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Potentiating role of palm oil (*Elaeis guineensis*) and its extracts in cadmium-induced alteration of aminotransferases in albino rats

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ABSTRACT

Aims: This study examined the effects of palm oil and palm oil extracts pre-treatment on the activities of serum and tissue aminotransferases in acute cadmium-induced toxicity. **Materials and Methods:** Six experimental groups were used and Group A served as the control, while Group B was administered 20 mg/Kg body weight of cadmium chloride orally and Groups C–F were administered 5 ml/Kg body weight of crude palm oil (CPO), silica gel extract (SGE), bleached extract, and unsaponifiable extracts of palm oil, respectively, for 28 days before administration of 20 mg/Kg body weight of cadmium chloride orally on the 29th day. Animals were sacrificed on the 29th, 30th, and 31st days within intervals of 12-, 24-, and 48-h post-cadmium administration for tissue and serum enzyme assay and liver, kidney, and muscle histological analysis. **Results:** There was a significant increase ($P < 0.05$) in the activities of alanine aminotransferase and aspartate aminotransaminase in the tissues (the liver, kidney, heart, and muscle) and serum of CdG relative to group CG, while the brain showed no relative significant ($P > 0.05$) changes in CdG relative to group CG after 12, 24, and 48 h. Although the activities of these enzymes also increased in groups CPO and SGE relative to group CG, it reduced significantly relative to those administered cadmium only (CdG). There was no significant alteration in the liver membrane integrity in all groups, and the pattern for increase in the activities of the aminotransferases was the liver, kidney, muscle, heart, serum, and brain after 12, 24, and 48 h. **Conclusions**: CPO and SGE pre-treatment were more potent for the modulation of tissue and serum aminotransferase enzyme activities in cadmium acute toxicity.

Keywords: Acute toxicity, aminotransferases, cadmium, palm oil, potentiate

INTRODUCTION

Cadmium is a heavy metal with the symbol Cd and atomic number 48. It occurs naturally in the earth crust and is relatively poor in abundance (64th among elements). While it occurs in air, water, soil as well as in tissues of plants and animals, it is not found in free state.[1-3] The presence of cadmium is primarily in ores of zinc, copper, or lead, their extraction and processing releases large quantities of cadmium into the atmosphere, hydrosphere and soil. It is non-biodegradable and accumulates in living system through active circulation by food chain[4,5] and readily poses threat as an environmental contaminant for the human environment.[6] It is classified as a human carcinogen by the North Carolina National Toxicology Program;[7] cadmium is a well-known heavy metal that causes damage to several human organs such as the kidneys, lungs, bones, liver, and testes.[8,9]

Palm oil is a vegetable oil with high content of tocopherols, tocotrienols, and carotenoids which act as potent antioxidants.[10,11] It is processed from the mesocarp of the fruits of the oil palm tree (*Elaeis guineensis* Jacq.) and has a rich source of natural antioxidants with anti-inflammatory properties. Crude palm oil (CPO) possesses 1% minor components; among them are carotenoids (α and ß carotene), Vitamin E (in the form of tocotrienols and tocopherol). CPO is considered to be the richest natural source of carotenoids.[12] Although present in small quantities, these minor constituents to a great extent are responsible for the healing or medicinal properties of palm oil. Carotenoids and tocols serve as biological antioxidants necessary for protection of cells and tissues from oxidative stress.[13] These natural antioxidants act as buffers against free radicals and are believed to play a protective role in cellular aging, atherosclerosis, cancer, arthritis, and Alzheimer's disease.[14,15]

Aminotransferases, also called transaminases, are present in most of the tissues of the body. They catalyze the interconversions of the amino acids and 2-oxacids by transfer

of amino groups. Transaminases are specific for the amino acid, from which the amino group has to be transferred to a keto acid. 2-oxoglutarate and glutamate couple serves as one amino group acceptor and donor pair in all amino transfer reactions.[16] Alanine aminotransferase (ALT)/Gpt1/ ALT catalyzes the reversible interconversion of L-alanine and 2-oxoglutalate to pyruvate and L-glutamate and plays a key role in the intermediary metabolism of glucose and amino acids.[17] Aspartate aminotransaminase (AST) catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health.[18,19]

The activities of both AST and ALT are high in tissues, especially the liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause the release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increased activities of these enzymes in the blood. Activity levels of 20–50 fold higher than normal are frequently seen in liver cell damage, but it may reach as high as 100 times in severe damage to cells. In myocardial infarction, high activity of AST is seen in serum. ALT activity is within normal range or slightly increased in uncomplicated myocardial infarction. The rise in AST is seen within 6–8 h of the onset of chest pain, the highest level at 18–24 h, and returns to pre-infarction levels by 4–5th days.[20] There are other superior markers available for myocardial infarction as AST lacks the tissue-specific characteristics and as its activity may also be increased in diseases of other tissues such as liver and skeletal muscles.[16,21]

Inhibition of the liver enzymatic function, through an increase in lipid peroxidation, production of reactive radicals, oxidative tissue damage, loss of membrane functions, and hepatic congestion have been reported as mechanisms of heavy metal-induced damage.[22] Cadmium (Cd) is able to generate reactive radicals leading to cellular damages and hepatotoxicity in humans and animals.[23,24] Since antioxidant supplementation has been found to be beneficial in metal toxicity and palm oil has been reported to have antitoxic and anti-hepatocarcinogenic effects,[25] this study investigated the ability of pre-treatment with CPO and various extracts of palm oil to potentiate the negative effects of cadmium-induced alteration of serum and tissue aminotransferases.

MATERIALS AND METHODS

Collection of Palm Fruits

Ripened palm fruits were obtained from an oil palm plantation located at Obiaruku, Delta State. The *Tenera* variety which was selected for this study was identified and authenticated at the Department of Botany, Delta State University, Abraka, and registered with the voucher number ID/2017/16807/ *Tenerra* Spp.

Extraction of Palm Oil

This was done according to the method of Twumasi *et al*.,[26] with little modification to sooth the method used by local palm oil producers in Abraka, Delta state, Nigeria**.**

A 10 kg freshly harvested ripe oil palm fruits were boiled in a 20 L pressure cooker for 4 h. Pulp mass was produced by pounding the boiled fruits using wooden pestle and mortar. This was then immersed in water (10 L) and stirred thoroughly for proper extraction. Filtrate was obtained by sieving with the use of 12 mm/15 mm pore size. This was subsequently poured into an aluminum cooking pot and boiled for 5 h. The heated mixture was allowed to cool and 2 L of cold water was added to the surface by a sprinkler. The palm oil which set on top of the aqueous portion of the boiled filtrate was scooped into a fresh container. The collected oil was heated gently for 10 min to remove traces of water.

Fractionation of CPO

Three palm oil extracts employed in this study were the unsaponifiable extract (UPE), silica gel extract (SGE), and bleached extract (BE).

Preparation of UPE

The method of Meloan as modified by Twumasi *et al*.,[26] was used with some modifications. To a 250 ml conical flask, 10 ml of the CPO was added and 10 ml of the CPO was added to 250 ml conical flask followed by and 15 ml of 20% sodium hydroxide and stirred using a magnetic stirrer. This was corked and connected to a vacuum pump. This was allowed to boil using a hotplate, and on thickening, the flask was disconnected from the vacuum pump while stirring continued until cooling was slowly achieved. It was then transferred into a 500 ml separating funnel followed by addition of 200 ml of hexane, shaken, and mounted on a retort stand to allow for settlement. Elution of the bottom layer (soap) was achieved by opening the tap while the upper layer was washed several times with distilled water until the wastewater tested neutral to phenolphthalein and was labeled and stored in a cold dry place until required.

Preparation of SGE

SGE of palm oil was carried out following the method described by Ahmad *et al*.,[27] A chromatographic glass tube of 3.0 cm in diameter was mounted on a retort stand, and to the bottom of the tube, a small glass wool was plugged, while silica gel of 70–230 mesh was used as the adsorbent was used to fill the column up to a height of 5.0 cm. Removal of air spaces was achieved by tapping the sides of the column, while a glass rood was used to press down the adsorbent and also flatten the surface. An eluting solvent (100 ml n-hexane) was then introduced into the column. 10 ml CPO was then dissolved in 30 ml of n-hexane and introduced into the column while the eluent was collected into a fresh conical flask until the adsorbent in the column became colorless. The collected eluent was then stored in a cool dry place.

Preparation of bleached palm oil extract (UPE)

The method described by Patterson[28] based on a ratio of 1:11 silica gel and CPO was used. 110 g of CPO was measured and poured into a beaker and heated on a hot plate. Stirring was achieved using a magnetic stirrer continuously until it reached 100°C. Thereafter, six drops of concentrated phosphoric acid were added to the CPO and immediately followed by the addition of silica gel (10 g). Addition of the adsorbent was done slowly so as to achieve a uniform mixture as well as

prevent bubble formation. The resulting mixture was again heated for an hour until it reached a temperature of 150°C to complete bleaching process. On the achievement of bleaching, the mixture was allowed to cool before the spent adsorbent was separated from the bleacher palm oil by filtration using Whatman No.1 filter paper. This was done in the oven for 22 h period at a temperature of 80°C.

Preliminary Analysis of Phytoconstituents of Palm Oil and Extracts

Determination of carotene in palm oil samples

The carotene content of the CPO was determined according to the method described by Coursey.[29] CPO (3 g) was dissolved in 20 ml hexane in a 50 ml volumetric flask. A spectrophotometer was used to measure the absorbance at 446 nm. Hexane was used as the blank solution. Using the absorbances obtained, the concentration (ppm) of carotene in the sample was calculated, using the following formula:

$$\text{Carotene} = (K_{Df} \times \text{absorbance [446 nm]} \times \text{volume [ml]}) / (100 \times \text{sample weight}_{[g]})$$

Where,
Volume = volume of hexane mixed with palm oil
K_{Df} (diffusion coefficient) = 383
The above procedure was repeated with other palm oil extracts.

Determination of Vitamin E (α-tocopherol) in palm oil samples

Total α-tocopherol was determined by AOAC method.[30] 50 ml of each sample for analysis was mixed with a 2:1 volume by volume mixture of ethanol-petroleum ether until the residues were observed to be free of oil, and these were then put into a separating funnel followed immediately by addition of 2.0 ml of ethanolic potassium hydroxide solution to aid the saponification of the oil. This (mixture) was allowed to settle before decanting off the ethanol layer but allowing the petroleum ether layer to be washed so as to remove soap and dried over anhydrous sodium sulfate. The resultant extract of 1.0 mL was put in screw cap test tube; 1.0 mL of bathophenanthroline, 0.5 mL $FeCl_3$, and 0.5 mL H_3PO_4 solutions were added and the absorbance was measured at 534 nm. Tocopherol content was extrapolated from the standard curve in Appendix I, and I.U. potency was calculated using the conversion method that 10 I.U. = 4.5 mg α-tocopherol.

Determination of free fatty acid (FFA) in the palm oil extracts

Determination of FFA contents in CPO and palm oil extracts was carried out by titration adopting the method described by Palm Oil Research Institute of Malaysia.[31] The CPO and the various extract samples were melted at 60–70°C and 10 g each weighed into separate 50 ml Erlenmeyer flasks, and ethanol (20 ml) and three drops of phenolphthalein were added. This was heated with an electric stove at a maintained and steady temperature of 40°C. The mixture was then titrated using 0.1 M NaOH while stirring continued until the first permanent pink color was observed. The volumes of NaOH for the titration were recorded while the percentage FFA in the sample was calculated, using the following formula:

$$\text{FFA\% as palmitic acid} = \frac{25.6 \times M\,(NaOH) \times V}{W}$$

Where,
M = Molarity of NaOH solution
V = Volume of NaOH solution used in ml
W = Weight of sample

Experimental Design

The effect of palm oil and palm oil extract pre-treatment on cadmium toxicity was studied after acute exposure. 72 male rats (Wistar strain) weighing between 180 and 200 g were used for this study. They were acclimatized for 2 weeks before dividing into six groups with 12 animals per group. The control group (A) received neither cadmium nor palm oil extracts, while the second group was treated with cadmium only (B) on the 29th day. Groups C-F were given palm oil fractions (5 ml/Kg body weight) by gavage based on an established lethal dose of undiluted palm oil >5 g/Kg body weight[32] using a 5 ml syringe for 28 days before cadmium exposure on the 29th day of the experiment. A single dose of cadmium in the form of $CdCl_2$ (20 mg/kg body weight) was administered orally by gavage using a 5-ml syringe on the 29th day to the rats in Groups B-F. The preparation of the cadmium chloride was carried out by dissolving 2 g of $CdCl_2$ in 200 ml of distilled water to produce a stock solution of 10 mg/ml. Based on the individual animal weights, however, the exact dosage administered to them was determined. It is important to note that the choice of the cadmium dose used for this study was made based on available information in literature that reports the lethal dose of oral cadmium chloride as 88mgKg-1 body weight and that a single dose 15.3mgKg-1 body weight of cadmium is able to cause deleterious effects in wistar rats within 24 h of administration as reported by the Agency for Toxic Substances and Disease Registry (ATSDR).[33] Control rats in Group A were administered orally by gavaging the same concentration of normal saline on day 29. All animals were allowed free access to commercial rat chow and water throughout the period of the experiment. Table 1 indicates the various treatment groups in the experiment.

Four animals from each group were sacrificed on the 29th day (12 h), 30th day (24 h), and 31st day (48 h) after cadmium oral administration; the choice of four animals for each assay replicate was made based on the resource equation on the reduction of animal wastage during experimental processes as developed by Festing[34] and Jatinder.[35] The equation is thus given as E = Total number of animals - Total number of groups, where E is the degree of freedom for the analysis of variance (ANOVA).

Collection and Preparation of Samples

At the end of various periods stated in the study, blood and tissue samples were collected from each rat after sacrifice. Each animal was sacrificed by cervical dislocation. The rats were dissected and the blood was collected through heart puncture by means of a hypodermic syringe and needle. The liver, kidney, heart, brain, and muscle were excised, weighed, and transferred into labeled containers. The blood from each rat was transferred immediately after collection into labeled plain tubes. Serum was obtained from the blood after clotting and eventual centrifugation at 3000 g and stored in a refrigerator at −4°C. The tissue homogenate of each organ was prepared in pre-chilled pestle and mortar using

Table 1: Experimental design for pre-treatment with palm oil and palm oil extracts before acute oral cadmium administration

Experimental group	Oral administration of palm oil and its extract				Acute administration of cadmium
	CPO	SGE	BE	UPE	
Group A (CG)	-	-	-	-	-
Group B (CdG)	-	-	-	-	+ (on day 29)
Group C (CPO)	+ (days 0–28)	-	-	-	+ (on day 29)
Group D (SGE)	-	+ (days 0–28)	-	-	+ (on day 29)
Group E (BE)	-	-	+ (days 0–28)	-	+ (on day 29)
Group F (UPE)	-	-	-	+ (days 0–28)	+ (on day 29)

The symbol (+) indicates the presence of the factor and (-) represents the absence of the factor under consideration, CPO: Crude palm oil, SGE: Silica gel extracts, BE: Bleached extract, UPE: Unsaponifiable palm oil extract

cold normal saline solution. The homogenate was subjected to centrifugation at 5000 g for 10 min, and the supernatant obtained was stored in a refrigerator at −4°C. All biochemical assays were done within 48 h of sacrifice.

Determination of Activities of Aminotransferases

Estimation of ALT and AST activity in the serum and tissue homogenates was carried out by the method of Reitman and Frankel.[36]

The assays were based on the following reactions of the enzymes.

$$\text{Alanine} + \alpha\text{-ketoglutaric acid} \xrightarrow{\text{ALT}} \text{pyruvic acid} + \text{glutaric acid}$$

$$\text{L-Aspartic acid} + \alpha\text{-ketoglutaric acid} \xrightarrow{\text{AST}} \text{oxaloacetic acid} + \text{L-glutamic acid}$$

In each of the cases, the keto acid produced was reacted with 2,4-dinitrophenylhydrazine to form the corresponding color hydrazine.

In each case, two test tubes were labeled, and to each, 0.2 ml of sample was added to 1 ml of ALT- and AST-buffered substrate. The mixture was incubated at 37°C for 30 min for ALT and 60 min for AST. At the end of the incubation periods, 1 ml of the color reagent (2, 4-dinitrophenylhydrazine) was added and further allowed to stand at room temperature for 30 min before sodium hydroxide (10 ml of 0.4 N) was then added, mixed, and allowed to stand for another 5 min. For the blank, 0.2 ml of distilled water was similarly treated following the same procedure. Absorbance of the sample was read against the blank at 540 nm using a spectrophotometer. The enzyme activities were extrapolated from a standard curve and expressed as unit/ml.

Histological Analysis of Tissues

Tissues were prepared using the method described by Zhang *et al*.[37] A known portion of the liver, kidney, and muscle of each rat was harvested and fixed in 10% formol saline for 48 h and processed for paraffin wax embedding with an automatic tissue processor by dehydrating through 70%, 90%, and 95% and two changes of absolute ethanol for 90 min each. Clearing was achieved through two changes of xylene for 2 h each; and infiltrating with two changes of paraffin wax for 2 h. Sections were cut at 5 μm with a rotary microtome. The sections were stained by hematoxylin and eosin (H and E) using the method of Odoula *et al*.,[38] examined, and photographed using a light microscope.

Method of Statistical Analysis

Analysis of data was done using Statistical Package for the Social Sciences version 21 (SPSS 21). The simple ANOVA was used, while *post hoc* analysis for comparisons across groups was done using Turkey HSD at $P < 0.05$ level of significance.

RESULTS AND DISCUSSION

Preliminary analysis of carotenoid, Vitamin E, and FFA content of the CPO and the various palm oil extracts used for pre-treatment of the experimental animals indicates that the highest contents of carotenoids were found in the UPE, while the least was in the bleached fraction. Although the carotenoid contents in the CPO and SGE were comparable to that of the UPE, the observed drop in carotenoid contents in bleached palm oil may have been as a result of thermal destruction of carotenes in palm oil and is in agreement with the study of Dongho *et al*.[39] who previously reported that carotenoid contents of palm oil were heat dependently depleted as temperature exposure increased. Vitamin E content in SGE was also observed to be significantly higher compared to CPO and other fractions (UPE and BE). This observation is indicative of the fact that most carotene and tocopherol contents in CPO are held bound to certain other components of the oil, thus making them not to be freely available. However, the use of saponification and the gel adsorption chromatographic technique in this study confirms earlier claims by Osei[40] that these techniques are effective for making available carotenoid contents and tocopherol contents inherent in palm oil. While FFA content was highest in the BE, it gives credence to earlier submissions by Idoko *et al*.,[41] that bleaching of palm oil contributes greatly to the destruction of primary antioxidants in palm oil compared to CPO [Table 2].

The result from this study revealed the elevation of tissue (liver, kidney, and heart) and serum ALT and AST activities in Tables 3 and 4 after 12-, 24-, and 48-h post-cadmium administration periods. Although this elevation was time-dependent relative to all the tissues, it was observed that the activities of ALT and AST peaked after 24-h post-cadmium administration. The result also revealed a non-significant impact on the elevation of the brain and muscle ALT and AST activities as there were no significant increases compared to

Table 2: Antioxidant and fatty acid content of palm oil and palm oil extracts

Parameter	Palm oil extracts			
	CPO	SGE	BE	UPE
Carotenoid (ppm)	1125.48±8.7[a]	1675.72±14.3[a]	540.08±6.7[b]	1920.34±10.7[a]
Vitamin E (α-tocopherol) (mg/ml)	177.78±14.46[a]	233.33±8.78[b]	44.44±6.89[c]	166.67±12.44[d]
Free fatty acid (%) V/V	30.96±5.89[a]	26.08±7.80[a]	56.56±8.43[b]	17.76±4.87[c]

All values are expressed as mean±SD, values sharing different alphabet superscript on the same row showed a significant difference with a indicating no significant difference from CPO, b indicating a significant difference from CPO but not from SGE, c indicating a significant difference from CPO and SGE, and d indicating a significant difference from CPO, SGE, and BE. CPO: Crude palm oil, SGE: Silica gel extract, BE: Bleached extract, UPE: Unsaponifiable extract

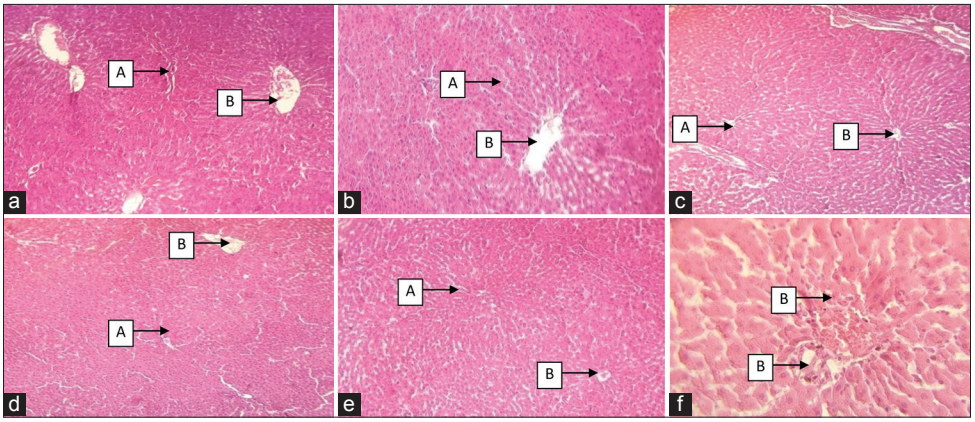

Figure 1: (a-f) Effect of palm oil and palm oil extracts on histology of the liver after 12-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/kg $CdCl_2$, (d) silica gel extract + 20 mg/kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/kg $CdCl_2$

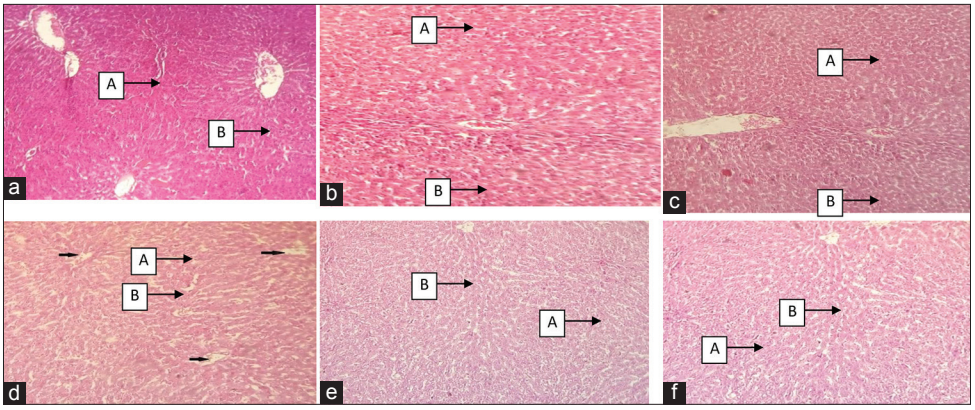

Figure 2: (a-f) Effect of palm oil and palm oil extracts on histology of the liver after 24-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil (CPO) + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

the control group. These observations are in line with the submissions of Asagba and Asagba and Obi,[42,43] who noted that cadmium administration had the ability to increase plasma AST and ALT activities in Wistar rats.

The results further revealed that the pre-treatment of rats with various palm oil extracts was able to modulate the observed increase in ALT and AST activities in the liver, kidney, and heart. Although there was observed increase in these activities relative to the control, these increase in activities were not elevated to the extent to which animals not pre-treated with the extracts were elevated. The trend in the modulation of organ and serum ALT and AST activities revealed that SGE and CPO had more effects in the reduction of ALT and AST elevation, while BE had the least efficacy followed by UPE. This observation is similar to the result reported by Eriyamremu *et al*.,[44] that the administration of palm oil significantly reversed the negative trends in the antioxidant enzymes and ATPases in ocular cadmium exposure. Substances such as CPO and its fractions that have high nutrient components are said to provide a great deal of scavenging capacities for pro-oxides arising from cadmium intoxication by alteration of its toxicodynamics and bioavailability,[44,45] and this is able to happen by possibly enhancing elimination of the metal from the tissues.[46-48]

Table 3: Effects of palm oil and palm oil extracts on activities of tissue and serum ALT of rats administered cadmium (units/L)

Tissues	Experimental groups *n*=4					
	A	B	C	D	E	F
12 h						
Liver % change	66.09±5.05^{a}	151.82±4.46^{b} (128)	77.93±5.12^{a} (17.9)	73.34±6.5^{a} (10.9)	130.22±3.27^{d} (97.0)	99.87±10.95^{e} (51.11)
Kidney % change	47.42±2.61^{a}	93.58±4.29^{b} (97.34)	61.42±2.71^{c} (29.31)	61.73±4.62^{c} (29.22)	82.74±4.23^{d} (74.48)	84.88±4.16^{d} (78.99)
Heart % change	41.77±2.88^{a}	48.44±3.29^{ab} (15.97)	48.07±3.64^{ab} (15.08)	47.34±4.50^{ab} (13.33)	49.38±2.34^{b} (18.22)	44.82±1.81^{ab} (7.30)
Brain % change	38.68±3.19^{a}	38.37±2.79^{a} (-0.80)	37.95±3.43^{a} (-1.88)	38.14±2.71^{a} (-0.13)	39.48±2.21^{a} (2.07)	35.67±4.97^{a} (-7.78)
Muscle % change	52.74±5.44^{a}	78.32±6.83^{b} (18.7)	68.43±2.94^{c} (22.92)	64.72±4.76^{c} (22.71)	65.56±8.09^{c} (24.31)	59.48±4.38^{ac} (12.78)
Serum % change	33.85±3.36^{a}	51.93±2.64^{b} (34.82)	40.52±3.47^{a} (16.46)	37.69±3.86^{a} (11.34)	50.47±4.31^{b} (32.93)	52.18±2.85^{b} (54.15)
24 h						
Liver % change	59.74±2.50^{a}	166.46±4.49^{b} (178.64)	82.35±3.68^{c} (37.84)	79.58±2.42^{c} (33.21)	137.00±6.27^{d} (129.32)	109.26±7.31^{e} (82.89)
Kidney % change	51.25±2.52^{a}	108.80±4.94^{b} (112.29)	85.28±2.10^{c} (34.03)	64.19±2.69^{d} (25.25)	95.88±3.40^{e} (87.08)	91.31±2.35^{ce} (78.17)
Heart % change	39.59±2.55^{a}	50.69±4.77^{b} (21.90)	51.82±1.16^{b} (30.89)	51.37±2.37^{b} (29.75)	52.50±2.31^{b} (32.61)	45.74±5.34^{b} (15.53)
Brain % change	37.81±2.81^{ab}	39.34±1.74^{ab} (4.05)	38.65±2.27^{ab} (2.22)	41.27±4.80^{b} (9.15)	37.80±3.72^{ab} (-0.026)	32.37±3.51^{a} (-14.39)
Muscle % change	54.47±4.45^{a}	87.20±8.36^{b} (60.09)	72.34±6.72^{c} (32.81)	68.27±6.74^{c} (20.21)	83.66±7.94^{d} (53.59)	64.68±6.83^{c} (18.74)
Serum % change	41.09±2.01^{a}	74.03±1.60^{b} (80.17)	49.39±2.58^{ac} (20.19)	45.99±1.72^{a} (11.93)	56.85±1.61^{c} (38.35)	57.93±1.75^{c} (40.98)
48 h						
Liver % change	64.47±8.50^{a}	164.64±7.49^{b} (60.84)	84.53±4.86^{c} (31.11)	82.85±4.27^{c} (22.18)	134.87±7.82^{d} (109.20)	111.62±5.13^{e} (74.13)
Kidney % change	49.52±6.25^{a}	100.72±6.34^{b} 51.2	87.84±4.13^{c} 43.62	58.19±2.96^{a} 17.51	98.55±3.08^{b} 99.01	82.44±3.52^{c} (70.11)
Heart % change	46.95±4.85^{a}	58.96±3.72^{b} (25.58)	54.22±1.16^{bc} (15.48)	48.73±4.67^{a} (3.79)	58.80±8.31^{b} (25.24)	48.40±6.44^{ac} (3.09)
Brain % change	38.46±4.18^{ab}	42.43±2.47^{ab} (10.32)	38.65±2.27^{ab} (4.94)	44.73±6.20^{b} (16.30)	38.60±4.27^{ab} (0.36)	37.73±4.17^{a} (-1.90)
Muscle % change	51.74±5.75^{a}	92.04±8.36^{b} (77.89)	68.44±4.27^{c} (16.70)	66.38±4.54^{c} (14.64)	78.72±3.46^{c} (52.14)	66.83±7.38^{c} (29.17)
Serum % change	40.92±4.01^{a}	68.36±4.60^{b} (40.14)	43.96±4.85^{a} (7.43)	42.09±2.62^{a} (2.78)	54.42±4.16^{c} (24.81)	48.37±2.56^{c} (18.21)

All values are expressed as mean±SD, values sharing different alphabet superscript on the same row showed a significant difference with a indicating no significant difference from Group A; b indicates significant difference from a but not from Group B; c indicates significant difference from all other groups but not from Group C; d indicates a significant difference from other groups but not from Group D, e indicates a significant difference from Group A and all other groups, while f indicates a significant difference from all other groups but not from Group F values expressed in bracket indicate percentage change in enzyme activities relative to control. A: Control, B: Administered 20 mg/kg body weight of $CdCl_2$, C: Crude palm oil (CPO) + 20 mg/kg $CdCl_2$, D=Silica gel extract (SGE) + 20 mg/Kg $CdCl_2$, E=Bleached extract (BE) + 20 mg/kg $CdCl_2$, F=Unsaponifiable extract (UPE) + 20 mg/kg $CdCl_2$

The elevation of ALT and AST activities from the study was consistent with evidences from previous studies on the ability of cadmium chloride to alter enzyme stability in experimental rats as it revealed an elevated tissue (liver, kidney, and heart) and serum ALT and AST activities within 12-, 24-, and 48-h post-cadmium administration when animals which administered only cadmium were compared to the control group that was neither pre-treated with palm

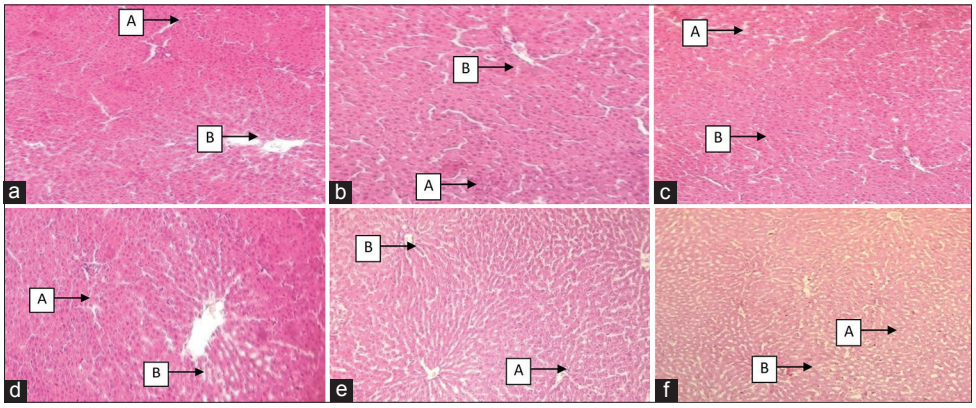

Figure 3: (a-f) Effect of palm oil and palm oil extracts on histology the liver after 48-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

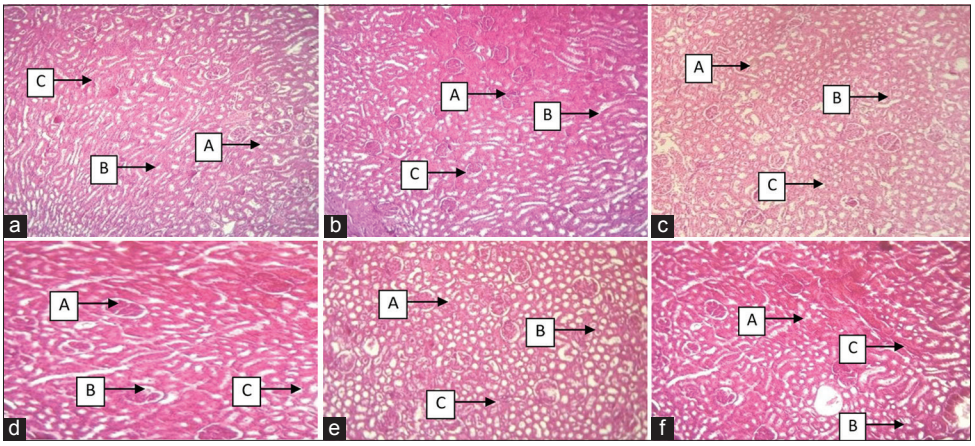

Figure 4: (a-f) Effect of palm oil and palm oil extracts on histology of the kidney of rats after 12-h post-cadmium administration (hematoxylin and eosin ×100). (a) control, (b) administered 20 mg/kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

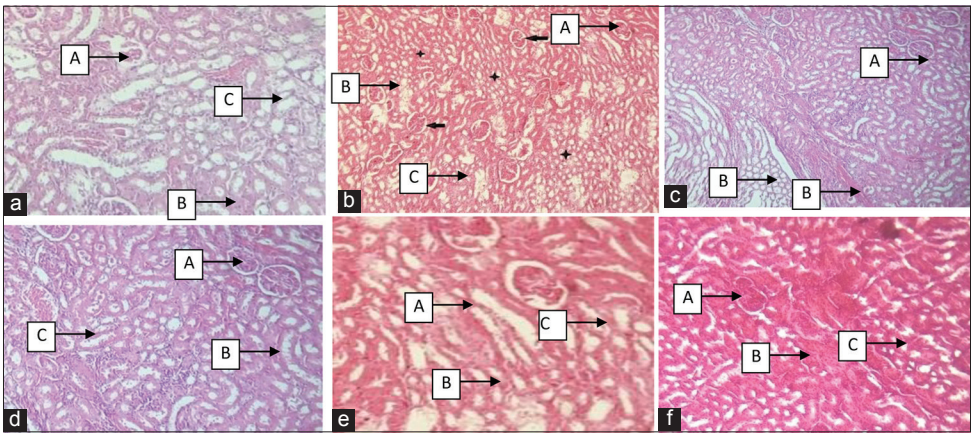

Figure 5: (a-f) Effect of palm oil and palm oil extract on kidney after 24-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

oil and its extracts nor administered cadmium after 12, 24, and 48 h of acute cadmium administration. There is no doubt that the justification for the increase in AST and ALT activities sterns from the need of the various organs to rise above the challenge accompanied with cadmium toxicity, this claim is also supported by the work of Asagba and Obi, Asagba and Eriyamremu, and Guilhermino *et al*.,[43,49,50]

Relative to organ specification, the effect of cadmium administration on the activities of ALT on organs was higher in the liver, followed by the kidney (liver>kidney>muscle>heart>serum>brain) at 95% confidence interval. This observation is justifiable considering the fact that the liver is the primary organ of metabolism of endogenous substances in the body. Likewise, information from literature notes that, in the metabolism of cadmium, the liver is one of the most susceptible organs after acute or chronic exposure to cadmium[42] which is followed closely to deposition in the kidney after a prolonged exposure.[51] Another justification for this trend in enzyme increase in the tissues in response to the cadmium intoxication is that the activities of these enzymes have been reported to be more in the liver and kidney compared to tissues such as the heart, brain, and muscle.[52,53] The availability of these enzymes in the serum in most cases is also reported to be responses to compromise in tissue ultrastructure.[17,49-51,54]

A careful study of the result trend in this study reveals that, of all the extracts used in the pre-treatment of the animals before cadmium administration, the BE had the least level of efficacy in protecting the rats from alteration in the stability of the ALT and AST enzyme activities relative to control. This observation may be justified from the reduced level of antioxidants of the BE as presented in Table 2. This submission is further given credence to by the investigations of Ani *et al*.,[55,56] that the consumption of thermally oxidized palm oil contributed greatly in the reduction of hematological parameters, increase in serum creatinine and urea as well as increased anemia in experimental rats.

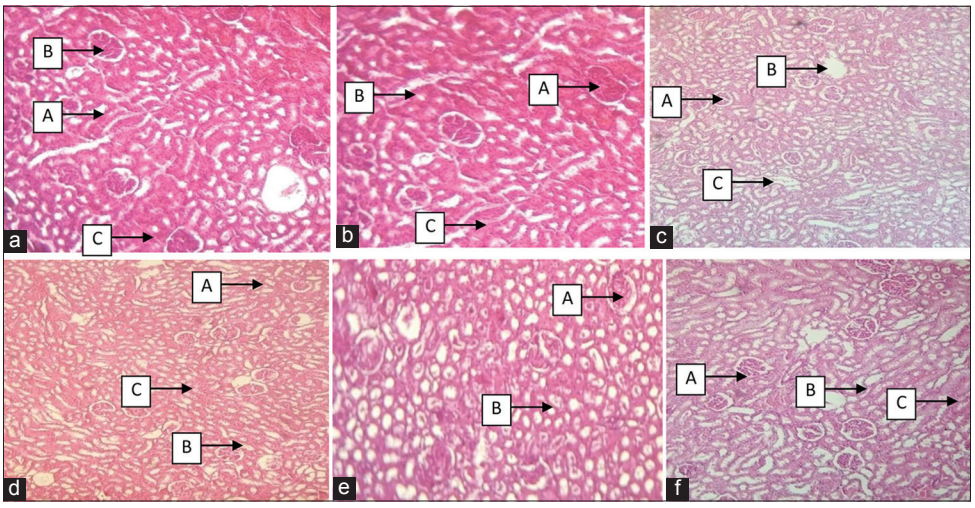

Figure 6: (a-f) Effect of palm oil and palm oil extracts on kidney after 48-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

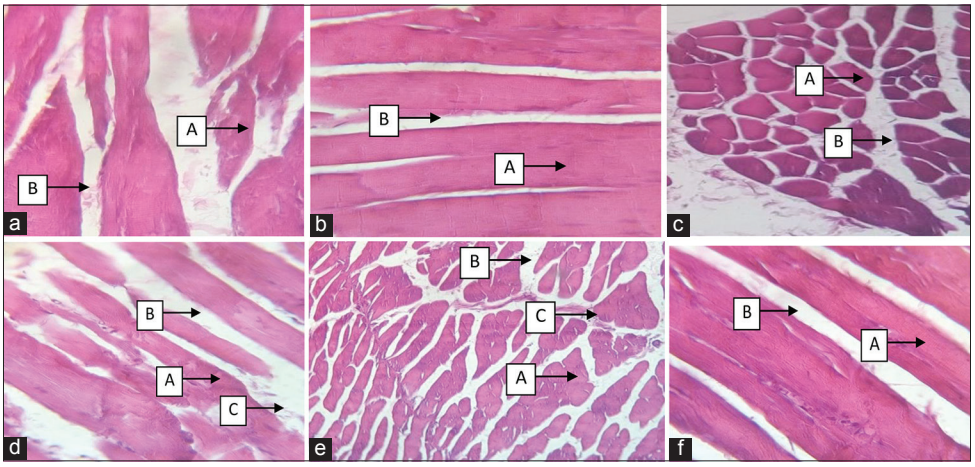

Figure 7: (a-f) Effect of palm oil and palm oil extracts on histology of the skeletal muscle tissue after 12-h post-cadmium administration (hematoxylin and eosin ×100). (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

From the histopathological perspective, this study put into consideration an investigation of the ability of palm oil and palm oil extracts to protect the rats from the disruption of membrane integrity in the liver, kidney, and muscle tissues of the rats. The rationale behind the study of membrane integrity in these selected tissues was influenced by the fact that the major sites of cadmium metabolism were the liver and kidney and remain very significant in the induction of the methalothione, a major transporter for heavy metals within the body.[51,57,58] The kidney, on the other hand, is a major site of filtration of several exogenous substances transported body fluids. Furthermore, there are strong evidence from research that has previously implicated cadmium in the alteration of liver and kidney homeostasis.[59-62] The skeletal muscle tissue, on the other hand, serves as a major source of storage for tissue proteins within the mammalian system and plays a vital role in the absorption and storage of several exorgenic metabolites that may hamper metabolic stability. The observations from Figures 1-3a-f revealed no deleterious effects in the liver in all groups revealing very clear and visible rows of normal hepatocytes radiating from central veins (A) and hepatic sinusoids (B) after the various periods of post-cadmium administration. Also reported in the histhopatological study of the kidney [Figures 4-6a-f] and muscle [Figures 7-9a-f] were no significantly observed negative effects of Cd administration in the kidneys and muscles of all the experimental groups indicated by very clear and visible glomeruli indicated by (A), renal tubules (B) renal arteries (C), while the muscle tissues also indicate very visible muscle fibers (A), blood vessels and perimysium (B), and nucleus (C) after 12-, 24-, and 48-h post-cadmium administration periods.

These observations were not in line with previous studies where changes in serum and organ enzyme activities have led to lesion and disruption of these organs in Cd-treated rats.[62-65] A justification for this observation may have come from the time and duration of exposure of the cadmium to the rats as well as the limitation of the H and E method used by this study to detect the barest minimum level of changes that may have occurred within the period of cadmium insult. Evidence available in literature notes that the toxic effects of cadmium in acute and chronic exposures are relatively time and dose dependent but is noticeable within 12–24 h of intoxication which was also observed by this study relative to alteration in enzyme activities compared to the control. The implication

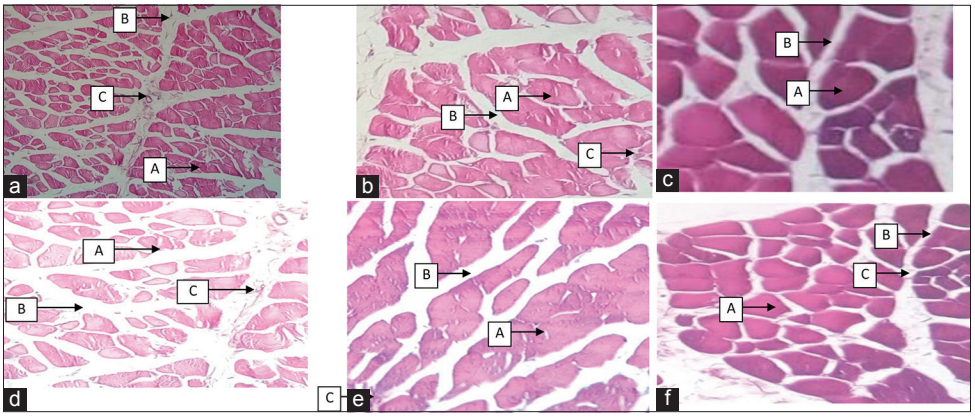

Figure 8: (a-f) Effect of palm oil and palm oil extracts on skeletal muscle tissue after 24-h post-cadmium administration. (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

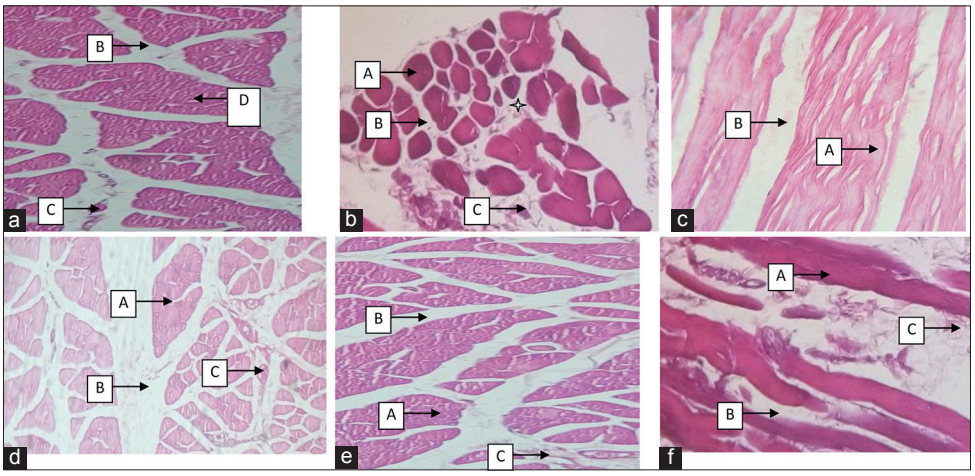

Figure 9: (a-f) Effect of palm oil and palm oil extracts on skeletal muscle after 48-h post-cadmium administration. (a) Control, (b) administered 20 mg/Kg body weight of $CdCl_2$, (c) crude palm oil + 20 mg/Kg $CdCl_2$, (d) silica gel extract + 20 mg/Kg $CdCl_2$, (e) bleached extract + 20 mg/Kg $CdCl_2$, (f) unsaponifiable extract + 20 mg/Kg $CdCl_2$

Table 4: Effect of palm oil and palm oil extracts on activities of tissue and serum AST of rats administered cadmium (units/L)

Tissues	Experimental groups (n=4)					
	A	B	C	D	E	F
12 h						
Liver % change	79.22±2.57^{a}	167.88±3.02^{b} (111.91)	89.30±7.25^{c} (12.7)	89.55±2.44^{c} (13.04)	141.54±5.39^{d} (78.67)	108.65±5.78^{e} (37.15)
Kidney % change	68.37±4.05^{a}	113.06±4.42^{b} (65.28)	77.30±2.52^{a} (13.06)	71.90±4.67^{a} (5.16)	94.61±4.05^{c} (38.38)	100.78±6.02^{c} (47.40)
Heart % change	52.77±3.38^{a}	55.23±2.51^{a} (4.66)	56.84±3.43^{a} (7.71)	59.62±3.51^{a} (12.98)	54.81±2.80^{a} (3.87)	55.21±2.60^{a} (4.66)
Brain % change	46.76±4.35^{a}	50.73±3.20^{a} (8.50)	49.09±3.57^{a} (4.98)	45.53±3.47^{a} (-2.69)	44.06±1.98^{a} (-5.77)	46.87±2.17^{a} (0.24)
Muscle % change	78.26±0.6.88^{a}	124.24±5.38^{b} (59)	102.34±7.84^{c} (31)	98.12±6.56^{c} (25)	96.64±4.96^{c} (23)	104.82±3.88^{c} (34)
Serum % change	44.11±1.92^{a}	61.63±2.38^{b} (49.91)	50.26±1.89^{a} (13.94)	46.38±2.12^{a} (2.38)	55.36±2.45^{b} (25.50)	61.42±2.41^{b} (28.18)
24 h						
Liver % change	78.67±3.13^{a}	192.27±3.60^{b} (144.41)	106.38±6.42^{c} (35.22)	91.29±5.22^{d} (16.04)	154.12±2.72^{e} (48.96)	126.92±3.16^{f} (61.33)
Kidney % change	63.98±3.58^{a}	125.80±3.94^{b} (78.58)	87.74±2.55^{c} (37.14)	74.67±3.05^{d} (16.71)	109.57±2.31^{e} (71.26)	99.96±3.43^{f} (56.24)
Heart % change	45.74±3.75^{a}	61.18±4.15^{b} (33.76)	63.22±2.18^{b} (38.21)	58.34±4.24^{b} (28.75)	63.80±3.29^{b} (39.48)	57.39±3.07^{b} (25.47)
Brain % change	45.19±2.64^{a}	50.70±2.95^{a} (12.19)	49.31±3.20^{a} (9.11)	50.17±2.16^{a} (11.02)	46.72±3.50^{a} (3.39)	49.50±2.38^{a} (9.54)
Muscle % change	74.62±0.5.83^{a}	130.42±7.80^{b} (74.77)	98.83±7.84^{c} (32.44)	104.21±7.66^{c} (39.65)	108.46±7.69^{c} (45.35)	100.28±4.85^{c} (34.39)
Serum % change	46.00±1.78^{a}	81.65±4.65^{b} (77.50)	59.05±3.39^{cd} (28.37)	54.50±1.82^{c} (18.48)	64.77±3.51^{d} (40.80)	63.11±3.65^{d} (37.20)
48 h						
Liver % change	82.74±4.13^{a}	184.72±4.06^{b} (123.25)	108.85±5.24^{c} (31.56)	86.90±7.46^{d} (5.03)	142.21±3.27^{e} (71.88)	120.24±3.26^{f} (45.32)
Kidney % change	65.89±4.85^{a}	120.68±6.40^{b} (83.15)	88.54±5.55^{c} (34.37)	76.76±4.56^{d} (16.50)	112.75±5.16^{e} (71.12)	94.68±4.30^{f} (43.69)
Heart % change	45.74±3.75^{a}	61.18±4.15^{b} (33.76)	63.22±2.18^{b} (38.22)	58.34±4.24^{b} (27.55)	63.80±3.29^{b} (39.48)	57.39±3.07^{b} (25.47)
Brain % change	47.84±3.75^{a}	49.78±3.56^{a} (4.05)	48.50±4.60^{a} (1.38)	51.74±4.61^{a} (8.15)	45.27±2.08^{a} (-5.37)	49.38±3.85^{a} (3.22)
Muscle % change	76.44±0.8.54^{a}	124.52±8.70^{b} (62.90)	100.38±4.48^{c} (31.32)	94.21±8.60^{c} (23.25)	115.64±8.79^{d} (51.28)	98.82±7.58^{c} (29.28)
Serum % change	48.00±2.87^{a}	88.56±6.45^{b} (84.50)	57.50±4.93^{cd} (19.79)	55.62±2.28^{c} (15.88)	66.87±3.51^{d} (39.31)	64.16±4.56^{d} (33.67)

All values are expressed as mean±SD, values sharing different alphabet superscript on the same row showed a significant difference with a indicating no significant difference from Group A; b indicates significant difference from a but not from Group B; c indicates significant difference from all other groups but not from Group C; d indicates a significant difference from other groups but not from Group D, e indicates a significant difference from Group A and all other groups, while f indicates a significant difference from all other groups but not from Group F. Values expressed in bracket indicate percentage change in enzyme activities relative to control. A: Control, B: Administered 20 mg/kg body weight of $CdCl_2$, C: Crude palm oil (CPO) + 20 mg/kg $CdCl_2$, D: Silica gel extract (SGE) + 20 mg/kg $CdCl_2$, E: Bleached extract (BE) + 20 mg/kg $CdCl_2$, F: Unsaponifiable extract (UPE) + 20 mg/Kg $CdCl_2$

of these submissions, therefore, is that the alteration of enzyme activities in the presence of endogenous poisons and contaminants may not necessarily imply the disruption of cellular ultrastructure.

CONCLUSION

The evidence revealed in this study leads to a conclusion that the pre-administration of palm oil and palm oil extracts confers protection to animals acutely administered cadmium and the modulation of activities of serum and tissue aminotransferases. Relative to specification, it was also observed that CPO and SGE conferred more protection to the tissues and prevention of alteration on the activities of tissue and serum aminotransferases, and this may be likened to the high level of phytonutrients in the form of carotenoids and tocotrienol contents in them.

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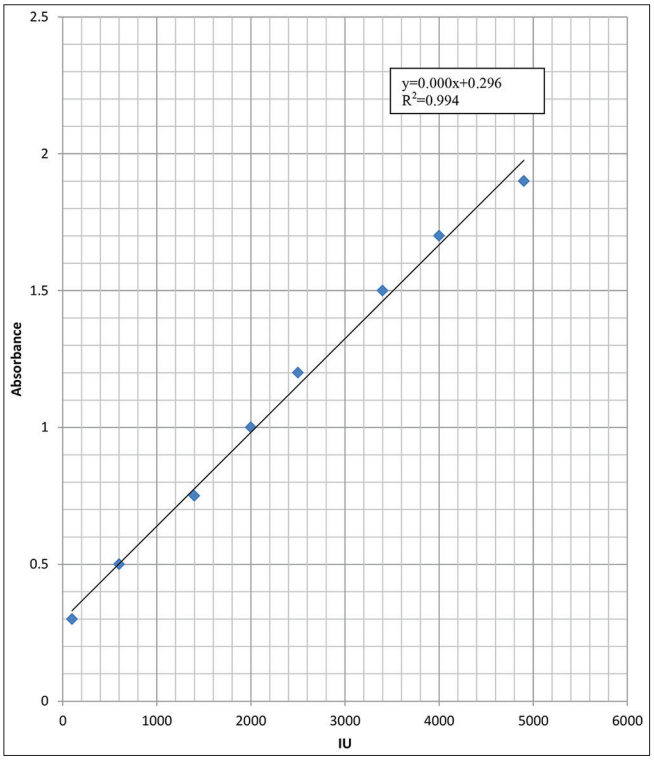
REFERENCES

1. Ricard AC, Daniel C, Anderson P, Hontela A. Effects of subchronic exposure to cadmium chloride on endocrine and metabolic functions in rainbow trout *Oncorhynchus mykiss*. Arch Environ Contam Toxicol 1998;34:377-81.
2. Amutha C, Subramanian P. Cadmium alters the reproductive endocrine disruption and enhancement of growth in the early and adult stages of Oreochromis mossambicus. Fish Physiol Biochem 2013;39:351-61.
3. Ting Z, Yu Z, DeLiang LI, Tiao X, LI J. Exposure of silver carp (*Hypophthalmichthys molitrix*) to environmentally relevant levels of cadmium: hematology, muscle physiology, and implications for stock enhancement in the Xiangjiang River (Hunan, China). Sci China (Life Sci) 2013;56:66-72.
4. Ezedom T, Asagba,SO. Effect of a controlled food-chain mediated exposure to cadmium andarsenic on oxidative enzymes in the tissues of rats. Toxicol Rep 2016;3:708-15.
5. Asagba SO. Alteration in the activity of oxidative enzymes in the tissues ofmalewistar albino rats exposed to cadmium? Int J Occup Med Environ Health 2010;23:55-62.
6. Merian, E. Metals and their Compounds in the Environment: Occurrence, analysis and Biological Relevance. New York: Weinheim' 1991. p. 469-79.
7. North Carolina National Toxicology Program. Tenth Report on Carcinogenesis. Department of Health and Human Health Services. Research Triangle Park Nc; 2000; 111-42-111-44.
8. Domingo JL. Metal-induced developmental toxicity in mammals: A review. J Toxicol Environ Health 1994;42:123-41.
9. Lee WK, Abouhamed M, Thevenod F. Caspase dependant and independent pathways for cadmium induced apoptosis in cultured kidney proximal tubule cells. Am J Physiol 2006;291:823-32.
10. Cottrell RC. Introduction: Nutritional aspects of palm oil. Am J Clin Nutr 1991;53:989S-1009.
11. Jianxun X, Zahir AS. Cadmium induced apoptosis in rat kidney epithelial cells involves decrease in nuclear factor- *Kappa* B activity. Toxicol Sci 2006;91:299-308.
12. Chew BP, Park JS. Carotenoid action on the immune response. J Nutr 2004;134:257-61.
13. Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. J Nutr 2001;131:369S-73S.
14. Goh SH, Loh TP, Huang JM, Choo YM, Hew NF. Antioxidants in palm oil: Their chemistry and some insights from feeding experiments on rabbits. In: Ong AS, Packer L, editors. Biological Oxidants and Antioxidants: Molecular Mechanisms and Health Effects. Champaign, Ill, USA: American Oil Chemists Society; 2001. p. 274-83.
15. Packer L. Protective role of vitamin E in biological systems. Am J Clin Nutr 1991;53:1050S-1055S.
16. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000;342:1266-71.
17. *Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: A narrative review and clinical perspective of prospective data. Hepatology 2010;52:1156-61.*
18. Goessling W, Massaro JM, Vasan RS, D'Agostino RB Sr, Ellison RC, Fox CS, *et al.* Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. Gastroenterology 2008;135:1935-44, 1944.e1.
19. Schindhelm RK, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ, *et al.* Alanine aminotransferase and the 6-year risk of the metabolic syndrome in caucasian men and women: The hoorn study. Diabet Med 2007;24:430-5.
20. Walker N, Lorimer AR. Coronary heart disease: Epidemiology, pathology and diagnosis. In: Lindsay G, Gaw A, editors. Coronary Heart Disease Prevention. A Handbook for the Health Care Team. 2nd ed. London: Churchill Livingstone; 2004. p. 311-56.
21. Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: Potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol 1999;94:1018-22.
22. Swapna G, Reddy AG. Effect of cadmium on organ biomarkers and evaluation of certain adaptogens in broilers. Toxicol Int 2011;18:47-9.
23. McGoldrick TA, Lock EA, Rodilla V, Hawksworth GM. Renal cysteine conjugate C-S lyase mediated toxicity of halogenated alkenes in primary cultures of human and rat proximal tubular cells. Arch Toxicol 2003;77:365-70.
24. Mishra M, Acharya UR. Protective action of vitamins on the spermatogenesis in lead-treated swiss mice. J Trace Elem Med Biol 2004;18:173-8.
25. Nwokocha CR, Owu DU, Ufearo CS, Iwuala MO. Comparative study on the efficacy of *Garcinia kola* in reducing some heavy metal accumulation in liver of wistar rats. J Ethnopharmacol 2011;135:488-91.
26. Twumasi P, Nsiah K, Osei EY. Treatment of lead-poisoned rats through oral administration of palm oil extracts. Afr J Biochem Res2014;8:43-51.
27. Ahmad AL, Chan CY, Abd-Shukor SR, Mashitah,MD. Recovery of oil and carotenes from palm oil mill effluent (POME). Chem Eng J 2008;141:383-6.
28. Patterson HW. Bleaching and Purifying Fats and Oils. Theory and Practice. Kalar Lumpur: AOCS Press; 1992. p. 45-65.
29. Coursey DG. The deterioration of palm oil during storage. J West Afr Sci Assoc 2000;7:101-22.
30. AOAC. Association of Official Analysis Chemist. Official Methods of Analysis of the Association of Official Analytical Chemiast. 17th ed. Gaithersburg: William Horwtiz; 2000.
31. Palm Oil Research Institute of Malaysia (PORIM). The PORIM Test Method. Kuala Lumpur: Palm Oil Research Institute of Malaysia; 1990.
32. Johnson W, Slaga TJ. Final report on the safety assessment of *Elaeis guineensis* (Palm) oil, *Elaeis guineensis* (Palm) kernel oil, hydrogenated palm oil and hydrogenated palm kernel oil. Int J Toxicol 2000;19:7-28.
33. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Cadmium. Draft for Public Comment. Atlanta, US: US Department of Health and Human Services; 2008.
34. Festing MF. Design and statistical methods in studies using animal models of development. ILAR J 2006;47:5-14.
35. Jatinder S. National centre for replacement, refinement, and

reduction animals in research. Experimental design/statistics. J Pharm Pharmacother 2012;3:87-9.
36. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
37. Zhang C, Tian X, Zhang K, Li GY, Wang HY, Wang JH, *et al.* Protective effects of *Foeniculum vulgare* root bark extract against carbon tetrachloride-induced hepatic fibrosis in mice. World J Gastroenterol 2017;23:5722-31.
38. Oduola T, Adeniyi FA, Adenaike FA, Ogunyemi EO, Bello IS, Idowu TO. Haematological response to intake of unripe *Caricapapaya* aqueous extract. IJMR 2009;1:20-5.
39. Dongmo FF, Annie NN, Adélaide DM, Florian S, Inocent G. Effect of heating and of short exposure to sunlight on carotenoids content of crude palm oil. J Food Process Technol 2014;5:314.
40. Osei EY. Treatment of Lead-poisoned Rats through Oral Administration of Palm Oil Extracts; Published M. Phil Thesis Submitted to the Department of Biochemistry and biotechnOlogy, Kwame Nkrumah University of Science and Technology; 2013.
41. Idoko O, Bwai MD, Emmanuel SA, Thomas SA. Effect of bleaching and degumming on the physicochemical properties and antioxidant activity of palm oil. Res J Eng Appl Sci 2013;2:343-5.
42. Asagba SO. Role of diet in absorption and toxicity of oral cadmium: A review of literature. Afr J Biotech 2008;8:7428-36.
43. Asagba SO, Obi FO. A comparative evaluation of the biological effects of environmental cadmium-contaminated control diet and laboratory-cadmium supplemented test diet. Biometals 2005;18:155-61.
44. Eriyamremu GE, Ojimogho SE, Asagba SO, Osagie VE. Palm oil induced changes in ocular tissue lipid peroxidation, antioxidant enzymes and ATPases of rabbits in cadmium toxicity. Food Chem Toxicol 2008;46:3155-8.
45. Tito A, Carola A, Bimonte M, Barbulova A, Arciello S, de Laurentiis F, *et al.* A tomato stem cell extract, containing antioxidant compounds and metal chelating factors, protects skin cells from heavy metal-induced damages. Int J Cosmet Sci 2011;33:543-52.
46. Nwokocha CR, Owu DU, Nwokocha MI, Ufearo CS, Iwuala MO. Comparative study on the efficacy of *Allium sativum* (garlic) in reducing some heavy metal accumulation in liver of wistar rats. Food Chem Toxicol 2012;50:222-6.
47. Fariss MW. Cadmium toxicity: Unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes.Toxicology 1991;69:63-77.
48. Bharavi K, Reddy AG, Rao GS, Reddy AR, Rao SV. Reversal of cadmium-induced oxidative stress in chicken by herbal adaptogens *Withania somnifera* and *Ocimum sanctum*. Toxicol Int 2010;17:59-63.
49. Asagba SO, Eriyamremu GE. Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet. J Nutr Environ Med 2007;16(1-3):267-74.
50. Guilhermino L, Soares AM, Carvalho AP, Lopes MC. Effects of cadmium and parathion exposure on hematology and blood biochemistry of adult male rats. Bull Environ Contam Toxicol 1998;60:52-9.
51. Nordberg GF, Jin T, Wu X, Lu J, Chen L, Lei L, *et al.* Prevalence of kidney dysfunction in humans - relationship to cadmium dose, metallothionein, immunological and metabolic factors. Biochimie 2009;91:1282-5.
52. Giannini E, Botta F, Fasoli A, Ceppa P, Risso D, Lantieri PB, *et al.* Progressive liver functional impairment is associated with an increase in AST/ALT ratio. Dig Dis Sci 1999;44:1249-53.
53. Anahat D, Randolph HS. Liver disease. In: Anesthesia and Uncommon Diseases. 6th ed. Pennsylvania: Saunders Elsevier; 2012. p. 162-214.
54. Chen F, Ding M, Castranova V, Shi X. Carcinogenic metals and NF-kappaB activation. Mol Cell Biochem 2001;222:159-71.
55. Ani EJ, Nna VU, Owu DU, Osim EE. Effect of chronic consumption of two forms of palm oil diet on serum electrolytes, creatinine and urea in rabbits. J Appl Pharm Sci 2015a;5:115-9.
56. Ani EJ, Owu DU, Osim EE. Causative factors and type of anaemia developed in rabbits following chronic consumption of thermally oxidized palm oil diet. J Med Sci 2015b;15:61-70.
57. Cadmium and Cadmium Compounds. IARC Monogr Eval Carcinog Risks Hum 2009;58:119-237.
58. Angshuman S, Geethanjali R, Vishnvardhan K. A brief interview on the effect of cadmium toxicity from cellular to organ level. Int J Bio-Tech Res 2013;3:17-36.
59. Imam AA, Usama MM, Ekbal TW, Mervat N. Effects of cadmium on some histopathological and histochemical characteristics of the kidney and gills tissues of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and Vitamin E. J Fish Aqu Sci 2013;8:553-80.
60. El-Refaiy AI, Eissa FI. Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi J Biol Sci 2013;20:265-80.
61. Dudley RE, Svoboda DJ, Klaassen C. Acute exposure to cadmium causes severe liver injury in rats. Toxicol Appl Pharmacol 1982;65:302-13.
62. Alkushi AG, Sinna MM, EL-Hady M, ElSawy NA. Structural changes in adult rat liver following cadmium treatment. Pak J Nutr 2018;17:89-101.
63. Okpoghono J, Achuba FI, George BO. Protective effect of *Monodora myristica* extracts on crude petroleum oil-contaminated catfish (*Clarias gariepinus*) diet in rats. Int J Vet Sci Med 2018;6:117-22.
64. Wang CS, Chang TT, Yao WJ, Wang ST, Chou P. Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. J Formos Med Assoc 2012;111:201-8.
65. Aulbach AD, Amuzie CJ. Biomarkers in nonclinical drug development. In: A Comprehensive Guide to Toxicology in Nonclinical Drug Development. 2nd ed. San Francisco, CA: Elsevier Science Publishing Co Inc.; 2017. p. 441-7.

APPENDIX



Appendix I: Standard calibration curve for levels of Vitamin E