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THE ROLE OF AQUEOUS EXTRACT OF *CITRUS SINENSIS* PEEL IN CADMIUM MEDIATED HEPATOTOXICITY

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ABSTRACT: Predisposing exposures to cadmium have been implicated in hepatotoxicity, hence the search for therapeutic interventions. Thirty Wistar rats were randomly grouped into six (1-6) with 5 rats each. Group 1 received only 5mg/kg body weight (b.wt) of Cadmium Chloride ($CdCl_2$) intra-peritoneally. Group 2 received feed and water *ad libitum*. Groups 3 and 4 received $CdCl_2$ followed by low and high dose (10mg/kg b.wt and 40mg/kg b.wt respectively) of Aqueous Extract of the Zest of *Citrus sinensis* (*AEZCs*). Groups 5 and 6 were pre-treated with low and high doses of the extract before $CdCl_2$. Extract administrations were orally and the experiment lasted for 8weeks. The livers were harvested, grossed and the liver to Bodyweight ratios (LW/BW) was determined shortly before being processed for histology and homogenate for SOD and MDA assay. Blood samples were collected for AST, ALT, ALP and total proteins (TP) analysis. Significant ($p<0.05$) differences in LW/BW, SOD, MDA, ALT, ALP, AST and TP were observed between groups 1 and 2. This was similarly noted between groups pre-treated and post-treated with AEZCs low dose as well as groups pre-treated and post-treated with high dose. Group 1 showed progressive non-alcoholic fatty liver disease. At the high dose, AEZCs restored LW/BW to near normal, moderated liver enzymes activities and increased serum protein. Furthermore, SOD concentrations were boosted, hence the decrease in lipid peroxidation. Histological evidence corroborates these findings, suggesting that AEZCs may detoxify the liver from cadmium toxicity at this dose.

INTRODUCTION: According to the World Health Organization (WHO) comprehensive study on the Environmental Burden of Disease, 2.2 million people die each year in all the 46 African countries, due to environmental risk factors [1]. Cadmium is a classified carcinogen that has been implicated in the development of cancer [2-3].

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Despite efforts by many countries and international agencies to reduce environmental exposures to cadmium through smoking, traffic pollution, and various industrial processes, its toxicities continue to pose a major health concern, especially in emerging industrial nations where environmental controls are still being developed [4-5].

Implicit here is the fact that the Nigerian populace is at constant risk of its adverse health effects [6]. Although, it is a widely distributed toxicant whose pathogenesis occurs primarily in the liver and kidney *via* oxidative stress pathway [7]. There has been considerable interest in utilizing natural plant sources in the development of the pharmaceuticals

as only a small number of reliable drugs from plant sources are available in practice for the treatment and management of liver toxicities [8]. More so, the accessibility and affordability of these drugs are some of the factors that have prompted over 80% of the populace in the developing countries to continually utilize plant products in handling primary medical problems [9]. *Citrus sinensis* is known as 'sweet orange' in English, '*Oroma'* in Igbo tribe in Nigeria belongs to the tree family of *Rutacea* and ranks as one of the most produced fruit crops in the world [10]. The Peels (zests) of *C. sinensis* represent between 50 to 65% of the total weight of the fruits and remain as the primary by-product that if not processed may become a waste, environmental pollutant and harborage for insects [11]. Studies have reported that *C. sinensis* peels possess anti-inflammatory, anti-bacterial and antioxidant properties [12-14]. Within our contemporary society, the health benefits of citrus fruit peels have not been adequately investigated against cadmium-induced liver toxicity even though it is a widely grown tree plant in Nigeria and many other tropical or subtropical regions. Thus, it is against this background, that the present study was designed.

MATERIALS AND METHODS: Fresh fruits of *Citrus sinensis* were collected from cultivated farmland in Abakaliki area of Ebonyi State. The fruits were identified and authenticated at the Department of Plant Science and Biotechnology, Faculty of Science, University of Nigeria, Nsukka, Enugu State, Nigeria. The Specimen identification number was UNN/14/345.

Plant Preparation and Extraction: Five hundred oranges were peeled with a zester or grater. The white portion of the peel under the zest was generally avoided by limiting the peeling depth. The zest was thoroughly rinsed in distilled water, air-dried at room temperature for 2 weeks and then pulverized into a powdered form. This is ideal for efficient extraction to occur since the extraction solvent makes contact with target analytes and particle sizes smaller than 0.5 mm [15]. The extraction was done using the cold maceration method as previously described [16]. The powdered *C. sinensis* zest was weighed and divided into two equal portions; A and B. Portion 'A' weighing 100g was soaked in a 1 Litre of distilled water inside a stoppered conical flask. The mixture was vigorously agitated at intervals of 30 min to enhance the proper dissolution of the *C. sinensis* zest powder. The mixture was then allowed to stand for 48 h at room temperature before filtration using a muslin cloth. The clear solution was then centrifuged at 3000 x/g for 5 min and the supernatant collected. The supernatants obtained were cleaned of traces of the plant particles using a Whatman no. 1 filter paper (24 cm). The filtrate was subsequently concentrated in a vacuum using a Rotary Evaporator to 10% of their original volumes at 40 °C. Further concentrated was done using a water bath at 37 °C into a solid mass (paste). This was stored in a desiccator and was used to prepare different fresh solutions of the extract in normal saline when required for administration. 10.5g of the paste was dissolved in 400ml of normal saline (Stock Solution).

Phytochemical Screening of AEZCs: The qualitative analysis of the phytochemicals in AEZCs was done using portion 'B' of the powdered zest of *C. sinensis*. The phytochemical screening for the presence of Alkaloids, Flavonoids, Saponins, Tannins, and phenols was done at the Project Development Institute, Enugu State, Nigeria according to a descriptive method [17].

Preparation of the Hepatotoxic Chemical: Cadmium chloride ($CdCl_2$) was used as the hepatic toxin for the experiment. It was obtained from the Chemical Laboratory of the Faculty of Science, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria. The chemical product is manufactured by British Drug House Chemicals, Poole, England. 500 mg of $CdCl_2$ was crushed and dissolved in 100 ml of distilled water (*i.e.* 1ml of the solution containing 5 mg of $CdCl_2$). The stock solution was shaken for proper dissolution and allowed to stand for some minutes before use.

Experimental Animals: Thirty (30) Adult Wistar rats (weighing 160-320g) were purchased from the Animal house of the Department of Anatomy, College of Medicine, UNN, Enugu State Campus (UNEC), Nigeria. The animals were housed in well ventilated wired cages and were allowed to acclimatize for one week in the Animal Facility of Department of Anatomy, UNEC. They were maintained under the standard photoperiodic

condition of 12 h light/dark cycle at a temperature of 27 °C - 30 °C and relative humidity of 50 ± 5 °C. The animals were fed with rat pellets (a product of Top Feed Ltd, Nigeria) and were allowed unrestricted access to drinking water.

Induction of Hepatotoxicity: Toxicity was induced by a single dose of 5 mg/kg body weight of cadmium intraperitoneally into the animals following reported toxicity ranges, exposure routes and concentrations [18-20].

Dose of AEZCs and Administration Route: a Selected dose of 10 mg/kg b.wt (low dose) was adopted from a related study [21] and four times of this dose; being 40 mg/kg body weight was given as high dose**.** These doses reflect percentage values below the $LD_{50.}$ Administrations were orally through gastric gavage.

Experimental Design: The rats were randomly divided into six (6) groups of five (5) rats each. They were pre-treated and Post treated with AEZCs following cadmium chloride administration. Group 1 served as the positive control and received 5mg/kg body weight (b.wt) of Cadmium Chloride ($CdCl_2$). Group 2 served as the normal control, received rat chow and Normal saline. Groups 3 and 4 received $CdCl_2$ followed by low and high doses of AEZCs (10 mg/kg b.wt and 40mg/kg b.wt) respectively. Groups 5 and 6 received low and high doses of AEZCs (10 mg/kg b.wt and 40mg/kg b.wt) before treatment with $CdCl_2$. The experiment lasted for 8 weeks.

Acute Toxicity Test: Prior to the commencement of this experiment, eight (8) Adult Wistar rats were used to evaluate the acute toxicity test for AEZCs. This study was conducted using the OECD guidance document for which a limit dose test of the 'up and down' procedure is required [22]. The animals were divided into two (2) groups of 4 rats each and were administered with different doses of the *C. sinensis* extract in two experimental phases for a period of 14 days. They were observed periodically for symptoms of toxicity or mortality, and the LD_{50} of AEZCs was subsequently determined following a descriptive procedure [23].

TABLE 1: ADMINISTRATION PROTOCOL OF AEZCs AND CADMIUM CHLORIDE

Groups	Evaluation	Administration	Treatment
Group 1	Effect of $Cdcl_2$ (Positive Control)	5mg/kg b.wt of $CDcl_2$ as single dose	Induce hepatotoxicity without treatment for 8 weeks
Group 2	Normal (Negative Controls)	2.5ml/kg b.wt of Normal Saline (N.S)	Treated daily for 8 weeks
Group 3	Ameliorative evaluation of the low dose of AEZCs	5mg/kg bwt $Cdcl_2$ as single dose + 10mg/kg bwt of AEZCS orally	After 2 weeks of receiving $CDcl_2$, rats were treated with the extract daily for 6 weeks
Group 4	Ameliorative evaluation of the high dose of AEZCs	5mg/kg bwt $Cdcl_2$ as single dose + 40mg/kg bwt of AEZCs orally	After 2 weeks of hepatotoxic induction with $Cdcl_2$, rats were treated with the extract daily for 6 weeks
Group 5	Protective evaluation of the low dose of AEZCs	10mg/kg bwt of AEZCs orally by gastric gavage + 5mg/kg bwt of $Cdcl_2$ as a single dose	Treat rats with extract daily for 2weeks. 24 h after last AEZCs dose, induce hepatotoxicity for 6 weeks with single doses of $Cdcl_2$ for 6 weeks
Group 6	Protective evaluation of the high dose of AEZCs	40mg/kg bwt of AEZCs orally by gastric gavage + 5mg/kg bwt of $Cdcl_2$ as a single dose	Treat rats with extract daily for 2 weeks. 24hrs after last AEZCs dose, induce hepatotoxicity with single doses of $Cdcl_2$ for 6 weeks

Animal Sacrifice and Sample Collection: At the end of the experimental period, all the animals were fasted overnight, anesthetized with diethyl ether and sacrificed *via* cervical dislocation. Blood was collected *via* cardiac puncture with the aid of a needle mounted on a 5 mL syringe (Hindustan Syringes and Medical Devices Ltd., Faridabad, India) following a midline incision. The samples were collected into tubes containing 2% sodium oxalate, centrifuged at 3000 rpm for 10 min using a tabletop centrifuge (P/C 03) and the serum extracted. The sera were separated and stored in aliquots at -25 °C for biochemical assays of specific liver enzymes. The liver was harvested after the abdominal incision, washed three times in ice-cold saline and blotted on ash-free filter paper for macroscopic inspection. The right lobe of each liver was excised, fixed in a 10% formal saline for

routine histological processing. Ribbon sections of 5µm thickness were obtained with a rotary Microtome (Lieca RM2125 RT V2.3 model) and stained with hematoxylin and eosin (H&E). Photomicrographs were taken after microscopic evaluation of the tissue under the light microscope. The left lobe of the liver tissues was homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer pH 7.4 using an Omni multi-sample automated homogenizer to properly enhance cell fractionation. The tissue homogenate was centrifuged at 10,000 rpm, for 15 min. The aliquots of the supernatant were collected in clean test tubes for the estimation of MDA and SOD activities using commercially available Analyse Gold Kits.

Estimation of Organ/Body Weight Ratio: The weights of the liver of each group and their respective body weights were taken with an electronic weighing balance. The estimation of the liver to body weight ratio was determined according to the method adopted in a descriptive study [24].

Estimation of ALP Activities: This was done by an optimized standard method described in a related study [25]. P-nitrophenyl phosphate is hydrolyzed to phosphate and p-nitrophenol in the presence of ALP.

Estimation of AST, ALT Activities and Total Protein Count: The measurement of AST and ALT activities in the serum was done using endpoint colorimetric diagnostic Kit (Randox; laboratories the UK) based on Reitman and Frankel method [26]. Total serum protein was estimated using the biuret reaction method in colorimetric protein assay techniques [27].

Determination of Malondialdehyde (MDA) Level in Tissues: Malondialdehyde (MDA), an index of lipid peroxidation was determined using a descriptive method [28]. The principle was based on the reaction of MDA with thiobarbituric acid-producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which was measured spectrophotometrically at 532 nm. MDA was calculated using the molar extinction coefficient for MDA TBA-complex of 1.56×10^5 $m^{-1}cm^{-1}$ and its concentration was expressed as micromole of MDA/gm liver tissue.

Determination of Superoxide Dismutase (SOD) Activity: Spectrophotometric estimation of the Superoxide dismutase (SOD) activity was adopted from a descriptive technique [29]. A single unit of SOD was defined as the amount of the enzyme required to inhibit the reduction of nitro blue tetrazolium (NBT) by 50% under specific conditions.

Preliminary Phytochemical Test: The presence of bioactive metabolites; saponins, tannins, terpenes, steroids, alkaloids, flavonoids in the extract was identified using standard phytochemical procedures and tests [30].

Ethical Approval: The experiment was done according to operational guidelines for the Institutional Animal Ethics Committee of the Nigeria National Health Research Ethics Committee on Control and supervision of Experiments on Animals. This was in conformity with recognized International, National and Institutional guideline [31]. IAEC approval no number 501/b/2008/NNHREC was obtained.

Data Analysis: The data generated were analyzed and expressed as mean ± Standard Error of Mean (SEM). Statistical differences in the mean between groups were analyzed using one way ANOVA and compared using paired student's t-test. The statistical significance level was established at a value of $P \leq 0.05$ with the aid of a statistical software package (SPSS) version 20.

RESULTS:

TABLE 2: PHYTOCHEMICAL CONSTITUENTS PRESENT IN AEZCs

Phytochemicals	*Citrus sinensis*
Alkaloids	++
Tannins	+
Phenols	+
Saponins	++
Flavonoids	+++

(+) = present, 50%, (++) = present, > 50% but < 80%, (+++) = present, >80%

As shown in **Table 2**, flavonoids were present in the highest amount while tannins and phenols were present in moderate amounts. The presence of Alkaloids and saponins were slightly above moderate amounts in the extract of the zests of *C. sinensis.*

TABLE 3: TOXICITY TEST OF AEZCs IN WISTAR RATS

Treatment Phases	Doses of AEZCs	Number of mortality
Phase one	10 mg/kg	0/8
	100 mg/kg	0/8
Phase Two	1000 mg/kg	0/4
	1500 mg/kg	0/4
	2000 mg/kg	0/4
	2500 mg/kg	¾

LD_{50} of AEZCs = v· 2000 × 2500 = 2236

Table 3 shows that there was no mortality or signs associated with oral toxicity such as significant loss of fur and skin lesions, diarrhea, salivation, tremors or coma except at 2500 mg/kg b.wt of AEZCs. The LD_{50} of the extract calculated as the square root of the multiplication of the highest dose that did not cause death and the highest dose that caused death was found to be greater than 2000 mg/kg body weight. The animals did not show any signs of aggression or unusual behavior on handling during the observation period.

TABLE 4: ANIMAL BODY WEIGHT, LIVER WEIGHTS AND LIVER/BODY WEIGHT RATIO

Groups	Initial Weight of rats (g)	Final Weight of rats (g)	Liver Weight of rats (g)	Liver/body weight ratio
1	275.57 ± 21.04	201.65 ± 13.25	12.75 ± 0.50	0.06*
2	318.40 ± 16.35	327.80 ± 39.78	8.47 ± 1.22	0.03
3	180.42 ± 18.24	231.50 ± 22.11	10.60 ± 1.57	0.05[a]
4	165.47 ± 10.72	215.10 ± 13.52	8.57 ± 0.38	0.04[b]
5	180.47 ± 12.67	221.10 ± 31.16	8.32 ± 1.87	0.04[b]
6	251.07 ± 45.90	194.40 ± 144.38	6.97 ± 4.69	0.04[b]

*,** represent significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared with the negative control (Group 2). [a, b] represents significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared with the positive control (Group 1). The values are means ± SD. n = 5 in each group

Table 4 shows that there was a significant increase in the liver/body weight (LW/BW) ratio in the positive control group of rats. A similar increase was also noted in the rat groups post-treated with a low dose of AEZCs after cadmium exposure. In contrast, the liver/body weight ratios in the rat group pre-treated with the low dose of AEZCs was significantly lower than that observed in the positive control. Significant decreases in liver /body weight ratios were also observed in the groups of rats that were pre-treated and post-treated with a high dose of AZECs.

TABLE 5: SERUM LIVER ENZYMES AND TOTAL PROTEIN LEVELS

Groups	ALT (u/l)	ALP (u/l)	AST (u/l)	Total protein (g/100ml)
1	83.80 ± 8.06*	537.05 ± 36.26*	66.71 ± 3.39*	6.36 ± 0.62**
2	47.85 ± 3.26	246.69 ± 0.72	33.79 ± 1.81	6.46 ± 0.43
3	72.90 ± 6.02[a]	496.28 ± 9.65[a]	52.43 ± 0.95[a]	7.12 ± 0.01[a]
4	62.63 ± 9.14[b]	373.87 ± 8.92[b]	45.27 ± 2.49[b]	6.95 ± 0.21[a]
5	73.24 ± 2.95[a]	420.31 ± 42.28[a]	61.03 ± 4.27[a]	7.28 ± 0.18[a]
6	60.30 ± 7.11[b]	309.66 ± 31.46[b]	40.53 ± 3.30[b]	6.88 ± 1.00[a]

*,** represent significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared to the negative control (Group 2). [a,b] represent significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared to positive control (Group 1). The values are means ± SD. n = 5 in each group

Table 5 shows that the positive control (Group 1) had significantly higher ALT, ALP, AST, and a low serum protein level than observed in the normal control (Group 2). Although, there was also a significant increase in ALT, ALP, AST in the rat groups post and pre-treated with a low dose of AEZCs (groups 3 and 5 respectively). Their level of serum proteins also increased significantly than that of the positive control. In contrast, the groups of rats post and pre-treated with the high dose of AEZCs (groups 4 and 6 respectively), showed a significant decrease in ALT, ALP, AST with a similar increase in the serum total protein.

TABLE 6: CONCENTRATIONS OF MDA AND SOD

Groups	SOD (U/mg pro)	MDA (nmol/mg pro)
1	30.65 ± 1.33*	4.77 ± 0.08*
2	19.89 ± 0.36	1.81 ± 0.06
3	24.83 ± 0.99[a]	3.77 ± 0.05[a]
4	20.83 ± 1.93[b]	2.51 ± 0.53[b]
5	20.82 ± 0.89[b]	3.76 ± 0.41[a]
6	26.30 ± 2.78[a]	2.48 ± 0.55[b]

*, **represent significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared to negative control (Group 2). [a,b] represent significant increases or decreases at $p < 0.05$ and ($p < 0.01$) respectively when compared to positive control (Group 1). The values are means ± SD. n = 5 in each group.

As shown in **Table 6**, the concentration of Malondialdehyde and superoxide dismutase activity in the positive control (Group 1) was significantly higher than in the negative control. There was also a significant increase in the concentration of malondialdehyde in the groups of rats pre-treated and post-treated with the low dose of AEZCs (5 and 3 respectively). This increase in the concentration of MDA in the groups pre-treated and post-treated with AEZCs was accompanied by an increase and a decrease in the activity of superoxide dismutase respectively. In contrast, there was a significant decrease in the concentration of malondialdehyde in the groups pre-treated and post-treated with a high dose of AEZCs (groups 6 and 4) with a concomitant surge and decrease in the activity of superoxide dismutase respectively. The fluctuations in the activities of superoxide dismutase are dependent on the degree of lipid peroxidation in the tissue.

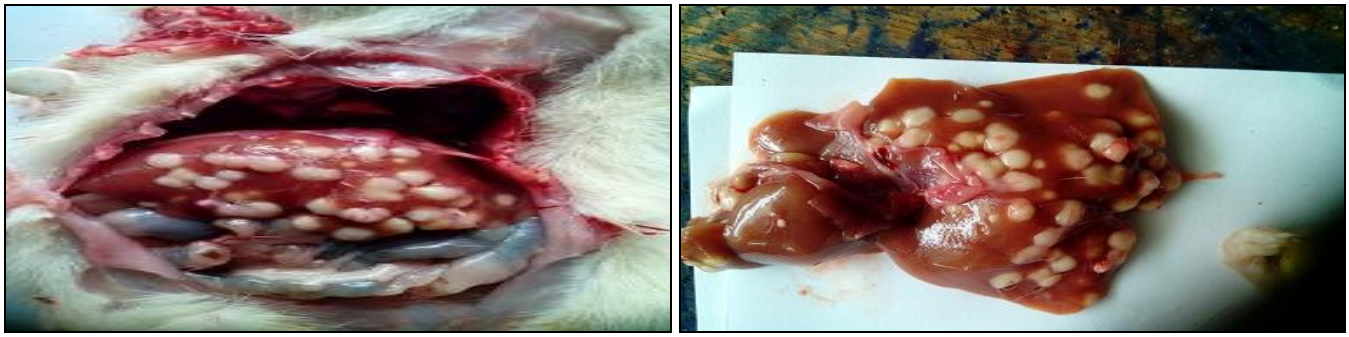
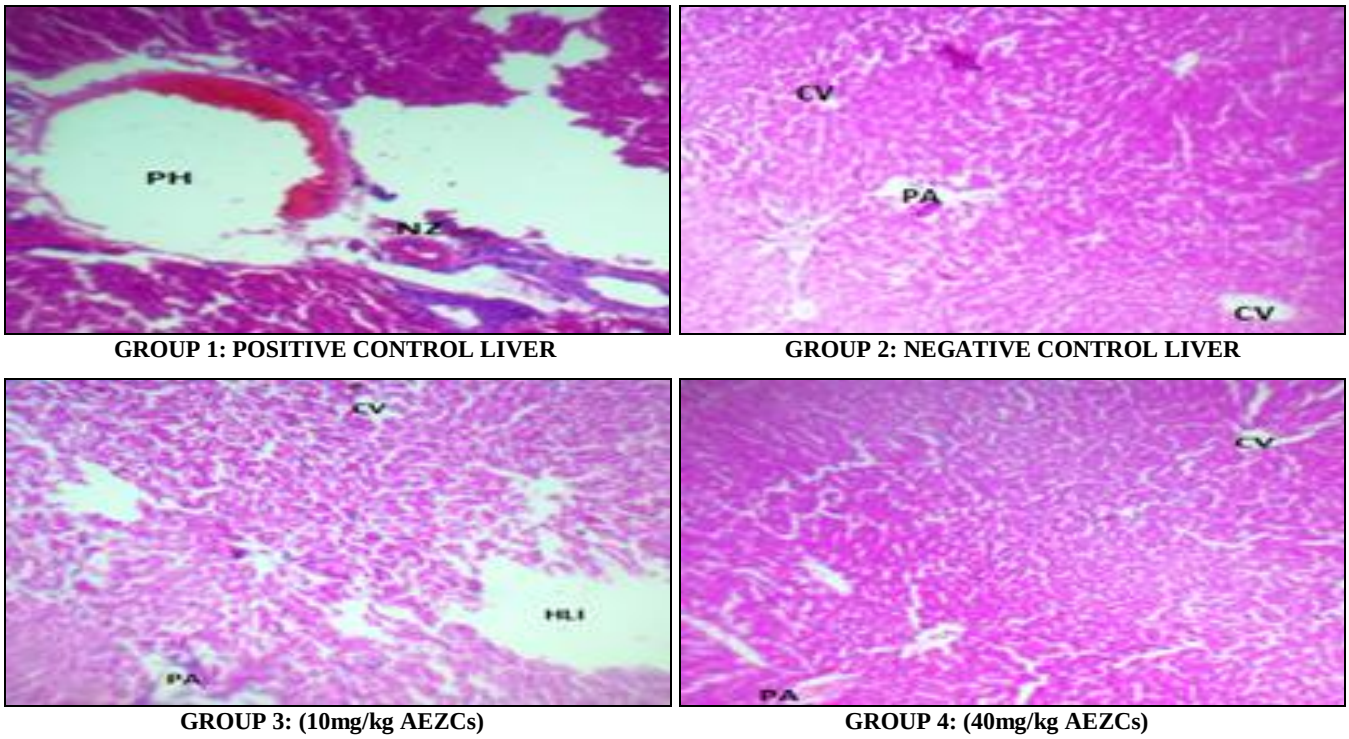


FIG. 1: GROSS APPEARANCE OF THE LIVER IN POSITIVE CONTROL GROUP. Fig. 1 shows a pathologic liver on chronic exposures to Cadmium. Nodular fatty deposits tissues were observed on the liver of the rats in the positive control group of rats.

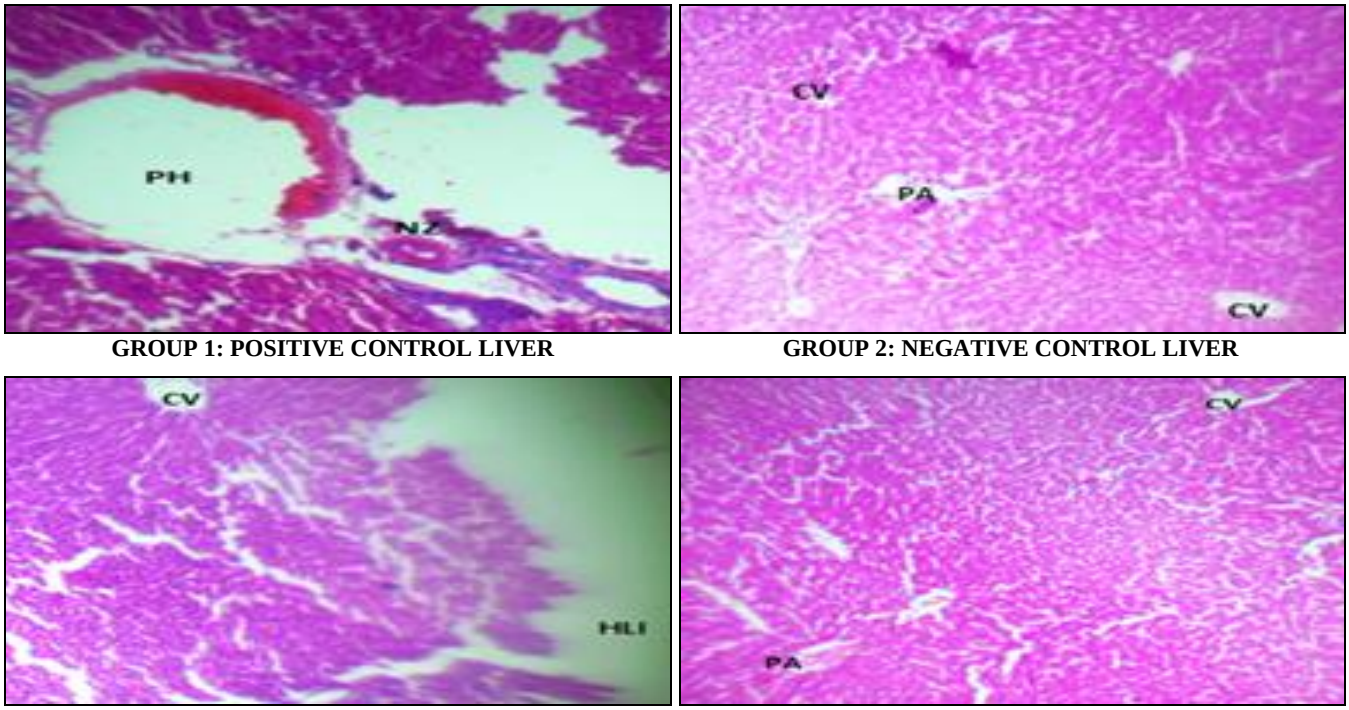
Histology: Photomicrographs of histological sections of the control groups of Wistar rats were compared with those of the treatment groups. Sinusoidal-hepatocytes' arrangement, cytoplasmic vacuolization in both peri-portal and central areas, necrosis and lipid infiltrations were assessed. Histological changes were used to assess the depth of hepatic damage and recovery following treatment with AEZCs.



GROUP 1: POSITIVE CONTROL LIVER **GROUP 2: NEGATIVE CONTROL LIVER**

GROUP 3: (10mg/kg AEZCs) **GROUP 4: (40mg/kg AEZCs)**

PLATE 1: HISTO-ARCHITECTURAL COMPARISON OF THE LIVER BETWEEN POST-TREATMENT GROUPS (LOW AND HIGH DOSE OF AEZCs) WITH POSITIVE CONTROL. CENTRAL VEIN (Cv) WITH RADIATING SINUSOIDS WITHIN THE HEPATIC PLATES; PORTAL AREA (PA), NECROTIC ZONES (NZ) AND PROMINENT HALOS OR EMPTY SPACES (PH) WITH LIPID INFILTRATIONS (HLI). H&E STAIN x400



GROUP 1: POSITIVE CONTROL LIVER

GROUP 2: NEGATIVE CONTROL LIVER

GROUP 5: (10mg/kg AEZCs)

GROUP 6: (40mg/kg AEZCs)

PLATE 2: HISTO-ARCHITECTURAL COMPARISON OF THE LIVER BETWEEN POST-TREATMENT GROUPS (LOW AND HIGH DOSE OF AEZCs) WITH POSITIVE CONTROL. CENTRAL VEIN (Cv) WITH RADIATING SINUSOIDS WITHIN THE HEPATIC PLATES; PORTAL AREA (PA), NECROTIC ZONES (NZ) AND PROMINENT HALOS OR EMPTY SPACES (PH) WITH LIPID INFILTRATIONS (HLI). H&E STAIN x400

DISCUSSION: Flavonoids are potent free radical scavenger and super anti-oxidants that confers anti-peroxidative properties in most plant extracts [32-33]. Interestingly, our study showed that flavonoids were present in the highest percentage proportion in AEZCs. Furthermore, the extract did not produce any mortality or toxicity signs in the animals except for doses beyond 2000 mg/kg body weight. This is similar to a safety report on oral LD_{50} of some extract [34].

In toxicological experiments, comparison of organ/body weights between treated and untreated groups of animals have been conventionally used to evaluate toxicity in target organs as well as an indicator of organ swelling, atrophy or hypertrophy [35-36]. The increase in the liver/body weight (LW/BW) ratios following the administration of cadmium chloride in the positive control group of rats suggest liver hypertrophy which may be attributed to homeostatic imbalances in solute selectivity and rate of tissue fluid uptake into the hepatocyte due to alterations in the cell membrane and protein channels. In contrast, ameliorative and protective benefits were associated with AEZC treatments. Furthermore, the presence of granular whitish deposits on the liver in the positive control group is indicative of the steatogenic potential of Cadmium as a tangible NAFLD risk factor. This corroborates report that prolonged exposure to a critical level of cadmium is associated with Non Alcoholic fatty Liver Disease (NAFLD) [37]. Serum aminotransferase activities (AST and ALT) are increased in hepatic injuries, providing an estimate of the amount of recent damage [38]. The specificity of alkaline phosphatase for liver damage is poor as other conditions may increase its serum level [39]. In this present study, the rise in the level of these enzymes, particularly ALT in the positive control group agrees with an earlier submission that enzymes leakages and increases in the blood are hallmarks of hepatocellular membrane damage [40].

AEZCs administration at both low and high doses protected and maintained liver function by moderating the metabolic activities of the liver enzymes. At the high dose, serum liver enzymes activities that are increasingly altered in oxidative stress conditions were significantly lowered. Thus, the ameliorative potential of AEZCs marked by the decline in liver enzymes activity after exposure to cadmium may indicate recovery as reported in a related study [41]. Though, this may not be a very strong prognostic sign in fulminant liver injury,

where there are major losses of functional hepatocytes. The relatively long elimination half-life (20 days) and ample storage pool of albumin limit the utility of serum protein estimation in the evaluation of chronic liver injuries[42]. However, lower serum proteins especially albumin indicates poor liver function caused by liver cirrhosis with or without accompanying ascites and oxidative stress [43]. In the positive control group, cadmium exposure significantly decreased the level of serum total proteins. A consequence of a decrease in serum albumin being the major plasma protein decreases plasma oncotic pressure, causing intravascular volume depletion and edema formation [44]. In contrast, there was an increment in the total protein in the rats pre-treated and post-treated with the low and high doses of AEZCs, therefore, suggesting its protective and ameliorative potency against Cadmium.

Although the presence of an injury can be clearly established on the basis of biochemical evidence, histologic examination of a liver biopsy specimen remains a definitive means of diagnosing the presence and type of liver injury [45]. In the positive control, there were focal losses of hepatic tissue (optical empty spaces) which suggests extensive destruction of the fenestrations on the luminal surfaces of hepatic endothelial cells lining the sinusoids. The endothelial cells appear to be the first cellular target for Cadmium-induced hepatocellular injury [46]. The normal structure and tortuous paths of hepatic sinusoids through the liver bed which allows efficient exchange of materials between the hepatocytes and blood is consequently disrupted. Specific sinusoidal membrane transporters such as ZIP8 have been reported to participate in cadmium uptake into the hepatocytes [47]. In the hepatocyte, cadmium forms complexes with small peptides and proteins via binding to sulfhydryl groups, in a process described as ionic and molecular mimicry [48-49]. These events may have aided in triggering a cascade of inflammatory mediators that promoted necrosis in the liver. The histopathological changes associated with cadmium exposure agree with previous reports [50-51]. Pre-treatment with doses of 10 mg/kg and 40 mg/kg AEZCs revealed that the liver parenchyma and associated vessels were preserved to near normal states. However, this potency was better defined at the extract high dose.

The lobular structure was generally maintained, and focal areas of necrosis were surrounded by viable hepatocytes that revealed the degree of degenerative changes. Considerable regeneration of the liver tissue evidenced by restorative changes in the peri-portal hepatocytes, sinusoidal arrangement, and portal area followed post-treatment with AEZCs. Again, this ameliorative potential of AEZCs on the liver was more visible at the high dose.

Spontaneous attacks by reactive oxygen species (ROS) like Superoxide on polyunsaturated fatty acids lead to variable degrees of lipid peroxidation, estimated by measuring the amount of malondialdehyde (a product of such polyunsaturated fatty acids peroxidation) [52]. These free radicals combine with nitric oxide to form the peroxynitrite, an oxidant which attacks biological membranes, causing cellular damage [53]. Antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are considered to be the first line of cellular defense against cellular oxidative damages. In the positive control group of rats, there was an excessive lipid peroxidation with an increase in hepatic superoxide dismutase (SOD) activities. This finding is in keeping with the report that excessive lipid peroxidation and an increase in superoxide dismutase (SOD) activities are hallmarks of oxidative stress [54].

From a clinical standpoint, *in-vivo* investigations of biomarkers of oxidative stress are of interest because they aid to evaluate the efficacy of treatment. Pre-treatment with AEZCs revealed a protective effect against cadmium on the liver. This finding is similar to a report on the protective effect of Citrus paradisci seed against cadmium [55]. AEZCs were insufficient to protect the hepatocytes from oxidative damage at the low dose due to persistently increased in MDA level and hepatic SOD activity. Following the post-treatment with AEZCs, biochemical evidence showed a marked change in SOD activity in relation to the MDA concentration. The observed decrease in MDA concentration as a function of the high dose of AEZCs. This supports a similar report on the ameliorative effect of the peel of *Citrus sinensis* against castration induced oxidative stress on the liver [56].

CONCLUSION: Hepatotoxicity from most environmental toxicants has posed a predominant health risk in various populations especially in sub-Saharan Africa. The treatment options are few, usually expensive, less accessible and not entirely devoid of side effects. This study sought to evaluate the effect of AEZCs on liver toxicity with a possible view of developing better alternatives to synthetic treatment products since the extract of the zest of *Citrus sinensis* contains important anti-oxidants that could combat the harmful effect of free radicals of oxidative stress and promote liver health.

From our findings, we submit that the results obtained in this research work, show that the aqueous the aqueous extract of the zests of *C. sinensis* may possess a protective and ameliorative potency against liver toxicity associated with cadmium in our contemporary environment.

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