

Functional and Histomorphological Assessments of Liver and Kidney in Matured Female Albino Wistar Rats Following Ingestion of *Citrullus lanatus* Seed Extracts

Original Research Article

ABSTRACT

Oil from different seeds in fruits has been exploited for nutritional and health benefits ranging from provision of essential nutrients for cellular metabolic processes to being an alternative route to the orthodox drugs in the quest for a better living. However, adverse consequences following consumption of this seed oils on both the liver and kidney are not well known. The present study was carried out to assess the integrity of the liver and kidney following the administration of different doses of *Citrullus lanatus* seed extracts (oil) to mature female albino Wistar rats. Twenty - four female albino Wistar rats weighing between 179g - 257g were randomly divided into four groups of six animals per group. Animals in group 1(control group) received normal rat chow placebo. The experimental groups 2, 3, and 4 received 932.0 mg, 1864.0 mg and 2796.0 mg seed oil extract / kg body weight in 0.2, 0.4 and 0.6 ml respectively for twenty-one (21) days by oral gavaging. The effects of treatment on the integrity of the liver and kidney were assessed by measuring the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) for liver and Na^+ , K^+ , Cl^- , HCO_3^- and Creatinine for kidney as well as the histomorphology of the liver and kidney for possible distortions. The results showed a dose-dependent decrease that was only significant ($p < 0.05$) in group 4 relative to the control group for ALT, while both AST and ALP showed non-significant ($p > 0.05$) changes. Furthermore, non-significant changes were recorded for Na^+ , K^+ , Cl^- , HCO_3^- and Creatinine. Histomorphologically, the liver and kidney integrity were not altered except for fatty infiltration of group 4 hepatocytes relative to control group and hyperchromasia of group 4 kidney relative to control. Generally, this study showed that *Citrullus lanatus* seed oil consumption results are of great advantage to liver and kidney tissues when consumed.

Keywords: *Citrullus lanatus*; Hepatocytes; Liver; Serum liver enzymes; Seed oils.

1. INTRODUCTION

Fruits contain a high proportion of water averaging 85%, fats, proteins in very small amount along with a fair proportion of carbohydrates content. (Pamplona - Roger 2008). A variety of fruits are consumed in Nigeria daily, forming an integral part of the diet. One such fruits that are commonly consumed in Nigeria is water melon (*Citrullus lanatus*).

C. lanatus is an important Cucurbit crop accounting for 7% of the world-wide areas devoted to fruits production. (Shaogui and Honge, 2013). Water melon belongs to the family Cucurbitaceae and about three (3) genera of cucurbitaceae bear the common name 'Melons'. They are Cucumis, *Citrullus* and *Cucumeropsis* (Shaogui *et al.*, 2013). The genus *Citrullus* includes *Citrullus lanatus*, which is one of the major fruits grown in the warmer part of the world. *Citrullus lanatus* is both edible and vine-

like fruit plant that plays a very important role in Africa as the water in the fruits is used to quench thirst.

Citrullus lanatus is an annual fruit that originates from the southern part of Africa (Shaogui *et al.*, 2013). It is mainly a dry season crop and requires only limited rainfall to fruit well. The fruits of *C. lanatus* vary in sizes from about 7cm in diameter to over 200cm. Their shape varies also in size from round to long marrow. The outside colouration of the fruit is usually a variegation of green stripes with the inside flesh coloured red or pink. (Shaogui *et al.*, 2013). Water melon plants flowers from July to August and the flowers are monoecious and pollinated by an insect. *Citrullus lanatus* is vulnerable to fungal and bacterial attack.

The seeds of *C. lanatus* are usually black or pale coloured (Maynard 2001), and are useful for oil production in most part of the world (Mandel *et al.*, 2005). The seeds and by extension the oil from the seeds have been documented to contain high lipids along with carbohydrates, proteins, vitamins, minerals, and secondary metabolites such as saponins, alkaloids, tannins, flavonoids etc essential for good health which has informed the need to assess the liver and kidney, organs directly involved in metabolism of the nutrients.

In view of the fact that the liver and kidney are major organs and tissues directly involved in metabolism of lipids and other nutrients in the body, the present study was carried out to assess the effect of administration at different doses of *Citrullus lanatus* on the integrity of the liver and kidney by measuring the activities of some serum enzymes viz (ALT, AST and ALP) for liver and Na^+ , K^+ , Cl^- , HCO_3^- and Creatinine for kidney as well as histomorphological examination of both tissues. Furthermore, recent studies have been carried out using male Albino Wistar rats, hence the use of female rats in this study to elucidate more the metabolic consequence of the ingested seed oil on the females liver and kidney respectively.

2. MATERIALS AND METHODS

2.1 Materials

The plant material used for the study was water melon (*C. lanatus*) seeds. The water melon seeds were taken from matured, fresh water melon fruits purchased from Mbak Item market in Uyo

LGA of Akwa Ibom State, Nigeria. The seeds were washed and sun-dried for a period of about one (1) week in the laboratory and stored in a cool dry place to avoid fungal growth. The seeds thereafter were ground. Chloroform extraction was performed (in a fume cupboard) by adding 200ml of chloroform to the grinded *C. lanatus* seeds. The resulting mixture was mashed very well before filtration with cheese cloth and later with white filter paper (Hoghman paper) to obtain a clear filtrate. The filtrate was then placed in a water bath at temperature of 60° C to evaporate the solvent. The evaporated extracts (oil) was then decanted into a clean container closed tightly and refrigerated till it was ready for use.

2.2 Experimental Design

Twenty-four matured female albino Wistar rats weighing between 179 g - 257g were purchased from the College of Health Sciences Animal House, University of Uyo, Uyo, Nigeria. The animals were housed in cages and allowed to acclimatise before the commencement of treatment. They were maintained under a 12 - hour light and 12-hour dark cycle with feeds and water *ad-libitum*. The animals were randomly grouped into four groups of six animals each. The grouping is as follows:

Group 1: Control group fed with commercial rat feed and water placebo

Group 2: Received 932.0 mg seed oil extract / kg body weight in 0.2 ml

Group 3: Received 1864.0 mg seed oil extract / kg body weight in 0.4 ml

Group 4: Received 2796.0 mg seed oil extract / kg body weight in 0.6 ml

At the end of the extract administration (21 days), all the experimental animals were fasted overnight and anaesthetized by dropping each animal in a transparent glass chamber saturated with chloroform fumes. Incisions were made on the thorax and blood samples were collected by cardiac puncture into sterile plain tubes for sera preparation and sample bottles contain 0.77m EDTA (anticoagulant). Serum samples were obtained from the clotted blood into sterile plain tubes after centrifugation at 200 rpm for 10 minutes using a bench top centrifuge (MSE Minor, England). Liver and kidney samples were also excised, preserved in buffered formalin and used for histomorphological studies.

A quantitative study of the activities of alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase

(ALP) was done using the relevant kits from Randox (Antrim, UK) while using acid-base titration for bicarbonate, electrolyte analyzer called Landwind LW E6OB for electrolytes determination. Sections from the liver and kidney were passed through the processes of fixation, dehydration, clearing, infiltration, embedding, sectioning, staining with haematoxylin and eosin. Photomicrographs were taken with a digital camera attached to a microscope at a magnification of x400.

2.3 Statistical Analysis

The data obtained were expressed as mean $\pm$ SD. The one-way analysis of variance (ANOVA) was used for comparison and the results were subjected to post hoc test using Turkey multiple comparisons. Test values of $p < 0.05$ were considered significant.

The study was approved by the research ethics committee of the postgraduate school, University of Uyo.

3. RESULTS

The results of the effects of *Citrullus lanatus* seed extracts on liver enzymes: Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline Phosphatase (ALP) are presented in Table 1, while the effects on kidney parameters viz: Electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) and Creatinine are presented on Table 2. Statistical analysis revealed that serum AST levels of all the test groups compared favourably with the Control group, with slight but non-significant ($p > 0.05$) increases in serum AST levels at higher doses (Group 3 and 4).

The results of ALT, as shown in Table 1., indicated that there was no change at the lower dose of extract (932.0mg/kg bwt) but the higher dose of extract (2796.0mg/kg bwt) produced significant ($p < 0.05$) decrease in serum ALT levels relative to the Control group.

Statistical analysis revealed non-significant ($p > 0.05$) changes in serum ALP in all test groups relative to the Control group. The results of the effects of *Citrullus lanatus* seed extracts on serum electrolyte and creatinine levels as presented in Table 2, revealed non-significant ($p > 0.05$) comparing the experimental group to the control.

Table 1. Effect of *Citrullus lanatus* seed extracts on serum liver enzyme levels of female albino Wistar rats

G R O U P S .	AS AST (μL)	ALT (μL)	ALP (μL)
1. C O N T R O L	10.91 $\pm$ 2.67	17.81 $\pm$ 2.51	39.40 $\pm$ 3.61
2. 932.0mg/Kg Bwt	8.51 $\pm$ 1.52	16.38 $\pm$ 2.72	40.52 $\pm$ 6.02
3. 1864.0mg/kg Bwt	11.68 $\pm$ 1.52	N d	39.58 $\pm$ 6.66
4. 2796.0mg/kg Bwt	13.39 $\pm$ 2.01	12.00 $\pm$ 1.56 ^a	44.17 $\pm$ 3.91

Values are expressed as mean $\pm$ SD. n=6.

Legends: a - Significantly different from control at $p < 0.05$, nd - Not done due to technical problems. AST- Aspartate amino transferase, ALT-Alanine amino transferase, ALP -Alkaline Phosphatase.

Table 2. Effect of *Citrullus lanatus* seed extracts on serum electrolytes and creatinine levels of female albino Wistar rats

Groups	Na^+ (Mmol/l)	K^+ (Mmol/l)	Cl^- (Mmol/l)	HCO_3^- (Mmol/l)	Creatinine (Mmol/l)
1. Control	140.33 $\pm$ 1.63	4.30 $\pm$ 0.51		103.00 $\pm$ 3.03	23.0 $\pm$ 1.00
2. 932.0mg/Kg Bwt	140.66 $\pm$ 2.50	4.45 $\pm$ 0.53		102.83 $\pm$ 3.50	19.0 $\pm$ 7.00
	81.72 $\pm$ 26.87				

	3. 1864.0mg/kg Bwt	141.60 ± 2.40	4.40 ± 0.45	103.40 ± 2.31	20.0 ±
5.00	91.78 ± 44.67				
	4. 2796.0mg/kg Bwt	142.16 ± 6.24	3.96 ± 0.39	100.83 ± 3.42	23.0
±2.00	90.32 ± 33.65				

Values are expressed as mean ± SD. n=6

4. DISCUSSION

The liver is an important tissue which plays a pivotal role in xenobiotic, glucose and lipid metabolism (Oluba, 2011). The function of the liver is commonly evaluated with respect to disease condition and injury by using liver enzyme markers. These liver enzymes are as follows; Aspartate Aminotransferase (AST) formerly called Serum Glutamate Oxaloacetate Transaminase (SGOT), Alanine Aminotransferase (ALT) formerly called Serum Glutamate Pyruvate Transaminase (SGPT), and Alkaline Phosphatase (ALP).

The enzyme Aspartate Aminotransferase (AST) is distributed in erythrocytes and tissues principally in the heart, muscle, liver and kidney. AST occurs as two isoenzymes, indistinguishable from standard AST assays, namely; the mitochondrial isoenzyme and the cytosolic isoenzyme. The results of AST, ALT and ALP levels in treated albino Wistar rats are presented in Table.

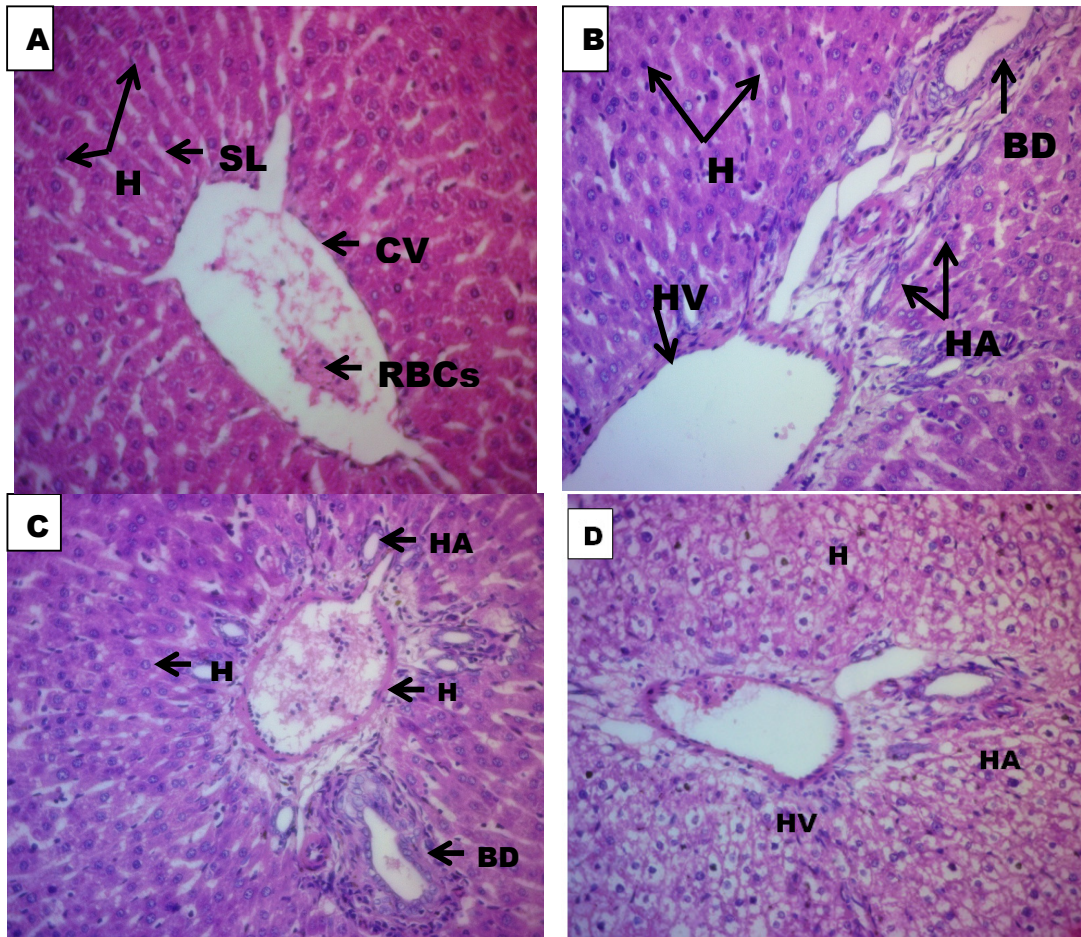
From this study, non-significant changes in AST levels in all test groups compared to the control were observed. The results of this study agree with the works of Oluba, *et al.*, (2011), who evaluated the effect of palm and egusi melon seed oils on serum lipid profile in rats fed a high-fat diet.

The enzyme Alanine Amino Transferase (ALT) compared to AST is localized in the cytosol of the liver and to a lesser level in other organs

(Sherlock, 1997). ALT catalyzes the transfer of an amino group from Alanine to keto group of oxoglutarate to form Pyruvate and glutamate. ALT is an indicator that is often used to confirm the hepatocellular injury, damage, and disease conditions such as hepatitis, cirrhosis, obstructive jaundice, and carcinoma.

The result of this study showed that ALT levels were generally reduced in all test groups with group 4 significantly reduced compared to control. This shows that *Citrullus lanatus* seed oil does not have an adverse effect on the liver integrity and this agrees with the report of Chilaka, Ifediba, and Ogamba (2005), on the evaluation of the effect of *Citrus sinensis* seed oil on liver enzymes in rats. Alkaline Phosphatases (ALP) are a group of isoenzymes that act to dephosphorylate a variety of molecules throughout the body. ALP is produced mainly in the membrane of cells lining bile ducts and bile canaliculi. ALP is released in response to accumulation of bile salt or Cholestasis. ALP is equally contributed from non-hepatic sources in the kidney, intestine, leucocytes, placenta, and bone as well as rises during pregnancy or in growing children. From the result of this study, ALP levels showed a non-significant difference comparing the test groups to the control.

The kidney is a major homeostasis organ responsible for the maintenance, balance and excretion of electrolytes (substances that become ionized in solution) and creatinine (a water-soluble molecule generated from muscle creatine metabolism). The results of the effects of



Histologic photomicrographs of Liver at magnification x 400 : (A) Control group, (B) Group 2 treated with 0.2ml (932.0 mg seed oil/kg body weight) , (C) ;Group 3 treated with 0.4ml (1863.0mg seed oil/kg body weight and (D); Group 4 treated with 0.6ml (2796mg seed oil/kg body weight),
Keys; Hepatocytes (H), Hepatic Vein(HV), Bile Duct (BD),Hepatic Artery (HA), Sinusoidal Lining (SL), Central vein(CV), Red blood cells (RBCs)

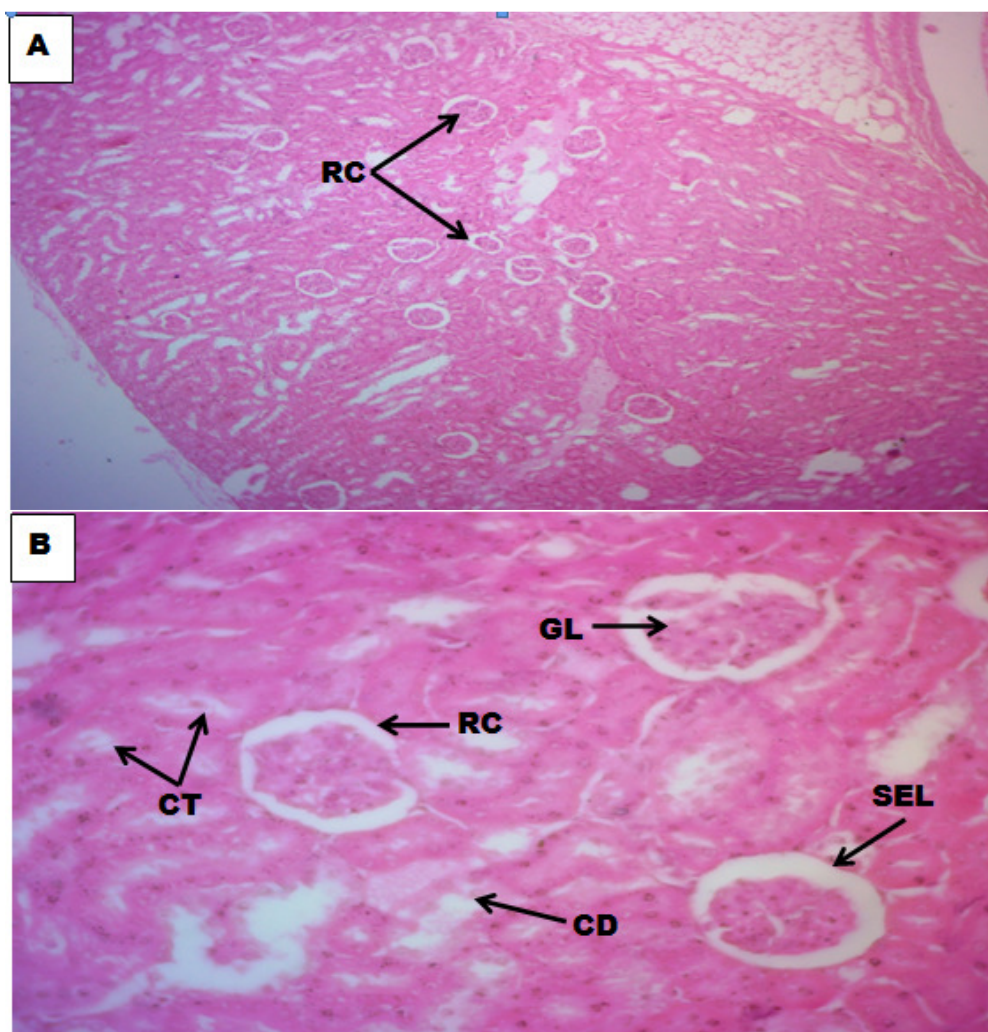


Plate I (panels A&B) - Histologic photomicrographs of kidney tissue of normal female albino Wistar rat without treatment with chloroform seed extract of *Citrullus lanatus* at magnification A (x100) and B (x400) stained with H&E method. Kidney tissue reveals normal appearance of renal corpuscle (RC)

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Collecting ducts (CD), Squamous epithelial lining (SEL), Glomerulus (GL)

Citrullus lanatus on electrolytes and creatinine levels are shown in Table 2. Electrolytes are vital for normal functioning of body cells and organ including that of the kidney and from the results of this study, the electrolytes assayed for in this study revealed non-significant ($p>0.05$) different comparing the experimental group to the control group. This findings which are in agreement with Omigie (2014) who worked on the effect of *Citrullus lanatus* seed extracts on serum electrolyte levels revealed that the kidney was not in distress nor suffered any form of toxicity as a result of the seed oil administered. Creatinine is a water-soluble molecule generated by muscle

cells during the metabolism of creatine for energy for muscular activities such as contraction and relaxation. The kidney excretes creatinine at a constant rate. Hence any change in creatinine excretion pattern is a reflection in kidney function. From the results as presented in Table 2, creatinine levels did not pose any threat to the kidney function as the were within the normal physiological range and were not significantly ($p>0.05$) different comparing the experimental group to the control group. This finding agrees with the work of Ali and Blunden (2003), on the effects of *Nigella sativa* seed extracts on serum creatinine levels.

Histomorphological evaluation of the liver and kidney as revealed by the micrographs showed that at lower dosages Group 2 and 3 liver and kidney tissues showed no different compared to the Control group except for Group 4 which received the highest dose were the hepatocytes were prominently filled with fat tissues and the

kidney convoluted tubule blurred with its nuclei prominently stained compared to the Control group which is in agreement with Oyewo *et al.* (2012), on the effect of aqueous extract of *Mangifera indica* where the lower doses show no distortion of kidney tissues compared to control.

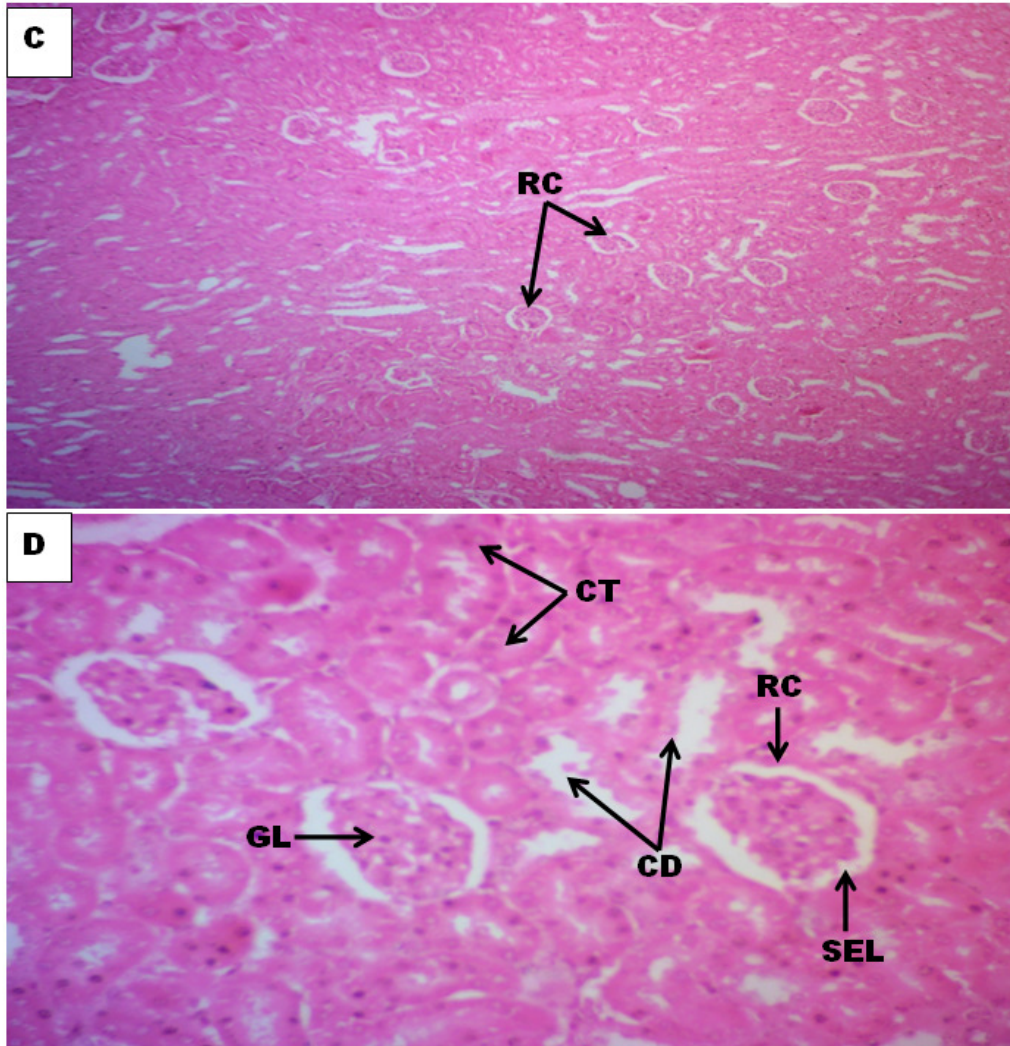


Plate II (panels C&D) - Histologic photomicrographs of kidney tissue of normal female albino Wistar rat without treatment with chloroform seed extract of *Citrullus lanatus* at magnification C (x100) and D (x400) stained with H&E method reveal normal appearance of renal corpuscle (RC) compared to control

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Collecting ducts (CD), Squamous epithelial lining (SEL) and Glomerulus (GL)

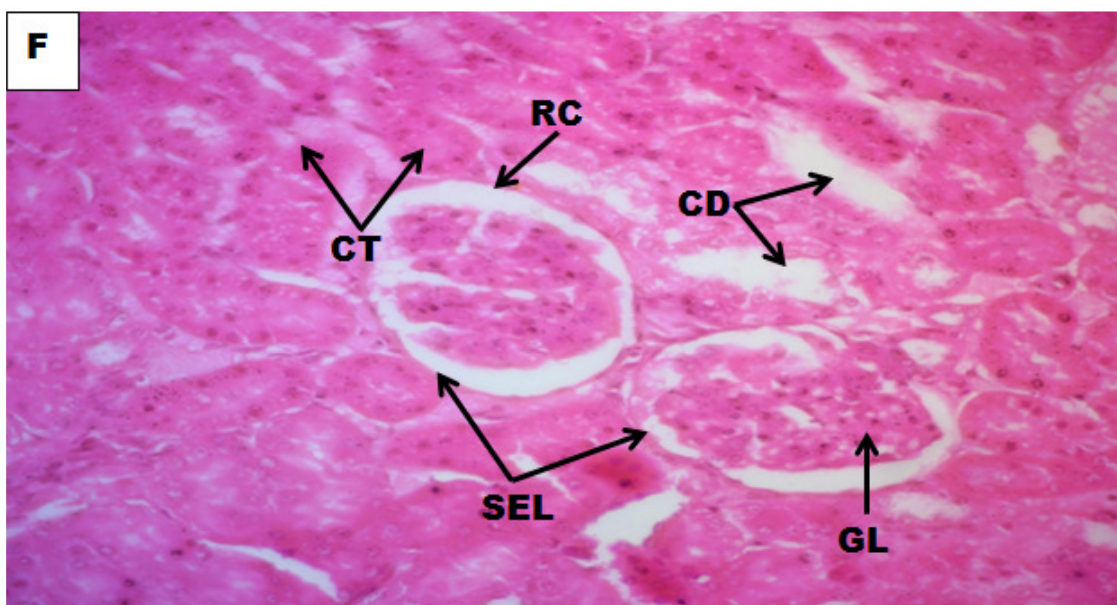
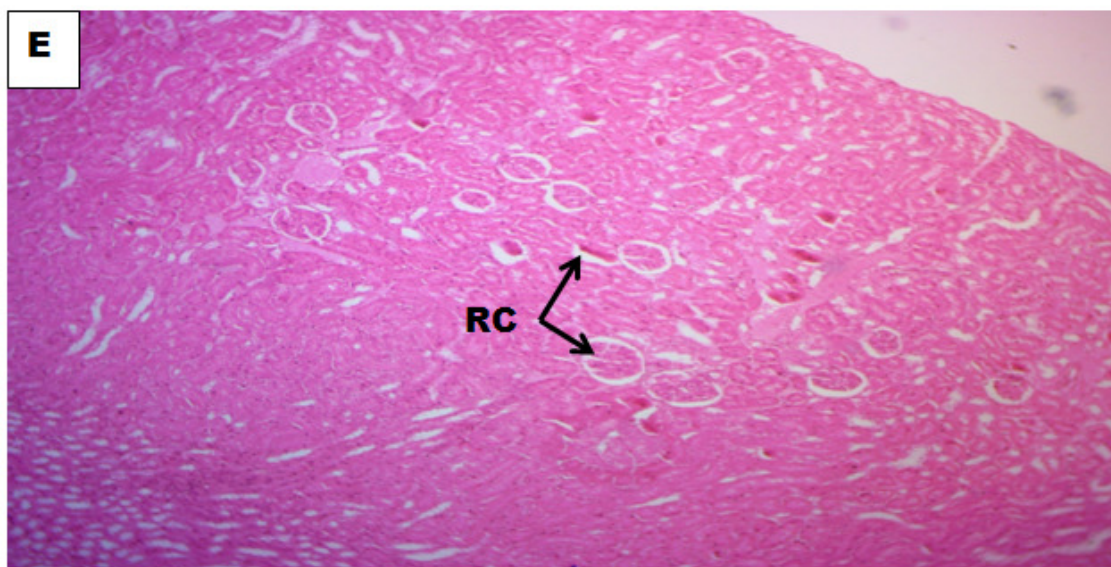


Plate III(panels E&F) - Histologic photomicrographs of kidney treated with 0.4ml (1864mg seed oil/kg body weight) of *Citrullus lanatus* at magnification E (x100) and F(x400) stained with H&E method revealed normal cellular appearance of renal corpuscle (RC) compared to control group

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Collecting ducts (CD), Squamous epithelial lining (SEL), Glomerulus (GL)

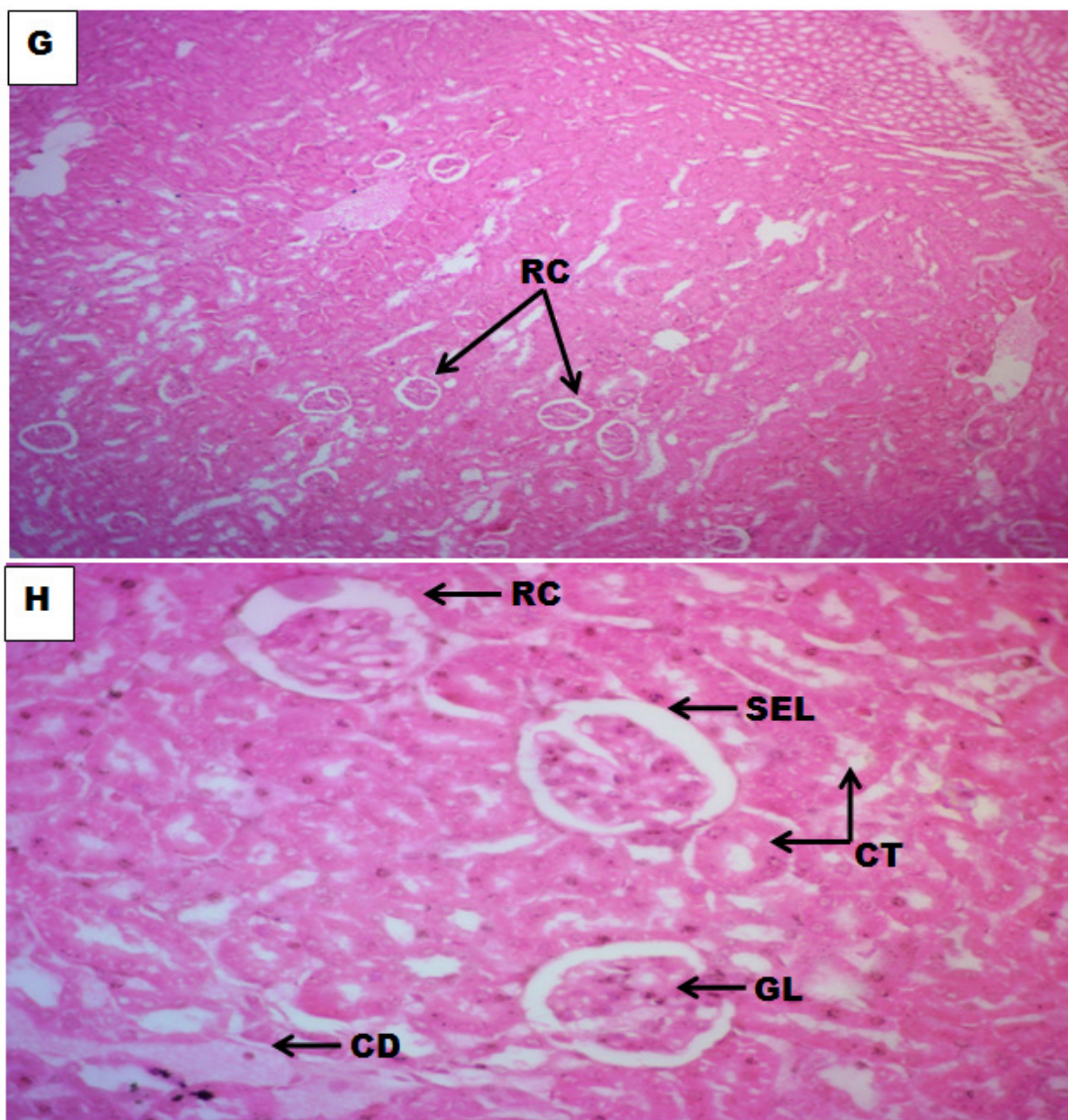


Plate IV (panels G&H) - Histologic photomicrographs of kidney treated with 0.6ml (2796.0mg seed oil/kg body weight) of *Citrullus lanatus* at magnification G (x100) and H(x400) stained with H&E method revealed blurred luminal surfaces of convoluted tubule (CT) and hyperchromasia of compared to control group

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Collecting ducts (CD), Squamous epithelial lining (SEL), Glomerulus (GL)

5. CONCLUSION

In conclusion, the results of the present study revealed that *Citrullus lanatus* seed extract (oil) consumption results in favourable liver and kidney function and may be hepatoprotective which is in agreement with the reports of Erhirhie and Ekene,(2013) on the medicinal values of *Citrullus lanatus*. Furthermore, the prominently stained hepatocytes with fatty tissues suggest

the need for regular monitoring of liver functions in the course of treatment and discontinuation of treatment if the substantial damage is indicated.

CONSENT:

It is not applicable.

ETHICAL DISCLAIMER

The study was approved by the research ethics committee of the postgraduate school, University of Uyo. The Postgraduate School of University did not issue any certificate for ethical clearance, rather they ensure that any research proposal is passed through the Research and Ethics Committee who clears the work before the postgraduate student commences the research. This work went through these processes in May 2013. In 2015 the dissertation was assessed and the student was awarded the degree.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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