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The effect of aqueous extract of *zest of citrus sinensis* (AEZCs) on cadmium chloride induced liver toxicity in wistar rats

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The potency of the peels of *Citrus sinensis* against cadmium induced liver damage has not been explored in our environment. 48 wistar rats were used for this study. The animals were randomly divided into eight (8) groups of five (5) rats each. Group A was the positive control and received 5 mg/kg body weight (bw) of cadmium chloride ($CdCl_2$) intraperitoneally as a single dose. Groups B and C received the aqueous extract of zest of citrus sinensis (AEZCS) at a low doses of 10 and 40 mg/kg bw respectively. Groups D and E received cadmium chloride, followed by low and high doses of AEZCS respectively. Groups F and G received low and high doses of AEZCS followed by CdCl2 while group H served as the normal control. Liver enzymes (AST, ALT and ALP) and serum total proteins were analyzed. The results showed significant (P<0.05) differences in the mean values of LV/BW, ALT, AST, total proteins, serum dismutase (SOD) and malondialdehyde (MDA) when the positive control group was compared with the normal control group (P<0.05). Histological sections of the negative control groups were significantly different from the positive control group but not from the groups treated with AEZCS at the high doses. Thus, AEZCS had ameliorative and protective health benefits at the high dose of 40mg/kg body weight.

Key words: *Citrus sinensis*, oxidative stress, cadmium chloride, hepatotoxicity.

INTRODUCTION

Citrus is widely grown in Nigeria and many other tropical and subtropical regions (Piccinelli et al., 2008) In terms of

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ranks, citrus ranks after banana as the world second fruit crop with more than 108 million tons (FAO Statistics, 2007). It originated from Southern China where it has been cultivated for many years but it is today grown commercially in tropical, semi tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Nicolosi et al., 2000). Various existing species of citrus are of useful benefits. They are *Citrus limon* (lemon), *medica* (citron), *aurantium (s*our orange), *C. paradisi* (grapefruit), *C. reticulata* (mandalain ,tangerine), *C. clementina an*d *C.sinensis* (sweet orange). Citrus sinensis is also referred to as sweet orange and belongs to the family *Rutacea* with a life span of over years (Geeta and Kalidhar 2010). *Citrus sinensis* is called sweet orange in English; the Yorubas, Igbos and Hausas call it *Osan, oroma and lemu* respectively (Etebu and Nwauzoma, 2014). The height of orange tree is generally 9-10 m, with large spines on the branches. Anatomically, the fruit consists of two distinct regions; the pericarp also called the peel, skin, zest or rind and the endocarp or the pulp and juice sacs (Geeta and Kalidhar 2010). The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell (Favela–Hernandez et al., 2016). The citrus zest or peels contain important phytochemical and nutritional elements such as calcium, coumarins, peptides, vitamin C (ascorbic acids), phenolic antioxidants: flavonoids, alkaloids, saponins, tannins, hesperidin and naringin, beta– cryptoxanthin (the pigment that gives oranges gloss paint) (Answar, 2014). While the flesh and nectar of these citrus sinensis fruits are usually consumed as food, herbalists have their peels as medicine for numerous maladies throughout history. The citrus peel extract is used for nurturing breast when added to soybean oil, honey, propolis and other substances. There are researches on the effects of citrus sinensis peel against toxicity induced by xenobiotics or mutagenic agents (Marc and George 1997).

Cadmium is a relatively rare element (0.2 mg/kg in the earth crust) that is not found in the pure state in nature. It occurs mainly in association with the sulphides ores of zinc, lead and copper. Cadmium compounds have varying degrees of solubility of the specific cadmium compound as well as its exposure concentration and route. Cadmium is more efficiently absorbed from the lungs than from the gastrointestinal tract (ATSDR, 1989). As a multi– target toxicant, it is transplanted in the blood and widely distributed in the body then accumulates primarily in the liver and kidneys after absorption. Its burden tends to increase in a linear fashion up to 50-60 years of age after which the burden remains somewhat constant (Goyer, 1991). Cadmium is a xenobiotics toxicant of environmental and occupational concern and it has been classified as ahuman carcinogen; inhalation of cadmium has been implicated in the development of emphysema, pulmonary fibrosis but the detailed mechanism by which cadmium induced adverse biological effect might be its central role in xenobiotics metabolism (Shin et al., 2003). Cadmium and many xenobiotics (drugs and environmental chemicals) are capable of causing some degree of liver toxicity. In US, xenobiotics–induced liver toxicity is implicated in 2-5% of the hospitalizations for jaundice; an estimated 15 -30% of the fulminant liver failure, and 40% of the acute cases in individuals older than 50 (Marc and George 1997). Fortunately, most drug-induced liver injuries resolve once the offending agent is withdrawn but mortality may be severe and prolonged recovery ensues. The overall mortality rate for drug_ induced liver injury is approximately 5% (Marc and George 1997). Therefore this research aimed at investigating possible ameliorative effect of aqueous extract of *zest of Citrus sinensis* (AEZCs) on cadmium chloride induced liver toxicity in wistar rats

MATERIALS AND METHODS

Plant collection and identification

The fresh fruits of *C. sinensis* were collected from a cultivated farm at Agbani, Enugu State, Nigeria. The zests of these fruits were obtained using a zester. They were identified and authenticated at the hebarium Unit of the Department of Crop Science University of Nigeria, Nsukka.

Chemicals, reagents and equipment

Chemicals/ reagents such as sodium hydroxide, potassium dihydrogen phosphate, hydrogen peroxide, trichloroacetic acid, hematoxylin, Eosin and sodium nitrate, hydrochloric acid etc. produced by Shijiazhuang Xinlongwei Chemicals .Ltd, Hebel China were purchased from Ogbete Main Market, Enugu, Nigeria They were of analytical grades of the highest purity. Standard laboratory equipment was used for this study. The sample bottles and kits for biochemical assay were purchased from Sigma Aldrich USA.

Preparation of the powdered zest of citrus sinensis

A total of 1000 oranges were peeled with a zester or grater while the white portion of the peel under the mesocarp was carefully avoided by limiting the peeling depth (Akunna et al., 2018). The zest was thoroughly rinsed with distilled water, dried at room temperature (27± 2°C) for 4 weeks and then reduced to powdered form by blending with a mechanical blender (Binatone Nigeria Ltd)

Preparation of the aqueous zest extract of citrus sinensis

400 g of the powdered sample of zest of *C. sinensis* was weighed and macerated in 1000 ml of distilled water and the mixture was thoroughly stirred after 8 h using a sterile glass rod. They were allowed to stand for 30 min before filtration using a muslin cloth. The filtrate was centrifuged at 3000 rpm for 10 min and the supernatant collected. The supernatant obtained was further cleaned off particles by suction using Whatman No 1 filter and cellulose paper. The extract was subsequently concentrated to dryness in vacuum at 40°C using a rotary evaporator (LE -10105)

Table 1. Phytochemical analysis of AEZCS.

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

(+)= Mildly present, (++)= Moderately present, (+++)=Highly present.

and stored in desiccators. Different fresh solutions of the extract were prepared in normal saline as vehicle.

Phytochemical analysis

The phytochemical analysis of the constituents present in the AEZCS was done according to the method of Rizk 1982 using Wagner's reagents.

Alkaloids

The aqueous extract of the peel was separately evaporated to dryness and the residue heated on a boiling water bath (Marshal Scientific isotherm 220) with 2N HCl (5 ml). After cooling, the mixture was filtered and the filtrate divided into two equal portions. One portion of each mixture was treated with a few drops of Mayer's reagent and the other with equal amounts of Wagner's reagent (Rizk, 1982). The samples were observed for the presence of turbidity or precipitation. A letter sign probably (S+) score was used to record if the reagent produced only a slight opaqueness; (E+) score, if the definite turbidity, but no flocculation was observed and (E+++) score, if a definite heavy precipitate or flocculation was produced (Surmaghi et al., 1992).

Flavonoids

According to the method described by Somolenski et al. (1972), the presence of flavonoid was confirmed when pink or magenta–red color developed within 3 min of treatment of 5 ml each of the extracts with a few drops of concentrated HCl and magnesium turnings.

Saponins

2.5 g each of the plant extract was further extracted with boiling water. After cooling, the extract was shaken thoroughly to froth and then allowed to stand for about 15-20 min. The saponin content classification using the method described by Kapoor et al., (1969) was followed:

No froth=negative
Froth less than 1 cm =weakly positive
Froth 1:2 cm high =positive
Froth greater than 2 cm high= strongly positive

Tannins

Each of the extract was further extracted by 10 ml of 0.9% NaCl solution; it was filtered and divided into 3 equal portions. Sodium chloride solution was then added to one portion of each of the extracts, 1% gelatin solution to a second portion and the gelatin salt reagent to a third portion. Precipitation with a latter reagent or with both the second and third reagent was used in indication of the presence of tannins. Positive test was confirmed by the addition of FeCl3 solution to the extract which gave a characteristic blue, blue–black, green or blue green color and precipitate (Segelman and Farnsworth, 1969).

Method of acute toxicity test (LD_{50})

The acute toxicity test (LD_{50}) was determined according to the method of Enegide et al. (2013). In this method, wistar rats were procured, acclimatized and were administered different doses of the AEZCS. The administration was done in phases ranging from phase 1 to a maximum phase 4. Mortality or morbidity was monitored every 2 h for 10 min and monitoring in each phase lasted for a maximum of 24 h. When there was no mortality, the experiment proceeded to the next phase as explained in Table 1. Later, the maximal dosage where no mortality occurred and the minimal dosage that caused mortality were summed up and divided by 2 and their square root determined in accordance with Enegide et al. (2013).

Experimental animals

40 adult wistar rats purchased from the breeding stock from Animal House Unit of the College of Medicine, Enugu State University of Science and Technology, Parklane Enugu, were used for this research work. The animals were housed in standard rat's cages with proper ventilation at 12 h light/dark cycle. They were allowed to acclimatize for 14 days under standard natural photoperiodic condition with access to food and water *ad libitium.* All experimental procedure involving the animal care were conducted in conformity with International, National and Institutional guidelines for the care and use of Laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (CCAC, 1985) and the guideline principles for research in Helsinki Declaration of 1979 was adhered to.

Experimental design

A total of 40 adult wistar rats were randomly divided into eight groups of five rats each as stated below.
Group A: received 5 mg/kg of $CdCl_2$ without treatment
Group B: received 10 mg/kg AEZCs orally for 8 weeks
Group C: received 40 mg/kg AEZCs orally by gastric lavage
Group D: received 5mg/kg $CdCl_2$ as single dose for 2 weeks+ 10 mg/kg AEZCS daily for 6 weeks
Group E: received 5mg/kg $CdCl_2$ as single dose daily for 2 weeks + 40 mg/kg AEZCS for 6 weeks
Group F: received 10 mg/kg AEZCS daily for 2 weeks + 5 mg/kg

$CdCl_2$ as single dose, 24 h after treatment with AEZCS
Group G: received 40 mg/kg AEZCS daily for 2 weeks + 5 mg/kg Cadmium chloride as single dose 24 h after treatment with AEZCS
Group H: received 10ml/kg normal saline for 8 weeks

Animal sacrifice and sample collection for analysis

The animals were observed daily for signs of toxicity during the experimental period. At the end of the treatment period, all animals were fasted for 12 h before sacrifice and organ harvest. The abdominal cavity was opened up following a midline abdominal incision made to expose the organ of study for harvest. Following its harvest, it was then washed thrice in ice cold saline and blotted on ash free paper for macroscopic inspection before being weighed with an electronic analytical and precision balance (Metler Nigeria ltd). The weight of the liver of each animal was taken. The estimation of the liver to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat as described by Ashafa et al. (2011)

Collection of blood sample

Blood samples were collected by cardiac puncture method under 25% Urethane anesthesia with the aid of a 5 ml hypodermic syringe (Hindustan syringes and Medical Devices Ltd, Faridabad, India). The blood samples were collected into tubes containing 2% sodium oxalate and centrifuged at 3000 rpm for 10 min using a table top centrifuge (P/C 03) (Model No. HR20, Zhengou, Henan China) and serum extracted. Sera were separated using cooling centrifugation and stored in aliquots at –25°C for biochemical assays of specific liver enzymes.

Method of determination of alkaline phosphatase activity

This was done by optimized standard method recommended by the Geseiischage fur Klinishche Chemic GSCC (1972).P –Nitrophenyl phosphate is hydrolyzed to phosphate and p– nitrophenol in the presence of ALP. A calculated amount of sample 0.01 ml in a test tube was mixed with the reagent (0. 5 ml) containing the substrate p nitrophenyl phosphate and brought to room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was then allowed to stand for 3 min and the absorbance read against 405 nm. The enzyme activity was calculated thus:

U/L= 3300 x Absorbance of the test sample at 405 nm/min MACRO

Measurement of alanine and aspartate aminotransferases (ALT and ALP)

The measurement of AST and ALT activities in the serum was done using end point colorimetric diagnostic kits (Randox; laboratories UK) based on the method of Ofem et al. (2014). The pyruvate produced by transmission reaction between L–alanine and ketoglutarate reacts with 2,4- dinitrophenyl hydrazine to give a colored hydrazone which represents alanine aminotransferease activity. The oxaloacetate hydrazine formed with 2, 4–dinitrophenyl hydrazine is used to measure aspartate aminotransferease (AST). Both ALT and AST were read at 540 nm wavelength. The enzyme activity was obtained from the table after plotting a graph of absorbance against enzyme activity as provided in the leaflet/ manual.

Measurement of total serum protein

A widely used method of measuring serum total protein is the biuret reaction. The principle of this reaction is that serum protein reacts with copper sulphate in sodium hydroxide to form violet “biuret complex”. The intensity of the violet color is proportional to the concentration of the proteins

Determination of the biochemical parameters of the oxidative stress

Preparation of the tissue homogenates for the biochemical assays

The liver specimen were weighed and homogenized separately with potter-Elvenhjem homogenizer. The liver tissue was homogenized in potassium phosphate buffer 10 Nm pH (7.2) for estimation of MDA level and SOD activity. The crude tissue homogenate was centrifuged at 10,000 rpm for 15 min in a centrifuge and the resultant supernatant was used for the different estimation using the Analyzer Gold Kits.

Determination of Malondialdehyde (MDA) level in tissues

The level of the tissue MDA was determined using the method of Ohkawa et al. (1979). Thus, 0.2 ml of the supernatant homogenate was pipetted out followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3. 5) and 1.5 ml of 30% thiobabituric acid. The volume was made up to 4 ml with distilled water. The test tube was incubated for one hour at 95^{o}C and then cooled. 1ml of distilled water was added followed by the addition of 5 ml of n– butanol pyridine mixture (15: 1 w/v). The tubes were centrifuged at 1000 rev per minute for 10 min. The absorbance of the developed pink color was measured spectrophotomically (DU 640B spectrophotometer) Beckman Coulter, Inc CA USA at 532 nm.

Determination of superoxide dismutase level in tissues

Tissue SOD enzymes activity was assayed utilizing the technique of Ekaterina and Bernard (2004). The total superoxide activity was determined at 500 nm by measuring the inhibition of xantine-xanthanic oxidase mediated reduction of 2-3-bis(2-methoxy-4-nitro-5-sulfophenyl-2H-tetrazolidium -5-carboxamide (XTT); 0.5Mm xanthine oxidase sufficient to produce a slope of 0.25,50µM XTT, 25°C. A single unit of the enzyme was expressed as 50% inhibition of nitrobluetetrazolium (NBT) reduction/min/protein.

Tissue processing

The right lobe of the liver was excised and fixed in 10% formaldehyde solution. Subsequently, it was dehydrated in increasing concentrations of alcohol (80-100% v/v). The tissue was then cleared in xylene, embedded in paraffin blocks and sectioned at 5 µm thickness on a Leica Rotary Microtome. The tissues were stained with hematoxylin/eosin (H & E). Photomicrographs of the liver tissue sections were then taken after evaluation of the tissue of the tissue histology under light microscope. The tissue architecture were assessed for nuclear variations, loss of normal architecture of the parenchymatous tissue, cytoplasmic vascularization in both peripartal and central areas, cellular degeneration and necrosis, fat globules formation and lipid infiltrations.

Table 2. Acute toxicity of AZECS in wistar rats.

Phases	Treatments (mg/kg)	Number of deaths
ONE	10	0/8
	100	0/8
TWO	1000	0/4
	1500	0/4
THREE	3000	0/4
	5000	4/4

LD_{50} OF AEZCS = $\sqrt{3000 x 5000}$ =3873 mg/kg.

Table 3. Animal body weights, liver weights and liver/body weight ratio (g).

Groups	Initial body wt	Final body wt	Liver weight (g)	Liver/body wt ratio
A	318.40±16.35	327.80±39.78	8.47±1.22	0.03
B	297.05±33.98	307.77±27.24	8.02±0.27	0.03
C	257.97±28.1	186. 60±14.77	6.25±4.25	0.03
D	180.42±18.24	231.50±22.11	10.65±1.57	0.05[a]
E	165.47±10.72	215.10±13.52	8.57±0.38	0.04[b]
F	180.47±12.67	221.10±31.16	8.32±1.87	0.04[b]
G	251.07±45.90	194.40±14.38	6.97±4.69	0.04[b]
H	275.57±21.04	201. 65±13.25	12.75±0.50	0.06*

*Represents significant difference at P<0.05 when compared with the negative control (Group H); [ab] represents significant difference at P<0.05 when compared with the positive control. Those without superscript show that there was no significant difference (P>0.05). Values were expressed as Mean ±SD, n=5.

Statistical analysis

Data obtained were analyzed using a Statistical Package for Social Sciences (SPSS Version 21) and were expressed as Mean ± standard error of mean (SEM) using One way Analysis of Variance (ANOVA) with Tukey Post–hoc test. P<0.05 was considered statistical significant difference

RESULTS

As Shown in Table 1, flavonoids had the highest amount in the extract of the zest of *C. sinesis* while tannins and phenols had the least. Alkaloids and saponins were present in moderate amounts. Table 2 shows that at varying doses of AEZCS there were no mortality or signs associated with oral toxicity except at the maximum dose of 3780 mg/kg body weight of AEZCS which resulted in a toxicity signs; significant loss of fur skin and lesions, diarrhea, salivation, tremors, coma and eventually death of the rats.

Table 3 shows that there was a significant increase (P>0.05) in the liver/body weight (LW/BW) ratios following the administration of cadmium chloride in the positive control group of rats. Similar increase was also noted in the liver and body weight ratio in the rat groups post treated with low dose of AEZCs after cadmium exposure. In contrast, the liver or body weight ratios in the rat group pretreated with the low dose of AEZCs was significantly lower than observed in the positive control group. Significant decreases in the liver /body weight ratios was also observed in the group of rats that were pretreated and post treated with the high dose of AZECS.

As shown in Table 4, the concentration of Malondialdehyde and superoxide dismutase activity in the positive control (Group A) was significantly higher (P>0.05) than the test groups. There was significant increase in the concentration of malondialdehyde of the groups of rats pre-treated and post-treated with the low doses of AEZCs (groups F and D respectively).The increase in the groups pre-treated and post –treated with AEZCS was accompanied by an increase and decrease in the activity of superoxide dismutase respectively. In contrast, there were significant decrease (P>0.05) in the concentration of malondialdehyde in the groups pre-treated and post-treated with the high dose of AEZCS (groups G and E) with a concomitant surge and decrease in the activity of superoxide dismutase respectively. The variations in the activity of lipid peroxidation in the tissue.

Table 5 shows the concentration of the serum liver enzymes. The ALT was more pronounced in groups A (83.80±8.00)u/L, followed by group F (73.24±2.95), Group D (72.90±6.02) u/l etc with the least value observed in group H (47.85±3.26). Group A has the maximum concentration of ALP level followed by groups

Table 4. Concentrations of Malondialdehyde and Superoxide dismutase on aqueous extract of zest of *Citrus sinensis* on cadmium chloride induced liver toxicity.

Groups	SOD (U/mg protein)	MDA (nmol/mg protein)
A	4.17±0.42	8. 5±1.01
B	22.62±2.14	1.01±0.17
C	18.8±0.33	1.15±0.26
D	24.83±0.99	3.77±0.05
E	20.83±1.93	3.76±0.41
F	26.30±2.78	2.48±0.55
G	30.65±1.33	4.77±0.08
H	19.89±0.36	1.81±0.06

Values were expressed Mean ±SD, n=5. Those without superscript shows that there was no significant difference (P>0.05) .

Table 5. Serum liver enzymes activity of aqueous extract of zest of *Citrus sinensis* on cadmium chloride induced liver toxicity.

Groups	ALT (U/L)	ALP (U/L)	AST (U/L)	Total protein (g/100 ml)
A	83.80±8.06*	537.05±36.26*	66.71±3.39*	6.36±0.62*
B	51.17±4.34	226.90±4.63	29. 51±1.69	8.12±0.44
C	60.11±11.32	215.86±6.44	29.2±1. 56	7.74±0. 60
D	72.90±6.02 [a]	496.28±9.65 [a]	52.43±0.95[a]	7.12±0.01 [a]
E	62.63±9.14 [b]	373.87±8.92 [b]	45.27±2.49 [b]	6.95±0.21 [a]
F	73.24±2.95 [a]	420.31±42.28 [a]	61.03±4.27 [a]	7.28±0.18
G	60.30±7.11 [b]	309.66±31.46 [b]	40. 53±3.30[b]	6.88±1.00[a]
H	47.85±3.26	246.69±0.72	33.79±1.81	6.46±0.43

* represents significant increase or decrease at P<0.05 when compared with the negative control (Group H); [ab] represents significant difference at P<0.05 when compared with the positive control.

D, F with values of 537.05±36.26, 496.28±9.65 and 420.31±42.28 u/l respectively. The least values were in groups C (215.86±6.44) u/L. Meanwhile similar pattern of their concentration was observed in AST concentration with group A, F, D and G having the least values respectively. In their total protein concentration, group B has the highest values followed by groups C and F with group A having the least concentration.

Histological results

Photomicrographs of the histological sections of the control groups of wistar rats were compared with those of the treatment groups. Histological changes were observed in the peri-portal hepatocytes, sinusoidal arrangement, portal area etc. These changes were used to assess the depth of hepatic damage and recovery following treatment with AEZCS as well as to substantiate evidences obtained in the biochemical analysis (Figures 1 to 8).

DISCUSSION

In the acute toxicity test, the animals did not show any sign of aggression or unusual behavior during handling throughout the 14 days observational period following oral administration of the different doses of the aqueous extract of the zest C*. sinensis*. The extract did not produce any mortality up to 3783 mg/kg body weight and this is not in keeping with similar finding of LD_{50} of citrus peel extract by Saalu et al. (2006). It is therefore suggestive that the zest extract of *C. sinensis* may not be toxic to health at the administered dose. Phytochemical analysis of AEZCS showed a highest percentage abundance of flavonoids. This finding is in keeping with the report by Lu and Foo (2004) that flavonoids are potent free radical scavenger and super anti-oxidant conferring anti-peroxidative properties in most plant extracts. The extract of zest citrus sinensis also showed increased proportions of alkaloids and saponins and this may also have contributed to the medicinal benefits of AEZCS. This is in agreement with the submission by

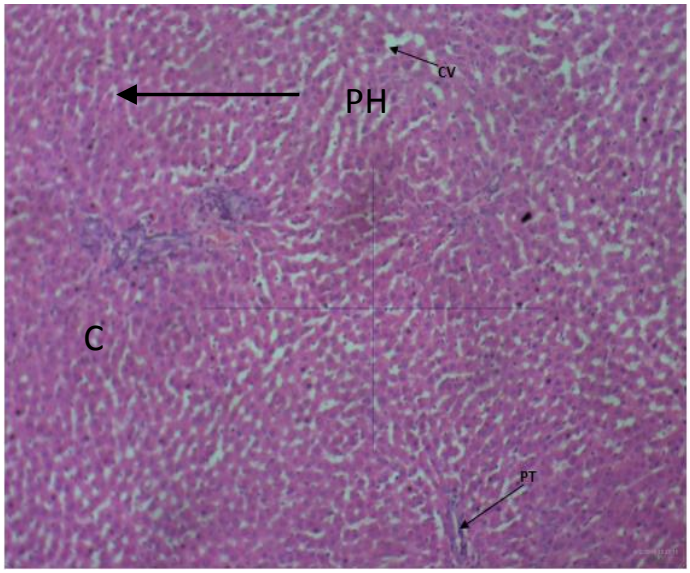

Figure 1. Photomicrograph of rat liver in positive control (Group A) shows hepatic cells without necrotic zones (NZ) and prominent halos (PH).Central Vein (CV). H & E stain x400.

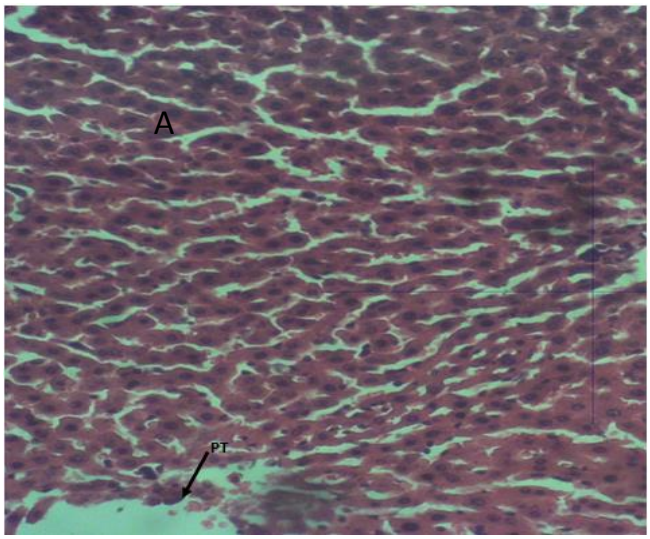

Figure 2. Photomicrograph of rat liver treated with low dose of AZECs alone (group B) Portal Area (PA), Hepatic plates (HP),vein (CV) with hepatic sinusoids. HE stain x400.

Milugo et al. (2013) that alkaloids and saponins contributes to health; being actively involved in body metabolism and development. Generally, our finding on the presence and varying proportions of phytochemical in AZECS is in line with that of Srividhya et al. (2013). The toxicological experiment, comparison of organ/ body weights between treated and untreated groups of animals have conventionally been used to evaluate toxicity in target organs (Peters and Boyd, 1966; Pfeiffer, 1968). In this study, the increase in the liver/body weight ratios following the administration of cadmium chloride in the positive control group of rats may be attributed to tissue swelling. This finding on the organ-body weight ratio is in keeping with Amresh et al. (2008) that toxic chemicals may contribute to organ swelling, atrophy or hypertrophy. Generally, our finding showed that of hepatocytic damage

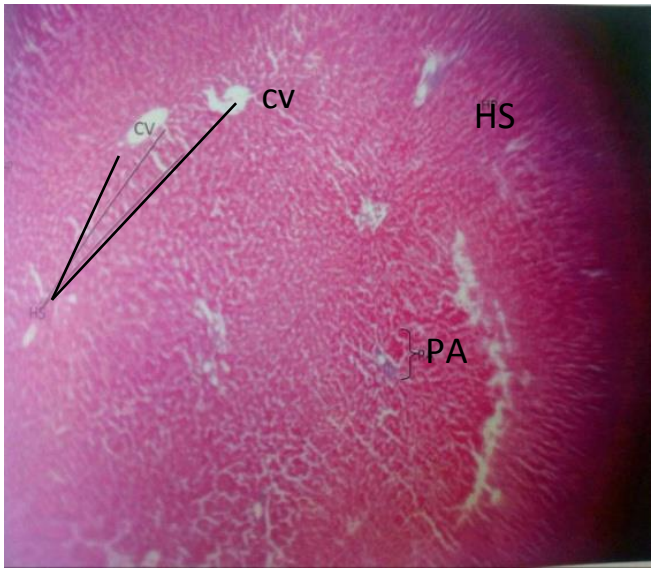

Figure 3. Photomicrograph of rat liver treated with low dose of AZECs alone (group C) showing preserved Central vein (CV) with hepatic sinusoids (HS), Portal Area Plates (HP),.HE stain x400.

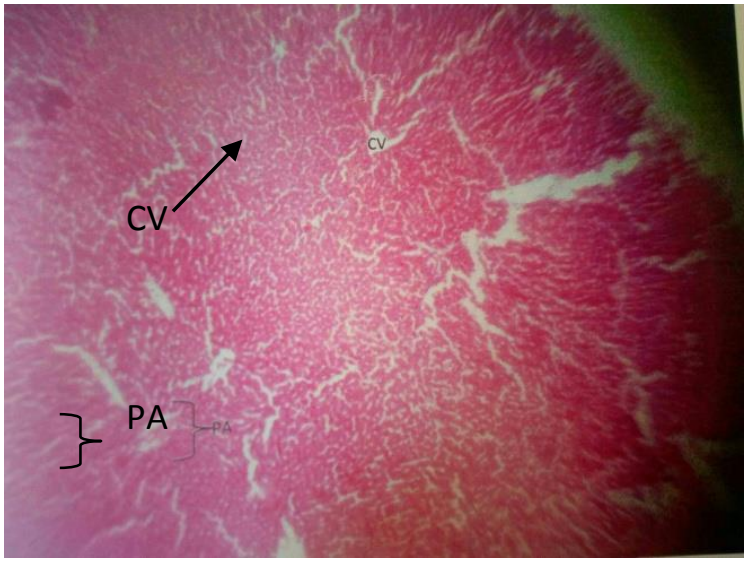

Figure 4. Photomicrograph of rat liver treated with cadmium chloride and shortly followed with low dose AZECS (Group D) showing Central vein (CV) and, Hepatic prominent Halos (PH).H & E stain x400.

relative to the animal body weight was minimal since the liver/body weight ratios in the groups treated with AEZCS before and after exposure to cadmium did not significantly differ from that of the negative control. Using the index, our finding on the ameliorative and protective benefit of AEZCs is in line with the work of Udoh et al. (2005) on the seed extract of *C. papaya.* When the liver was exposed to cadmium chloride, there were nodular deposits of whitish brown substances believed to be adipose tissues of the liver. The positive control groups of rats shed light on the potential impact of chronic exposures to environmental toxicants like cadmium on the liver health and in particular on the presence of diffused portal area (PT) and sinusoidal dilations. These

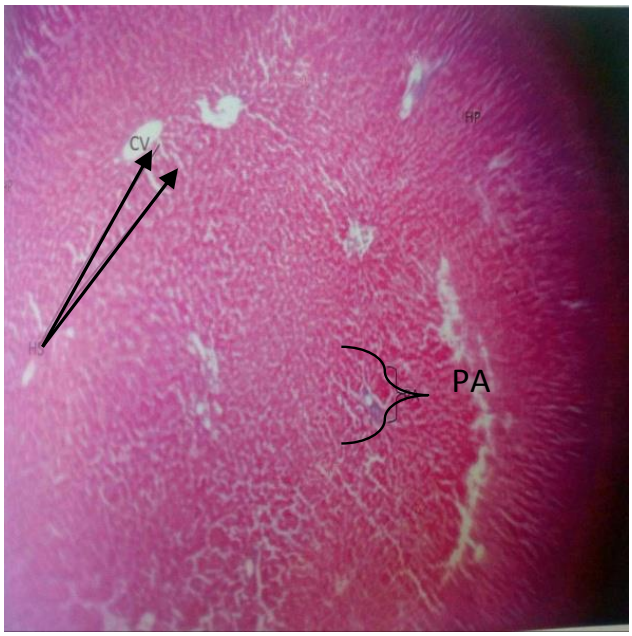

Figure 5. Photomicrograph of rat liver treated with Cadmium chloride and shortly followed with low dose of AZECs (Group E) shows distinct Portal area (PA), Central vein (CV)and Hepatic sinusoids which were otherwise distorted in the photomicrograph of the positive control. H & E stain x400.

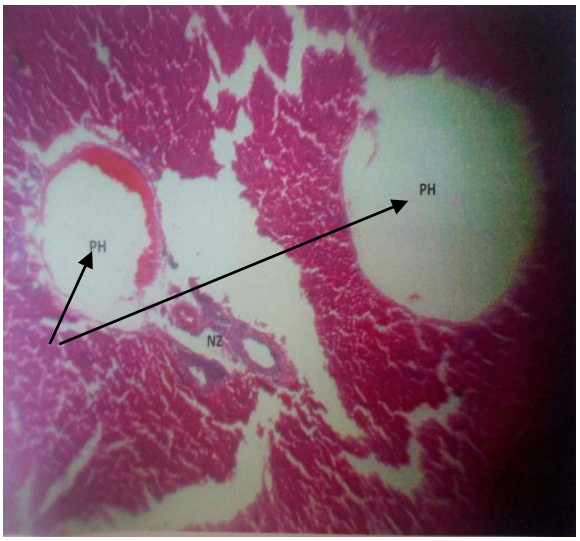

Figure 6. Photomicrograph of rat liver treated with low dose of AZECS before Cadmium chloride administration (Group F) showing Central vein (CV) and prominent Halos (PH) H & E stain x400.

toxic contaminants have a great steatogenic potential and needs to be considered tangible as risk factors as a result of cadmium chloride toxicity. Suzuki et al. (2014) reported that the factors that mediate occurrence of liver disease are oxidative stress, tissue hypoxia, and immune response and membrane alterations. This present study collaborates the report by Omar et al. (2013) that prolonged exposure to critical level of cadmium is

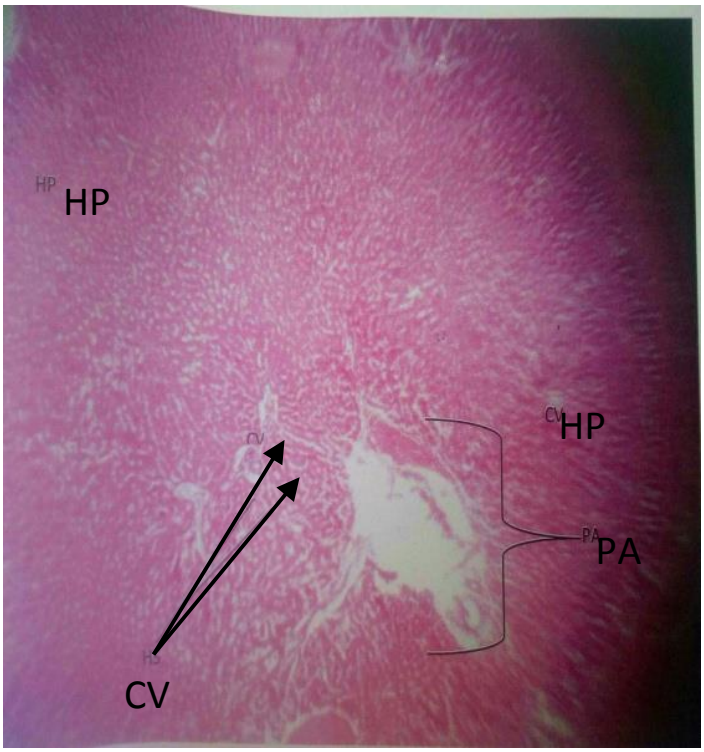

Figure 7. Photomicrograph of rat liver treated with low dose of AZECS before Cadmium chloride administration (Group G) shows Hepatic plates (HP), Central vein (CV and radiating hepatic sinusoids (HS) and Area (PA) which are in contrast to the photomicrograph of the positive control but similar to that of the negative control. H & E stain x400.

associated with incidences of liver diseases. The effect of AEZCS on the histological appearance of the liver shows tortuous, dilated hepatic sinusoids lined by a discontinuous layer of fenestrated endothelial cells that also exhibit fenestration and discontinuous basal lamina. The structure and the tortuous path of hepatic sinusoids through the liver bed allow for an efficient exchange of materials between the hepatocytic and blood. According to Yamano et al. (2000), hepatic endothelial cells might be the first cellular target for cadmium induced hepatocellular injury. Our findings on the histopathological changes associated with cadmium chloride exposure are in consonance with the report of El-Sokkary et al. (2010)

There was extensive destruction of fenestrations on the luminal surface of endothelial cells which supports the findings of Kuester et al. (2002). This condition is marked by the extrusion of damaged endothelial into the capillary lumen, producing local ischemia and subsequent activation of Kupffer cells as well as polymorphonuclear neutrophils (PMN) infiltration. These events trigger a cascade of inflammatory mediators that promote necrosis. These cellular changes may have also resulted in apoptosis since studies in rats, mouse or human hepatocytes show that apoptosis play a role in cadmium hepatotoxicity (Lasfer et al., 2008; Yu et al., 2011).

The liver histology of the rats pretreated with doses of 10 and 40 mg/kg prior to the exposure to cadmium revealed significant differences in their liver histology. However, only high dose of AEZCS showed better histoarchitectural preservation of the parenchyma. Our work supports the findings of Saalu et al. (2006) on the protective effect of high dose of 10 and 40 mg/kg after exposure to cadmium revealed significant attenuating changes in the liver histology. However, this ameliorative change in the liver parenchyma was better observed at the high dose of 40 mg/kg. Our work supports histological studies on dose dependent ameliorative effect of the Citrus peel extract (Parmar and Kar, 2008; Saalu et al., 2006).

Biochemical changes of the liver in our positive control group of rats which received a single dose of cadmium chloride without treatment with AZECS revealed excessive lipid peroxidation with an increase in superoxide dismutase (SOD) activities. This finding is in line with the report by Watkins (1992) that excessive lipid peroxidation using MDA index and an increase in superoxide dismutase (SOD) activities are hallmark of oxidative stress. These biomarkers of oxidative stress

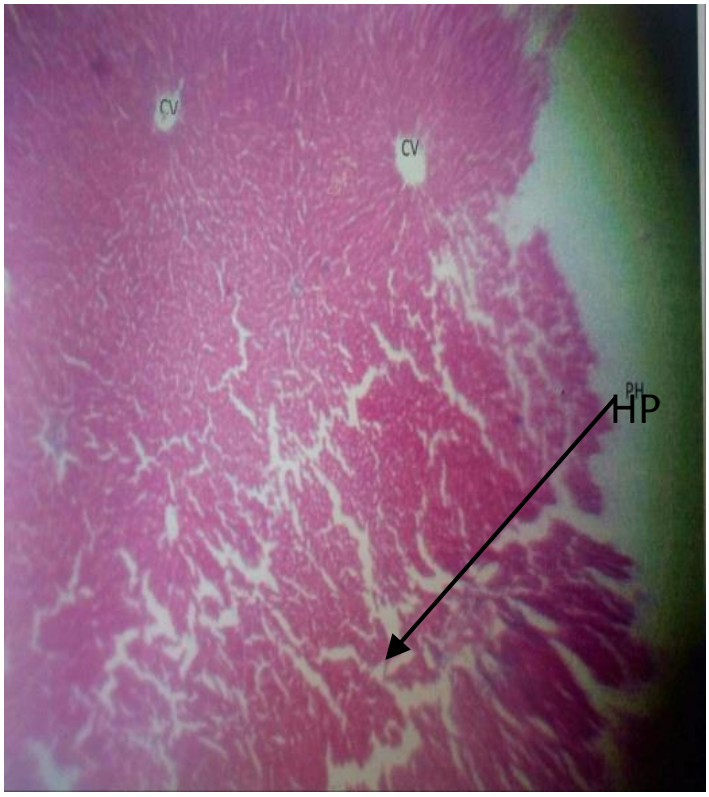

Figure 8. Photomicrograph of rat liver in negative control group showing normal hepatic plates (HP) with well preserved cytoplasm and nucleus, Portal Area (PA), Hepatic sinusoids (HS), Portal radiating with Central vein (CV),towards the periphery. H & E stain x400.

were also observed in the rats pretreated with low dose and high dose of AEZCS before cadmium exposures. Sustained increase in SOD and MDA levels suggested that AEZCS may be unable to protect the hepatocyte from oxidative damage at low doses; the biochemical role of AEZCS to better protect the liver against cadmium toxicity may be associated with the high dose. This supports report on protective efficacy of high dose of AEZCS against testicular damage (Saalu et al., 2006).

Following the post-treatment of cadmium toxicity with low and high dose of AEZCS, biochemical evidence showed an ameliorative change in SOD activity and MDA level. Our findings showed a decrease in MDA concentrations after treatment of toxicity with the high dose of 40 mg/kg of AEZCS being more effective. This supports similar report by Nada et al. (2014) on the ameliorative effect of peel of citrus sinensis against castration induced oxidative stress on the liver.

Hence, the effect of the AEZCS on the liver enzymes and total protein concentration showed that the liver injury induced through cadmium chloride administration culminated in increased concentrations of Alanine aminotransferease (ALT), Aspartate aminotransferease (AST) and alkaline amino phosphatase (ALP). The rise in levels of these enzymes particularly ALT, is in concordance with report in hepatocellular membrane damage (Ahmadizadeh et al., 2013). AEZCS administration at both lower and higher doses protected and maintained the liver function by moderating the activities of serum liver enzymes. Although this potency was more efficient at the high dose AEZCS at our higher dose, significantly lowered the serum liver enzymes activities which is increasingly altered in oxidative stress conditions as reported by Friedman et al. (1996). Thus, the ameliorative potential of AEZCS supports the findings by Kaplan (1993) that a decline in liver enzymes activity after exposure to toxic agents usually indicates recovery. Though, this may not be a strong prognostic sign in fulminant liver injury, where there are major losses of functional hepatocyte. In the positive control group of rats, cadmium exposure significantly decreased the level of serum total proteins. The abnormal decrease has been associated to damage in the hepatic sinusoids and blood vessels in oxidative stress condition (Friedman et al., 1996). A consequence of decrease in serum albumin being the major plasma protein is a shift of blood from the intravascular to interstitial spaces and peritoneal cavity resulting in intravascular volume depletion and edema

formation (Busher, 1990). However, in the groups of pretreated and post treated with low and high doses of AEZCS before and after exposure to cadmium, there was a noticeable increment in the total protein suggesting its protective and ameliorative potency against cadmium induced serum protein depletion

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 synthetic treatment products since the extract of zest of *C. sinensis* contains important phytochemical constituents. From the results obtained in the research, the extract of the zest of *citrus sinensis* significantly protected and ameliorated liver associated with cadmium in our study environment. Hence, this research recommends that attempt should be made to insert the extract as residues in dietary supplements and in drug formulation for treatment of liver dysfunction associated with cadmium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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