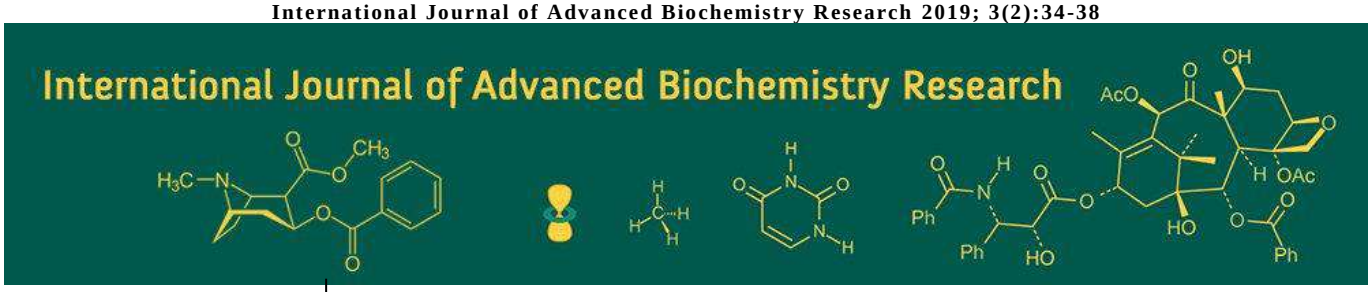

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Evaluation of the potency of ethanol leaf extract of *Persea americana* in the treatment of carbon tetrachloride (CCL_4) induced renal damage

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Abstract
The aim of this study was to evaluate the potency of ethanol leaf extract of *Persea americana* as a treatment option for renal damage. Freshly harvested leaves of *P. americana* were processed into fine powder. 500 g of powdered plant sample was soaked in 70% ethanol for 72 hr and afterwards, sieved and concentrated. Twenty five adult male wistar rats were divided into five groups of five rats each. Group 1 was the normal control and was fed normal rat chow and water only. Group II was the negative control and was induced renal damage without treatment. Group III-V were induced renal damage and treated with 100, 300, 500 mg/kg b.w of extract orally respectively. Animals were sacrificed, after which blood sample was collected and kidney tissue harvested and analysed using standard procedures. The serum urea and creatinine levels of rats administered CCL_4 only was significantly ($P< 0.05$) higher than that reported for their treated counterparts. However, the urea and creatinine levels of rats treated with different doses of extract was not significantly (P>0.05) from that reported for the normal control. There was a significant ($P< 0.05$) body weight increase among treated groups contrary to the observation made on group II which was induced renal damage without treatment. In conclusion, ethanol extract of *Persea americana* leaf has the potential to ameliorate renal damage.

Keywords: Creatmine, *Persea americana*, kidney, urea, body weight

Introduction
Air and water pollutions are largely implicated in human exposure to harmful substances. The susceptibility of the kidney to damage resulting from toxic substances is high owing to its large surface area, high blood flow, high metabolic activities and active re-absorption and concentrations of toxins. It is worthy to note that diverse forms of kidney injuries are associated with toxic substances. Kidney Disease is a globally acknowledged public health problem which is directly linked to kidney failure, cardiovascular disease and ultimately premature death [1]. An estimated 10% of the world's populations are victims of this problem [2].

Dialysis and kidney transplantation which are the most commonly subscribed treatment options are implicated in pitfalls such as immunological rejection of kidney graft, immune suppression and its attendant consequences [3].

Plants and herbs have been a veritable source of food and folk medicine for mankind since prehistoric times. The pharmaceutical industries have also relied extensively on plants to develop synthetic drugs [4]. Over 70% of the world's populations rely on plant based therapies to meet their health needs. They are considered affordable, readily available and safe for human consumption [5].

Persea americana, a smallish evergreen tree with a grey trunk is a member of the *Lauracea* family. It is native to Central America [6]. In Nigeria, it is commonly known as avocado pear [7]. Various parts of *P. americana* such as such as the root, bark, fruit, seed and leaf are employed in the treatment of diseases ravaging mankind in many tropical and subtropical countries [8].

Some of the diseases which had been treated with the aforementioned plant include diarrhea, dysentery and toothache etc. [9]

Despite the fact that the outcome of previous studies had established the usefulness of different parts of *Persea americana* in the management of different health conditions, there is paucity of data on its potency in the treatment of kidney diseases. Hence, the imperativeness of this study is determined.

Material and Methodology

Collection and Processing of Plant Material

Mature green leaves of *Persea americana* which were harvested from a family compound in Uli, Ihiala Local Government Area of Anambra State were subsequently identified and authenticated at the herbarium unit of the Department of Botany, Nnamdi Azikiwe University Awka Anambra State. The leaves were thoroughly washed with clean water and afterwards dried at room temperature. The dried leaves were subsequently ground and sieved to fine powder.

Animals

Adult male wistar rats weighing 130-200 g were housed in plastic cages in the Animal House of the Department of Human Physiology, College of Health Sciences Anambra State University and were fed rat chow and water *ad libitium*. They were acclimatized for two weeks before experiment.

Extraction of Plant Material

Exactly 200 g of the powdered leaf sample was soaked in 500 mL of 70% ethanol in an airtight conical flask for 72 hr and stirred intermittently at room temperature. This was followed by the filtration first through a double layered muslin cloth and then filtered through Whatman No. 1 filter paper, the residue was evaporated to dryness with the aid of the rotary evaporator under reduced pressure at 40-50°C to obtain extract [10].

Median Lethal dose 50% (LD50)

Three groups of three rats per group were used in the experiment to determine the LD_{50} of extract. The various groups were separately administered with 10, 100 and 1000 mg/kg of extract orally. The rats were observed for 24 hr for effects of toxicity. In the absence of mortality in any of the groups, another three groups of one rat each were each administered with 1600, 2900 and 5000 mg/kg of extract separately. The animals were observed for 48 hr for signs of toxicity [11].

Induction of Renal Damage

Animals were administered with 1 mL/kg body weight CCL_4 (Sigma Aldrich, USA) intraperitoneally in 30% v/v olive oil for induction of renal damage [12].

Experimental Design

Group I: was fed with rat chow and water *adlibitum*.

Group II: was administered with CCL4 only without treatment.

Group III: was administered with CCL4 before 100 mg/kg of *P.amaericana* leaf extract

Group IV: was administered with CCL4 before 300 mg/kg of *p. amaericana* leaf extract.

Group V: was administered with CCL4 before 500mg/kg of *p. amaericana* leaf extract.

Administration of leaf extract lasted for 21 days after which the animals were euthanized by administering 2 µg/kg medetomidine. Blood sample was subsequently collected via cardiac puncture and centrifuged at 4 °C, 500×g for 15 min to obtain serum. The kidney of each animal was excised, washed with ice-cold water to get rid of debris before being stored in 10% formalin (Sigma Aldrich, USA) for histopathological evaluation Burki *et al*. [13].

Determination of Serum Creatinine and Urea

Diagnostic kit (AMP Krenngasse 12, 1810 Graz, Australia) was used in the analysis of serum creatinine and urea with the aid of the manufacturer's guidelines.

Histopathological Study

Harvested kidney sample was fixed and dehydrated in alcohol (90%). The kidney tissue was further processed as described by Burki *et al*. [13].

Results

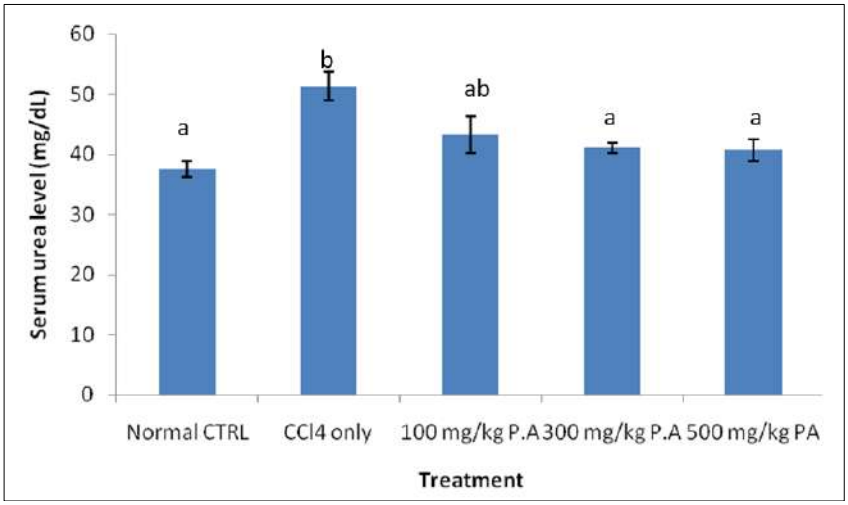

Fig 1: Serum urea level of rats induced renal damage prior to treatment with ethanol leaf extract of *P. americana*

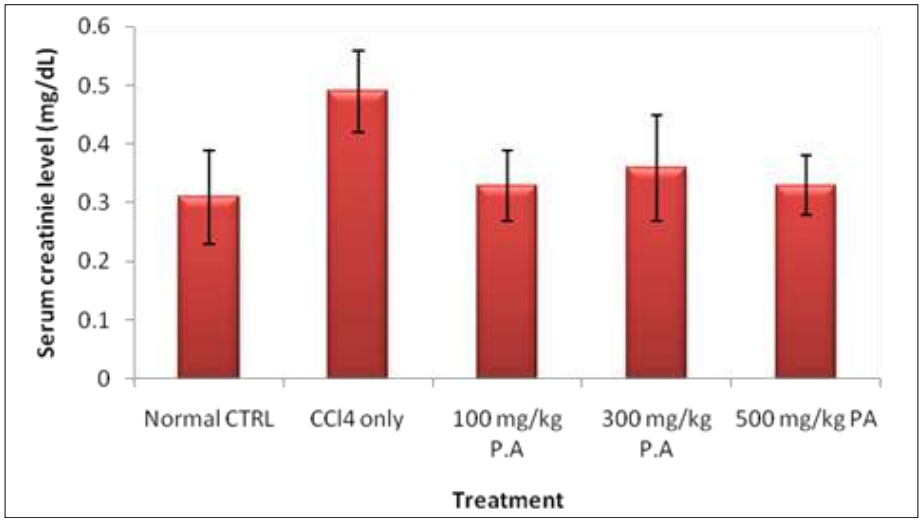

Fig 2: Serum creatinine level of rats induced renal damage prior to treatment with ethanol leaf extract of *P. americana*

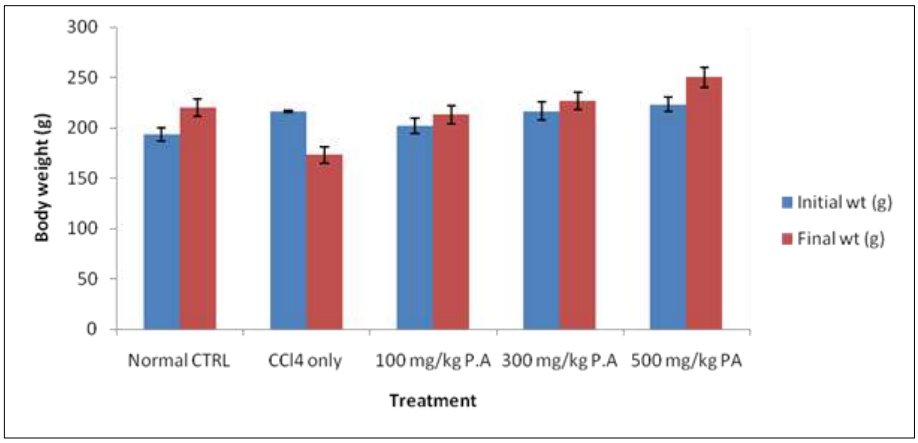

Fig 3: Body weight of rats induced renal damage prior to treatment with ethanol leaf extract of *P. americana*

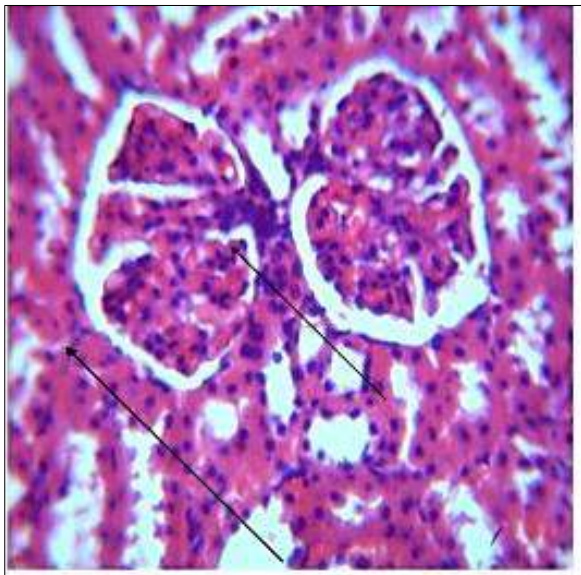

Plate 1: Photomicrograph of kidney of the control group showing normal renal architecture. (X400)(H/E)

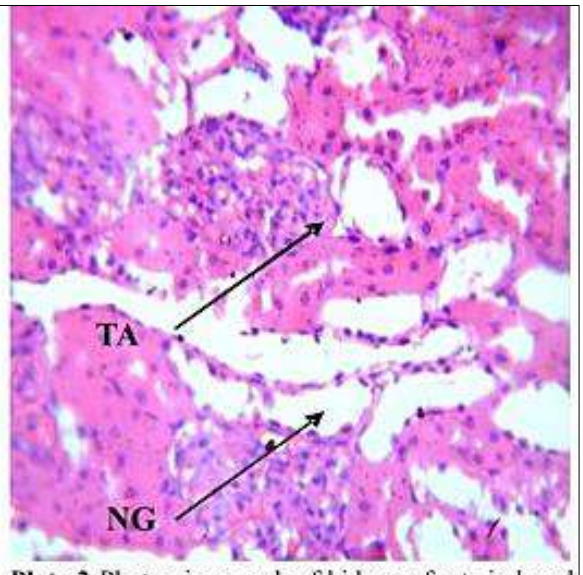

Plate 2: Photomicrograph of kidney of rats induced with renal without treatment showing severe degeneration of renal tissue with necrossed glomeruli (NG) without bowman space , and tubular atrophy(TA)

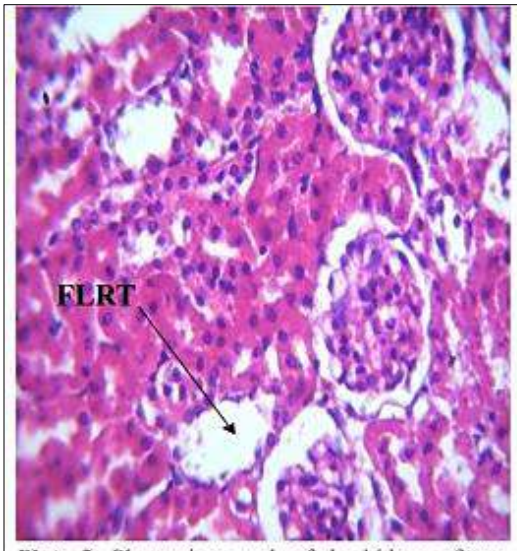

Plate 3: Photomicrograph of the kidney of rats administered 100 mg/kg b.w of ethanol leaf extract of *P. americana* (x400) (H/E) showing mild focal loss of renal tissue (FLRT).

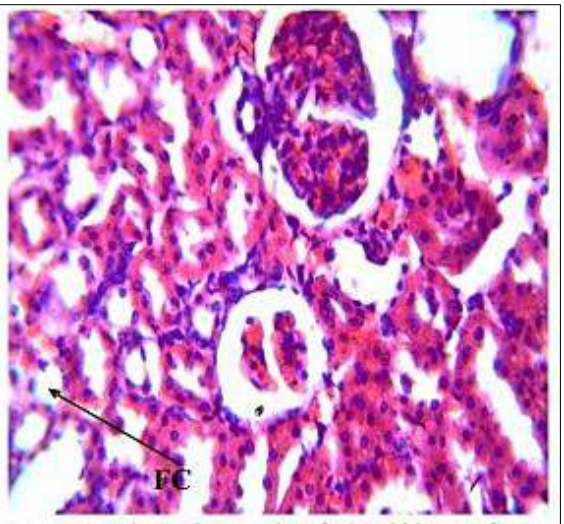

Plate 4: Photomicrograph of the kidney of rats administered 300 mg/kg b.w of ethanol leaf extract of *P. americana* (x400) (H/E) showing moderate regeneration of renal tissue with mild fatty change (FC) and focal area of intra renal hemorrhage (FAIRH).

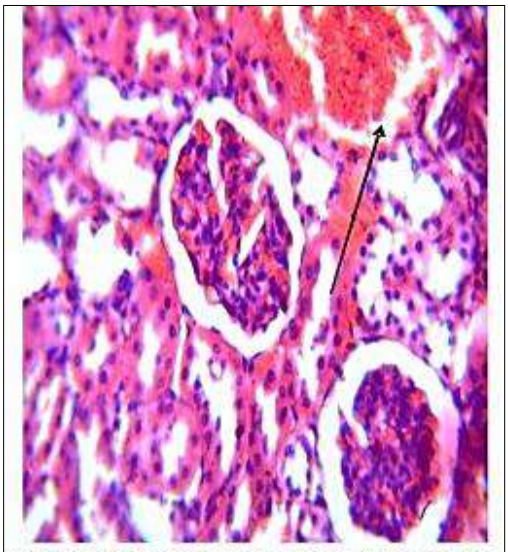

Plate 5: Photomicrograph of the kidney of rats administered 500 mg/kg b.w of ethanol leaf extract of *P. americana* (x400) (H/E) showing moderate regeneration of renal tissue with mild fatty change (FC) and focal area of intra renal hemorrhage (FAIRH).

Discussion

Countries of the world rely on plant based therapies to treat diverse human ailments. In developing countries, over 80% of the population depends on medicinal plant preparations to meet their health needs [14]. Figure 1 shows the serum urea level of rats with experimentally induced renal damage treated with ethanol leaf extract of *Persea americana*. The serum urea level of rats administered CCL_4 without treatment was significantly ($P< 0.05$) higher than that reported for its treated counterparts. However, there was no significant ($P> 0.05$) difference in the urea level of rats treated with different doses of extract. Figure 2 shows the serum creatinine level of rats with experimentally induced renal damage treated with ethanol leaf extract of *Persea americana* indicating a non significant ($P> 0.05$) difference in the serum creatinine level of rats treated with different doses of extract likewise the control. However, the serum creatinine level of rats administered with CCL_4 was significantly ($P> 0.05$) higher than that reported for their treated counterpart. The ameliorative effect of the extract on the damaged kidney could be attributed to the presence of the phytochemicals reportedly present in the leaf. This result is consistent with the finding of Folorunsho *et al.* [15] which established that ethanol leaf extract of *Persea americana* attenuated renal damage associated with streptozotocin induced diabetic rats. Figure 3 shows the body weight of rats with experimentally induced renal damage treated with ethanol leaf extract of *P. americana* indicating a significant ($P< 0.05$) body weight increase among treated groups same as was observed on their control counterpart. However, a contrary observation was made on Group II which was induced renal damage without treatment. The irreversible weight loss observed on Group II could be attributed to a declination of the glomerular filtration rate in chronic kidney disease which is associated with a significant reduction in food intake and consequently weight loss [16].

Conclusions

In conclusion, ethanol leaf extract of *Persea americana* has the potential to ameliorate renal damage and could be considered a viable candidate for the development of a potent therapy for the treatment of kidney disease.

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