20*	34.96 ± 8.07*

Values expressed as mean± standard deviation (values were taken in duplicates)

*- Significantly high compared with control ($p<0.05$), #- Significantly low compared with control ($p<0.05$)

RBC- Red blood cell, HGB- Hemoglobin, HCT-Hematocrit, MCH- Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration

Table 2. Effects of Cashew nut shell methanol extract (CNSME) on Superoxide dismutase and Catalase activities in Lungs and Liver of rats

Treatment Groups	Superoxide dismutase (U/mg protein)		Catalase (U/mg protein)	
	Lungs	Liver	Lungs	Liver
Control	3.21 ±2.31	7.43±5.97	2.37 ± 0.09	0.83 ± 0.01
A (50 mg/kg)	6.79 ±3.43*	4.37±1.57#	1.33 ± 0.14#	1.98± 0.31*
B (100 mg/kg)	4.96 ±1.66*	4.25±1.84#	1.55 ± 0.45#	1.72 ± 0.00*
C (150 mg/kg)	7.10 ±1.80*	3.71±2.61#	1.05 ± 0.00#	1.59 ± 0.01*
D (200 mg/kg)	5.64 ±1.93*	2.19±0.66#	1.52 ± 0.32#	1.83 ± 0.21*
E (250 mg/kg)	7.14 ±1.31*	4.40±0.49#	1.58 ± 0.11#	1.67 ± 0.04*
F (300 mg/kg)	7.37 ±3.87*	5.24±2.18#	1.49 ± 0.01#	1.36 ± 0.11*
H (350 mg/kg)	5.35 ±2.62*	3.60±1.90#	1.33 ± 0.21#	1.83 ± 0.16*
I (400 mg/kg)	5.36 ±2.11*	5.28±1.82#	1.31 ± 0.02#	1.93 ± 0.05*

*- Significantly high compared with control ($p<0.05$), #- Significantly low compared with control ($p<0.05$)

Table 3. Effects of Cashew nut shell methanol extract (CNSME) on Glutathione-S-transferase and Glutathione peroxidase activities in Lungs and Liver of rats

Treatment Groups	Glutathione-S-transferase (U/mg protein)		Glutathione peroxidase (U/mg protein)	
	Lungs	Liver	Lungs	Liver
Control	6.58 ± 2.09	6.10 ± 1.98	2.53 ± 0.63	2.08 ± 0.00
A (50 mg/kg)	5.80 ± 1.11	5.87 ± 1.88	2.66 ± 1.01	2.04 ± 0.11
B (100 mg/kg)	5.72 ± 2.17	5.77 ± 1.06	2.41 ± 0.42	2.06 ± 1.00
C (150 mg/kg)	6.36 ± 0.99	6.15 ± 2.46	2.46 ± 1.22	2.05 ± 1.10
D (200 mg/kg)	5.61 ± 1.67	5.87 ± 0.32	2.58 ± 0.09	2.15 ± 0.91
E (250 mg/kg)	5.96 ± 1.09	5.86 ± 1.98	2.51 ± 0.91	2.33 ± 0.08
F (300 mg/kg)	5.99 ± 2.50	6.38 ± 1.31	2.71 ± 0.31	2.22 ± 1.01
H (350 mg/kg)	5.86 ± 1.89	6.22 ± 2.00	2.67 ± 0.98	2.28 ± 0.22
I (400 mg/kg)	5.77 ± 1.00	5.92 ± 1.65	2.45 ± 1.00	2.45 ± 0.04

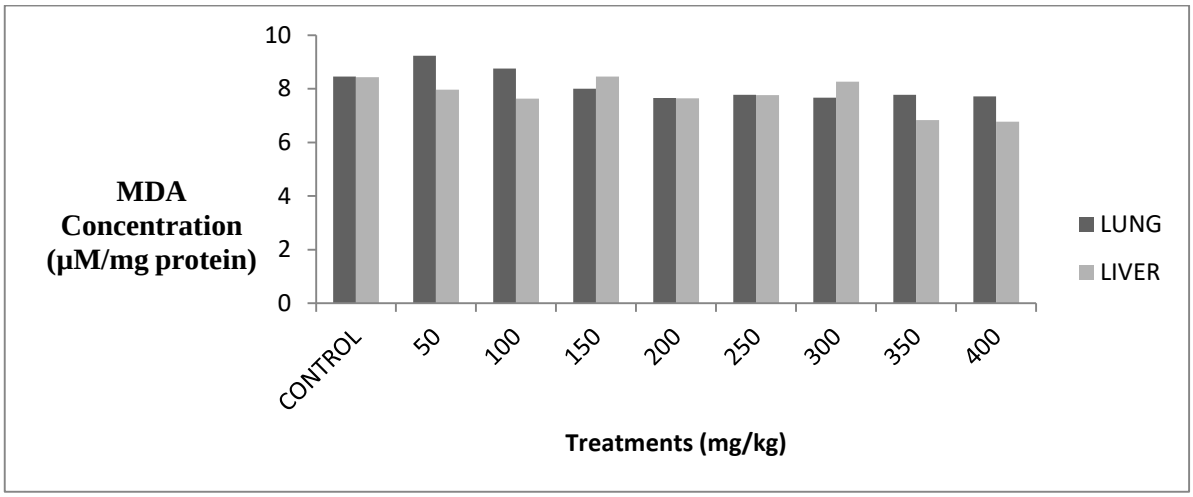

Figure 1. Effects of Cashew nut shell methanol extract (CNSME) on Malondialdehyde concentrations in Lungs and Liver of rats

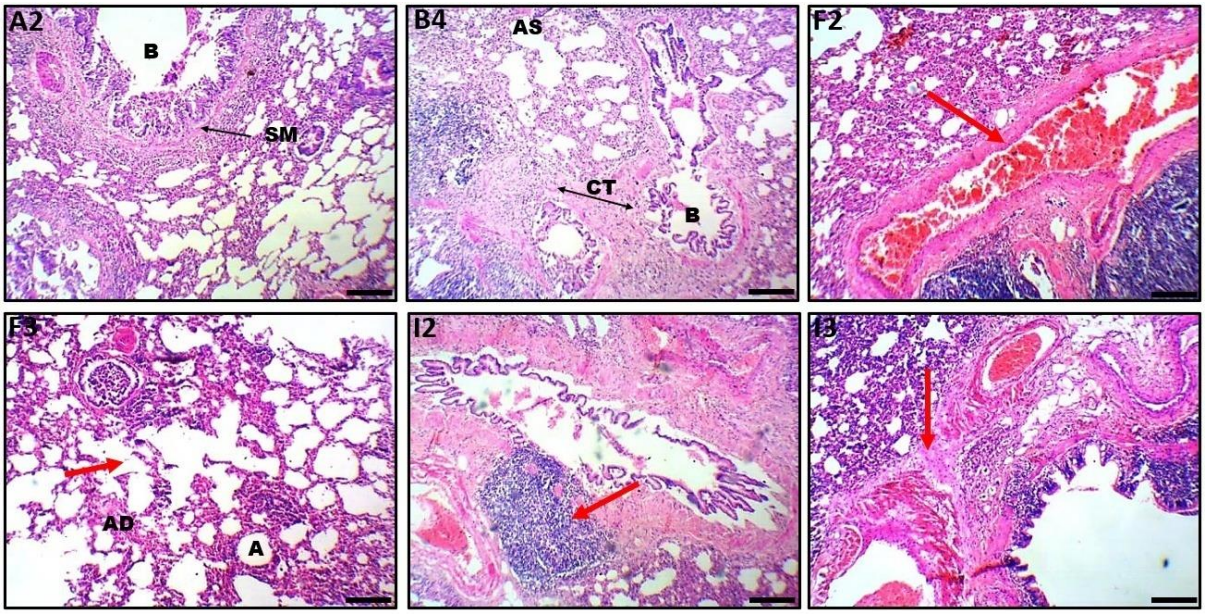

Figure 2. Effects of Cashew nut shell methanol extract (CNSME) on cyto-architecture of Lungs in experimental rats (Scale bar: 25µm). Representative groups: A2 (Control), B4 (50 mg/kg), F2 (250 mg/kg), F3 (250 mg/kg), I2 (400 mg/kg) and I3 (400 mg/kg). Features indicated: Alveoli (A), Alveoli duct (AD), Alveoli sac (AS), Smooth muscle layer (SM), Connective tissue layer (CT). Areas with morphological alteration are indicated with red arrows.

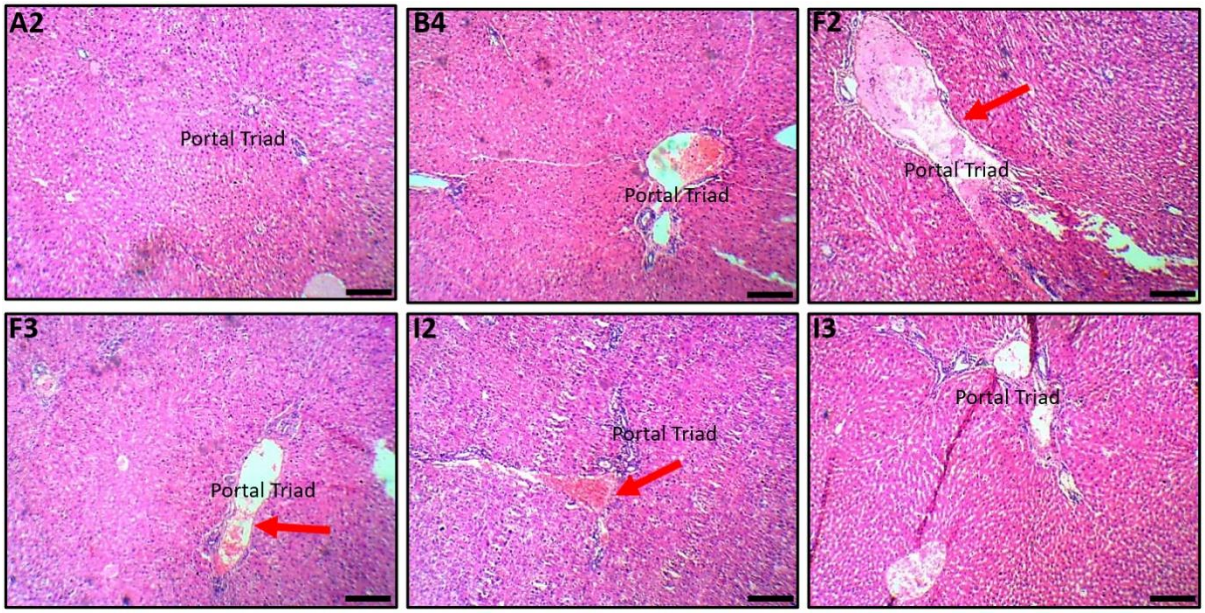

Figure 3. Effects of Cashew nut shell methanol extract (CNSME) on cyto-architecture of Liver of experimental rats (Scale bars: 25µm). Representative groups: A2 (Control), B4 (50 mg/kg), F2 (250 mg/kg), F3 (250 mg/kg), I2 (400 mg/kg) and I3 (400 mg/kg). Areas with morphological alteration are indicated with red arrows.

DISCUSSION

Cashew (*Anacardium occidentale*) is an evergreen tree, widely distributed all over the world and has great medicinal, nutritional and economic values [36]. Studies have shown that cashew nut shell extract is rich in several compounds including flavonoids, anacardic acid, cardol, beta-sitosterol and cardanol [14]. The presence of these and many other compounds in the shell could be responsible for the myriads of biological activities exhibited by the extract [3, 18, 19, 21]. The present study investigated the impacts of oral administration of cashew nut shell methanol extract on the hematological, antioxidant and histopathological indices in experimental rats.

The hematological examination of the rats treated with CNSME indicated that the extract caused a slight decrease in the RBC count among the treatment groups compared with control rats. However, the MCH and MCHC levels were significant increased, whereas the hemoglobin and hematocrit levels were not significantly influenced. [28] reported that a methanol extract of cashew nut shell lowered the RBC, PCV, hemoglobin, and blood platelet counts in experimental animals. In a related study, a reduction was reported in total WBCs count, while platelets and granulocytes counts were elevated [27]. Dysregulation of antioxidants and apoptosis have been linked to the parthenogenesis of hematopoietic disorders [37 - 39].

Under normal physiological conditions, there is a balance between the generation and detoxification of free radicals from the body. However, when the detoxifying capacity of the antioxidant system is overwhelmed by generation of reactive oxygen species, oxidative stress occurs, leading to oxidation of biomolecules [40 - 42]**.** These biomolecules are naturally protected from such oxidation by several mechanisms involving enzymes such as SOD, catalase, GPx and GST [43, 44]. Oxidative changes to biomolecules in living systems contribute to the development of many free radical-mediated diseases of the respiratory, nervous and circulatory systems [45].

The mechanisms involving the activities of superoxide dismutase, catalase and glutathione peroxidase form the first line of defense against antioxidant imbalance. The activity of SOD in lungs was increased, but reduced in the hepatic organ. However, catalase activity was lowered in lungs, but elevated in the hepatic organ of the rats by the doses of CNSME. Catalase is an important enzyme of the peroxisome, responsible for catalyzing detoxification of hydrogen peroxide to water and oxygen [46]. The observed elevation in the activity of SOD coupled with a reduction in that of catalase in the lungs is an indication of possible accumulation of superoxide anion, which could not be properly detoxified by catalase to form water and oxygen. This condition signifies possible toxicity of the extract in the lungs of the rats. Oxidative processes have been implicated in the development of pulmonary problems, such as lung cancer, asthma and chronic obstructive pulmonary disease (COPD) [47]. Interestingly, the lungs and respiratory tracts are naturally endowed with strong intracellular and extracellular antioxidant defense mechanisms against oxidative injury [48]. However, in the hepatic organ, the observed reduced activity of SOD coupled with increased catalase activity could lead to a condition of antioxidant imbalance and oxidative stress in this organ.

Recent *In-vivo* studies have pointed out that similar doses of Cashew nut shell extract could induce oxidative injury in insect and rat models [14, 27]. The extract exerted no significant effects on the levels of GST, GPx and

Malondialdehyde in the two organs. As an alternative pathway to catalase activity, hydrogen peroxide is also reduced by glutathione peroxidase (GPx) in the presence of reduced glutathione (GSH), to form water, thereby preventing lipid peroxidation [43, 49]. GPx has been reported to exert greater anti-oxidative potential than both superoxide dismutase and catalase [50].

The present study has also demonstrated that administration of CNSME in rats induced loss of pulmonary parenchyma in the lungs at doses as high as 250 and 400 mg/kg. Low doses of the extract showed well-outlined alveoli and bronchi, with distinct layering of pulmonary cells, comparable to the control rats. Serra et al [51] reported the potential of cashew nut shell to induce lung injury in mice model. However, an earlier study by Petry et al [52] showed that supplementations with anacardic acid (a major component of cashew nut shell) exhibited protection against inflammatory and oxidative processes in mice lungs. High doses (250 and 400 mg/kg) of the extract were also found to induce heamoragic congestion in the venules, pyknotic hepatocytes, infiltrated sinusoids and fibrosis in the hepatic organ of the rats. However, the low dose (50 mg/kg) of the extract and controls showed hepatocytes with normal central venules and sinusoids without infiltration. The present study has earlier noticed possible accumulation of superoxide anions, due to an increased SOD activity coupled with a decreased catalase activity in the lungs. However, in the liver, reduced SOD activity could cause inefficient dismutation of superoxide radical, resulting in oxidative stress in this organ. The histopathological derangements observed in both lungs and hepatic organs in the present study may be due to oxidative stress, particularly at high doses of the extract. Some of our recent studies have suggested that high doses of cashew nut shell methanol extract could potentially induce pathologic derangements in brain and testicular tissues of experimental rats [27].

In conclusion, the present study has shown that although, the extract of cashew nut shell may not adversely affect the red blood cell groups, the high doses could induce cytological damage in lungs and liver of rats via oxidative imbalance.

ETHICAL APPROVAL

The Ethical Committee of the Ladoke Akintola University of Technology, Ogbomoso, gave an approval to the authors prior to the conduct of this Study.

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