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Effect of Ethanolic Extract of Avocado (*Persea americana)* Fruits Peels on Serum Hepatic and Renal Indices in Male Wistar Albino Rats Fed with Crude Oil Contaminated Diet

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ABSTRACT: This study was aimed at investigating the effect of ethanolic extract of *Persea americana* fruit peels on serum hepatic and renal indices in crude oil contaminated diet stimulated toxicity in male Wistar rats using appropriate standard methods. The findings of this study revealed that the treatment of rats exposed to contaminated diets with the varying doses of *P. americana* fruit peels extract had no statistically ($p>0.05$) significant effect on the AST, ALT and ALP compared to those exposed to only crude oil contaminated diet. However, there were observed significant decrease in ACP activities in crude oil extract treated group when compared to the crude oil control group. Conversely, glucose, total protein, albumin and globulin levels showed no significant difference across the crude oil extract treated group when compared to the crude oil control group. Also it was observed that there was a significant decrease ($p<0.05$) in levels of serum urea, potassium, calcium and chloride in the crude oil extract treated group when compared to the crude oil control group in a dose dependent manner. These activities potentiated by the ethanolic extract of *P. americana* fruit peels could be attributed to its medicinal properties.

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The toxicity of crude oil cuts across various organs in the body, and this could be ascribed to the composition of crude oil. Crude oil contains an array of free radical inducing compounds such as cadmium, arsenic, lead, polycyclic aromatic hydrocarbons (PAHs) etc (Fowzia and Fakhruddin, 2018). These xenobiotic found in crude oil, mediate various form of cellular aberration ascribed to various mechanism of actions of its individual components. Some of the toxic effect of these xenobiotic could be spontaneous or long time effect of bioaccumulation (Chinedu and Chukwuemeka, 2018). Whether spontaneous or via bioaccumulation mechanism the composition of crude oil induce oxidative stress, a condition in which the level of oxidant exceeds the threshold of the pro-oxidant in the cells (Patel *et al*., 2020; Holme *et al*., 2019). This condition is marked by antagonistic cellular activities such as uncontrolled regulation of metabolic activities of influx and efflux of materials in and out of the cell, distortion in the regulation of key metabolic enzymes, cellular genetic activities of; replication, transcription and translation. Vulnerability of human exposure to crude oil toxicity are hardly inevitable due to the mode of entry into the body through inhalation, ingestion, skin absorption (primary contact) or secondary contact (food borne chain activities) (Onwurah *et al*., 2007; Mordi *et al*., 2021).Several studies have been carried out using various medicinal plants in addressing crude oil toxicity. Achuba and Ichipi-Ifukor (2020) and Achuba

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(2018) reported that the methanol leaf extract of *Vernonia amygdalina* attenuated for the serum alterations of hepatic enzymes and renal indices, tissues antioxidants enzymes, oxidase enzymes and energy metabolizing enzymes caused by crude oil toxicity in Wistar rats. Onakurhefe *et al.* (2020) reported that the use of various blends of crude extract of *Elaeis guineensis* ameliorated genotoxicity indices such as fragmented DNA and micronucleated erthyrocytes caused by crude oil. *Persea americana* commonly known as avocado have been used since time immemorial in folklore medicine, with various parts of the plants such as the pulps, leaves, stems, peels, seeds are used to prepare various decoctions of local herbal remedy, link to pathological conditions associated with inflammation, infections, carcinogens, radical activity etc. Their application in addressing this disease conditions are attributed to the relative abundance of phytonutrients such as polyphenol, carotenoids, tocopherols which have been identified to be present by previous authors (Dabas *et al*., 2013; Gouegni and Abubakar, 2013; Hurtado-Fernández *et al*., 2014). Hence, the objective of this paper was to investigate the Effect of Ethanolic Extract of Avocado (*Persea americana)* Fruits Peels on Serum Hepatic and Renal Indices in Male Wistar Albino Rats Fed with Crude Oil Contaminated Diet

MATERIALS AND METHODS

Materials: All reagents used for this study were of analytical grade.

Equipment: Refrigerating centrifuge, GCMS chromatography Uv-Vis Spectrophotometer, Water bath, etc.

*Collection of Plant Material and Identification:*Fresh fruits peels of *Persea Americana* were collected from Agha Street in Obiaruku, Delta state Nigeria. The study plant specimen was authenticated and identified at the Department of Botany, Delta State University, Abraka-Nigeria.

*Animals:*Sixty-four (64) healthy male Wistar albino rats weighing between 100 and 120g were obtained from the Animal House, Faculty of Basic Medical Science, Delta State University, Abraka. They were acclimatized for two (2) week. The rats were maintained on growers mash (Top feed, Premier feed mills Co. Ltd, Ibadan, Oyo State) and water *ad libitum.*

*Extract Preparation:*The Fresh fruits of *P. americana* were washed with distilled water to remove debris and pulp were then removed to obtain the peels, which were then air dried for two weeks, till a constant weight was obtained and then reduced to coarse powder using a manual grinder. 100g of coarsely powdered peels was extracted with 400ml of ethanol (95%v/v) using cold maceration for 24hours. The extract was then filtered through cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman No. 1 filter paper. The resulting extract was then concentrated at 50^{o}C in a rotary evaporator for 2hours. The resulting concentrate contains two layers of the extract and oil. The oil part was then removed by using diethyl ether in ratio of 1:1 of the extract. The resulting non-oil fraction was then subjected to dryness in a water bath maintained at 50^{o}C and evaporated to dryness to yield a dark brown mass. The obtained extract was stored at 4^{o}C until when required for use.

*Experimental design:*In the experiment, a total of sixty-four (64) male Wistar albino rats were used. They were randomly divided into eight (8) groups containing eight (8) rats in each group. All these animals were acclimatized for fourteen (14) days before experimental exposure of twenty eight (28) days. The animals were housed in plastic (polypropylene) cages using paddy husk bedding. The rats were provided with grower‘s mash and water *ad-libitum*. The experimental animal models were grouped as follows:

Group A: control that received normal feed and water only.
Group B: rats fed with normal diet, that received 100mg/kg of ethanol extract of peels of *P. americana*
Group C: rats fed with normal diet, that received 200mg/kg of ethanol extract of peels of *P. americana*
Group D: rats fed with normal diet, that received 300mg/kg of ethanol extract of peels of *P. americana*
Group E: rats fed with normal diet, that received 200mg/kg of Vitamin C
Group F: rats fed with crude oil contaminated diet only (4.0ml/100g)
Group G: rats fed with crude oil contaminated, that received 100mg/kg of ethanol extract of peels of *P. americana*
Group H: rats fed with crude oil contaminated, that received 200mg/kg of ethanol extract of peels of *P. americana*
Group I: rats fed with crude oil contaminated, that received 300mg/kg of ethanol extract of peels of *P. americana*
Group J: rats fed with crude oil contaminated, that received 200mg/kg of vitamin C
Crude oil contamination proportions were prepared as prescribed by Achuba, (2019).

Biochemical assays: Collection and preparation of sera samples and tissue homogenate: The study period

lasted for 28days after which the rats were fasted for 12 hrs. This was followed by the cervical decapitation of the rats. Each rat was placed on dorsal surface, and a laparotomy wascarried out to expose the internal organs. Blood samples were collected in plain tubes and allowed to clot and the serum separated by centrifuging at 3000rpm for 10min. The sera samples were then subjected to various biochemical analyses. The samples of sera samples were stored at 4^{o}C until they were required for use.

*Determination of biochemical parameters:*Serum alanine transaminase and aspartate transaminase (Reitman and Frankel 1957), alkaline phosphate was carried out according to the methods of the Deutsche Gesellschaft fur klinische Chemie DGKC (1972), total protein and albumin (Tietz, 1995; Doumas*et al.* 1971), bilirubin (Malloy and Evelyn, 1937), Urea (Weatherburn 1967), creatinine (Bartels and Bohmer, 1972), sodium (Maruna 1958; Trinder, 1951), chloride (Skeggs and Hochestrasser 1964), potassium (Terri and Sesin 1959), calcium (Cali *et al*., 1972),bicarbonate (Forrester *et al.*, 1976)

RESULTS AND DISCUSSION

The use of natural products in medicine and for the management of toxicities is increasingly been encouraged and explored by scientists' world over (Mordi *et al*., 2021; WHO, 2019). In the past five years, several scientists have attempted to show the increased relevance of plant-based products of great economic importance and value, and some other plant-based products regarded as wastes in the management of food chain mediated petroleum toxicities (Achuba et *al*., 2018a; 2018b; Achuba and Offor, 2020; Okpoghono, *et al*., 2018; Onakhurefe *et al*., 2020a; 2020b; 2022; Auruoren, *et al*., 2022). Findings in this study revealed highly elevated levels of serum enzyme activities of liver (AST, ALT, ACP and ALP) following crude oil contaminated diet consumption.

Table 1: Effect of *P.americana* ethanolic extract on serum liver function parameters of rats fed petroleum contaminated diets

Group	AST(UL^{1})	ALT(UL^{-1})	ACP(UL^{-1})	ALP(UL^{-1})
A	56.29±5.61^{a}	42.25±1.61^{ac}	5.03±0.54^{a}	109.48±6.29^{a}
B100	54.64±0.92^{a}	40.52±0.65^{a}	5.02±0.77^{a}	108.46±6.30^{a}
C200	54.21±5.57^{a}	40.19±1.34^{a}	5.12±0.78^{a}	110.05±8.79^{a}
D300	56.35±1.63^{a}	39.71±2.06^{a}	5.28±0.51^{a}	113.49±2.58^{a}
EVIT C	55.54±6.39^{a}	41.12±0.80^{a}	4.69±0.98^{a}	103.89±7.70^{a}
FCO	68.39±2.49^{b}	56.37±6.49^{b}	12.16±1.40^{b}	147.96±6.93^{b}
G CO+100	63.88±2.22^{ab}	53.59±6.14^{bc}	8.54±1.50^{c}	138.51±3.93^{b}
H CO+200	64.24±1.94^{ab}	53.90±5.70^{b}	7.67±0.85^{ac}	138.19±2.79^{b}
ICO+300	65.27±2.07^{ab}	54.15±6.33^{b}	8.53±0.69^{c}	137.47±3.45^{b}
JCO+VIT C	60.25±1.01^{ab}	45.53±3.28^{a}	7.13±0.86^{ac}	132.81±9.84^{b}

All values are expressed as Mean± Standard deviation. Values with the same alphabet superscript on the same column indicates there is no significant difference (p>0.05). Values followed by different alphabet superscript on the same column indicates there is a significant difference (p<0.05).

***Key**: A= Normal control; B= 100mg/Kg P. americana Extracts; C= 200mg/Kg P. americana extract; D= 300 mg/kg P. Americana extract; E= 100mg/Kg Vit. C; F= Crude oil contaminated diet (4.0ml/Kg of feed) (COCD); G= COCD +100mg/Kg P. americana Extracts; H= COCD+200mg/Kg P. americana Extracts; J= COCD+300mg/Kg P. americana Extracts; J= COCD+Vit. C*

These observations are in agreement with previous studies by Okpoghono *et al*., 2018a; Achuba and Ichipi-Ifukor, 2020). These liver enzymes are well known markers for liver function as their increasing concentration within the blood system have been severally implicated in the disruption of liver architectural stability. It is of importance to note that rise in alkaline phosphatase has been implicated in metabolic bone diseases as well as the liver. In the case of the acid phosphatase however, its rise has been implicated in the uncontrolled increase in cell differentiation and is also used as an early marker for carcinogenesis (Halaby *et al*., 2001; Kong *et al*., 2013). It is also of importance to note that petroleum hydrocarbon has been implicated in several forms of cancer development (Peng *et al*., 2010) thus in giving credence to the above hypothesis, we observed a serious level of drop in the serum glucose levels in rats fed petroleum contaminated diet which may be related to increased level of glucose consumption implicated by a possible uncontrolled differentiation of the hepatocytes in response to hepatocellular carcinoma. Serum total protein levels are important indicators for a functioning liver as it is known as a sum of two important proteins albumin and globulin (Khanna *et al*., 2017). These proteins which are known to be synthesized in the liver are known to fall seriously in supply within the serum once the liver has been compromised. In another development and in relation to our earlier postulation of a possible uncontrolled hepatocellular differentiation, it is important to note that in liver undergoing energy crises protein metabolites are known to be mobilized towards the continued synthesis of glucose so as to meet up the increasing energy demand from the cells (Litwack, 2018; Rui, 2010).

Table 2: Effect of P .americana ethanolic extract on serum liver function parameters of rats fed petroleum contaminated diets

Group	Glucose(gdl^{-1})	Total Protein (gdl^{-1})	Albumin (gdl^{-1})	Globin(gdl^{-1})
A	132.05±5.88^{ac}	6.34±0.74^{a}	4.38±0.80^{a}	2.82±0.18^{a}
B100	132.24±3.59^{ac}	6.29±1.19^{ab}	4.44±0.91^{a}	2.95±0.28^{a}
C200	132.08±3.01^{ac}	6.29±0.73^{ab}	4.13±1.11^{a}	2.96±0.08^{a}
D300	131.50±2.98^{ac}	6.25±0.76^{ab}	4.49±0.98^{a}	3.18±0.28^{a}
EVIT C	133.81±2.51^{ab}	6.22±0.72^{ab}	4.48±0.88^{a}	3.05±0.36^{a}
FCO	120.54±0.78^{c}	4.20±0.42^{b}	2.95±1.15^{a}	1.46±0.07^{b}
G CO+100	125.36±1.46^{ac}	4.32±0.73^{ab}	3.22±0.47^{a}	2.01±0.12^{b}
H CO+200	124.80±6.17^{ac}	4.88±0.19^{ab}	3.22±0.58^{a}	2.39±0.41^{b}
ICO+300	128.06±5.56^{ac}	4.88±0.13^{ab}	3.04±0.28^{a}	2.09±0.03^{b}
JCO+VIT C	131.32±3.16^{ac}	6.67±0.56^{a}	4.50±0.83^{a}	2.95±0.17^{b}

All values are expressed as Mean± Standard deviation. Values with the same alphabet superscript on the same column indicates there is no significant difference (p>0.05). Values followed by different alphabet superscript on the same column indicates there is a significant difference (p<0.05)

This may have accounted for the shortfall in total protein as well as albumin and globulin found in the serum. Though the *P. americana* fruit peels extract was not able to modulate the uncontrolled rise in serum liver functional enzymes such as (ALT and AST), their ability to reverse to near normal the rise in ACP, ALP and drop in total protein, albumin, globulin and glucose gives an insight on their possible role as anticancer agent. These assertions is given greater credence as the treatment of rats without petroleum contaminated diet using varying doses of *P. americana* showed no significant difference in relation to the control as well as the standard antioxidant vitamin C.

Table 3: Effect of *P.americana ethanolic* extract on kidney function parameters of rats fed petroleum contaminated diets

Groups	Serum creatinine (mg/dl)	Serum Urea (mg/dl)
A	2.14±0.16^{a}	18.5±0.80^{ac}
B100	1.89±0.08^{a}	16.4±0.99^{a}
C200	1.77±0.15^{a}	16.5±0.86^{a}
D300	1.96±0.19^{a}	16.5±0.58^{a}
E VIT C	1.96±0.07^{a}	19.0±0.94^{ac}
FCO	3.56±1.06^{b}	27.8±3.33^{b}
GCO+100	2.86±0.18^{ab}	22.1±1.06^{c}
HCO+200	2.89±0.13^{ab}	22.3±1.30^{c}
ICO+300	2.67±0.22^{ab}	21.8±1.07^{c}
JCO+ VIT C	2.07±0.13^{a}	18.9±1.02^{ac}

All values are expressed as Mean±SD. Values with the same alphabet superscript on the same column indicates there is no significant difference (p>0.05). Values followed by different alphabet superscript on the same column indicates there is a significant difference (p<0.05)

Table 4: Effect of *P.americana* ethanolic extract on kidney function parameters of rats fed petroleum contaminated diets

Groups	Serum Na+(mg/dl)	Serum K+ (meq/L)	Serum Ca2+ (meq/L	Serum Cl- (meq/L	Serum HCO_3 (Mmol/L
A	90.0±3.85^{a}	5.21±1.06^{a}	6.85±0.20^{ac}	12.6±0.94^{ab}	24.6±1.18^{a}
B100	82.9±6.57^{a}	4.87±0.37^{a}	6.30±0.49^{a}	10.6±0.59^{a}	22.6±1.36^{a}
C200	84.2±6.51^{a}	5.30±0.70^{ac}	6.43±0.46^{a}	10.66±0.59^{a}	21.3±0.68^{a}
D300	85.9±5.28^{a}	4.75±0.37^{a}	5.95±0.46^{a}	10.5±0.56^{a}	21.8±1.86^{a}
E VIT C	87.8±2.28^{a}	5.47±0.63^{a}	5.47±0.65^{a}	11.4±1.40^{a}	21.3±2.30^{a}
FCO	120±10.56^{b}	8.47±0.56^{b}	13.01±2.70^{b}	14.8±0.82^{b}	32.1±1.50^{b}
GCO+100	112±6.02^{b}	7.06±0.47^{bc}	9.68±1.53^{bc}	12.8±0.90^{ab}	30.0±1.10^{b}
HCO+200	110±5.54^{b}	6.89±0.15^{ac}	8.97±0.49^{a}	12.0±0.54^{a}	28.2±1.49^{b}
ICO+300	112±5.49^{b}	6.82±0.65^{ac}	9.03±0.13^{a}	11.9±0.24^{a}	28.7±1.50^{b}
JCO+ VIT C	92.9±6.31^{a}	6.75±0.26^{ac}	7.62±0.56^{a}	10.7±0.67^{a}	22.4±1.70^{a}

All values are expressed as Mean±SD. Values with the same alphabet superscript on the same column indicates there is no significant difference (p>0.05). Values followed by different alphabet superscript on the same column indicates there is a significant difference (p<0.05).

As in earlier studies, it was observed that petroleum contaminated diet led to the disruption of renal embolism. Increase in serum urea and creatinine are linked to drop in glomerular filteration and is a well-known marker for kidney malfunction (Ogbeke *et al*., 2016; Achuba, 2018a; Achuba and Offor, 2020). Increase in serum sodium, potassium and calcium have been linked to the breakdown of several electrogenic pumps within the kidney and liver owing to the increased disruption of ATPASE enzymes within these tissues and are in agreement with the reports of Achuba, (2018a); Orisakwe, (2004) and Uboh (2009). Likewise, the rise in serum chloride ions is in agreement with Ita and Edaga, (2016), it does not agree with Achuba (2018a) that reported a drop in serum chloride ions following consumption of crude oil tainted diets. Like in the study of Achuba, (2018a; 2018b); Achuba and Offor (2020); Onakurhefe *et al*., (2021), the ability of the *P. americana* extracts like the standard antioxidant to modulate to the barest minimum serum urea, creatinine, potassium and

calcium is indicative that the fruit peels has some kidney functional enhancing capabilities.

Conclusion: The administration ethanolic extract of *P. americana* fruits peels attenuated for the biochemical deficits of hepatic and renal functions caused by the intake of crude oil contaminated diet. This findings support the medicinal values attributed to *P. americana*.

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