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Haematological profile and nephroprotective effect of ethylacetate extract of *Persea americana* (Avocado pear) seed in acetaminophen induced wistar rats.

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ABSTRACT

Hematological profile and the nephroprotective activity of ethyl acetate extract of *Persea americana* seeds was investigated in albino rats. Twenty-five (25) rats (125 - 176 g) were randomly grouped into five. Rats in group 1 served as control; group 2 were orally administered with 750mg/kg acetaminophen; animals in group 3 were fed simultaneously with 750 mg/kg acetaminophen and 200mg/kg silymarin; while, groups 4 and 5 were administered with 200 mg/kg and 400 mg/kg of *Persea americana* seed extract respectively, for 14 days. Results of haematological analysis, for groups 3,4 and 5, revealed significant ($p<0.05$) elevation in the concentration of white blood cells and platelets and a decrease in the levels of Hb, RBC, Neutrophil, Monocytes, Eosinophil and PCV as compared to groups 1 and 2. Results on renal function parameters showed that acetaminophen treatment caused nephrotoxicity as evidenced by marked elevation in serum urea and creatinine. Co-administration of *Persea americana* seed extract decreased the rise in these parameters in a dose dependent manner. Serum electrolyte indices also indicated increase in K^+ and Mg^{2+} concentrations, and a decrease in Ca^{2+} concentration for groups 3, 4 and 5. These results revealed that the use of the extract in phytotherapy may produce adverse side effects such as anaemia while ameliorating nephrotoxicity.

INTRODUCTION

Recognition of drug-induced nephrotoxicity as a significant contributor to kidney disease including acute kidney injury (AKI) and chronic kidney disease (CKD) has gained increasing momentum in recent times. Nephrotoxicity constitute a whole gamut of disorders reflecting damage to different nephron segments as a consequence of individual drug mechanisms. Consequences of drug toxicity might include both glomerular and tubular injuries leading to acute or chronic functional changes. Significant paracetamol-induced hepatotoxicity usually triggers nephrotoxicity. Renal insufficiency is reported to occur in 12% of patients exposed to paracetamol toxicity [1]. After oral administration, about 63% of paracetamol is metabolized via glucuronidation and 34% via sulphation primarily in the liver. The water-soluble metabolites consisting of these metabolic pathways are excreted via the kidney. Paracetamol toxicity creates acute tubular necrosis, which is one of the main causes of acute renal failure [2]. Serum urea and creatinine levels may be indicators of acute tubular necrosis induced by paracetamol [3]. The assessment of haematological parameters provide information on inflammation, necrosis, various infections of visceral organs, presence of stress factors [4] as well as the extent of deleterious effect of foreign compound including plant extract on the blood [5].

Different parts of plants are traditionally used in different countries for mitigation of drug or toxin induced hepatic and renal disorders. The fruit of *Persea americana*, commonly known as avocado (other names include; alligator pear or butter fruit), is an edible fruit from Central America which is easily adaptable in tropical regions [6]. The avocado has an olive-green peel and thick pale yellow pulp that is rich in fatty acids such as linoleic, oleic, palmitic, stearic, linolenic, capric, and myristic acids. This fruit is normally used for human consumption, but it has also been used as a medicinal plant [7]. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including antioxidants, and hepatoprotective activities [8]. Avocado is one of the plants that have been widely used in ethno-medicine. In Nigeria, the leaf has

various local names such as Ewépia (Yoruba), Akwukwo Ube oyibo (Igbo), and Ganyenpiya (Hausa). The plant is usually 20m (60 ft.), tall and the leaves are 12.5cm long in alternate arrangement. The flowers are greenish yellow and bear a pear-shaped fruit [9]. Avocado fruits have culinary and nutritional values. The leaves have been shown to possess anti-inflammatory, anti-convulsant, anti-diabetic, and vasorelaxant activities [10]. Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, larvicidal, fungicidal and hypolipidemic [6]. However, there has been little or no documented evidence of avocado seed extract as a nephroprotective agent. Hence the study aimed to evaluate the haematological profile and the nephroprotective activity of aqueous extract of *Persea americana* seeds in a rodent model of actaminophen induced nephrotoxicity.

MATERIALS AND METHODS

Experimental animals

Healthy Wistar albino rats of both sexes weighing between 125-176g were purchased from the animal house, Department of Biochemistry, University of Port Harcourt, Nigeria. The animals were housed in cages and fed with commercial feed and water *ad libitum.*

Collection / preparation of plant extract

Fresh fruits of avocado were obtained from the Fruit Garden Market, in Mile1 Port Harcourt, Nigeria. The fruits were identified at the Herbarium (Voucher Number: UPH/C/102) in the Department of Plant Science and Biotechnology, University of Port Harcourt.

The fruits were cut open to remove seeds and seeds sliced into pieces and air dried before being pulverized with and electrical grinding machine. The pulverized plant sample was extracted in ethyl acetate using soxhlet apparatus to obtain the extract. After the extraction, the extract was concentrated using rotary evaporator and the concentrated extract was weighed and stored in an airtight container in the refrigerator until required for analysis.

Experimental procedure

This research was designed to last for 14 days.The animals were placed into five (5) groups of five rats per group. Group 1 animals served as control and received distilled water and animal feed only. Group 2 were administered with acetaminophen (750mg/kg) only. Group 3 animals were administered with acetaminophen + silymarin (200mg/kg), group 4 were given acetaminophen + extract (200mg/kg) and group 5 were administered with acetaminophen + extract (400mg/kg). The rats were starved after the last administration on the 14th day and sacrificed the next day by cervical dislocation under mild diethyl ether anaesthesia. Blood samples were collected separately into labelled plain and EDTA bottles, for measurement of urea, creatinine and haematological parameters, respectively. Animals were dissected and kidneys were harvested, weighed and processed for histological evaluation.

Determination of haematological parameters

Whole blood collected into EDTA bottle was used to assay for packed cell volume (PCV), and total white blood cell (WBC),

Table 1: Effect of ethyl acetate extract of *Persea americana* seeds on Hematological parameters

Group	Group 1 (distilled water+ Feed)	Group 2 (Acetaminophen - 750mg/kg)	Group 3 (Acetaminophen + Silymarin 200mg/kg)	Group 4 (Acetaminophen +Extract 200mg/kg	Group 5 (Acetaminophen +Extract 400mg/kg)
PCV	40.00±0.41^{a}	39.33±0.33^{a}	38.60±0.40^{a}	38.25±0.85^{a}	38.67±1.76^{a}
Hb	13.33±0.14^{a}	12.58±0.53^{a}	12.88±0.12^{a}	12.34±0.46^{a}	12.02±0.67^{a}
RBC	6.00±0.17^{a}	5.65±0.39^{a}	5.78±0.12^{a}	5.44±0.28^{a}	5.28±0.40^{a}
WBC	5.57±0.23^{a}	6.08±0.68^{a}	9.40±0.59^{b}	9.40±0.59^{b}	8.20±0.53^{b}
Platelets	270.00±7.07^{a}	246.67±14.53^{b}	360.00±20.00^{c}	330.00±11.55^{d}	330.00±20.00^{d}
Neutrophils	25.67±2.3^{a}	38.33±2.03^{c}	27.33±1.45^{a}	25.67±3.48^{a}	34.67±1.45^{b}
Lymphocytes	70.00±2.8^{a}	57.33±3.71^{b}	69.00±1.00^{a}	66.67±4.41^{a}	61.60±1.03^{ac}
Monocytes	3.00±0.41^{a}	3.67±1.20^{a}	2.75±0.25^{a}	2.67±0.33^{a}	3.00±0.58^{a}
Eosinophils	1.25±0.25^{a}	2.00±0.00^{a}	1.67±0.33^{a}	1.67±0.33^{a}	1.00±0.00^{a}

Results are expressed as mean ± SEM (n=5). Values across a row with different superscripts are significant (p<0.05).

lymphocyte and neutrophil counts, eosinophil using standard laboratory techniques [4]

Determination of Kidney indices

Blood sample was centrifuged for 15 min at 3000 rpm to separate the serum which was later stored in a refrigerator. The Serum levels of urea and creatinine were then assayed. Creatinine was measured using alkaline picrate method and Urea concentration was determined by the diacetyl monoxime method using assay kit from Randox laboratories [11]. Determination of serum potassium ,magnesium and calcium concentrations were done using reagent kit [12] .

Histological examination of the kidneys

Histopathological examination was by the method of Tietz [13] with slight modification. The kidneys of rats in all the five groups were fixed in 10% neutral formalin for 48 to 72 hours. The tissues were trimmed and processed for a routine histopathological examination.

The kidney tissues were embedded into paraffin wax and 4-5µm thick sections were cut. All tissue sections were stained with haematoxylin and eosin (H&E) and then examined under a light microscope.

Statistical analysis

Data was represented as mean ±standard error of mean (SEM) of triplicate determinations. The results were analysed by one-way analysis of variance (ANOVA) and, where applicable, Bonferroni's Post-hoc comparison was used to determine significant values. The differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 2: Effects of ethyl acetate extracts of *Persea americana* seeds on renal function parameters.

	Urea (mmol/L)	Creatinin (mmol/L)	Potassium (mmol/L)	Magnessium (mmol/L)	Calcium (mmol/L)
Distilled Water + Feed	7.25 ± 0.38^{a}	83.86 ± 3.05^{a}	4.26 ± 0.27^{a}	2.10 ± 0.03^{a}	$2.16 = 0.25^{a}$
Acetaminophen only (750mg/kg)	7.34 ± 0.85^{a}	103.65 ± 7.68^{b}	3.75 ± 0.25^{b}	2.10 ± 0.04^{a}	$2.65 = 0.03^{b}$
Acetaminophen + Silymarin (200mg/kg)	7.26 ± 0.77^{a}	83.38 ± 1.20^{a}	4.74 ± 0.23^{a}	2.18 ± 0.06^{a}	$2.60 = 0.03^{b}$
Acetaminophen + Extract (200mg/kg)	7.30 ± 0.51^{a}	83.86 ± 5.41^{a}	4.34 ± 0.39^{a}	1.96 ± 0.05^{a}	$2.66 = 0.02^{b}$
Acetaminophen + Extract (400mg/kg)	6.28 ± 0.10^{b}	82.96 ± 0.86^{c}	4.44 ± 0.34^{a}	2.08 ± 0.05^{a}	$2.56 = 0.12^{b}$

Results are expressed as mean ± SEM (n=5). Values down the column with different superscripts are significant ($p<0.05$).

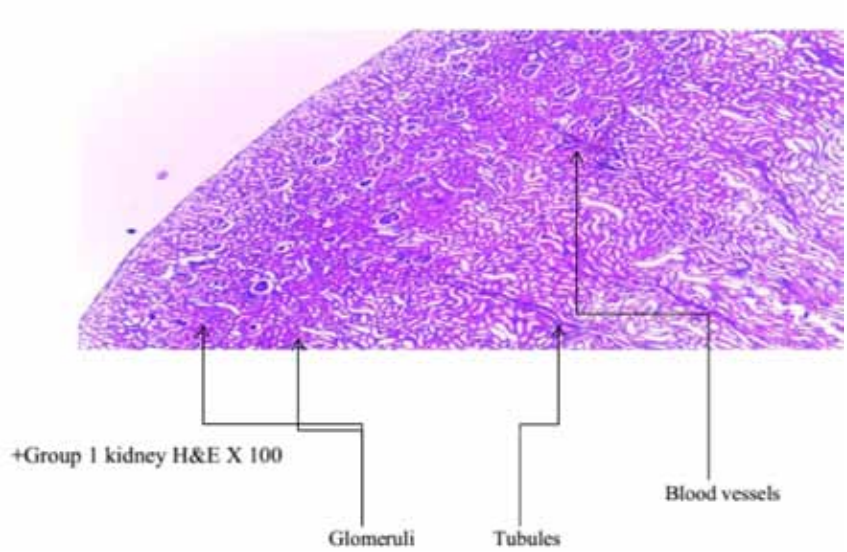

Fig. 1: Control slide of the kidney shows normal histological features.

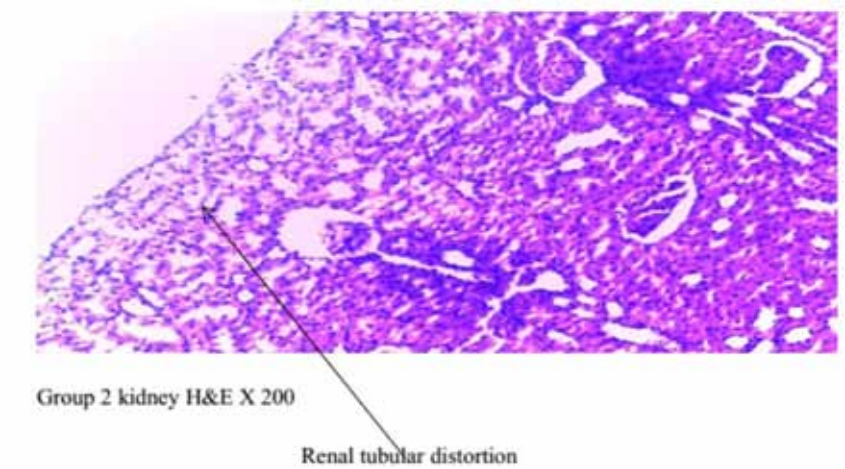

Fig. 2: Histological slide from the kidney, shows tubular damage

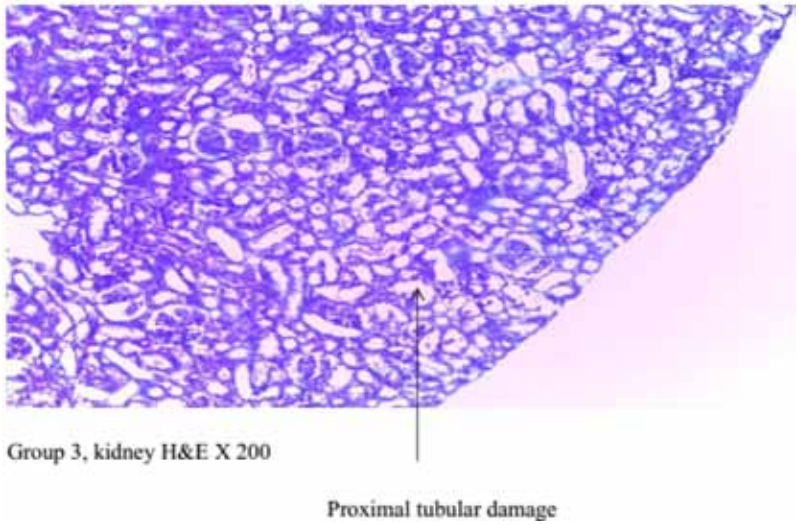

Fig. 3: Histological slide shows mild tubular damage..

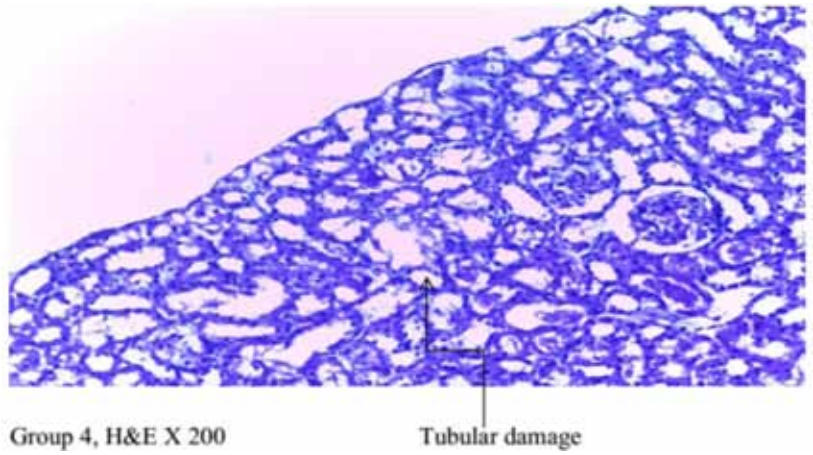

Fig. 4: Histological slides from the kidney shows tubular distortion and epithelial slourghing.

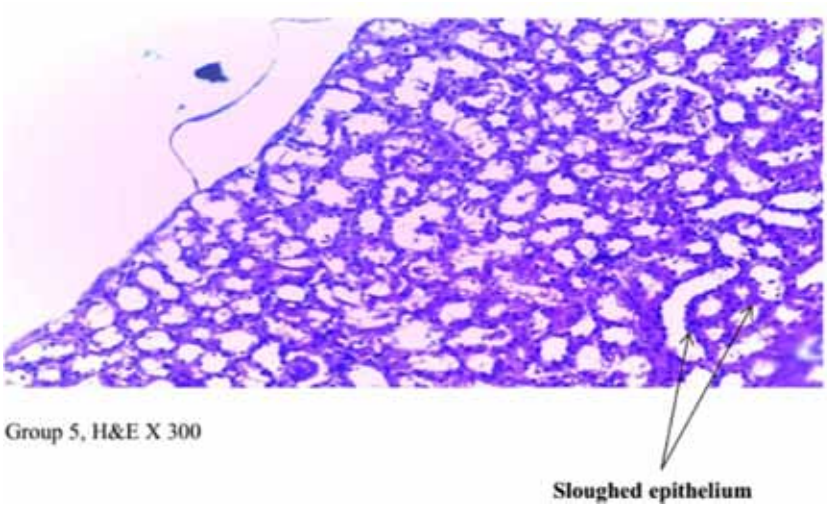

Fig. 5: Histological slides show sloughed epithelium and tubular casts.

DISCUSSION

Results *of haematological profile* (Table 1) revealed that groups 3, 4 and 5 showed significant ($p<0.05$) elevation in the concentration of white blood cells and platelet counts as compared to groups 1 and 2. However, there was a decrease in the levels of PCV, Hb, RBC, Neutrophil, Monocytes and Eosinophil for the extract treated groups when compared with the negative group. RBC, Hb and PCV were slightly affected by the treatments in the current study. In other words, the absence of significant ($p<0.05$) changes in these parameters could be due to the short time exposure or the acetaminophen toxicity in which the destruction in erythrocyte membrane most likely resulted in direct removal of the damaged erythrocytes rather than remaining in circulation [14]. The assessment of the haematological parameters in wistar rats is a valuable tool for monitoring the effect of plant extract on animal blood chemistry [15]. The significant increase ($p<0.05$) in WBC following the administration of the ethyl acetate seed extract of *P.americana* suggests that the administration of acetaminophen and some components of the extract may increase the production of some regulatory factors or interfered with the sensitivity of the committed stem cells; responsible for the production of WBCs. Also increase in neutrophil concentration following acetaminophen toxicity has been reported as being necessary for host defence through removal of necrotic cells [16]. Significant reduction in neutrophil levels as seen in groups 3, 4 and 5 may indicate amelioration of acetaminophen toxicity. Similarly acetaminophen toxicity has been reported to cause apoptosis of human proliferative lymphocyte [17] leading to significant reduction observed for group 2 compared to group 1. Reversal of this trend in groups 4 and 5 may also result from mitigation of acetaminophen toxicity by the ethyl acetate extract of *P. americana.*

Moreso, the slight reduction in RBC following the administration of *P. americana* on wistar rats treated with 200mg/kg body weight and 400mg/kg body weight is an indication that the extract might prevent RBC synthesis through the inhibition of erythropoesis in the bone marrow. These also suggest that the extract can induce anaemia possibly by causing bone marrow depression through inadequate production of RBC and ultimately cell death [15]. Increase in platelet indicates that coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). The changes, resulting from the administrations of the extract doses, may be due to the physiochemical constituents of the extract, which is in line with the report by Neboh *et al.,* [18].

Results on renal function parameters (Table 2) showed that acetaminophen treatment may have caused nephrotoxicity as evidenced by marked elevation in serum urea (7.34±0.85 mmol/l) and creatinine (103.65±7.68mmol/l) when compared to the normal control group. Co-administration of *Persea americana* seed extract with acetaminophen decreased the rise in these parameters in a dose dependent manner. Serum electrolyte analysis also revealed that groups 3, 4, and 5 produced a significant ($p<0.05$) increase in the K^+ and Mg^{2+} concentrations, and a decrease in the Ca^{2+}. However, the change in the potassium concentration of group 4 (extract; 200mg/kg) and group 5 (extract; 400mg/kg) was not significant but are dose dependent; this may be due to the short period of exposure. Results of the serum electrolyte concentrations indicate impaired kidney function or renal function-related diseased states. This present result corroborates the studies of some researchers [19, 20].

The measurement of urea and creatinine is considered a tool for clinical diagnosis of renal dysfunction following acute and chronic injury. These markers are end products of various metabolic pathways that are excreted in the urine via glomerular filtration whose serum levels are an indicator of renal functions [21]. The high significant increase ($p<0.05$) in the serum creatinine upon acetaminophen toxicity exposure may possibly be as a result of cellular damage due to the excess free radical production [22]. The reduction in glomerular filtration may be due to a decrease in the number of functional nephrons [23].

The Histology of the kidney (Figures 1-5) revealed that the control group (group 1) rats had intact and normal histological features with normal blood flow. Photomicrograph of group 2 (acetaminophen 750mg/kg) kidney showed tubular damage (lack of blood flow & oxygen to the tissues). Figure 3, showed mild tubular damage, this group was administered with silymarin (200mg/kg).

Figure 4, photomicrograph of the kidney section from rats treated with lower dose (200mg/kg) of the seed extract showed tubular distortion and epithelial sloughing (shedding off dead epithelial cell tissue); whereas, Figure 5, treated with higher dose (400mg/kg) of the seed extract showed tubular casts which may be due to the higher dose of extract administered to the animals, therefore increased the rate of epithelial sloughing. Results of this study confirmed that acetaminophen at a dose of 750mg/kg produced significant nephrotoxicity as evidenced by histological changes of the kidneys that include tubular necrosis, dilatation of tubules, degeneration of tubular epithelial cells with casts in the tubular lumen, cell infiltration in interstitium, marked congestion of the glomeruli and extensive necrosis with alteration of corresponding biochemical parameters as shown by increase in the serum urea and creatinine. The present histological and biochemical findings in actaminophen treated group correlates with previous report [24]. Pretreatment with ethyl acetate extract of *P. americana* provided marked nephroprotection against actaminophen induced renal damage in rats as evidenced by significant reduction in biochemical parameters. This is supported by histopathological evaluation; concurrent administration of *P. americana* appeared to mitigate the severity of the acetaminophen-induced renal necrosis, resulting in the preservation of the tubular histology by significantly reducing the histopathological damages compared to acetaminophen treated groups. The signs of regeneration of tubules were also seen, which was prominent at highest dose. Restoration of the structure of glomerulus and renal tubules in *P. americana* treated groups provides a direct evidence for nephroprotective activity of this extract.

CONCLUSION

Findings from this study provides scientific evidence for the nephroprotective effects of orally administered ethyl acetate extract of *P. americana* in acetaminophen induced renal damage; *as well as a manifestation that the use of the extract in phytotherapy may produce adverse side effects such as anaemia.*

Conflict of Interest

The authors declare no conflict of interest.

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