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Cashew Leave Ethanolic Extract on the Histology of Select Organs of Wistar Rats: a Clinical Study of the Effects on the Liver, Kidney and Small Intestine

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CASHEW LEAVE ETHANOLIC EXTRACT ON THE HISTOLOGY OF SELECT ORGANS OF WISTAR RATS: A CLINICAL STUDY OF THE EFFECTS ON THE LIVER, KIDNEY AND SMALL INTESTINE

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ABSTRACT

Background: Cashew leaves are leaves of the cashew tree. They are oval with fine pronounced veins and midribs. Each leaf is 10 to 20cm long, by 7 to 12cm in width. Cashew leaves are botanically classified as *Anacardium Occidentale*. Cashew leaves are best eaten when they are young and are a purplish green at the tips and a brighter green on the bottom. When young, the leaves have a texture like hard spinach. They have a tangy and astringent taste. Cashew leave is consumed in Nigeria and other parts of the world as a refreshing drink and used for culinary purposes. Cashew leaves are used medicinally in several cultures. In Africa, it is used to treat diabetes and malaria. In Java, it is used as a paste to treat skin conditions.

Methods: Twenty (20) male wistar rats of average weight of 172.6g± 4.8 (Means ± S.E.M) were assigned to a control group (A) and test group (B, C and D, E). They were administrated with varying ethanolic concentrations (200 to 800mg/kg) of the extract for 28days daily. On day 29, all rats were sacrificed by cranial-spinal dislocation. The kidney, liver and small intestine were excised, grossed, and fixed in 10% neutral buffered formalin solution for 48hrs prior to histological processing. Sections were obtained at 3-5 microns and stained with hematoxylin and Eosin and Periodic Acid Schiff solution for light microscopy.

Results: The acute toxicity of *Anacardium Occidentale* dose (L.D50) tolerable by the rat is 4,525mg/kg body weight. No changes were observed grossly and histologically. However, the empirical measurement showed significant reduction in weight in low dose treated animals (group B and C) and negligible weight loss in high dose (Group D and E) treated animals. The histopathology of the Kidney, Liver and Small intestine after the administration of *Anacardium Occidentale* leaves extract, showed no toxic effects exerted on the wistar rat.

Conclusion: This study suggests that since there is no toxic effect exerted on the Kidney, Liver and Small intestine, *Anacardium Occidentale* leave remains suitable for consumption (as a pleasure drink, for culinary purposes, in delicacies or for therapeutic use).

Keywords: Cashew Leave Extract, Effects of Cashew Leave Extract on the histology of organs of wistar rats.

METHODS

Aim, Design and Setting of the study: This study was to examine the effects of Cashew *(Anacardium occidentale)* leave ethanolic extract on the histology of the Kidney, the Liver, and the small intestine of wistar rats. The study also examines the empirical and physical measurement of wistar rats treated with oral administration of the ethanolic leaves extract after a 28days.

Only 20 male wistar rats were used during this study. No female wistar rats were used during this study. The reason is because female rats undergo change in their physiology and can often be pregnant. These changes often alter the result of the work done and can change the main course or objectives of the work leading also to a false result.

This study was conducted at the department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, Igbinedion University Okada, Edo State, Benin-city, Nigeria. Animal acclimatization, extract preparation and administration, Grossing, Tissue Processing, Sectioning, Staining, Microscopy, and photomicrography lasted for three months (May, June, and July 2017).

Materials

Twenty (20) in-bred white rats of both sexes (Rattus norvergicus) of about 2-4 months old and an average body weight of 130.3-213.9g, were selected into five (5) groups of 4 rats per cage and labelled as cages A, B, C, D and E.
The animals were housed in wire gauze cage with saw dust as beddings to acclimatize, in the animal house of the Department of Biochemistry, Igbinedion University Okada for 2 weeks, under standard condition of temperature (25±5ºC) and a light/dark periodicity of 12:12 hours. Enough food (Standard Top Feed®) and water was provided *ad libitum*.

The leaves of Anacardium Occidentale were plucked from *Anacardium Occidentale* tree. The leaves were identified and authenticated at Igbinedion University Teaching Hospital, Okada, Edo state, Benin-City.
After proper identification of the plant, it was washed thoroughly with tap water and allowed to air dry under the sun for some days. The dried leaves were pulverized into uniform powder using a household blender and sieved to obtain a fine powdered particle. Thereafter, 700grams of the pulverized sieved, fine powdered particle was measured using a weighing balance. Seven liters (7L) of commercially purchased ethanol was measured and added with a measuring cylinder. Heat was applied for 10 minutes at 100ºC in a hot air oven. The heated, boiled mixture was transferred to the GFL Shaker (No 3017 MBH, Germany) while the mixture was mechanically agitated for 8hours. It was left on the bench for 12 hours before filtration under suction pressure with a Whatman's filter paper. Filtrate was concentrated under reduced pressure in a rotary evaporator (Buchi, Switzerland), lyophilized (Christ Alpha 1-2LD, Germany) and stored at 4ºC for experimental use (Odigie, 2014). The process was repeated to obtain a total yield of about 500gm for experimental use.

Methods

The median lethal dose (L.D50) of *Anacardium Occidentale* leaf aqueous extract was determined in the rat using *Lorke's Method (1983)* presented in *(Madara et al., 2010)* and was further modified in the present study.

The method described by (Ajiboso et al., 2007) was used to determine body weight of experimental rats. Individual rats were monitored for daily gain in body weight using digital electronic balance (Gilbertian, Italy). Gain in weight was calculated from the relationship given below: Daily gain in weight=Final day weight-initial day weight, while the average mean weight 172.6g was noted.

Average weight of rats = 172.6g

This study was conducted in 2 phases:

Phase 1: In the first phase, sixteen rats were grouped into four groups (1, 2, 3, and 4) with four rats in each cage. Phase 1 had the following concentration to the rats 200mg/kg, 400mg/kg, 800mg/kg, 1600mg/kg of test substrates. The animals were placed under observation for signs of toxicity which includes but were not limited

to paw licking, salivation, stretches, weakness, reduced activities, dullness, sleep, respiratory distress, coma, and death in the first (4hrs) and subsequently daily for 7days.

Phase 2: This phase was grouped in (5, 6, 7, and 8) with four rats in each cage/group. Phase 2 had the following concentration 3200mg/kg, 12800mg/kg, 25600mg/kg, 51200mg/kg of the test substrate. The animals were placed under observation for signs of toxicity and mortality for the first critical four (4hrs) and thereafter daily for 7days.

Both phases were separately and orally administered (the ethanolic extract) to the mice in each of the test groups. Each of the mice in the control group were then allowed free access to food and water were observed over a period of hours for signs of acute toxicity.

Each rat in group A, B, C, D and E (n=5) were picked on at a time with a hand towel and a dose of 200, 400, 600 and 800mg/kg body weight (b.w) respectively were administered orally using a sterile 5ml syringe. Nonetheless, group A served as the control. Each animal was weighed before and after administering the test extract. The experiment lasted for 28days; oral treatment was given while on the 29; all animals were sacrificed by cranial-spinal dislocation. The Kidney, Liver and Small intestine of the animals (treated and untreated groups) were excised and examined grossly. After that it was processed histologically by the automatic tissue processor (Hestion-ATP7000 Tissue Processor-Germany).

Behavioral signs of acute toxicity were observed in experimental animals and were noted, such as diarrhea, watery stool, hair loss, stretching, reduced activities, dullness, restlessness, paw licking and salivating.

The number of deaths (caused by the extract) with the period was noted and recorded. Log dose response plots were constructed for the plant's extracts, from which the L.D50 of the leaf aqueous extract was determined. The L.D50 was calculated according to Lorke's method using the formulae:

$L.D50=\sqrt{}$ (minimal tolerated dose) × (maximal tolerated dose)

Acute toxicity of *Anacardium Occidentale* leaves extract was found to be L.D50 = ≥ 4,525mg/kg b.w (oral). *(Njoku et al., 2011)* using modified *Lorke's method (1983).*

The animals were weighed before and after administering the crude extract in order of 200mg/kg, 400mg/kg, 600mg/kg, and 800mg/kg body weight with regards to the L.D50 of 4525mg/kg body weight, *(Njoku et al., 2011)* Using oral administration for 28days. On the 29 days, all animals were sacrificed by cervical dislocation; the Kidney, the Liver and Small intestine was excised and preserved in 10% Neutral Buffered Formalin and transported to the Laboratory for tissue processing using the automatic tissue processor.

The select organs such as the kidney, liver, and small intestine from each group of each animal were fixed in 10% Neutral Buffered Formalin for 48hrs following grossing at 3-5mm in diameter. Subsequently, they were dehydrated in graded concentration of ethanol (ascending grades), cleared in xylene, and embedded in paraffin wat for sectioning. After that, sections of about 3-5mm in thickness were obtained using the Hertz rotary microtome (Leica RM2255, Cambridge Mode) and mounted on glass slides. Staining of the section was according to Hematoxylin and Eosin technique and Periodic Acid Schiff technique and was examined using Swift® binocular microscope with an inbuilt lightening system (Olympus, England). All histopathology changes observed in kidney, liver and small intestine sections were observed by the pathologist. *(Avwioro, 2010; Baker et al., 2001).*

Protocols for Hematoxylin and Eosin:

a. Deparaffinize tissue slides in xylene.
b. Hydrate in decreasing grades of alcohol (100%, 95%, 90%, 70%).
c. Stain in Coles hematoxylin 3-5minutes
d. Rinse in distilled water
e. Differentiate in 1% HCL in 70% alcohol, briefly (5seconds).
f. Rinse in distilled water, Blue in Scott's water for 5 minutes.
g. Counter stain with 1% eosin solution for 2 minutes.
h. Rinse in distilled water, Dehydrate in increasing grades of alcohol (70%, 90%, 95%, 100%), rinse in

water. Clear in xylene. Mount with synthetic medium. Observe under the microscope.

Protocol for Periodic Acid Schiff technique:

a. Deparaffinize tissue slides in xylene.
b. Hydrate section in decreasing grades of alcohol (100%, 95%, 90%, 70%).
c. Rinse in water, oxidize sections in 1% Periodic Acid Schiff for 5 minutes.
d. Wash sections thoroughly in running tap water, place in Schiff reagent for 15minutes.
e. Wash in lukewarm water for 5 minutes, counter stain nuclei with Erlich's hematology for 1minute.
f. Wash sections in water for 5minutes, dehydrate sections in increasing grades of alcohol (70%, 90%, 95% and 100%). Clear sections in xylene, mount in synthetic medium. *(Avwioro, 2010; Baker et al., 2001).*

Photomicrography and Statistical Analysis

Olympus photomicroscope (Opticshot-2; Nikon, Tokyo, Japan) was used at x40 magnification. Data were presented as Means ± S.E.M and significance were determined at $p<0.05$ using one way analysis of variance (ANOVA) and differences between pairs of means within the five groups of rats (treated and untreated) were made by using the Students Newman-Keuls test.

Table 3: Empirical and Physical measurements of wistar rats treated with oral administration of *Anacardium Occidentale* leaf extract for 28days.

CAGES	DOSE in mg/kg b. w	Mean Average weight before administration of extract.	Mean Average weight after administration of extract.	Physical weight loss or gain	Activities/dullness
A (Control)	000	181.95	194.41	↑↑	-
B	200	147.38	145.23	↓	±
C	400	178.45	170.15	↓	±
D	600	173.73	164.63	↓	+
E	800	181.25	159.81	↓↓	++

Average weight of rats = 172.6g ± 4.8 (Mean ± S.E.M)

Key to histological scoring

↑ Slight increase in weight

↑↑ Marked increase in weight

↓ Slight weight loss

↓↓ Severe weight loss

± Presence of features

\+ Intermediate features

++ Marked presence of features

\- Absence of features

Table 2: Experimental Design for oral administration of *Anacardium Occidentale* leave extract in 20 wistar rats.

GROUP	NUMBER OF RATS	AVERAGE WEIGHT (kg)	DOSAGE (mg/kg) body weight	ROUTE OF ADMINISTRATION	NUMBER OF DAYS
A (Control)	4	181.95kg	-	Oral	28days
B	4	147.38kg	200	Oral	28days
C	4	178.45kg	400	Oral	28days
D	4	173.73kg	600	Oral	28days
E	4	181.25kg	800	Oral	28days

Table 1: Determination of acute toxicity dose of *Anacardium Occidentale* leave extract in wistar rats (oral administration).

GROUP	NUMBER OF RATS	DOSE (MG/KG)	RESULTS	NUMBER DIED/ NUMBER USED	% MORTALITY
Phase 1					
A (Control)	4	200	-	0/4	0
B	4	400 (minimum)	-	0/4	0
C	4	800	±	0/4	0
D	4	1,600	+	0/4	0
Phase 2					
E	4	3,200	++	0/4	0
F	4	12,800	+++	0/4	0
G	4	25,600	++++	0/4	0
H	4	51,200(maximum)	+++++	0/4	0

L.D50 = √ minimal tolerated dose × (maximal tolerated dose)
Where: minimal tolerated dose = 400mg

Maximal tolerated dose = 51,200mg

√ (400×51,200) = 4,525.4

Therefore, L.D50 = ≥ 4,525mg/kg b.w (oral).

Key: mode of scoring

±	One of the signs of acute behavioral toxicity
+	Two of the signs of acute behavioral toxicity
++	Three of the signs of acute behavioral toxicity
+++	Four of the signs of acute behavioral toxicity
++++	Five of the signs of acute behavioral toxicity
+++++	More than six of the signs of acute behavioral toxicity
b. w	Body Weight

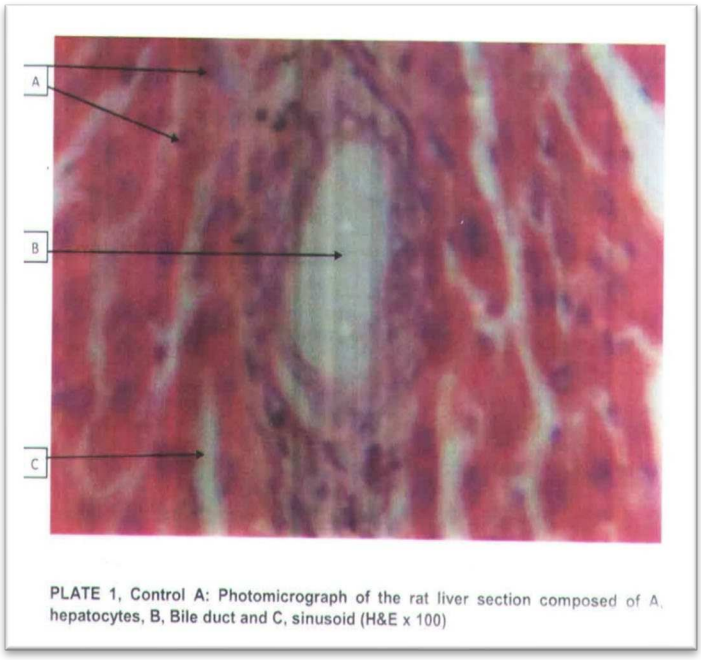

PLATE 1, Control A: Photomicrograph of the rat liver section composed of A. hepatocytes, B, Bile duct and C, sinusoid (H&E x 100)

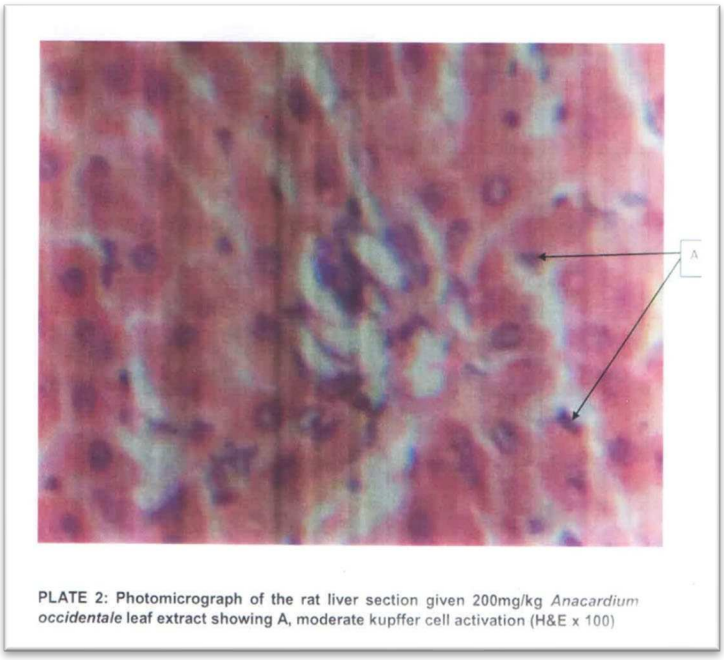

PLATE 2: Photomicrograph of the rat liver section given 200mg/kg *Anacardium occidentale* leaf extract showing A, moderate kupffer cell activation (H&E x 100)

Plate 1 & 2: Photomicrograph's, showing the control and moderate Kupffer cell activation of the rat liver section given 200mg/kg only of *Anacardium Occidentale* leave extract respectively.

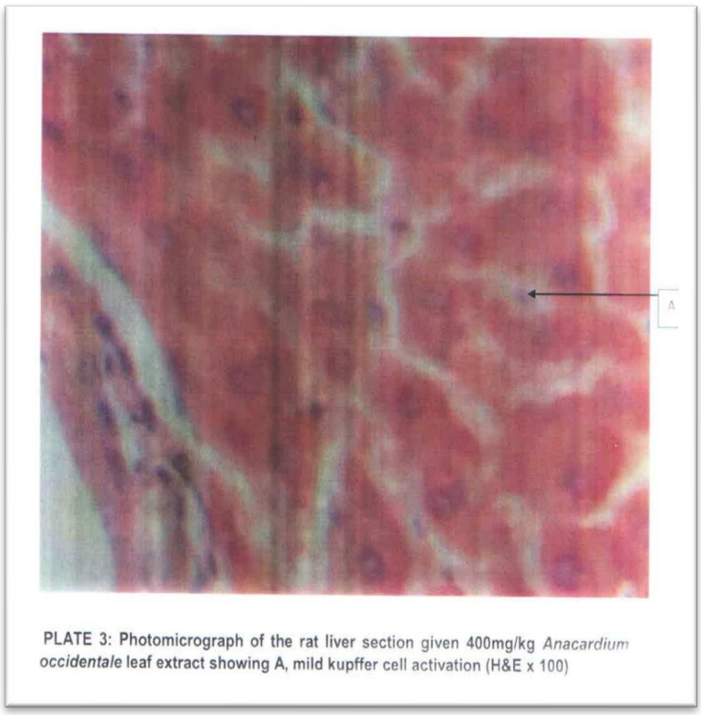

PLATE 3: Photomicrograph of the rat liver section given 400mg/kg *Anacardium occidentale* leaf extract showing A, mild kupffer cell activation (H&E x 100)

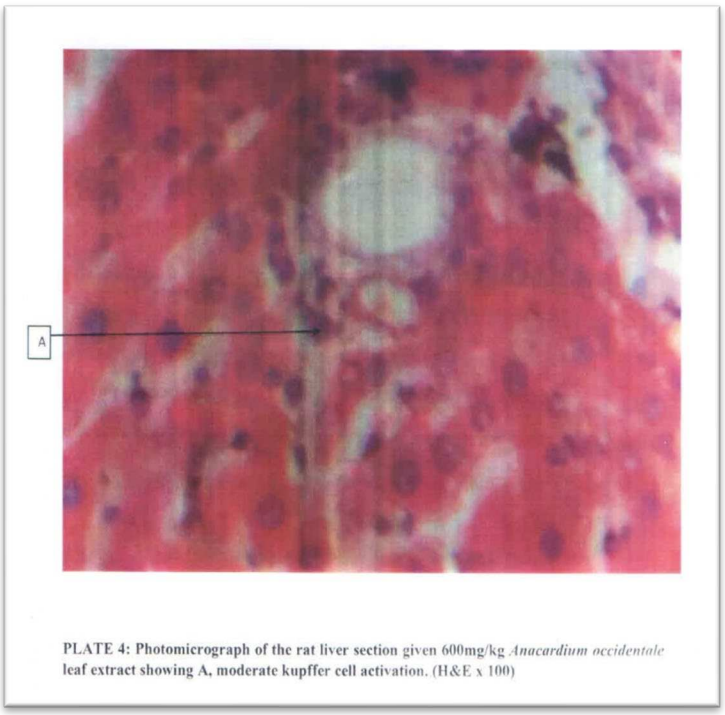

PLATE 4: Photomicrograph of the rat liver section given 600mg/kg *Anacardium occidentale* leaf extract showing A, moderate kupffer cell activation. (H&E x 100)

Plate 3 & 4: Photomicrograph's, showing mild and moderate Kupffer cell activation of the rat liver section given 400 and 600mg/kg of *Anacardium Occidentale* leave extract respectively.

PLATE 5: Photomicrograph of the rat liver section given 800mg/kg *Anacradium occidentale* leaf extract showing A, mild kupffer cell activation. (H&E x 100)

Plate 5: A photomicrograph, showing mild Kupffer cell activation of the rat liver section given 800mg/kg of *Anacardium Occidentale* leave extract.

CONCLUSION

Cashew (*Anacardium Occidentale*) tree has been used medicinally worldwide. The bark, leaves and shell oil of the plant are used medicinally to treat different ailments. *(Agedah et al., 2010).* All parts of the plant (apple, bark, leaves, gum, nut, and roots) have different ethno-medicinal uses for both man and animals alike *(Ramiakajato et al., 2001).* Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects *(Oyesomi and Ajao, 2011).* However, investigation on the phytochemical constituents of the plant extracts also revealed, the presence of bioactive ingredients in the cashew leaves like carbohydrates, tannins, saponins, resins, alkaloids and flavonoids add to their antimicrobial activities. The phytochemical screening analysis on *Anacardium Occidentale* leaves has showed the presence of high concentrations of tannins in the aqueous extract of the leaf *(Abulude et al., 2010).* This could probably account for the effective action of aqueous form compared to the methanolic extract in the inhibitory activity *(Varghese et al., 2013).* Contrary to the above findings, deleterious effects were observed in the present study thereby suggesting that dose relativity may be responsible for the results obtained in this study. It is evident that *Anacardium Occidentale* has anti-protozoan properties *(Obembe et al., 2012)* and anti-microbial properties *(Patrick et al., 2013)* which complements our earlier studies on this plant.

Behavioral changes observed in the wistar rats after oral administration of the Cashew ethanolic extract.

Dullness and restlessness were observed amongst all treated animals but was marked in the high dose treated animals in (Cages C and D). the empirical measurements in the low dose treated animals little or no weight loss compared to the severe weight loss observed in the high dose treated animals. Upon grossing, the organs of the high dose treated animals showed slight variation in coloration while sizes and consistency were intact. Meanwhile, the mean weight of the liver (Cages B, C, D, E and E) was 0.45g.

The cut surface of the liver was smooth and shiny (Control group A), while the color was reddish brown. The outer and inner surface of the kidney was smooth and shiny, same as control (A), while the color was dark-reddish brown on the cut surface and brown on the exterior surface of the kidney. The cut surface of the small intestine was light brown with patches of greyish coloration on the lining of the wall of the small intestine (Group D and E). For the treated group (B, C, D, and E); the cut surface was also smooth like the control group but dull, especially the high dose treated animals (600 and 800mg/kg body weight in rats).

However, the histopathological findings revealed evidence of the activation of Kupffer cells resulting in the localization of the immune system and deleterious effects on the sinusoids. The activation of Kupffer cells in the sinusoids' appeared very prominent in high dose treated liver sections (Plate B and D). However, comparisons were made with the control group (A) without test administration (Plate 1).

Furthermore, in the histopathological findings, there was absence of toxic effect on the kidney (Plate B, C, D, and E); However, the glomerulus, the tubules, and the interstitial spaces, appeared to be more histologically enhanced and distinct in high dose treated animals than in control group (A).

Furthermore, there was presence of deleterious effects on the small intestine. The histopathological findings of the small intestine revealed evidence of muscularis mucosa congestion (Plate B and C), moderate mucosa congestion (Plate E) and normal mucosa architecture (Plate D). Comparison was also made with the control group (A).

Effects of the Cashew ethanolic extract on the histology of the selected organs.

This present study showed that administration of the ethanolic extract of Anacardium Occidentale resulted in histopathological changes in the liver, kidney and small intestine when administered in varying doses (200, 400, 600, 800mg/kg body weight) of the extract in wistar rats.

The results of the hematoxylin and eosin stain (H and E) reactions revealed that with increasing dose of ethanolic extract of Anacardium Occidentale, there was varying degrees of activation of Kupffer cells in the sinusoids of the liver. The liver sinusoids are lined with two types of cells, sinusoidal endothelial cells and phagocytic Kupffer cells. *(Berg et al., 2010).*

In 400mg/kg body weight and 800mg/kg body weight concentration, the activation of the Kupffer cells were

noted as mild while in 200mg/kg body weight and 600mg/kg body weight concentrations, the activation of the Kupffer cells were noted to be moderate. The difference in the immunologic response to the concentration administered, could be due to the varying immune system of the rats before administration of the ethanolic extract *(Bramstedt, 2006).* This could also mean that, in 200 and 600mg/kg body weight, (Group B and E), the immune system of the rats before administration was weak compared to after administration of the dosage where the Kupffer cells became active (moderate) in response to the ethanolic extract induced in rats. From previous research work carried out on the role of Kupffer cells in the pathogenesis of liver disease, it was noted that, Kupffer cells are the first cells exposed to materials absorbed from the gastrointestinal tract. The ability to eliminate and detoxify microorganisms, endotoxins, degenerated cells, immune complexes, and toxic agents (e.g., ethanol) is an Important physiological function of the Kupffer cells *(Nolan 1981).* Therefore, comparing this with the present response of the Kupffer cells to the ethanolic extract in this study, the reaction observed can be said to be physiological and not pathological. This is because, the ethanol concentration used in the preparation of the extract is a toxic agent and triggers the activation of Kupffer cells in response to the toxic agent (ethanol) induced. This further proves that besides the ethanol used in the extraction of *Anacardium Occidentale* leave, the leave (*Anacardium Occidentale*) itself is not a toxic agent. Which further explains, that using a different means of extraction (aqueous extraction) would not have triggered the activation of Kupffer cells. In other words, the extracts administered to (Groups B and E) 200 and 600mg/kg body weight only served as a boost to the immune system of the rats, enabling elimination of the toxic agent (ethanol) introduced into the liver through blood circulation. In comparison of these findings in moderate activation (200 and 600mg/kg) of Kupffer cells to previous research work carried out on the ethanolic extract, it can be said that the Kupffer cells only reacted to the presence of ethanol as a toxic agent in the liver. However, the same could also be applied to group C and E (400 and 800mg/kg body weight) having mild activation of the Kupffer cells in which the immune system of the rat before administration was already said to be active and after administration of the concentrations, there was only a mild boost to the presence of ethanol (toxic agent) in the extract. This agrees to the previous studies carried out in anti-microbial activities regarding the hepatic stage of the malaria parasite Lifecyle. Whereby *Anacardium Occidentale* leaves has been reported for its amoebicidal, antioxidant and astringent properties. *Anacardium Occidentale* can be used to treat malaria *(Razalia et al., 2008).* As reported by *Obambe et al., (2012)* and *kunle et al., (2013),* cashew has been used extensively in the treatment of malaria. The tannins that is presence in the leaf extract of cashew was said to be responsible for the anti-microbial activity against plasmodium spp. Which is the causative organism of malaria. However, the mechanism of action is not yet known *(Obambe et al., 2012).*

The Periodic Acid Schiff Stain (a special stain) was used as a confirmation of the result and to rule out artifact that may be mistaken as hepatocytes and Kupffer cells.

Furthermore, the result of the hematoxylin and eosin (Hand E) staining reaction for the kidney, revealed that, with increasing dose of ethanolic extract (200, 400, 600 and 800mg/kg body weight) of *Anacardium Occidentale*, there was no presence of any deleterious effect of the ethanolic extract on the globurelli, tubules and the interstitial spaces of the kidney. Whereas from early studies carried out on dogs *(Chiakoff et al., 1948),* investigators observed several striking alterations after increased ethanolic extract administration. The basement membrane of the glomerulus became abnormally thickened and was characterized by cell proliferation included enlarged and altered cells in the kidney tubules. This was probably because a different experiment animal was used and a very high dosage of ethanolic extract was administered compared to this presence study. In another study, Van Thiel, and colleagues (1997) compared kidney structure and function in ethanol-fed and controlled rats. The ethanol-fed group experienced kidney swelling and significantly reduced kidney functions. Therefore, comparing this past study with this present study, the result obtained from this study differed from the previous study due to the high ethanolic extract concentrations administered to rats compared to the concentrations used in this study. Reasons for this difference could also be because the rats were fed for a longer duration compared to the duration of feeding in this study. Therefore, this further suggest that *Anacardium Occidentale* leaf is not toxic to the histology of the kidney despite the use of ethanol. These findings are also in agreement to earlier research works done on urinary insufficiency which is a medical condition in which the kidney fails to adequately filter waste products from the blood *(medicine plus, 2012). Omotosho et al., (2012)* found that the ethanolic extract of *Anacardium Occidentale* (EAO) has a cleansing effect on the kidney.

The Periodic Acid Schiff stain (A special stain) was used as a confirmation of the result and to determine whether the cytoplasm of the tubules involved in filtration, reabsorption, and secretion processes, was affected during administration which also proves to be normal.

In addition to this, the result of the hematoxylin and eosin (H and E) staining reaction for the small intestine revealed that with increasing dose of the ethanolic extract (200, 400, 600, and 800mg/kg b. w) of *Anacardium Occidentale* there was varying degrees of congestion.

The muscularis mucosa is a thin layer of smooth muscles that supports the mucosa and provides it with the ability to move and fold. *(Gary et al., 2009).* For 200mg/kg and 400mg/kg, there was mild muscularized mucosa congestion which also exhibited mild signs of inflammation of the result of the ethanolic concentration used. While, in the high dose, (600 and 800mg/kg) there was more effect on the mucosa and mucosa architecture of the small intestine. The mucosa consists of the cryptic and villi, primarily response for absorbing digested nutrients *(silk, 1974).* The effect of ethanolic extract of *Anacardium Occidentale* on the histology of the small intestine led to normal and moderate congestion in the mucosa architecture and mucosa respectively. Therefore, the varying degrees of congestion can be considered as a physiologic reaction due to the presence of high concentration of ethanol in the small intestine. From previous research works carried out on ethanolic extract and alcohol there was several alterations of the small intestinal histology and function after increased administrations which also resulted to diarrhea and absorptive dysfunction *(person, 1991).* This hereby suggests that by using an aqueous extract rather than an ethanolic extract, there would be no signs of congestion in the muscularis mucosa and mucosa, and this also indicates that *Anacardium Occidentale* leaf possess no toxic effect. The present findings agree with the previous research carried out in gastrointestinal tract disorder for diarrhea. Whereby, with the exemption of ethanol, the leaves of cashew which are a rich source of tannins a group of plant chemicals with documented medicinal activities demonstrated in anti-inflammatory and anti-astringent effect, are effective in treating diarrhea *(Abdul garfa et al., 2008).* Banso and Adeyemo (2007) had earlier reported that tannins isolated from the medicinal plants possessed remarkable toxic activity against bacterial and fungi and may assume pharmacological importance in the future. Furthermore, the present findings are also in agreement with the research work carried out for dyspepsia (a condition of impaired digestion). Which *(talley et al., 2005)* reported that the leave of cashew can be used in the treatment of dyspepsia. The ability of the cashew leave has also been attributed to the presence of flavonoids found in the leave of cashew *(Jellin et al., 2002).* Thus, the agreement can be clearly pointed out when coparing the evidence of the presence findings (using ethanolic extract) with the previous research work (using and aqueous extract), on *Anacardium Occidentale* in gastrointestinal tract disorder (diarrhea and dyspepsia) of the small intestine, it can be said that ingestion of high dose of ethanolic *Anacardium Occidentale* extract is more likely to cause not only congestion but alterations in the mucosa architecture and inflammatory effect on muscularis mucosa.

The Period Acid Schiff (a special stain) was used as a confirmation of the result and to rate the extent of the damage of the ethanolic extract on the epithelial mucin, goblet cells and mucosa glands of the small intestine, as PAS is used to detect epithelia mucin and glycogen in tissue *(Avwioro, 2010).*

The result obtained in this study revealed that the ethanolic extract of *Anacardium Occidentale* at low and high doses (200 and 600mg/kg body weight in wistar rats) adversely affects the liver. In addition, the hepatocyte (Kupffer cells) of the liver of the wistar rat that received a low dose (200mg/kg b. w) and high dose (600mg/kg b. w) of the ethanolic extract showed more activation of the Kupffer cells to the toxic extract. With the present result, it is impossible that the functions of the liver as a major metabolic and immunologic organ may be adversely affected if caution is not taken into consideration.

However, the results obtained in this study also revealed that *Anacardium Occidentale* leave extract at varying concentrations (200, 400, 600, and 800mg/kg b. w) had no adverse effects on the kidney of the wistar rats but however, resulted to an enhanced histological feature of the kidney. Therefore, it is further impossible that the functions of the kidney as a major role in filtration, absorption, secretion, and excretion may be adversely affected if proper caution of the duration of administration is not taken into consideration.

Conclusively, the results also obtained in this study of *Anacardium Occidentale* leave extract at different concentrations revealed, more adverse effect on the muscularized mucosa, mucosa architecture, and mucosa of the small intestine of wistar rat. Therefore, it is impossible that the functions of the small intestine as a major role in the support of the mucosa and the absorption of the digested nutrient may be adversely (toxically) affected if the method of extraction is not taken into consideration.

DECLARATION

Ethical Approval and Consent to Participate

This clinical research study and the consent to participate in this clinical research was granted and approved by the board of Medical Laboratory Science Council of Nigeria.

The laboratory animals used in this research were obtained from the Animal house of the Department of biochemistry, faculty of life sciences, Igbinedion University Okada, Edo state, Benin-city, Nigeria, and the experiments conducted on these laboratory animals was approved by the Clinical department of Basic Medical Sciences, Igbinedion University Teaching Hospital, Okada. Nigeria.

The care of the laboratory animals was carried out in compliance with the policies outlined in the 'Guide for the Care and use of Laboratory Animals, published by the US National institute of Health *(NIH Publication No. 85-23, revised 1996)*.

The botanical plants used as extracts in this research study was obtained from the cashew tree at a student residential area and was approved by research experts at the biomedical science department. The leaves were identified, authenticated, and processed at Igbinedion University Teaching Hospital, Okada, Edo state, Benin-City, Nigeria.

Ethical Statement

We ensured that all research was conducted in accordance with ethical principles and confidentiality. We also ensured that all research conducted was in accordance with the approval of the board of Medical Laboratory Science Council of Nigeria. The ethical care of the laboratory animals was duly observed with an ethics of respect for animals, and independent knowledge.

Consent for publication

The research was independently carried out by the author and co-supervised by another clinical scientists. The authorization of publication is based on the rights of the author who conducted this research.

Availability of data and material

The availability of data used in this research study was easily obtained during and after the completion of the research. The materials used in this research study was easily accessible within the geographical area of the institution.

Competing Interest

There was no competing interest involved in this research study.

Funding

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Author's Contribution

The author was solely involved in the processing of the plant for the ethanolic extract and in the manufacturing of the ethanolic extract. The author was involved in the monitoring and observation of the laboratory animals before and after oral administration of the ethanolic extract. Furthermore, the author also contributed to the documentation, compiling, and interpretation of the data obtained from this research.

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