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PROTECTIVE PROPERTIES OF ETHANOLIC EXTRACT OF *PERSIA AMERICANA* SEED ON THE OVARY OF POTASSIUM ALUMINIUM SULPHATE INDUCED TOXICITY IN FEMALE WISTAR RATS

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ABSTRACT

Introduction: Potassium aluminium sulphate is a toxic chemical commonly used in the purification of water. This is an old method of water purification but it is still common in some parts of West Africa. This study evaluates the protective effect of *persea americana* on potassium aluminium sulphate induced toxicity on the ovary of female wistar rats. **Materials and Methods:** Thirty (30) female wistar rat weighing between (150-200) were divided into six(6) groups, with five (5) animals in each group, The groups were designated as A, B, C, D, E and F. Group A served as the control, Group B received potassium aluminium sulphate only (2.3ml), Group C received *Persea americana* only (0.8ml), Group D and E, received potassium aluminium sulphate at same dose for one week (2.3ml) and were treated with 0.2ml and 0.8ml of *P. americana* respectively for three weeks while Group F recieved 2.3ml of potassium aluminium sulphate and 0.8ml of *Persea americana* extract concurrently for four weeks. At the end of the experiment, the rats were sacrificed, their ovaries were harvested and their morphological differences were observed under light microscope. **Results:** Group A and C showed normal ovarian features, Group B showed ovarian cortex with ovarian cysts, focal area of haemorrhage within the theca interna and oocyte, Group D showed mild healing with ovarian cysts and clearing of follicular fluid. Group E showed mild healing with focal area of haemorrhage and Group F showed moderate healing with ovarian cyst and mild area of haemorrhage. **Conclusion:** The extract of *P. americana* seed has a protective effect against toxicity in the ovary.

KEYWORDS: *Persia americana*, Ovary, wistar rats, weight.

INTRODUCTION

The potential hazards of heavy metals to human are of immense importance as man is continually exposed through natural events that leads to bioaccumulation across food chain. One of such heavy metals is potassium aluminium. Potassium aluminium sulphate is an inorganic salt with the molecular formula $KAl(SO_4)_2$.[1] Potassium aluminium sulphate forms a solid, white powder at room temperature. It is a hygroscopic material which absorbs water when expose to air.[2]

Potassium aluminium sulphate has been known to have many industrial applications and is an important part of many products created by the pharmaceutical, cosmetic and food industrial because of its astringency property.[3] It is also used in the manufacture of paper, dyes, glue and explosives. Additionally, it is used in water purification because it helps to remove particulate matter, used to speed up the hardening of concrete and plaster and acts as a catalyst in various chemical reactions.[4] However, cities have to carefully filter and monitor levels of alum left behind because of its toxicity.[5] Its toxicity could result to symptoms such as colic, dryness of the skin, flatulence, burning pains in the head relieved by food, heartburn and an aversion to meat. Later symptoms include paralytic muscular condition, loss of memory and mental confusion.[5] 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.[4,6]

P. americana, commonly known as avocado belongs to the family Lauraceae. It is an edible fruit that was originated from Central America but now grown easily in tropical and sub-tropical regions.[7] In Nigeria, it is known as Ube oyibo (foreign pear) in Ibo speaking communities, "apoka or Igba" in Yoruba.[8] 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. The yellow pulp is normally consumed and has also been used as a medicinal plant.[8] During processing of the pulp, the seed is discarded as solid agro waste; however, in some countries including Nigeria it is milled and incorporated into foods due its numerous ethno medicinal uses in the management of various ailments such as diabetes, liver problem and inflammation.[9] Its chemical composition is comprised of phytosterols, triterpenes, fatty acids, and two new glucosides of abscisic acid.[10] Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, analgesic, fungicidal, hypolipidemic, and recently amoebicidal and giardicidal activities.[11-14] This study is aimed at ascertaining the ameliorating effect of *P. americana* seed on potassium aluminium sulphate induced toxicity in the ovary of female wistar rats.

MATERIALS AND METHODS

Animals

Thirty (30) female albino rats weighing between 150-200 g were purchased from the animal house of the Faculty of Basic Medical Sciences Chukwuemeka Odumegwu Ojukwu University, Uli. The rats were acclimatised for 14 days with a 12 h dark/light cycle, fed standard grower feed, and allowed free access to drinking water *ad libtum*. All protocols were approved by the ethical committee of the College for animal care and use in compliance with the National regulation for animal research.

Preparation of Extract

Fresh seeds of *P. americana were* procured from a market in Anambra State. They were identified at the herbarium units of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The seeds were sliced, washed and dried under ambient temperature. The dried seeds were crushed to a coarse powdery form using laboratory blender. 800 g of the powder was macerated in 4L of ethanol and allowed for 48 hours inside mechanical shaker. After maceration the mixture was sieved using a clean white cloth and further filtered using filter paper into a clean beaker. The filtrate was later concentrated using rotary evaporator into a jelly-like/paste-like form and stored in refrigerator for future use.

Reagent

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

Experimental Design

The thirty female rats were weighed and randomly allocated into six (6) groups of five (5) animals. The groups were designated as a group A, B, C, D, E and F and were distributed as follows;

Group A served as the control group and was administered 2 ml/kg body weight of distilled water.

Group B received 2.3 ml/kg body weight of Potassium aluminium sulphate only

Group C received 0.8 ml/kg body weight of *P.americana* seed extract only.

Group D received 2.3 ml/kg body weight of Potassium aluminium sulphate for seven days and then treated with 0.2 ml/kg body weight of *P. americana* seed extract.

Group E received 2.3 ml/kg body weight of Potassium aluminium sulphate for seven days and then treated with 0.8 ml/kg body weight of *P. americana* seed extract.

Group F received 2.3 ml/kg body weight of Potassium aluminium sulphate and 0.8 ml/kg body weight of *P. americana* seed extract concurrently.

The extract was administered orally between the hours of 8-10am daily and treatment lasted for four weeks. The animals were weighed before anaesthetized by chloroform inhalation and dissected 24 hours after the last dose. The ovaries of the rats were harvested and fixed in 10% formal saline for histological examination.

Histopathological Examination

The tissues of the ovaries were processed by passing them through histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting and 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.

Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) test followed by multiple comparisons using Least Significant Difference (LSD). The levels of significance were considered at $P<0.05$ and data was expressed as Mean ± SD.

RESULTS

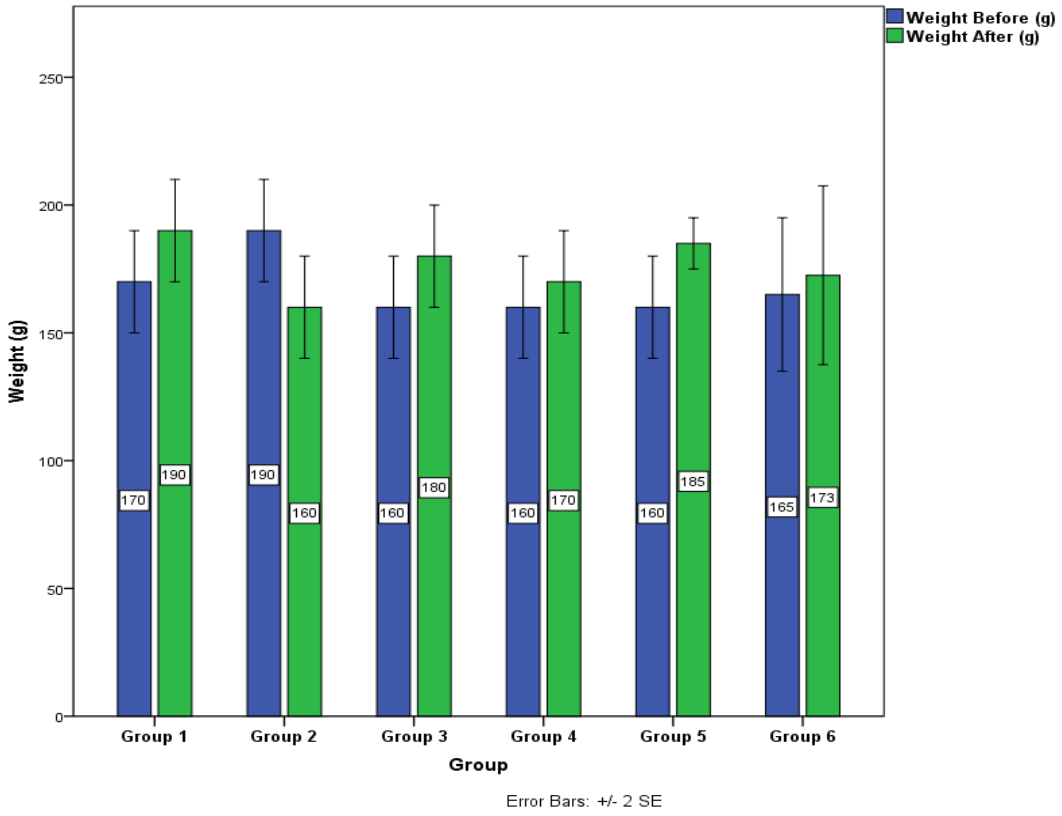

Figure 1: Effect of ethanolic extract of *P. americana* seed on the body weight of potassium aluminium sulphate induced toxicity in female wistar rats.

From the result in figure 1 above, there was an insignificant increase in the body weights of the animals in groups A, C, D, E and F when compared to their weight before treatment. Group B however had an insignificant decrease in body weight when compared to the weight before treatment.

Histopathological Examination

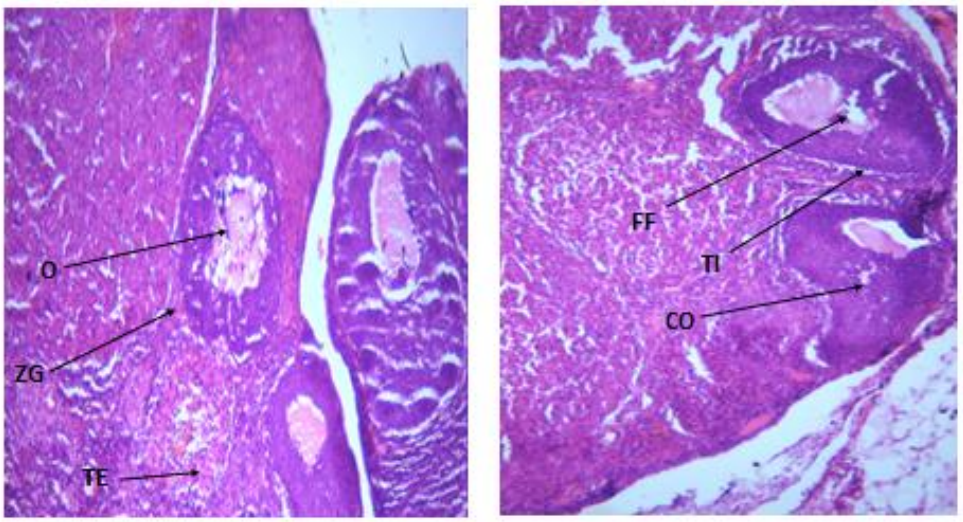

Figure 2: Photomicrograph section of group A animals showing normal ovarian features with the presence of zona granulosa (ZG), theca externa (TE), oocyte (O), follicular fluid (FL), Cumulus oophorus (CO) and theca interna (TI).

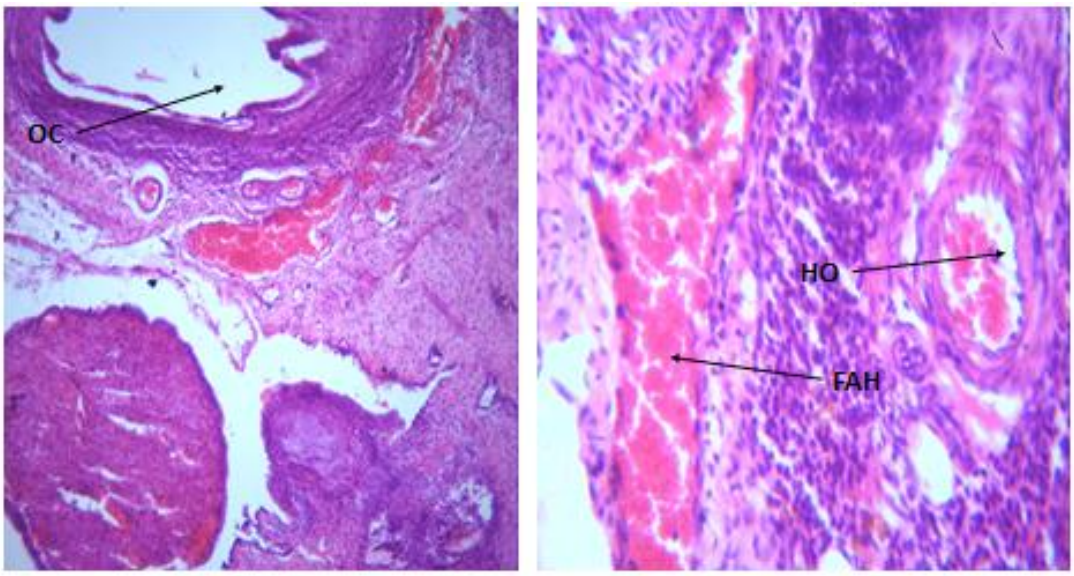

Figure 3: Photomicrograph section of group B animals showing ovarian cortex with ovarian cysts (CO), focal area of haemorrhage (FAH) within the theca interna and oocyte.

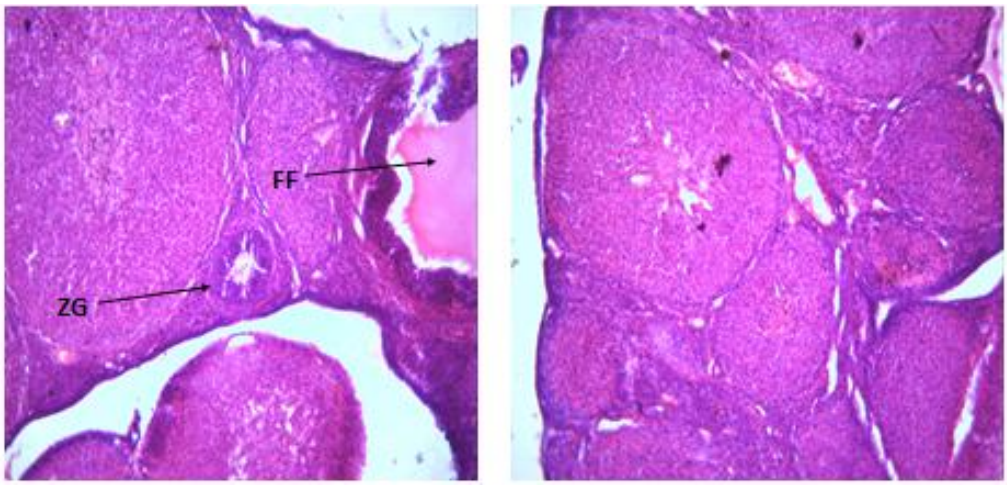

Figure 4: Photomicrograph section of group C animals showing normal ovarian features with follicular fluid (FF) and zona granulosa (ZG).

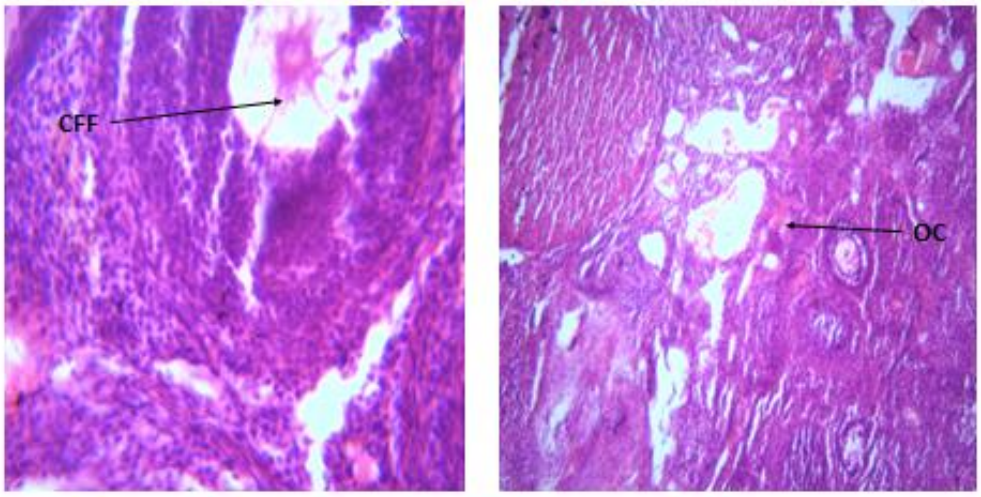

Figure 5: Photomicrograph section of group D animals showing mild healing with ovarian cysts (OC) and clearing of follicular fluid (CFF).

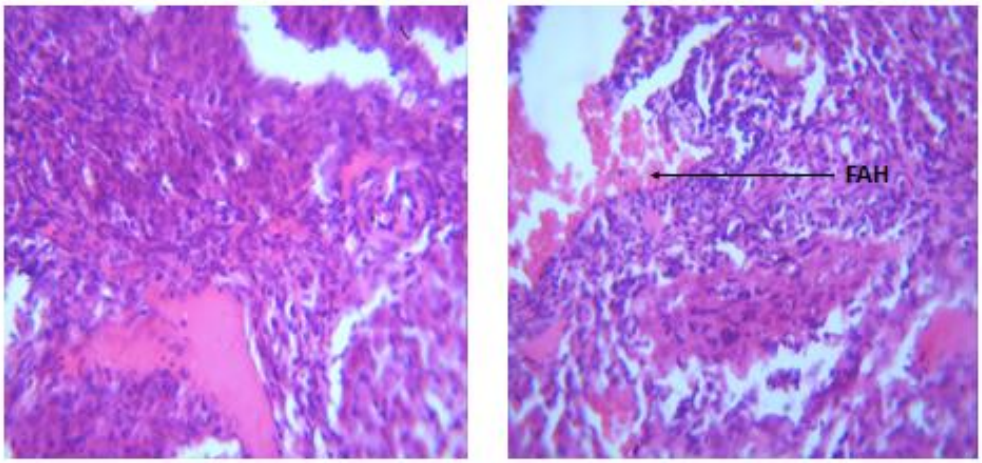

Figure 6: Photomicrograph section of group E animals showing mild healing with focal area of haemorrhage (FAH).

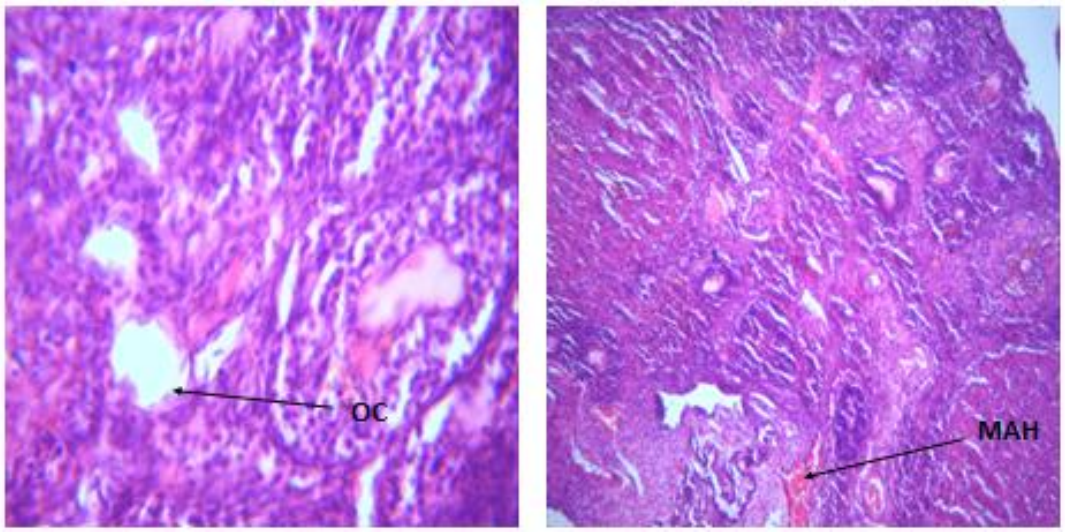

Figure 7: Photomicrograph section of group F animals showing moderate healing with ovarian cyst and mild area of haemorrhage (MAH).

DISCUSSION

Some low income countries with poor clean water supply make use of potassium aluminium sulphate as a convenient alternative in the treatment of water underestimating the underlying dangers it poses to health.

From this study, the toxic effect of potassium aluminium sulphate was indicated by a decrease in body weight in group B. However, there was an increase in body weight in the other groups treated with *P. americana*. The decrease in body weight could be as a result of loss of appetite due to toxicity whereas the increase in body weight could be as a result of the antioxidant property of *P. americana* which ameliorated the toxic effect of potassium aluminium sulphate. This result contradicts the report of Brai et al.[15] who reported a significant decrease in body weight of rats treated with *P. americana*.

Histopathological findings revealed distortions in the cytoarchitecture of the ovary administered potassium aluminium sulphate, with group B having ovarian cortex with ovarian cysts and focal area of haemorrhage. However, there was a mild to moderate healing effect after treatment with *P. americana* with group F having a more protective effect against the toxicity of potassium aluminium sulphate. This could be as a result of the bioactive compounds present in the seed which helps fight against free radicals.[7]

CONCLUSION

Findings from this study indicate that potassium aluminium sulphate is toxic to the reproductive system and the ethanolic extract of *P. americana* seed has a protective effect against its toxicity. The use of this chemical therefore, should be discouraged in the treatment of water.

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