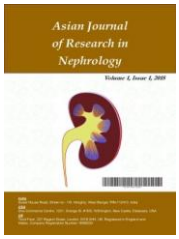

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Evaluation of the Protective Effect of Ethanolic Extract of *Persea americana* Seed on Potassium Aluminium Sulphate Induced Nephrotoxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluates the effect of *Persea americana* on potassium aluminium sulphate induced toxicity in the kidney of female wistar rat.
Methodology: Twenty five (25) female wistar rats weighing between 150-200 g were randomly divided into five (5) groups of five rats each. The groups were designated as groups 1, 2, 3, 4 and 5. Group 1 served as the control, group 2 received 2.3 ml of potassium aluminium sulphate only, group 3 received 0.8 ml of *P. americana* only, Group 4 received 2.3 ml of potassium aluminium sulphate only for 7 days and treated with 0.2 ml of *P. americana* while group 5 received 2.3 ml of potassium aluminium sulphate only for 7 days and treated with 0.8 ml of *P. americana*. The administration was given via oral route and lasted for a period of 28 days. At the end of the experiment, the rats were sacrificed, the kidneys harvested for histological examination and blood sample collected for

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creatinine and urea analysis. Their morphological differences were observed under a light microscope.
Results: The histological changes revealed coagulated necrosis of glomeruli and renal tubules and fatty changes in group 2. Treatment with *P. americana* restored the renal architecture to normal. There was significant increase (P<0.05) in mean serum levels of urea in groups 2, 4 and 5 when compared to group 1 and a significant decrease (P<0.05) in the mean serum levels of urea in groups 1, 3 and 4 when compared to group 2. Furthermore, there was a significant increase (P<0.05) in the mean serum levels of creatinine in groups 2, 4 and 5 when compared to group 1 and a significant decreases (P<0.05) in the mean serum levels of creatinine in groups 1 and 3 when compared to group 2.
Conclusion: The results indicated a nephrotoxic effect of postassium aluminum sulphate with *P. americana* exerting a protective effect against it.

Keywords: Persea americana; potassium aluminium sulphate; alum; nephrotoxicity.

1. INTRODUCTION

Potassium aluminium sulphate also known as potash alum or potassium alum is an inorganic salt with the molecular formula $KAl(SO_4)_2$ that is predominantly produced in the dodecahydrate form ($AlK(SO_4)_2*12H_2O$) [1]. It is the most important member of the generic class of compounds called alums, and is sometimes simply called alum [2]. It is a hygroscopic material which when exposed to air, absorbs water [3]. It has long been used purification of water and paper sizing to improve durability and ink receptivity. Potassium aluminium sulphate has been known to be an important part of many products created by the pharmaceutical, cosmetic and food industries because of its astringency property (ability to constrict body tissues, and restrict the flow of blood) [2]. Some antiperspirants or deodorants contain potassium aluminium sulphate, which acts by clogging, closing or blocking the pores that release sweats under the arm. Due to its active ingredient being aluminium, it is most times linked to cancer [4]. Previous researches have reported its toxic effect on many organs of the body such as the kidney, liver, testes, brain, parathyroid gland and gastrointestinal tract [5-7].

P. americana, commonly known as avocado belongs to the family Lauraceae. It is an edible fruit which originated from Central America but now grown easily in tropical and sub-tropical regions [8]. In Nigeria, the plant and its fruits are known as Ube oyibo (foreign pear) in Ibo speaking communities, “apoka or Igba” in Yoruba [9]. The fruit has an olive-green peel and thick pale yellow pulp that is normally consumed by humans and has been used as a medicinal plant globally [9]. The seed is discarded during processing of the pulp as solid agro waste, however, some countries including Nigeria it is milled and incorporated into foods due its numerous ethno medicinal use in the management of various ailments such as diabetes, liver problem and inflammation [10]. It may also be of interest to industries as a source of bioactive compounds since its chemical composition comprises of phytosterols, triterpenes, fatty acids, and two new glucosides of abscisic acid [9,11]. Several biological activities of *P. americana* have been reported such as antioxidant [12], antihypotensive [13], anticonvulsant [14], analgesic and anti-inflammatory [15] and recently amoebicidal and giardicidal activities [16]. Ozolua et al. [17] reported the acute and sub-acute toxicity of aqueous extract of *P. americana* and they found it to be safe up to a dose of 10g/kg body weight.

This study aimed at ascertaining the protective effect of *P. americana* seed against potassium aluminium sulphate induced toxicity in the kidney, using wistar rats as an experimental model.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty five (25) female albino rats weighing between 150-200g were procured from the animal house of the Department of Anatomy, Chukwuemeka Odumegwu Ojukwu University, Anambra State. They were kept in standard cages under normal temperature (27-30^oC) in the animal house of the Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu University. The ethical committee of the College for animal care and use approved the study

design in compliance with the National regulation for animal research. The animals were acclimatized for a period of two weeks before treatment and were fed with guinea feed and water *ad libitium*.

2.2 Preparation of the Extract of *P. americana*

Fresh seeds of *P. americana* was procured from a local market in Uli, Anambra State. The seeds were authenticated at the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. The seeds were washed to remove dirt, sliced into pieces and dried under ambient temperature. It was then grounded using laboratory blender to a coarse powdery form. 800g of the powder was macerated in four (4) litres of ethanol sealed and allowed for 48hrs. It was then filtered using a clean white cloth and the filtrate was concentrated using a rotary evaporator. The filtrate was further dried using a laboratory oven into a jelly-like form and stored in refrigerator.

Potassium aluminium sulphate was purchased from the market and 2 g of it was dissolved in 20 ml of distilled water to give a concentration of 100 mg/ml.

2.3 Acute Toxicity Test of *P. americana*

This test was carried out using the method of Lorke [18]. Three groups of three rats each were used in the first phase and were administered 10mg/kg, 100mg/kg and 1000mg/kg of *P. americana* orally and were observed for mortality for 24 hours. In the absence of mortality, second phase commenced and the animals were divided into four groups of one animal each. Each group received doses of 1200mg/kg, 1600mg/kg, 2900mg/kg and 5000mg/kg of *P. americana* respectively and were observed for 24 hours.

LD_{50} was calculated using the formula:

$LD_{50} = \sqrt{(a \times b)}$

Where,

a = Highest dose that gave no mortality
b = Lowest dose that produced mortality

2.4 Experimental Design

The twenty five (25) animals were weighed and randomly allocated into five groups of five animals. The groups were designated as groups 1-5 and the administration was given as follows;

Group 1 served as the normal control and were administered 2ml/kg of distilled water
Group 2 received 2.3ml of Potassium aluminium sulphate only
Group 3 received 0.8ml of the extract of *P. americana* only
Group 4 received 2.3ml of potassium aluminium sulphate only for seven days, after which they were treated with 0.2ml of ethanolic extract of *P. americana*
Group 5 received 2.3ml of potassium aluminium sulphate only for seven days, after which they were treated with 0.8ml of ethanolic extract of *P. americana*

The Administration was given orally between the hours of 9-10am daily and lasted for a period of 28 days. Twenty four (24) hours after the last administration, the animals were weighed and sacrificed by cervical dislocation. The rats were dissected by a central median incision into the abdominal and cardiac cavity to expose the abdominal and cardiac contents. Blood samples were then collected by cardiac puncture using sterile syringes with needles and put into tubes without an anti-coagulant. It was then centrifuged at 3,000rpm for 10minutes using bench top centrifuge (MSE, Minor, England). Analysis on blood serum creatinine and urea were determined using randox kit method. The kidneys were harvested and fixed in 10% formal saline for histological examination.

2.5 Histological Examination

The tissues were processed for easy study under light microscope by passing them through the normal histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting, staining. Fixation was carried out in 10% formal saline and dehydration was carried out in ascending grades of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and then cleared in xylene after which embedding in paraffin wax was carried out. Sections of about 3-5µm was obtained using a rotatory microtome. The sections were later deparaffinised, hydrated and stained using haematoxylin and eosin (H&E) dye. They were later mounted using neutral dibutylphthalate xylene (DPX) medium for microscopic examination at x150 magnification.

2.6 Statistical Analysis

Data was analysed using Statistical Package for Social Sciences (SPSS) software (V20, USA).

The results were presented as Mean ± Standard Deviation (SD). One way Analysis of Variance (ANOVA) was used to determine the significance of difference in the means of all parameters followed by Post hoc multiple comparison using Least Significant Difference (LSD). The results were considered statistically significant at $P<0.05$ level of significance.

3. RESULTS

3.1 LD_{50} of *P. americana*

The LD_{50} of *P. americana* was calculated to be 2154.07mg/kg using the formula given in the methodology.

Table 1. Phases of the acute toxicity level test of *P. americana*

	Dosage mg/kg body weight	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/1
Group 2	2900	1/1
Group 3	5000	1/1

3.2 Changes in Serum Level of Creatinine and Urea

The changes in serum level of creatinine and urea are summarized in Table 2. There was significant increase ($P<0.05$) in mean serum levels of urea in groups 2, 4 and 5 when compared to group 1 and a significant decrease ($P<0.05$) in the mean serum levels of urea in groups 1, 3 and 4 when compared to group 2. Furthermore, there was a significant increase ($P<0.05$) in the mean serum levels of creatinine in groups 2, 4 and 5 when compared to group 1 and a significant decrease ($P<0.05$) in the mean serum levels of creatinine in groups 1 and 3 when compared to group 2.

Table 2. Serum levels of creatinine and urea

Groups	Creatinine (mg/dl)	Urea (mg/dl)
1	1.40 ± 0.24^{b}	25.50 ± 0.71^{b}
2	4.10 ± 0.14^{a}	53.00 ± 1.61^{a}
3	1.85 ± 0.07^{b}	33.00 ± 1.04^{b}
4	3.10 ± 0.04^{ab}	54.00 ± 1.41^{a}
5	3.30 ± 0.14^{a}	52.00 ± 1.14^{a}

Values are expressed as means ± SD. Values with different superscripts in a column are significantly different (P<0.05).

3.3 Histopathological Finding

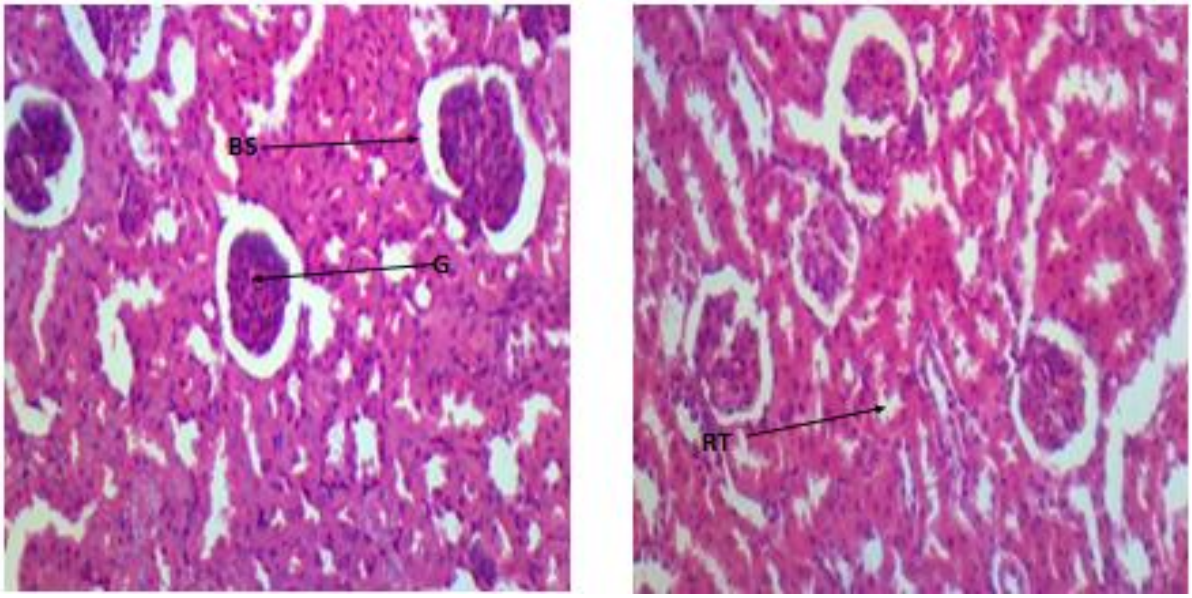

Plate 1. Photomicrograph of the normal control section of the kidney of rats administered with 2 ml/kg distilled water only showing normal glomeruli (G) and renal tubules (RT)

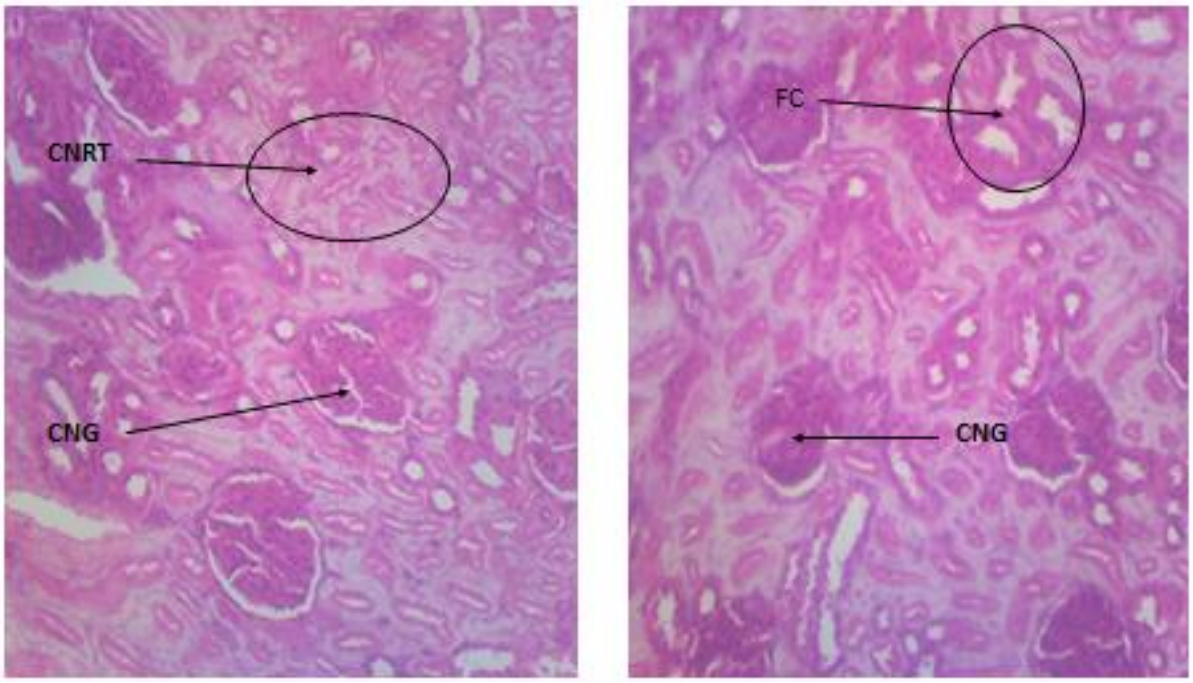

Plate 2. Photomicrograph of kidney tissue of rats administered with 2.3ml of potassium aluminium sulphate showing severe distortion of the renal architecture with coagulated necrosis of glomeruli (CNG), coagulated necrosis of renal tubules (CNRT) and fatty changes (FC) in some area

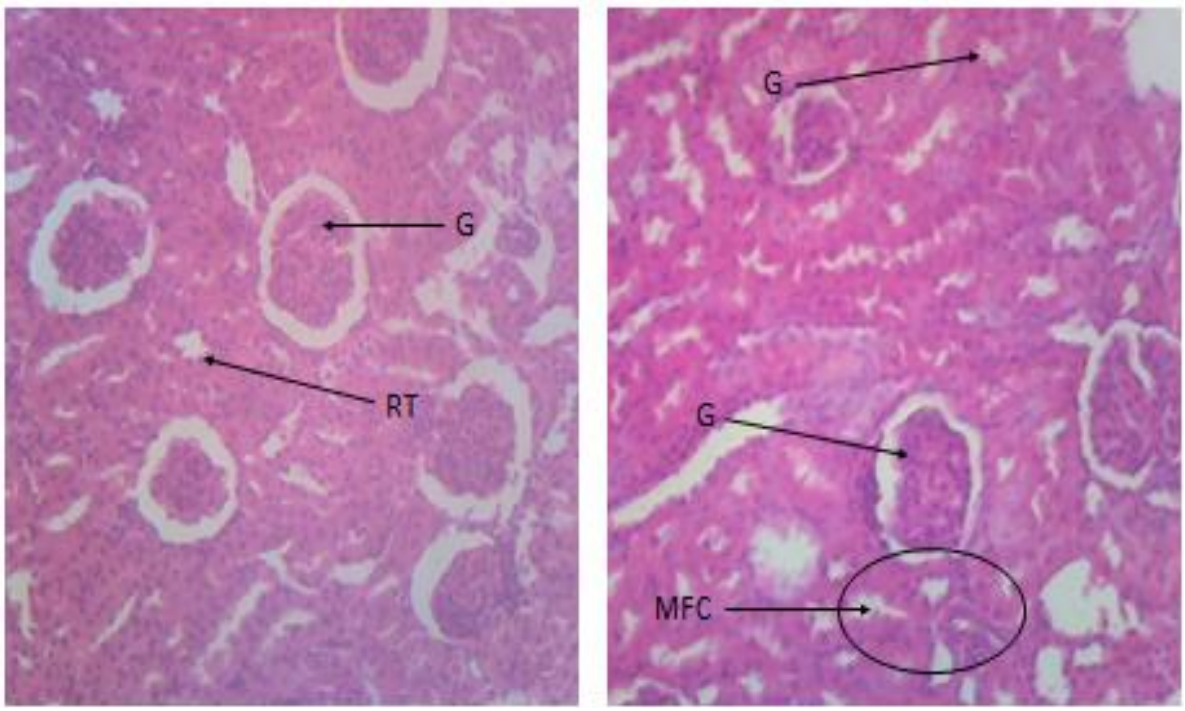

Plate 3. Photomicrograph of kidney tissue of rats administered with administered with 0.8mls of *P. americana* showing normal renal tubules (RT) and Glomeruli (G). However there are areas of mild fatty change (MFC)

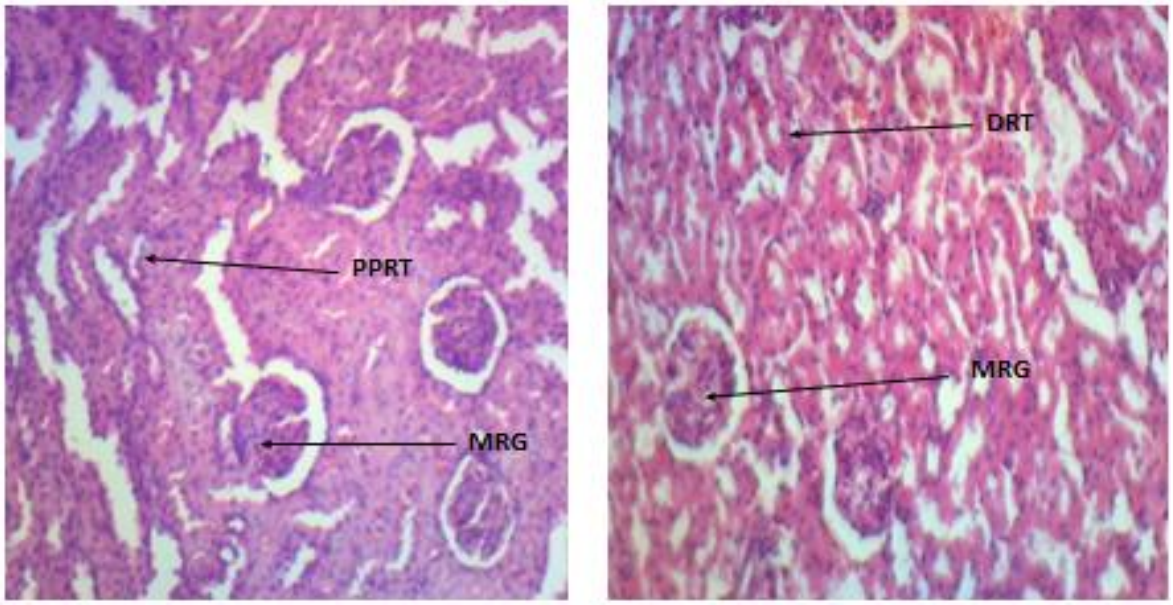

Plate 4. Photomicrograph of kidney tissue of rats administered with 2.3 ml of potassium aluminium sulphate and treated with 0.2 ml of *P. americana* showing poorly perfused renal tubules (PPRT), mild regenerated glomeruli (MRG) and dilation of the renal tubules

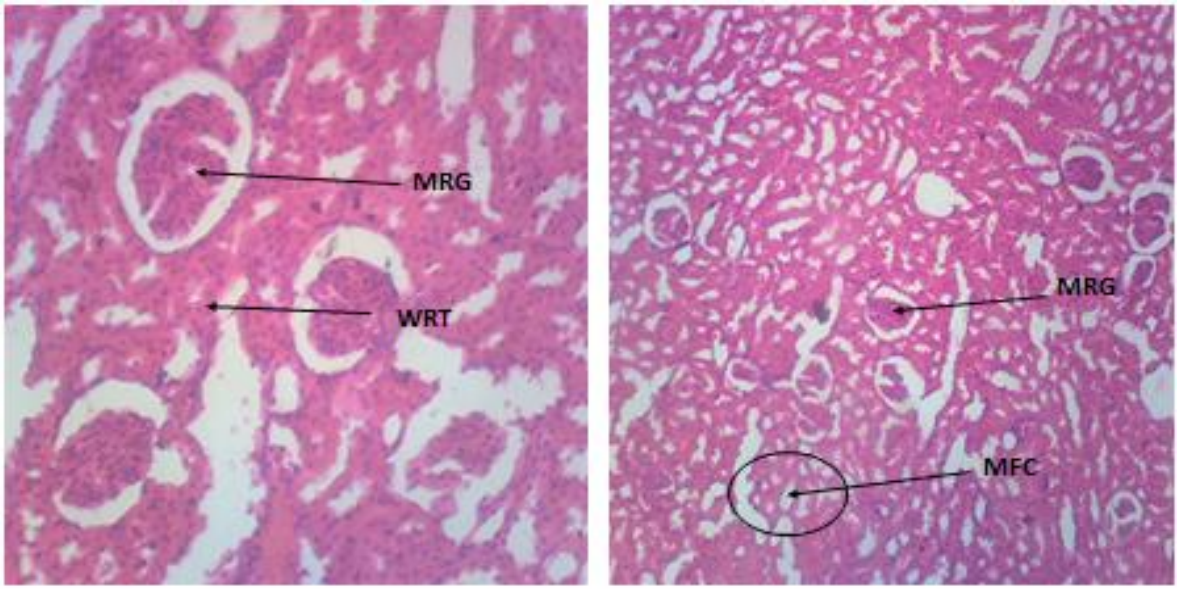

Plate 5. Photomicrograph of kidney tissue of rats administered with 2.3 ml of potassium aluminium sulphate and treated with 0.8 ml of *P. americana* showing well regenerated renal tubules (WRT), moderate regenerated glomeruli (MRG). There are areas with moderate fatty change (MFC)

4. DISCUSSION

In some countries with poor clean water supply, people tend to use potassium aluminium sulphate as a convenient alternative in treatment not knowing the underlying dangers it poses to health. Daily routine dosing of alum revealed marked nervous system involvement including frenzies, salivation, shivering, involuntary watery diarrhoea and finally recumbence [19].

The toxic effect of potassium aluminium sulphate was indicated by the increase in urea, creatinine, necrotic glomeruli and tubules. This probably

could be due to renal damage by the toxic effect of aluminium thereby causing leakage of these biomarkers from the kidney to the bloodstream or could be due to the reduction in the efficiency of the clearance function of the kidney. Enzyme activity levels in serum and tissues are often used as marker to ascertain toxic effects of administered foreign compounds to experimental animals [20]. A rise in serum creatinine or urea level is an indication of renal toxicity. This is in line with Medani et al. [6] who reported renal insufficiency which was indicated by increased urea, creatinine, total protein, decreased albumin concentrations and necrotic, haemorrhagic injured renal tubules. Treatment with different concentrations of *P. americana* seed ameliorated the toxic effect in a dose dependent manner, with the higher dose having a more protective effect. This could be due to the presence of its chemical constituents like Carotenoids, flavonoids, vitamins E which serve as antioxidant that scavenges free radicals curbing the damage mechanism of potassium aluminium sulphate in the kidney [21].

5. CONCLUSION

Findings from this study indicate that potassium aluminium sulphate is a nephrotoxic chemical and consumption of ethanolic extract of *P. americana* seed has a protective effect against its toxicity in a dose dependent manner. Therefore, the use of this chemical in the treatment of water should be discouraged.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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