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Effect of Aqueous Extract of Persea Americana on the Histology of the Liver of Albino Rat

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Abstract: *The Persea Americana (Avocados) is a tree native to the Caribbean. High avocado extract intake has been shown to have effect on serum cholesterol levels. Specifically, after a seven day diet rich in Persea Americana leaf extract. The aim of the study was to investigate the effect of aqueous leaf extract of Persea Americana on the histology of the Liver. A total of 25 albino rats weighing 160-200g were used for the study. The rats were divided into five groups of five rats each. They were treated with aqueous extract of Persea Americana for a period of 28 (twenty eight) days. Group A received normal saline (control). While groups B, C and D were treated with the extract daily at the doses of 250, 500 and 750mg/kg weight respectively for 28 days. Liver sections were processed and stained with hematoxylin and eosin. Histogical changes observed were hepatic degeneration in the liver parenchyma with severe congestion at high dose of the extracts. The sections also showed swollen hepatic cells with vacuolation and dilated sinusoidal spaces packed with RBCs. These observations are comparable to the findings of other workers who reported scattered non suppurative inflammation in the Liver. The histological changes observed were consistent with the biochemical and gross pathological changes reported earlier. Further toxicity studies (sub-acute and chronic) of the crude extract and organic solvent portions needs to be carried out using different animal models in order to evaluate the long term effects of the extract.*

Keywords: *Persea Americana; aqueous leaves extract; liver; rats; histological changes*.

I. Introduction

Persea Americana is a tree native to the Carribeans. The name"avocado" also refers to the fruit of the Kupa shell that contains a pill (hard seed which may be egg shaped or spherical). High Persea Americana (avocado) extract intake has been shown to have effect on serum cholesterol levels. Specifically, after a seven day diet rich in Persea Americana leaf extract.Nigerian herbalists use the aqueous seed extract for the management of hypertension [1]. The fruit of Persea Americana is eaten in many parts of the world and has been shown to possess medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids. These are believed to modify the fatty acid content in membranes of vital organs, especially the heart [2]. The carotenoid content of Persea Americana has been reported to play significant role in reducing cancer risk [3].The aqueous leaf extract has also been demonstrated to possess analgesic and anti-inflammatory activities [4].

Other medicinal properties of Persea Americana are wound healing [5] and hepato-protection [6]. Avocado toxicity was reported as early as 1942 in California [7].The leaves, bark, seeds and skin of the fruit are toxic and leaves remain toxic after dried. In mammals that have been experimentally fed dried leaves suffered cell death of the mammary glands which are commonly observed clinical finding in livestock, including goats and cattle, known to have ingested avocado plant parts [8]. Avocado leaves are very toxic to rabbits as it produced severe degenerative cellular changes in vital organs such as liver, kidneys and heart causing high mortality[9]. The aim of the study therefore was to investigate the effect of aqueous leaf extract of Persea Americana (avocado) on the histology of the Liver.

II. Methodology

2.1 Extract Preparation

The leaf of mature Persea Americana was collected from Sardauna Local Government Area of Taraba State and was identified at the Department of Botany, Faculty of Sciences, University of Maiduguri. The leaves were thoroughly washed and dried in the laboratory for a period of ten days. The dried leaf was pounded to a fine powder using pestle and mortar and then stored in a dry cylinder. The powdered leaf was extracted with

water using soxhlet extraction method for six hours. The solution obtained was concentrated in the oven at a temperature of 45^{o} C and further dried into a fine powder at a temperature of 50^{o} C.

2.2 Experimental Animals

Twenty- five (25) male albino rats weighing 160-200g and aged between 9-12weeks were used for the study. The rats were obtained from the animal house of the Department of Anatomy, University of Jos and allowed toacclimatize in the animal house of the Department of Human Anatomy, College of Medical Sciences, Universityof Maiduguri, for 2 weeks prior to experimentation. They were kept in properly ventilated cages, where beddingwas replaced every two days, at a room temperature of about 27oC and 12 hour light/dark cycle. The rats werefed with growers' marsh and water from tap *ad libitum*.

2.3 Experimental Design

The rats were divided into five groups of five rats each. They were treated with aqueous extract of Persea Americana for a period of 28(twenty eight) days. Group A received normal saline (control). While groups B, C and D were treated orally by the use of the orogastric tube sterilized with K-Y gel before use with the extract daily at the dose of 250, 500 and 750mg/kg weight respectively for 28 days. At the 29^{th} dayeach rat was anaesthetized with cotton wool soaked in chloroform, and then supported on dissecting bed in dorsal decubitus position; the limbs wereabducted and held with pins to keep the muscles in position. Scalpel blade, sharp scissors were then used to cutand open up the abdominal region, using forceps the kidneys were held and removed and transferred immediately into already prepared 10% formalin in specimen bottles.

2.4 Histological Tissue Processing

The fixed Livertissues were sectioned (5-micron thickness) and Liver sections were processed using the routine light microscopic techniques and stained with hematoxylin and eosin. Histological examinations were supplemented by biochemical assay analysis.Experimental protocols and procedures used in this study were approved by the Ethical Committee of the University of Maiduguri, Borno State, Nigeria. They also conform to the guidelines in the 'Principles of Laboratory Animal Care' [10].

2.5 Statistical Analysis

Numeric data obtained from the study were expressed as the mean value ± standard error of mean (SEM). Differences among means of control and treated group were determined using statistical packageInstant, Version 3, Graphpad Software Inc. [11]. A probability level of less than 5% ($P < 0.05$)was considered significant.

III. Results

3.1 Gross Anatomical Observation

Following administration of the aqueous extract of Persea Americana orally to the rats, it resulted to dehydration, rough hair coat and anorexia (fail to eat for some hours) and the faecal matter of the experimental groups became slightly watery two (2) days after commencement of the extract administration. However, these faecal matters and gross changes returned to normalcy after the fourth day. There were significant increases in weight at the low dose (250mg/kg)administration from days 14 through 28 with respect to the control group (Table 1). The results show that there is no significance difference in serum aspartase aminotransferase (ASAT). The mean values were 78.00±16.02, 68.00±17.46 and 46.25±12.09Mmol/L in groups treated with 250, 500 and 750mg/kg body weight of extract respectively. The mean value was 70.00±8.04Mmol/L.

The result of alanine aminotransferase (ALAT) shows significant difference ($P<0.05$) in group treated with 750mgkg body weight of extract. While in the groups treated with 250 and 500mgkg, there is no significant difference (Table 2). There are no significant differences in serum alkaline phosphatase (Alk. Phos), serum total cholesterol, Conjugated bilirubin, total protein and total bilirubin ($P>0.05$). The results of the serum albumin show significant ($P<0.05$) changes between the treated groups and the control (Table 2)

The liver of rat treated with 250mgkg of extract showed moderate bile duct hyperplasia, cloudy swelling of hepatocytes, mild nuclear cell degeneration (Figs. 3, 4 and 5). Rats treated with 500mgkg of extract showed moderate areas of mononuclear cell infiltration, congestion, interstitial inflammatory cells and mild nuclear cell degeneration (Fig. 6, 7 and 8). Treatment of rats with 750mgkg of extract resulted in enlarged peri-portal vascular channel and congestion, severe areas of focal inflammatory cells, interstitial haemorrhage, severe nuclear cell degeneration, vacuolar cell and necrosis of the hepatocytes (Fig.9, 10, 11 and 12).

Table 1: Effect of the aqueous extract on the weight of the albino rats

Dose (mg/kg)	Day 7	Day 14	Day 21	Day 28
Saline	211.60±28.89	211.60±28.89	211.60±28.89	211.60±28.89
250	256.80±19.56	282.78±22.78*	280.53±26.85*	305.63±27.62**
500	220.83±31.86	228.53±23.30	235.23±24.25	244.83±8.62
750	197.66±20.54	211.28±33.05	213.93±40.84	227.63±40.03

*P<0.05, **P<0.01 Significant increase compared to control.

Table 2: Effect of aqueous extract of Persea americana on serum marker enzymes in albino rats

Dose (mg/kg)	ASAT Mmol/L	ALAT Mmol/L	ALK. PHOS Mmol/L	T/B (g/l)	TC µl/L	CB (g/l)	TP (g/l)	ALB (g/l)
saline	70.00±8.04	25.75±2.75	269.75±39.74	8.50±1.29	2.58±0.41	5.25±1.50	66.25±15.74	33.25±4.19
250	78.00±16.02	22.50±3.70	267.25±26.11	8.00±0.82	2.03±0.05	4.00±0.82	69.75±8.14	31.75±1.26*
500	68.00±17.46	23.25±2.87	278.25±42.58	5.50±1.29	2.10±0.42	2.75±0.96	64.25±6.34	31.25±4.03*
750	46.25±12.09	19.75±2.63*	243.25±65.28	6.00±1.41	2.03±0.47	3.25±0.94	49.75±8.66	23.25±6.08*

*P<0.05 significant decrease compared to control.

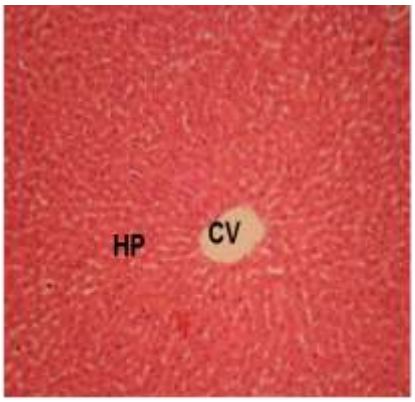

Fig. 1 Photomicrograph of control rat liver showing normal hepatocytes radiating away from the central vein H&E x200

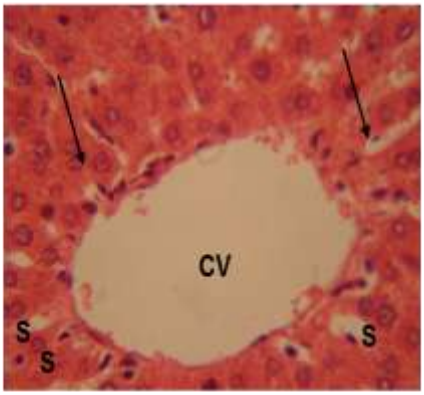

Fig. 2 Photomicrograph of control rat liver showing normal hepatocytes (arrows) radiating away from the central vein (CV) and clear sinusoids (S) H&E x400.

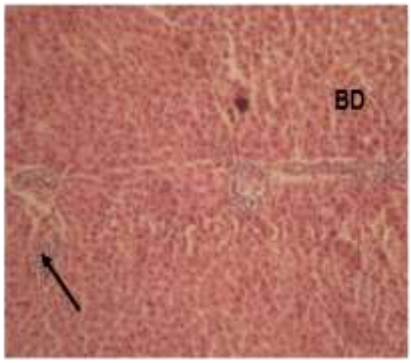

Fig. 3 Photomicrograph of rat liver treated with 250mgk-1 extract showing moderate bile duct hyperplasia and focal areas of mononuclear cell infiltration (arrow) H&E x200.

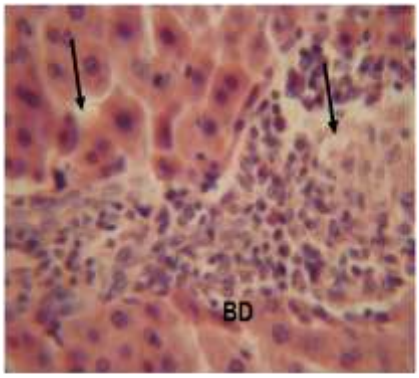

Fig. 4 Photomicrograph of rat liver treated with 250mgk-1 extract showing moderate bile duct hyperplasia (BD) and mild hepatic degeneration (arrows) H&E x400.

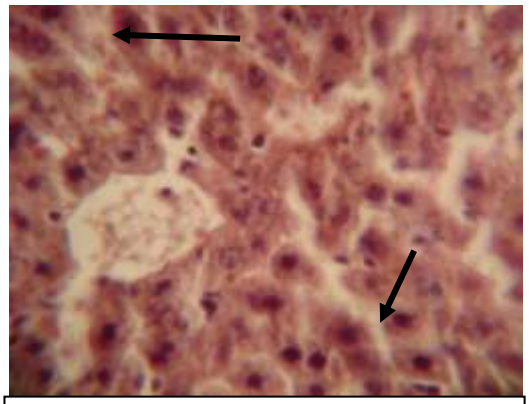

Fig. 5 Photomicrograph of rat liver treated with 250mgkg of extract showing cloudy hepatocytes, mild nuclear cell (arrows) H&E x400

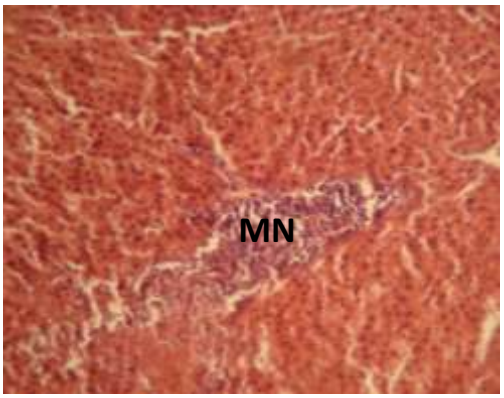

Fig. 6 Photomicrograph of rat liver treated with 500mgkg of extract showing moderate areas of mononuclear cell infiltration (MN) H&E x100

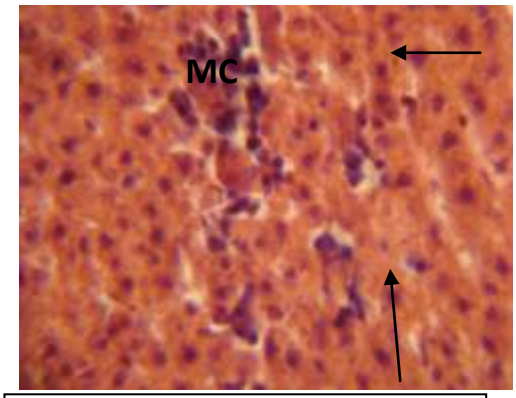

Fig. 7 Photomicrograph of rat liver treated with 500mgkg of extract showing interstitial inflammatory cells (MC) and mild nuclear cell degeneration (arrows)

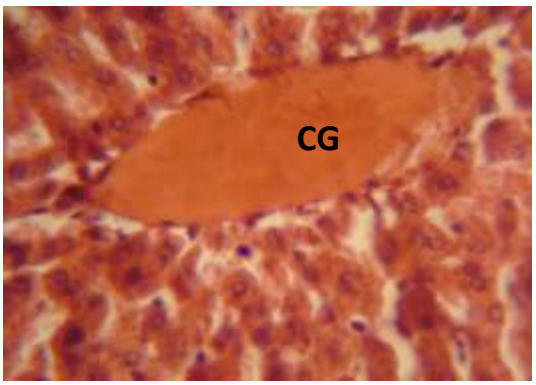

Fig. 8 . Photomicrograph of rat liver treated with 500mgkg of extract showing moderate congestion (CG) H&E x400

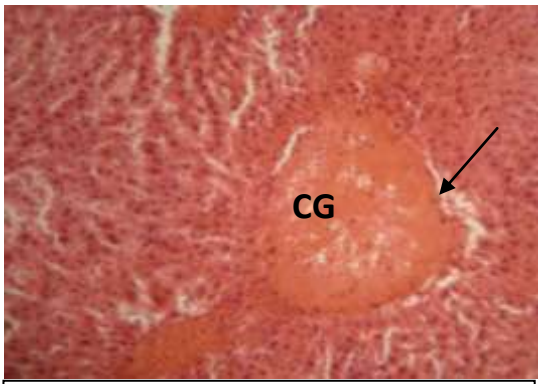

Fig. 9 Photomicrograph of rat liver treated with 750mgkg of extract showing enlarged peri-portal vascular channel (arrow) and congestion (CG) H&E x100

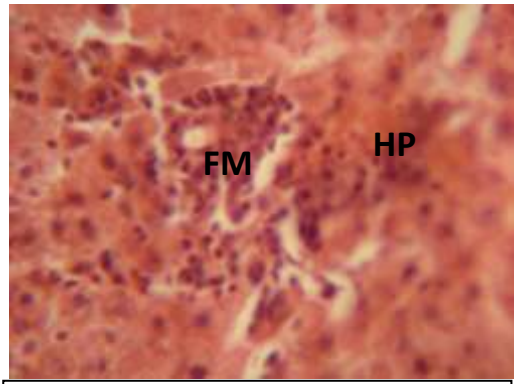

Fig. 10 Photomicrograph of rat liver treated with 750mgkg of extract showing severe areas of focal inflammatory cells (FM) and necrosis of the hepatocytes (HP) H&E

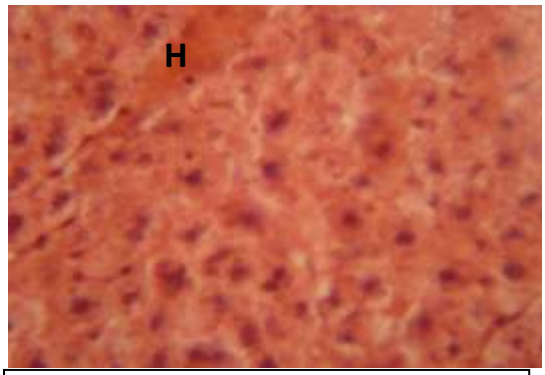

Fig. 11 Photomicrograph of rat liver treated with 750mgkg of extract showing interstitial haemorrhage (H), severe nuclear cell degeneration H&E x400.

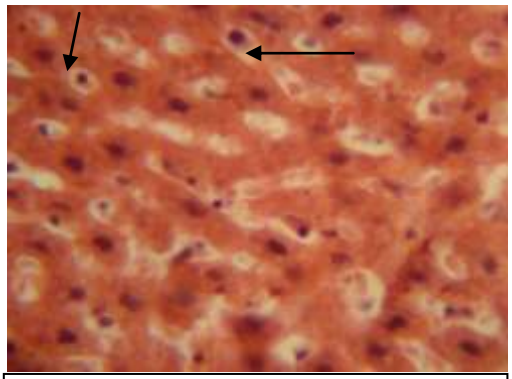

Fig. 12 Photomicrograph of rat liver treated with 750mgkg of extract showing moderate vacuolar cell (arrows) H&E

IV. Discussion

Histogical changes observed in the present study were hepatic degeneration in the liver parenchyma with severe congestion at high dose of the extracts. Other observations include swollen hepatic cells with vacuolation and dilated sinusoidal spaces packed with RBCs.

These observations are comparable to the findings of other workers [12, 13, 14 and 15] who reported scattered non suppurative inflammation in the liver. The histological changes observed were consistent with the biochemical and gross pathological changes reported earlier [16].

Avocado poisoning has been a source of controversy since the fruits are reported to be loaded with nutrients, but there are documented evidences 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 avocado leaves, bark, skin or pit in large quantity [8 and 17].Researchers have shown that avocados extracts improved calcium absorption in rats and addition of avocado to salsa significantly improved lycopene, lutein and carotenes absorption in healthy human subjects [18 and 19]. Lopez et al., [20] observed that after a seven-day diet rich in avocados, hypercholesterolemia patients showed a 17%, 22% and 22% decrease in total serum cholesterol levels, LDL and triglycerides respectively with 11% in-crease in HDL. However leaves are toxic to rabbits although the toxicity varies with the variety [21]. [9]Observed that avocado leaves are very toxic to rabbits as it produced severe degenerative cellular changes in vital organs such as liver, kidneys and heart causing high mortality. He suggested that rabbits should not be fed with avocado leaves which cause hepatic degeneration throughout the hepatic parenchyma with severe congestion. Hepatic cells were swollen with vacuolation and ruptured at many places. Sinusoidal spaces were dilated and packed with RBCs. Bile ducts revealed mild hyperplasia of mucosal epithelium [9]. Further, it should also be borne in mind that the avocado plant must be planted away from livestock enclosures to avoid accidental poisoning in other species of animals[9]; this study is consistent with the present study on albino rats which also reported similar Liver degenerative changes.

V. Conclusion

Based on the results and biochemical analysis, it can be concluded that avocado leaves are toxic to albino rats because of the severe degenerative cellular changes observed in the liver tissue. Albino rats should not be fed with avocado leaves. Further toxicity studies (sub-acute and chronic) of the crude extract and organic solvent portions needs to be carried out using different animal models in order to evaluate the long term effects of the extract.

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Competing Interests

Authors Have Declared That No Competing Interests Exist.

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