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Effects of Aqueous Extract of Stem Bark of *Persea americana* (Avogado) Plant on the Kidney of Adult Wistar Rats at Different Doses.

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ABSTRACT
Persea americana is used in the treatment of several diseases, therefore this study is aimed at determining the effects of 30 day administration of the aqueous extract of the stem bark of *Persea americana* plant on the kidney of Wistar rats. 30 adult Wistar rats were divided into five groups of six rats per group. Group A rats were fed with water and growers' mash only, while groups B, C, and D and E were administered with 100mg/kg, 500mg/kg, 750mg/kg and 1200mg/kg body weight of aqueous extract of *Persea americana,* respectively. Orogastric tube was used for daily administration of the extract to the rats. Rats were sacrificed on the 31st day. The kidneys were removed, weighed and processed for histopathological analysis. Blood samples are also collected for urea and creatinine estimation. The results from the study showed that there was no remarkable change in the histological and biochemical integrity of the kidney except at the dose of 1200mg/kg in group E where there was an abnormal elevation of urea level. It can be concluded that at doses of 100mg/kg, 500mg/kg, 750mg/kg, and 1200mg/kg of *Persea americana* stem extract, there was no damaging effects on the kidney structure and function histologically except biochemically at 1200mg/kg.

Keywords: Persea Americana, Kidney, Urea, Cretinine, stem bark

INTRODUCTION
Avocado (*Persea americana* Mill) belongs to the Lauraceae family composed of 50 genera and approximately 2,500–3,000 species[1,2]. It is a medium to large tree, 9-20 m in height. The avocado fruit has been given different names, depending on the country where it grows: Alligator in Florida, Xiene in Mexico, Palta in Colombia and Ecuador, Abacoteiro in Brazil, Avocado pear or (Ube Beke) in Nigeria, and simply pear in Cameroon[3]. Avocado fruit development period is quite extensive, between 6 and >12 months from flowering to maturity [4]. Liu et al., [5] reported high total sugar concentrations (up to 44%, 40 and 22% of total dry weight) in the flesh, seed, and peel of Persea americana cv.

The fresh leaves have been consumed in the form of aqueous infusion or decoction for influenza, bronchitis, menstruation pain, diabetes, rheumatism and also externally; hair tonic in Ecuador and as well. The leaves have been employed as antimalarial in Nigeria [6]. Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, larvicidal, hypolipidemic, fungicidal, amoebicidal and giardicidal activities [7,8,9,10]. The avocado seed is discarded in majority of the countries, although in some countries such as Niger, it is consumed [11,12]. It is commonly eating in Nigeria because of its nutritional constituent.

What turns avocados unique compared to other fruit is the presence of C_7 sugars (e.g., mannoheptulose and perseitol) instead of C_6 sugars as main phloem transported sugars and as respiratory substrates [13,14].

Due to the widespread use of *Persea americana* and limited information on the toxic effects of repeated administration to cure ailments such as malaria in Nigeria, this study was put together to determine the effects of the aqueous extract of the stem of this plant on the kidneys of rats associated with 30-day repeated orogastric administration in order to ascertain its safety.

MATERIALS AND METHODS
Experimental Animals: Thirty (30) adult Wistar rats weighing 180-250g used in this study were obtained from the Animal House, Department of Anatomy, University of Benin. The animals were placed in rat cages kept in a well ventilated room and allowed free access to standard feed and clean tap water under room temperature throughout the acclimatization and experimentation period. All procedures involving laboratory animals complied with National Academy of Sciences 1996 guidelines on handling of experimental animals and ethical approval was obtained from the animal ethics committee.

Preparation of Plant Sample/Extract: The plant sample, stem bark of *Persea americana* was collected within the premises of the University of Benin Junior

Staff Quarters and it was then identified by a plant taxonomist from the Department of Plant Biology and Biotechnology, University of Benin, Benin city as *Persea americana*. The stem bark was air-dried for two weeks and then pulverized to powder level, using the British Milling Machine. The powdered sample weighed 600g and it was then macerated in a jar with distilled water (1.2 liters) with constant shaking and stirring. Next, filtration was done to separate the residue from the filtrate using filter paper, conical flask and funnel. The filtrate was then concentrated to paste level using water bath and crucible. The crude extract was then preserved in a sample bottle, inside a refrigerator.

Experimental Design: Thirty (30) adult Wistar rats were randomly selected into a control group (group A) and four treatment groups (B, C, D, E,) each containing five animals each (n equals 5 per group). The animals in each cage were given Growers mash, manufactured by Premier Feed Mills co Ltd (a subsidiary of flour mills of Nigeria Plc.) and water. Group A, control was given feed and water only, Group B was given 100 mg/kg body weight of *Persea Americana extract*, Group C was given 500 mg/kg body weight of *Persea americana* extract only, Group D: 750 mg/kg body weight of *Persea americana* extract, while Group E was given 1200mg/kg body weight of *Persea americana* extract only.

Orogastric tube was used for daily administration of the extract to the rats. The experiment lasted for 30 days and the weight of the Wistar rats were taken weekly and recorded. On the 31st day, the animals were sacrificed.

Histopathological Studies: The kidneys were removed from their fascia via a midline incision on the anterior abdominal wall and their wet weight recorded immediately after dissection to avoid drying. Blood samples were taking for biochemical analysis. kidney from each rat were fixed immediately in 10% formal saline for histopathology examination. The tissue slices were dehydrated in ascending grades of alcohol: 70%, 90% and 100%v/v. Finally, they were cleared in xylene, embedded in paraffin wax, sectioned in microtome at 5um thickness. They were mounted on the glass slide, stained in Harris hematoxylin for 5 minutes, differentiated in 1% acid alcohol, blued in Scotch's tap water for a minute and finally stained in eosin for 5 minutes. The slides were then examined under light microscope for pathological lesions by a consultant pathologist. Photomicrographs were taken at x100 and x40 magnifications using a photomicroscope (LEICA microscope DM, 350; with SCOPEMED DIGITAL CAMERA).
Assay of biochemical markers for kidney damage test for urea and creatinine was done using standard procedure.

Statistical Analysis: The data obtained from all the groups were compiled and statistically analyzed using ANOVA method. The results were analyzed using Graph Red prism version 5.0 statistical software. Data was expressed as (mean + SEM). A statistical significance was taken at probability level of less than 5% ($P < 0.05$).

RESULTS

Statistical Analysis

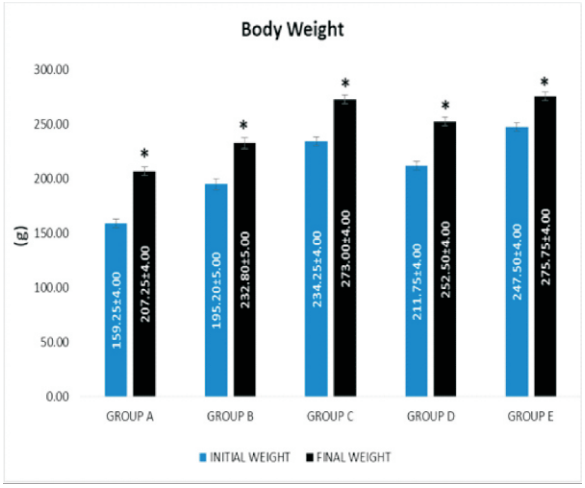

Figure 1: Chart showing the comparisons between initial body weight and final body weight.*significantly different from the initial body weight

There was a statistically significant increase of body weight in all the groups when the initial body weights were compared with the final body weight.

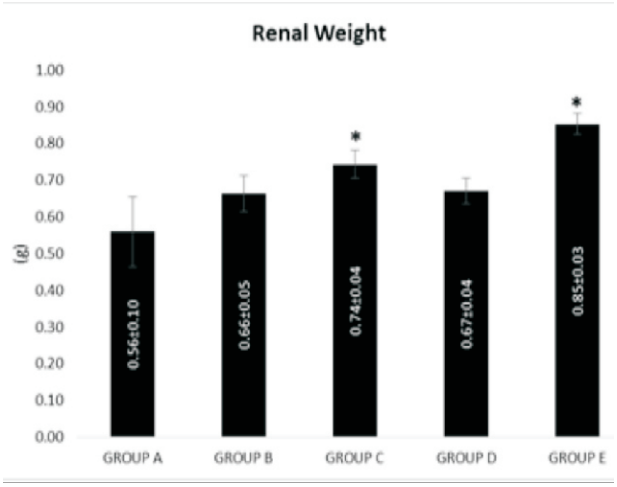

Figure 2: Chart showing renal weight across all the groups.*significantly different from the control group
There was a statistically significant increase of renal weight in groups C and E, when compared with the control group.

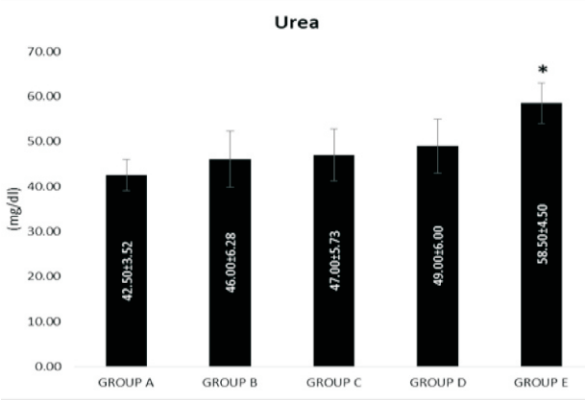

Figure3: Chart showing the levels of urea across all the groups.*significantly different from the control group
There was a statistically significant increase of urea in group E, when compared with the control group. There was no statistical difference in other groups when compared to the control

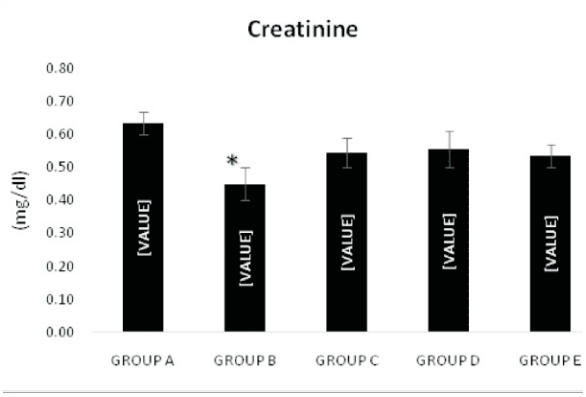

Figure4: Chart showing the levels of creatinine across all the groups.*significantly different from the control group
There was a statistically significant decrease of creatinine in group B, when compared with the control group.

Effect of aqueous extract of *Persea americana*stem on body weight: Aqueous extract of *Persea americana* stem caused a statistically significant increase ($P<0.05$) of body weight in all the groups when the initial body weights were compared with the final body weight. There was also a significant increase in renal weight in groups C and E, when compared with the control group.

Effect of aqueous extract of *Persea americana*stem on creatinine level: Aqueous extract of *Persea americana* stem causes no significant statistical changes ($P<0.05$) of serum creatinine level in all the groups when compared with the control group.

Effect of aqueous extract of *Persea americana*stem on urea level: Aqueous extract of *Persea americana* stem causes no significant statistical changes ($P<0.05$) of serum urea level in group B, C and D when compared with the control group. Furthermore, there was increased statistical significant change ($P<0.05$) in urea level of group E when compared to control group.

Histological effect of aqueous extract of *Persea americana*stem bark: Plate 1: Photomicrograph of cross section of kidney of control group (Group A) administered with water. Section shows normal glomeruli, tubules, interstitial space, arcuate artery and interlobular vein (H&E x 40).
Plate 2. Photomicrograph of cross section of kidney of rat administered with 100mg/kg weight of *P. americana*; Section shows no histopathological changes when compared with the control(H&E x 40).
Plate 3.Photomicrograph of cross section of kidney of rat administered with 500mg/kg weight of *Persea americana*; Section shows no histopathological changes when compared with the control (H&E x 40).
Plate 4.Photomicrograph of cross section of kidney of rat administered with 750mg/kg weight of *Persea americana*; Section shows no histopathological changes when compared with the control (H&E x 40).
Plate 5.Photomicrograph of cross section of kidney of rat administered with 1200mg/kg weight of *Persea americana*; Section shows no histopathological changes when compared with the control (H&E x 40).

Histological photomicrograph

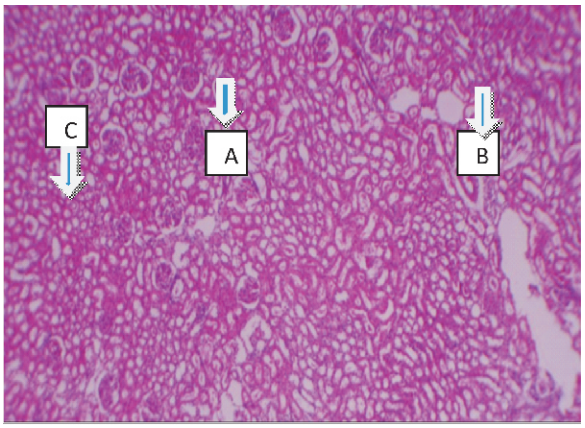

Plate 1: Photomicrograph of cross section of kidney of control group (Group A) administered with water. Section shows A, normal glomeruli, B, normal interstitial space, C, normal tubules when compared with the control (H&E x 40).

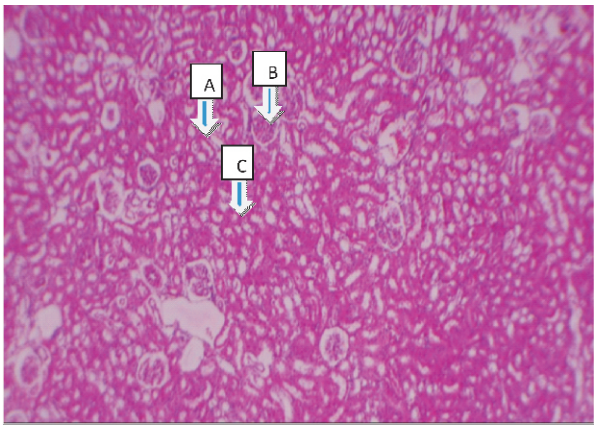

Plate 2: Photomicrograph of cross section of kidney of rat in group B administered with 100mg/kg weight of *P. americana*; Section shows A, normal glomeruli, B, normal interstitial space, C, normal tubules when compared with the control (H&E x 40).

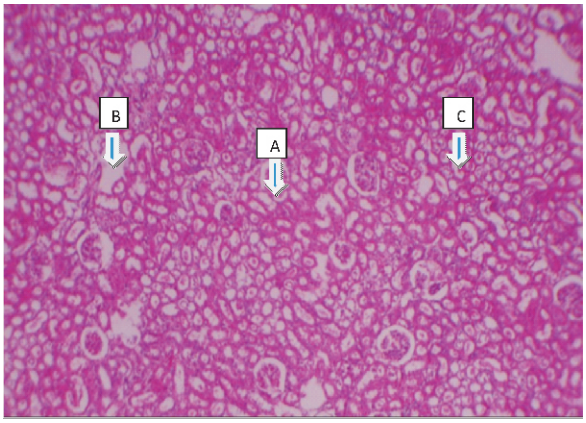

Plate 3: Photomicrograph of cross section of kidney of rat in group C administered with 500mg/kg weight of *Persea americana*; Section shows A, normal glomeruli, B, normal interstitial space, C, normal tubules when compared with the control (H&E x 40).

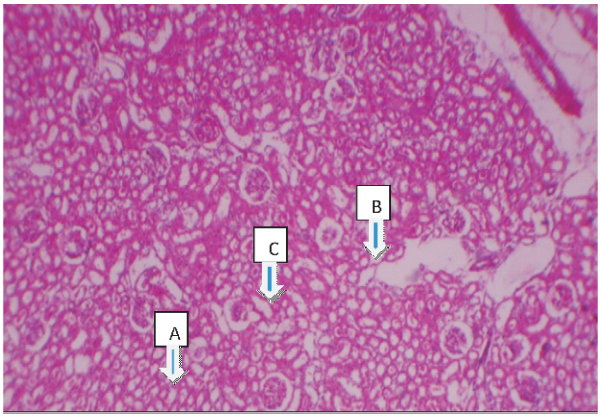

Plate 4. Photomicrograph of cross section of kidney of rat group D administered with 750mg/kg weight of *Persea americana*; Section shows A, normal glomeruli, B, normal interstitial space, C, normal tubules when compared with the control (H&E x 40).

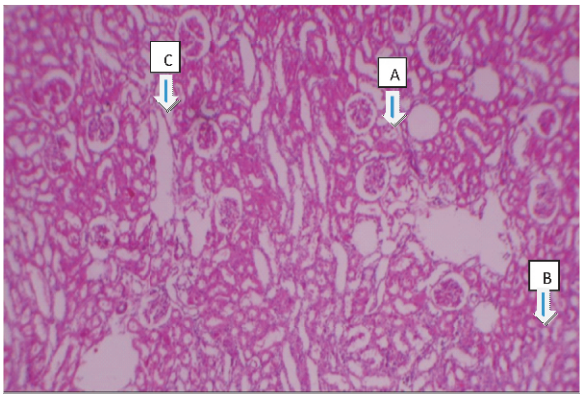

Plate 5. Photomicrograph of cross section of kidney of rat administered with 1200mg/kg weight of *Persea americana*; Section shows A, normal glomeruli, B, normal interstitial space, C, normal tubules when compared with the control (H&E x 40).

DISCUSSION

It has been discovered that toxicity level varies with the variety[15].Amaza *et al.,* studied the effect of aqueous extract of *P. americana* on the histology of the kidney of albino rats. Theyconcluded that aqueous leaf extract of *P.americana* have effects on the of the kidney histology. They discovered that *P. americana*causes dose-dependent tubular necrosis in the cortex, hydrophobic change in the lumen of the tubules and mild inflammatory cells. This research work not in accordance with our work as depicted in our histological photomicrograph where we discovered that at 100mg/kg, 500mg/kg, 750mg/kg, and 1200mg/kg of *P. americana* stem extract there was no damaging effects on the kidney glomerulus and tubules histologically.

Egbuonu *et al.,*[16] examined the effect of ethanolic extract of *P. americana* seeds on monosodium glutamate induced kidney damage in adult rats. They concluded that *P. americana* seed was able to interact with monosodium glutamate and prevent the damage caused by the monosodium glutamate on the kidney of rats. This experimental result is in accordance with our study where we postulate and confirmed that *P. americana* extract is not toxic to the wistar rats at the given dosages(100, 500, 750, and 1200mg/kg) histologically.

Also, It has been stated that animals such as cats, dogs, cattle, goats, rabbits, rats, birds, fish, and horses can be severely harmed or even killed when they consume the *P. americana*leaves, bark, skin[17]. Oelrichs *et al.*[17]*,* also reported that *P. americana*leaves contain a toxic fatty acid derivative known as persin which in sufficient quantity can cause equine colic and without veterinary treatment can lead to death. Their report may be parallel to our findings because there was no history of loss of any Wistar rats during the course of administration of the extract to the rats for 30 days at 100mg/kg, 500mg/kg, 750mg/kg, and 1200mg/kg.

Researchers have also investigated the biochemical and pathological changes associated with *P. americana*leaves poisoning in rabbits. Serum SGOT and SGPT were found to be elevated followed by the changes in concentration of sodium, phosphorus and chloride. Such biochemical changes can be correlated with the gross pathologic observation in various organs which includes fluid filled pericardium, congested liver[18].

The result of Ayub *et al.,*[18] is not in accordance with our work histologically at the dosage of 100mg/kg, 500mg/kg, 750mg/kg, and 1200mg/kg, where we discovered that at our own administered doses the structural architecture of the renal tissue was still intact. The variation in our results and previous one done by Ayub *et al.,*[18] might arises due to differences in the elementary constituent of the extracts occasioned by atmospheric condition of each zone and species variation. For the renal function test, both urea and creatinine levels in our present work are all in normal range for all the groups except at 1200mg/kg where there was an elevation of blood urea level when compared with the control group .This result for the group that was given 1200mg/kg is supported by previous work done[18]. Administration of *P. americana*seed extract in an experiment by Egwaoje*et al.,*[19] resulted in a significant increase in urea levels, which is consistent with this study. High levels of these parameters have been linked to renal injury.

Also, there was a progressive increase in the weight of the rats in all the groups when compared with the control rats. This showed that the extract is not toxic to the tissue of the rats. This result is supported by earlier work done[20]. They stated that aqueous seed extract from *P. americana* can increase the renal weight of an adult rats fed with it.

CONCLUSION

This study therefore indicates that moderate consumption of *Persea americana* stem bark has no remarkable effects on kidney structure and function. However, it possesses the capacity to interfere with normal kidney function at higher doses, due to its dose-dependent increase in serum urea concentration, which increases the risk of uremia, an early sign of kidney damage. Therefore, it is recommended that further toxicity studies of the crude extract, preferably at higher doses, need to be carried out using different animal models in order to evaluate the long-term effects of the extract.

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