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Histopathological effect of aqueous extract of *Persea americana* seed on alloxan- induced diabetic rats

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Abstract
Persea Americana (avocado) is widely grown in parts of South East, Nigeria, where it is used as a medicinal plant in the treatment of several ailments by alternative medical practitioners. This study was on the pharmacology of its seed in diabetes treatment. The study therefore suggested the histopathologic effects of *P. americana* seed extracts on the liver and kidney of alloxan-induced diabetic albino rats. One hundred gram (100g) of the sample was extracted with 1L of water using the maceration method. The rats were induced with diabetes by intraperitonal (IP) injection of 150mg/kg body weight of alloxan monohydrate solution and treated with different doses of the extract (200 and300 mg/kg.b.wt.) and insulin for 21 days. On Day 21 of the treatment regime, the animals were sacrificed and the necessary organs (liver and kidney) were harvested. The harvested organs were processed according to paraffin wax embedding technique, sectioned at 5µmand stained by the haematoxylin and eosin for light microscopy. Results obtained in alloxan-treated rats showed evidence of necrosis, vasculation and infiltration of the hepatocytes by inflammatory cells. In the kidney, there was mild infiltration by inflammatory cells in alloxan- treated rats. However, treatment of the alloxan- treated rats with extracts of *P. Americana* restored the histoarchitecture of both the damaged liver and kidney to normal as the control rats suggesting that *P. Americana* extracts reversed the histopathological damage that occurred in alloxan-induced diabetic rats. It is concluded this study seems to provides a pharmacological basis for the traditional use of *P. americana* seeds extracts in the management of Diabetes mellitus and raises the possibility of its potential clinical usefulness.

Keywords: *P. americana*, avocado, histopathology, alloxan, diabetes

Introduction
Medicinal plants are used extensively in Nigeria for traditional treatment of all forms of diseases. Usually, different forms of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects. Medicinal plants are known to contained active chemical components which at certain quantities can become harmful to the consumer. Results of many sub-chronic toxicity tests of various plant extracts showed that the liver and kidneys are the major organs usually affected (Ifeoma and Oluwakanyinsola, 2013) [9]. Hepatotoxic and nephrotoxic effects are to be expected, since the liver acts as the main detoxifying organ for chemical substances, while the kidney is a principal route of excretion for many chemical substances in both their active and inactive forms (Abdulrahman *et al.,* 2007) [1]. This is true because of the metabolic importance both organs play in the body. The liver being the center of metabolism is first compromised when the liver is faced with excess toxins. In a bid to detoxify these toxins, the liver could be compromised or overwhelmed. In the case of the kidney, it's a key excretory organ of the body. After detoxification, excess waste materials would be transported to the kidney for onward excretion, with excess of waste materials, the pressure on the kidney would be increased. This increase in kidney pressure adversely increases the tubular traffic and can in turn lead to tubular erosion. This tubular erosion can lead to kidney damage if left unchecked. Liver injury associated with the use of herbal medicine ranges from mild elevation of liver enzymes to liver failure often requiring a new transplant; and carcinogenesis (Maurer, 2015). Established hepatotoxic phytochemicals include podophyllin, eugenol, neoclerodanediterpenes, among others (Pak *et al.,* 2004; Chitturi and Farrel, 2000; Seeff, 2007) [17, 4, 21]. Neurological effects such as convulsions may arise following acute systemic exposure to some phyto medicines; while cerebrovascular accident, encephalopathy and psychosis can become evident in sub acute, sub chronic and chronic tests for toxicity (Seeff, 2007) [21]. It is important to note also that the presence of high levels of metals in the herbal

medicine can contribute to neurotoxicity (Choi, 2005) [5].

Various studies have reported on the effects of different medicinal plants on the liver kidney and other body organs (Item *et al.,* 2010; Rajalakshmi *et al*., 2014; Teoh *et al.,* 2010; Sally, 2007) [11, 9, 23, 20]. Adequate knowledge of the effects of these medicinal plants on the body organs can lead to correct protocol for their safe utilization in order to obtain the maximum health benefit with lower side effects. A study on the effects of methanol extract of *Azadirachta indica* leaves on the histology of liver and kidney of wister rats by Katsayal *et al.,* (2008) [13] concluded that the methanol extract of *A. indica* leaves caused liver and kidney histopathological damages of Wister rats, when the animals were administered with the extract at doses of 1000 and 2000 $mgkg^{-1}day^{-1}$ for 28 days. Rajalakshmi *et al*., (2014) [9], reported that at doses 400 and 600mg/kgb. wt, crude extracts of *Clitoria ternatea* (blue and white leaf extracts), *Solanum nigrum* (blue and red berries leaf extracts), and *Aloe vera*, showed no histological damage to the liver of experimental mice over 15- day study period. Mohamed *et al.,* (2010) reported a positive result on the hepato-ameliorative effect of *A. indica* leaves extract against mercuric chloride environmental pollution. They concluded that *A. Indica* leaves extract showed hepatoprotective properties; the leave extracts being able to reverse cellular injury caused by mercury exposure. Item *et al.* (2010) [11] studied the anti-diabetic mechanism of combined *Vernonia amydalina* and *A. indica* extracts by evaluating their effects on the histology of the pancreas and livers of normal and diabetic rats and reported a recovery/reversal of liver and pancreatic damage in streptozotocin-induced diabetic rats. Another report by Teoh *et al.,* (2010) [23] showed the protective effect of *Momordica charantia*, a bitter gourd known for its anti-diabetic properties, on the kidneys of streptozotocin-induced diabetic rats. Iweala and Oludare (2011) [12] also reported the ability of *Spondias mombin* and *Parinari polyandra* to also reverse liver damage induced by alloxan.

It is absolutely important that plants or drugs must be certified to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used (Sally, 2007) [20]. This study therefore seeks to evaluate possible histopathological effects of aqueous extract of *P. america* seed on alloxan -induced diabetic rats.

Materials and Method

Sample Collection and Preparation

Samples of ripe avocado pear (*P. amercana*) were purchased from New Market in Enugu metropolis. The succulent fleshy part of the fruit was removed to obtain the seed. The seeds were minced by means of a grater and dried to a constant weight in an oven at 50ºC. It was then ground to powder using a mill and then stored in a container. One hundred (100g) of the sample was extracted with 1000ml of water using the maceration method. 100 g of dried, ground sample materials were soaked in water (100%) for 5 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were filtered using Whatman (No.1) filter paper. The filtrates obtained were concentrated under vacuum using a rotary evaporator at 40 °C and stored at 4 °C until when needed for analysis.

Design and Conduct of Experiment

Twenty eight (20) apparently healthy male rats of body weights ranging between 160-220g were used for the experiment. The rats were housed in metal cages groups of four in a photoperiod cycle of 12h:12h (Light and dark), at room temperature (28 °C). The rats were fed with standard laboratory diet and tap water for a period of one week for acclimatization. Groups II, III, IV, V, VI and VII: were induced with diabetes by the intraperitonal (IP) injection of 150mg/kg body weight of alloxan monohydrate solution. The rats were assigned into seven (7) groups of four (4) rats per group as shown below;

Group I- Normal Control rats (negative control)

Group II- Diabetic Control rats (positive control)

Group III- Diabetic rats treated with insulin (1unit of u40/50g kg. b.wt./day).

Group IV-Diabetic rats treated with 200mg/ kg. b.wt water extract.

Group V- Diabetic rats treated with 300mg/kg. b.wt. water extract.

Induction of Diabetes

The baseline blood glucose levels of the rats were determined before they were induced with diabetes by intraperitonal (IP) injection of 150mg/kg body weight of alloxan monohydrate solution (Yanerday and Colae, 1998) [25]. The blood glucose levels of the animals were determined using a glucometer (Tyson Bio Evolve glucometer) and subsequently on a weekly basis for 21 days of administration of the extracts. The body weights of the rats before diabetes induction, was recorded.

Administration of Extracts

The prescribed doses of avocado seed extracts of were orally administered to the rats daily, for 21 days of experiment. After diabetes induction and at 7 days intervals throughout the extract administration body weights of the rats were measured.

For the histological study, they animals were euthanized and the necessary organs (liver and kidney) harvested. These organs were fixed in 10% formal saline for 12hours.They were prepared according to paraffin wax embedding technique, stained with haematoxylin and eosinfor light microscopy.

Results

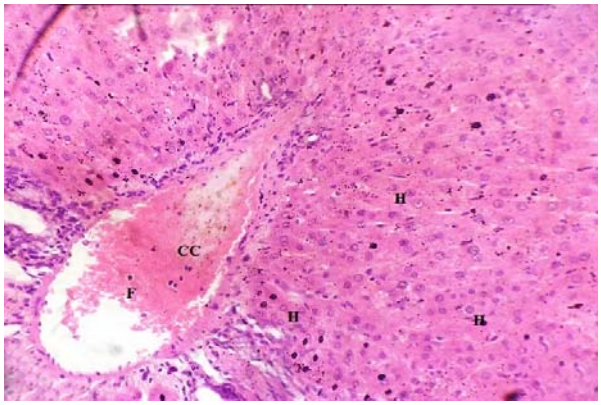

Fig 1: Section of liver tissue of normal rat (Group 1) showing normal hepatocytes (H), Normal central canal (CC), and Frank RBC (F), Stain= H/ E, Mag.: x200

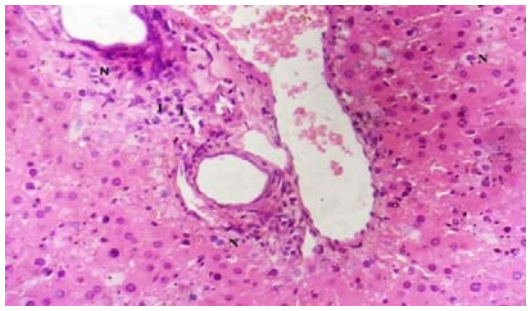

Fig 2: Section of liver tissue of untreated diabetic rat (Group 2) showing liver damage by alloxan. Note the infiltration of numerous inflammatory cells (I) and necrotic cells (N). Stain= H/E, Mag.: x200

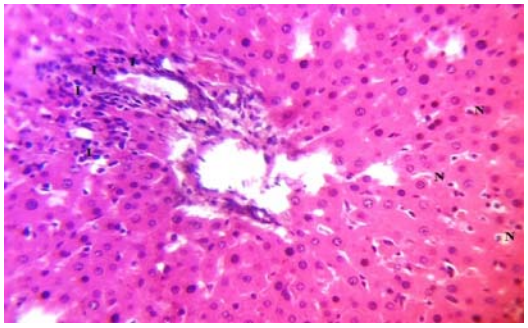

Fig 3: Section of liver tissue of diabetic rat treated with 1unit of 40µ/50g b.w./day dose of insulin (Group 3) following liver damage by insulin. Note the infiltration of inflammatory cells (I) at the peri-vascular area, necrotic cells (N) were also few. Stain= H/E, Mag.: x 200

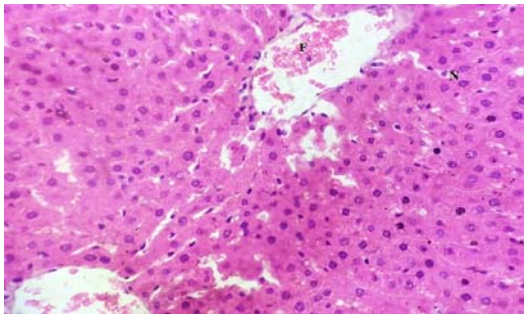

Fig 4: Section of liver tissue of diabetic rat (Group 4) treated with 200mg/kg b.w. of water seed extract of avocado following liver damage induced by alloxan. Note presence of Frank Rbcs in the central area vein (F), and necrotic cells (N) Stain= H/E, Mag.: x200

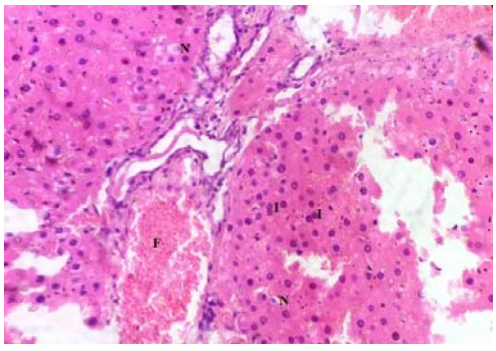

Fig 5: Section of diabetic rat (Group 5) liver tissue treated with 300mg/kg b.w. water seed extract of avocado following liver damage by alloxan. Frank red blood cells (F) are seen in the central area; inflammatory cells (I) and few necrotic cells (N). Stain= H/E, Mag.: x200

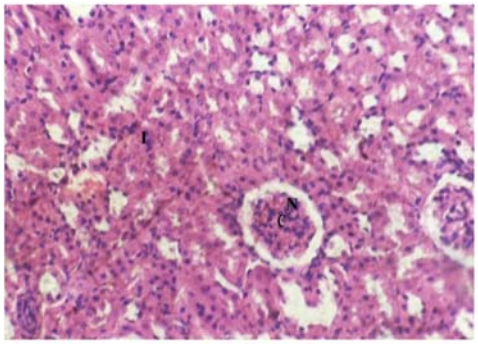

Fig 6: Cortico- medullary of kidney tissue of normal rat (Group 1). Note presence of normal kidney tubules (N), and renal corpuscles (C). Stain= H/E Mag.: x200

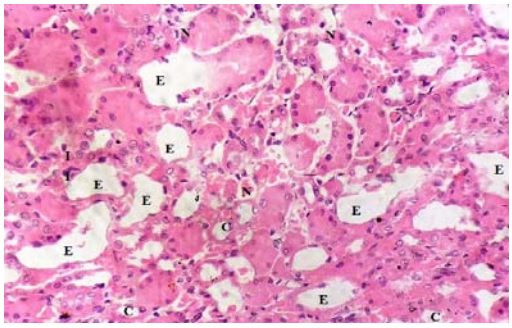

Fig 7: Section of medullar of untreated diabetic rat (Group 2) showing kidney damage by alloxan. Note the infiltration of numerous inflammatory cells (I), numerous tubular casts (C) and proximal convulatedtubular erosion (E) Stain= H/E, Mag.: x200

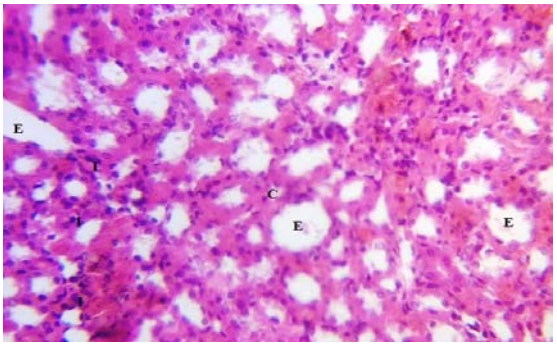

Fig 8: Section of kidney tissue of diabetic rat treated with 1unit of 40µ/50g b.w./day dose of insulin (Group 3) following kidney damage induced by alloxan. Note the presence of few inflammatory cells (I), few tubular cats (C) and decreased tubular erosion (E) Stain= H/E, Mag.: x200

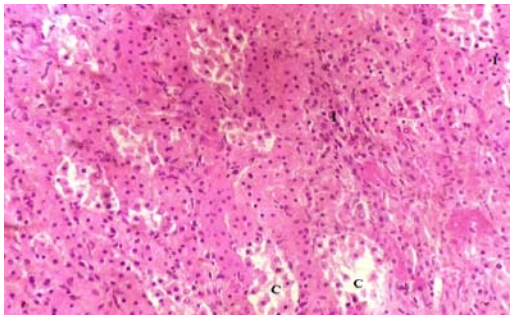

Fig 9: A section of kidney tissue of diabetic rat (Group 4) treated with 200mg/kg b. w. water seed extract of avocado following kidney damage by alloxan. Note the infiltration of inflammatory cells (I), and minimal tubular cats (C) Stain= H/E, Mag.: x200

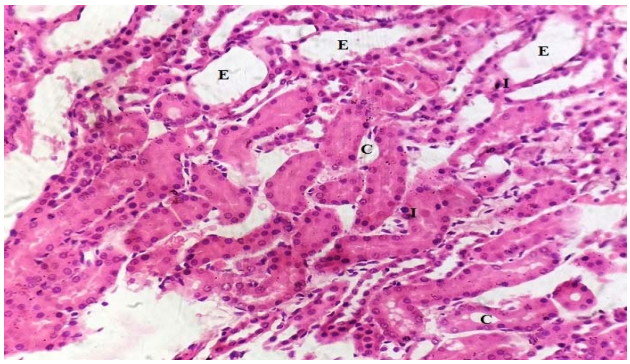

Fig 10: Section of diabetic rat (Group 5) kidney treated with 300mg/kg b.w. water seed extract of avocado following kidney damage by alloxan. Note the distribution of eroded tubules (E), presence of tubular casts (C), few inflammatory (I) cells are also seen. Stain= H/E, Mag.: x200

Histological results from this study showed that both the kidney and liver of the diabetic rats showed high levels of tissue damage when compared to the untreated controls shown in Fig. 1. Fig. 2 shows high number of necrotic cells in the liver of the untreated diabetic rat in Group II. Infiltrations of inflammatory cells within the liver cells were also evident. This inflammatory cell is an evidence of infiltration of the liver cells by toxic substances. These inflammatory cells seem to have been mobilized in order to assist the already compromised liver tissue. The kidney sections of the alloxan induced diabetic rats as shown in Fig. 6 were filled with tubular cats. Sections of the kidney (Fig. 6) were visibly eroded. This tubular erosion is as a result of macromolecules being washed away with toxic materials as the kidney struggles to excrete the excess toxic wastes from the liver into the kidney. This damaging effect of alloxan has been documented (Ezejiofor *et al.,* 2013) [8]. As shown in Fig. 6, many renal tubules of the rat kidneys showed marked degenerative lesions due to the effect of alloxan. This seems justifiable as the renal tubules are particularly sensitive to toxic influences. Renal tubules have high oxygen consumption and vulnerable enzyme systems, and have complicated transport mechanisms that may be used fortransport of toxins and could be damaged by such toxins. Also the tubules are in contact with toxic chemicals during their excretion and elimination by the kidneys (Tisher and Brenner, 1989) [24]. These results also align with earlier reports (Koechel *et al.*, 1984; Damjanov, 1996) [14], that many chemicals have a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubules. The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells (Shimizu *et al.*, 1996) [22]. Renal medullary necrosis occurs as a primary manifestation of renal disease. The mechanism of which is poorly understood, but it seems to involve a vascular change. However, one possible mechanism for the tubular lesions was the direct toxic effect on the cell function (Alden and Frith, 1992) [2].

As shown in Figs. 3, 4, 5, 8, 9 and 10, as treatment was introduced; the kidney and liver tissue (Groups III, IV, and V) sections were evidently restored to normal. It seems the *P. americana* seed has tissue-protective effect which can be observed by its ability to restore and reverse the damaged tissues of diabetic rats. This is shown by the ability of the extract treated groups presenting minimal necrotic and inflammatory cells, which is a clear evidence of healing of damaged tissue cells. Similar effects were reported by earlier studies on avocado seed. (Ezejiofor *et al.,* 2013; Camberos *et al.,* 2013) [8, 3].

On comparison with the insulin treated Group, it is observed that the hitherto damaged liver cells were almost completely restored as indicated by the presence of minimal necrotic cells (Fig. 3). However, presence of inflammatory cells concentrated at the perivascular area was observed, and the eroded kidney tubules were fewer compared with the untreated rat (Fig. 3). The groups treated with various doses of avocado seed extract as shown in Figs. 9 and 10, (Groups IV and V) could be seen to have almost recovered to normal when compared with the untreated group. This tissue tissue-protective effect of *P. americana* can be observed by its ability to restore and reverse the already damaged tissues of alloxan- induced rats, and this observed effect is in agreement with previous reports (Ezejiofor *et al.,* 2013; Edem *et al.,* 2009; Zarah and Dungca, 2015) [8, 9, 26]. Studies by Edem *et al*., (2009) [7] also observed the restorative effect of *P. americana* extracts on the pancreatic islet cells of alloxan induced diabetic rats. Mahadeva *et al*., (2011) [15] also demonstrated the insulin-stimulative and anti-oxidative properties of *P. americana* fruits on streptozotocin-induced diabetic rats, and thus revealing their tissue-protective nature. This restorative effect also corroborates with studies of Imafidon and Amaechina, (2010) [10] reporting that avocado seed extracts repaired liver damage of hypertensive rats. However, it was observed that as the dose of avocado seed extracts was increased, its tissue repair property was getting reduced as shown in Fig.10. This is not in agreement with the report of Zarah and Dungca, (2015) [26], that the repair was better with increase in extract dosage. The decreased tissue repair properties with increasing dosage observed in this study could be as a result of the closeness of the dose to its lethal dose (500 mg/kg) as reported by Camberos *et al*., (2013) [3].

Conclusions

This study seems to provide a pharmacological basis for the traditional use of *P. americana* seeds extracts in the management of *Diabetes mellitus* and raises the possibility of its potential clinical usefulness. However, further studies into its pharmocotoxicity would be necessary before clinical recommendations could be made.

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