Original Research

Volume 5, Issue 2, pp 162-176, September, 2023

ACHIEVERS JOURNAL OF SCIENTIFIC RESEARCH

Opecn Access Publications of Achievers University, Owo

Available Online at *www.achieversjournalofscience.org*

Subchronic Administration of Okra (*Abelmoschus esculentus* Moench L) leaf Reversed TBARS and Improved Functional Indices of Hepatic and Renal Functions in Male and Female Wistar Rats

Borokini, F.B.[1], Oladipo, G.O.[2], Komolafe O.Y.[3], Oladipo, M.C.[2] and Ajongbolo, K.F.[3]

[1]Department of Chemistry, University of Medical Sciences, Ondo City, Ondo State. Nigeria.

[2]Applied Clinical and Computational Biochemistry Laboratory, Department of Chemical Sciences, Achievers University, Owo, Nigeria.

[3]Department of Science Laboratory Technology, Faculty of Applied Sciences, Rufus Giwa Polytechnic, PMB 1019, Owo, Ondo State, Nigeria.

Corresponding Author E-mail: Oladipo402@yahoo.com

Submitted: July 12, 2023, Revised: August 25, 2023, Accepted: September 02, 2023, Published: September 28, 2023

Abstract

This study was designed to evaluate the sub-chronic effects of *Abelmoschus esculentus* Moench L leaf on liver and kidney functions indices in male and female Wistar rats. Ethanolic leaf extract of Okra plant (ELEOP) was prepared and administered to male and female WIstar rats in the following doses-10, 100, 1000, 2900 and 5000 mg/kg BWT to initially observe within 24 hours the autonomic, behavioral and neurological changes, and more importantly the lethality of the extract (LD_{50}), followed by sub-chronic evaluation within 28 days. The spectrophotometric procedures to determine the activities of ALT, ALP and AST was conducted to evaluate effects on the liver function. And the effect on kidney functions was evaluated by the determinations of the concentrations of blood urea nitrogen and creatinine in female and male rats. The effects of the treatment on the histology of the kidney and liver tissues were examined. Also determined were the extent of the inhibition of lipid peroxidation using concentration of MDA generated and degeneration of protein mass using assay of total protein determination in the liver and kidney. Ethanolic extract of Abelmoschus esculentus Moench L leaf had an LD50 of 1250 mg/Kg BWT in female Wistar rats and 2333.33 mg/Kg BWT in male Wistar rats. This was evident from the effects it had on the indices of kidney and liver functions. The quantitative evaluation of phytochemical composition of the leaf extract revealed caffeic acid as the most predominant, other phytochemicals included coumarin, quercetrin, quercetin, kaempferol, gallic acid and chlorogenic acid. These are phytochemicals of antioxidative importance but that are required endogenously in limited amounts.

Keywords: *Abelmoschus esculentus* (Okra) leaf; Acute toxicity; LD_{50}; HPLC-DAD

1.0 Introduction

The nutritional and pharmaceutical uses of plants are as old as man. The advent of civilization has caused massive drift from the folkloric use of this plants for the treatment of ailments, and the knowledge on the use of these plants was faintly passed down the generations as most was communicated orally but hardly written down. Plants have been used for the prevention,

treatment and management of diseases for a very long time without the knowledge of the toxic implications when used beyond doses that could impaired physiological status (Shafaei, 2011).
Investigation into the acute toxicity of a substance is the first step to determine the safety of the substance (Lorke, 1983) prior to therapeutic investigations. Abelmoschus esculentus (Okra) leaf has sparse scientific information, a review reported that a decoction of the leaf is demulcent, diuretic and emollient, it was used in the treatment of catarrhal infections, dysuria and gonorrhea (Kumar *et al.*., 2013). The fruit had enjoyed several studies into the fertility effects in males (Bello *et al.*., 2017), effects of the seed on human cancer cell lines (Chaemsawang *et al.*., 2019) and the nutritional qualities and health benefits (Gemede *et al.*., 2014). The infusion of the root can be used for the treatment of syphilis, cuts, wounds and boils (Kumar *et al.*., 2013). The seed has antispasmodic and sudorific properties (Kumar *et al.*., 2013). There was no report on the toxicology study of the Okra leaf and it is expedient to research into the safety of the use of Okra leaf prior to the therapeutic studies that would ensue.

2.0 Materials and Methods

2.1 Preparation of Sample

The okra (*Abelmoschus esculentus* Moench) leaf was washed with water to remove components and air-dried to a constant weight. The dried leaves were then pulverized using Beltone Luinohun Blender/Miller III (model MS-223, Taipei, Taiwan). The powdered material was stocked in a sealed plastic container from which 1000 g was mixed with 1.0 L of ethanol: water (80:20) and stirred for 48 h at room temperature. This was then filtered with a sieve of considerable pore seize. The filtrate was concentrated using rotary evaporator and freeze drying machines to give dried residue (dark green cake).

2.2 Quantification by HPLC-DAD

Chromatographic analyses were carried out under gradient conditions using C18 column (4.6 mm x 250 mm) in reverse phase, packed with 5μm diameter particles; the mobile phase was water containing 1% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Silva *et al.*. (2014) with slight modifications. fb (1,2,3,4,5,6,7,8) extracts mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the fb (1,2,3,4,5,6,7,8) extracts were analyzed at a concentration of 15 mg/mL. The flow rate was 0.6 mL/min, injection volume 50 μl and the wavelength were 270 for gallic acid, 278 nm for coumarin, 327 nm for chlorogenic acid and caffeic acid, and 365 nm for quercetin, quercitrin, kaempferol and rutin. All the samples and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.250 mg/ml for quercetin, quercitrin, kaempferol, coumarin and rutin; and 0.030 – 0.300 mg/ml for gallic, caffeic and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: $Y = 13480x + 1257.5$ ($r = 0.9998$); coumarin: $Y = 11983x + 1196.9$ ($r = 0.9997$); chlorogenic acid: $Y = 11786x + 1267.1$ ($r = 0.9991$); caffeic acid: $Y = 13048x + 1345.6$ ($r = 0.9995$); rutin: $Y = 12478x + 1194.9$ ($r = 0.9997$), quercitrin: $Y = 13641x + 1178.4$ ($r = 0.9997$), kaempferol: $Y = 11458x + 1269.4$ ($r = 0.9998$) and quercetin: $Y = 12783x + 1195.8$ ($r = 0.9996$). All chromatography operations were carried out at ambient temperature and in triplicate.
The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Silva *et al.*., 2014).

2.3 Experimental Design

Adult male and female Wistar rats, weighing 210±20 g were purchased from experimental Animal Care Center (University of Ilorin, Kwara State. Nigeria). All animals were maintained under controlled conditions of temperature (22±1°C), humidity (50-55%) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 14 days before the start of the experiment. Animals had free access to rat chow and drinking water. All experimental procedures including euthanasia were conducted in accordance with the Ethical Regulation and Guide for the Care and Use of Laboratory Animals of the Federal University of Technology, Akure, Nigeria.

Sub toxicity was determined by daily oral exposure of animals for 28 days of varying doses of the ELEOP as suggested by Lorke *et al.*. (1983) approach to practical acute toxicity testing.

The experiment was designed such that the animals were divided as follows with 5 animals in each group:

Group 1 (CF): Female rats' control;
Group 2 (10F): 10 mg/Kg BWT of ELEOP on female rats;
Group 3 (100F): 100 mg/kg BWT of ELEOP on female rats;
Group 4 (1000F): 1000 mg/kg BWT of ELEOP on female rats
Group 5 (1600F): 1600 mg/kg BWT of ELEOP on female rats;
Group 6 (2900F): 2900 mg/kg BWT of ELEOP on female rats;
Group 7 (5000F): 5000 mg/kg BWT of ELEOP on female rats;
Group 8 (CM): Male rats' control;
Group 9 (10M): 10 mg/kg BWT of ELEOP on male rats;
Group 10 (100M): 100 mg/kg BWT of ELEOP on male rats;
Group 11 (1000M): 1000 mg/kg BWT of ELEOP on male rats;
Group 12 (1600M): 1600 mg/kg BWT of ELEOP on male rats;
Group 13 (2900M): 2900 mg/kg BWT of ELEOP on male rats; and
Group 14 (5000M): 5000 mg/kg BWT of ELEOP on male rats.

2.4 Tissues Preparation

Animals in each group were completely anaesthetized and then sacrificed by cervical decapitation. The liver and kidney were carefully removed and weighed using digital electronic balance. Blood samples were collected via cardiac puncture into non-anticoagulant tubes. The tissues were rinsed in ice-cold 1.15% potassium chloride solution and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) using a Teflon homogenizer. The homogenized tissues were centrifuged at 3000 g for 10 min at 4ºC.

2.5 Biochemical Analyses

2.5.1 Determination of Total Protein Concentration

This was carried out using the manufacturer protocol of Randox Total Protein Kit. 1ml of reagent R1(Sodium hydroxide (100 mmol/l), sodium-potassium tartrate (16 mmol/l), Potassium iodide (15 mmol/l) and copper II sulphate (6 mmol/l)) was added to 0.02 ml of the test sample, the mixture was incubated at 25 o C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Total Protein Concentration = ((Abs Sample/ Abs Standard) x standard concentration.

2.5.2 Determination of MDA Concentration

Thiobarbituric Acid Reactive Substances (TBARS) assay kit (Randox) was used to measure the lipid peroxidation product MDA equivalent. One hundred microliters of homogenate was mixed with 2.5 mL reaction buffer (provided by the kit) and heated at 95°C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation product MDA levels are expressed in terms of nmoles MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric chromophore (1.56×105/M/cm).

2.5.3 Determination of Aspartate Amino Transferase Activity in Serum

Activity of AST was evaluated using manufacturer protocol of Randox AST Kit based

on the principle of Reitman and Frankel (1957). Diluted sample (0.1 mL) was mixed with 0.5 mL of R1 (phosphate buffer (100 mmol/L, pH 7.4), l-aspartate (100 mmol/L), and α-oxoglutarate [2 mmol/L]) and the mixture incubated for 30 min at 37°C after which 0.5 mL of R2 (2, 4-dinitrophenylhydrazine (2 mmol/L]) was added to the reaction mixture and allowed to stand for another 20 min at 25 0 C. Then, 5.0 mL of NaOH (0.4 mol/L) was added and the absorbance was read against the reagent blank after 5 min at 546 nm. The activity of AST in homogenate was obtained following the extrapolation of absorbance value on AST standard curve.

2.5.4 Determination of Alanine Amino Transferase Activity in Serum

Assay of alanine amino transferase (ALT) activity was carried out using the manufacturer protocol of Randox ALT Kit based on the principle described by Reitman and Frankel (1957). Reagent1 (0.5 mL) containing Phosphate buffer (100 mmol/L, pH 7.4), l-alanine [200 mmol/L), and α-oxoglutarate (2.0 mol/L) was added to a test tube already containing 0.1 mL of serum sample and the mixture was incubated at 37°C for 30 min. Then, 0.5 mL of R2 containing 2, 4-dinitrophenylhydrazine (2.0 mmol/L) was added and the mixture incubated again at 20°C for 20 min. Finally, 5 mL of NaOH was added. The mixture was allowed to stand for 5 min at room temperature and the absorbance was read at 546 nm. The activity of ALT in the homogenate was obtained from a standard curve.

2.5.5 Determination of Alkaline Phosphatase Activity in Serum

Assay of alkaline phosphatase (ALP) activity was carried out according to the procedure provided by Randox Kit Manufacturer which is based on the method of Englehardt (1970). ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP reacted with p-nitrophenyl phosphate. Exactly 1.0 mL of the reagent (1 mol/L, pH 9.8 Diethanolamine buffer, 0.5 mmol/L $MgCl_2$; substrate: 10 mmol/L p-nitrophenolphosphate) was added to 0.02 mL of sample and then mixed. The absorbance was read for 3 min at intervals of 1 min at a wavelength of 405 nm.

Calculation

ALP activity was determined using the formula:

U/l = 2760 × A405 nm/min.

2.5.6 Determination of Creatinine Concentration

The creatinine colorimetric assay was conducted according to Randox laboratories limited manual. Equal volume of 35mmol/L picnic acid and 0.32mol/L sodium hydroxide was mixed from the working solution. 2.0ml of the working solution was added to 1.0ml of the sample and differently to standard (1.0ml). It was mixed and allow to stand for 30 seconds. Absorbance was read at 492nm wavelength.

Calculation

$$\frac{\text{Absorbance of sample x Concentration of standard mg/ml}}{\text{Absorbance of standard}}$$

2.5.7 Determination of Urea Nitrogen

The urea nitrogen was estimated according to Teco diagnostic limited procedure. About 100µL of diluted serum (serum 1:4 in either normal saline or PBS) supernatant was pipetted into the microplate wells; then 150µL of Urease. Plate was tapped gently 3-4 times to mix sample and enzyme and incubated for 15 minutes at room temperature. Then 100µL of Alkaline Hypochlorite was added to each well and incubated for 10 minutes at room temperature. The absorbance of each sample was measured in duplicate at 620 nm.

The urea concentration (dilution factor = 5) in the well can be determined using the equation:

Blood Urea Nitrogen concentration = dilution factor x (Average absorbance –y-intercept)/slope

2.6 Histopathology Analysis

Excised liver and kidney tissues were stored separately in well labelled containers containing 10% formalin, sealed and sent for analysis in the Department of Anatomy, University of Ibadan, Oyo state (Nigeria). The tissues were fixed on slides and stained for histological study.

2.7 Statistical Analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

3.0 Results and Discussion

The use of plants and plants extract for medicinal purposes has been ongoing for thousands of years and it has been the source of most useful therapy in both herbalism and folk medicine (John-Dewole *et al..*, 2012). Phytochemicals produce a definite physiological action on the human body and they are non-nutritive but act as a protective shield against diseases.

The results revealed the acute toxicity effects of the ethanolic leaf extract of Okra plant (*Abelmoschus esculentus* Moench L.) (ELEOP). The study revealed that the LD_{50} values of ELEOP were 1250 mg/Kg BWT and 2333.33 mg/Kg BWT in female and male rats respectively. The experimental rats were sluggish after the assimilation of extract at doses above 1600 mg/kg BWT. Mortality was recorded among the male and female rats administered 2900 mg/Kg BWT and 1600 mg/Kg BWT of the extract respectively.

The effects of ELEOP on the functions of the liver and kidney in female and male rats were determined by; the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT); concentrations of blood urea nitrogen and creatinine; and concentrations of total protein and malondialdehyde (MDA) in the kidney and liver homogenates.

The activity of AST (Fig.1) in blood of female rats increased as the dose of ELEOP increased from 10 to 5000 mg/Kg BWT compared to the control groups (CF) (27.25 U/L). The activity of AST was inhibited in a dose-dependent manner in male administered with ELEOP (10 to 5000 mg/kg BWT).

The effects of ELEOP on ALP enzyme activity (Fig.2) obtained from the female rats administered groups revealed increase in the activity of ALP with corresponding increase in dose (10-5000 mg/Kg BWTF) of the extract. Contrarily, the administration of the ELEOP in male rats significantly ($p \leq 0.05$) inhibited the activity of ALP as the dose of ELEOP increased from 10 to 5000 mg/Kg BWTM.

The increase in ALP activity in female rats with the increase in the dose of ELEOP confirmed increased damage to hepatocytes compared to the male counterpart with dose-dependent decrease of ALP activity suggesting safe effects of administration of ELEOP to hepatocytes of the male albino rats.

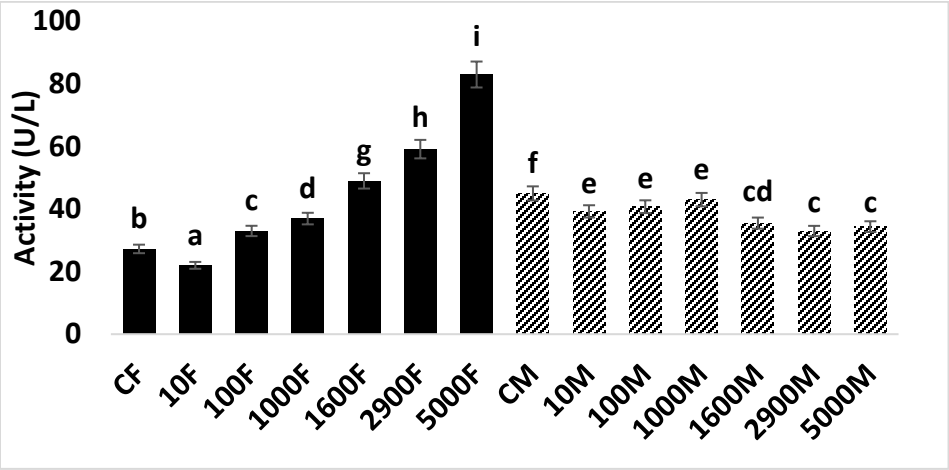

Figure 1: The activity of AST in the blood of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

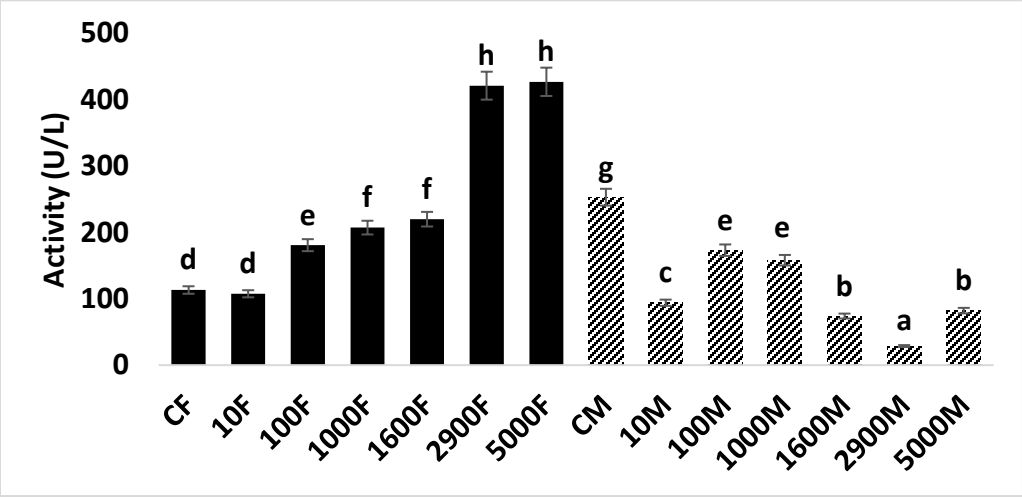

Figure 2: The activity of ALP in the blood of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

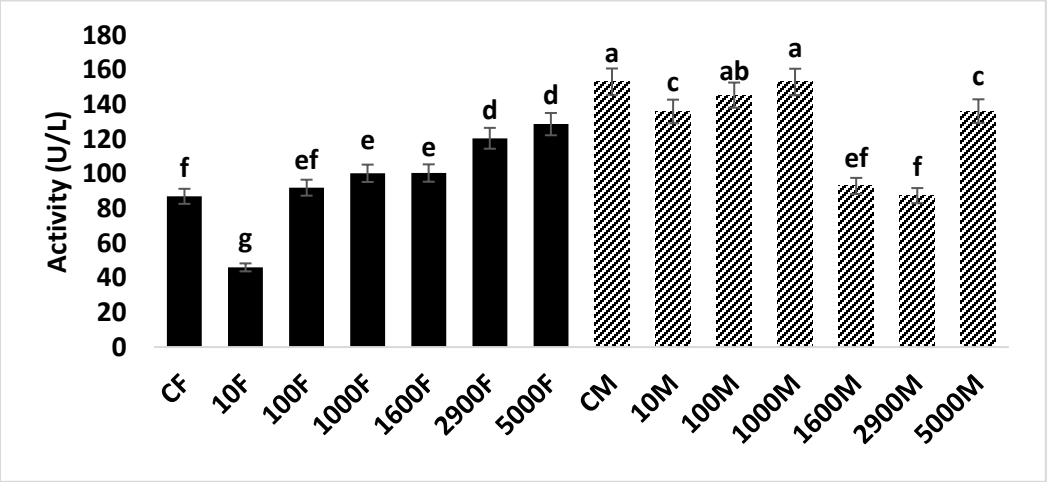

Figure 3: The activity of ALT in the blood of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

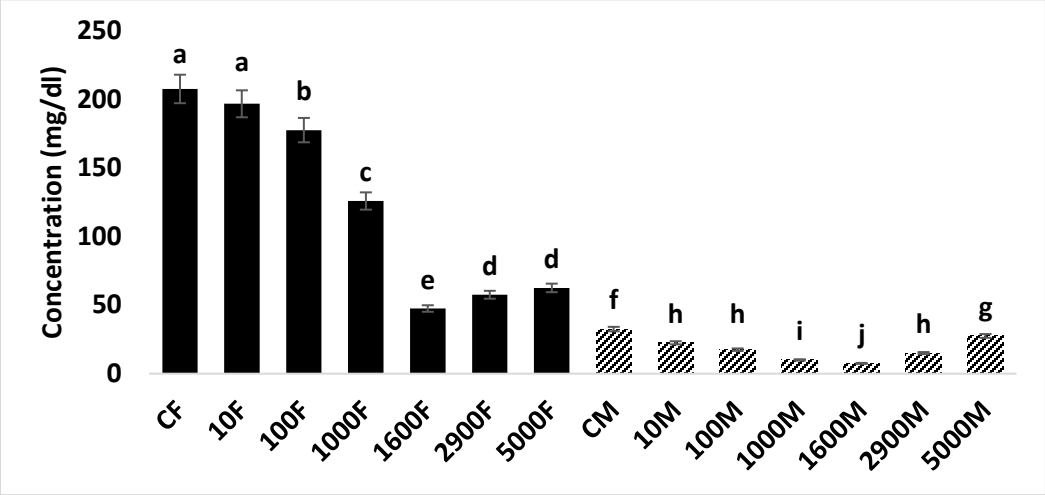

Figure 4: The concentration of blood urea nitrogen (BUN) in the blood of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

The activity of ALT (Fig. 3) in the female rats increased in ELEOP-dose-dependent manner. Activity of ALT in the male rats groups significantly ($p \leq 0.05$) decreased as the dose of ELEOP increased from 1000 to 2900 mg/Kg BWT, and significant ($p \leq 0.05$) increase at 5000 mg/kg BWT. The concentration of BUN (Fig. 4) in female rats significantly ($p \leq 0.05$) decreased as the dose of ELEOP increased from 10 to 1600 mg/kg BWT and a significant rise in BUN concentration at 2900 and 5000 mg/kg BWT. Concentration of Blood Urea Nitrogen (BUN) decreased as the dose of ELEOP decreased from 10 to 1600 mg/kg BWT and rised between 2900 and 5000 mg/kg BWT.

The concentration of Creatinine (Fig. 5) significantly ($p \leq 0.05$) decreased with increase in the dose of ELEOP (10-5000 mg/Kg BWT) in female rats compared to the female control rats. The concentrations of creatinine decrease in a dose dependent manner (10-5000 mg/Kg BWT). The concentration of creatinine before and after treatment was significantly higher in male rats was higher than in female rats ($p \leq 0.05$).

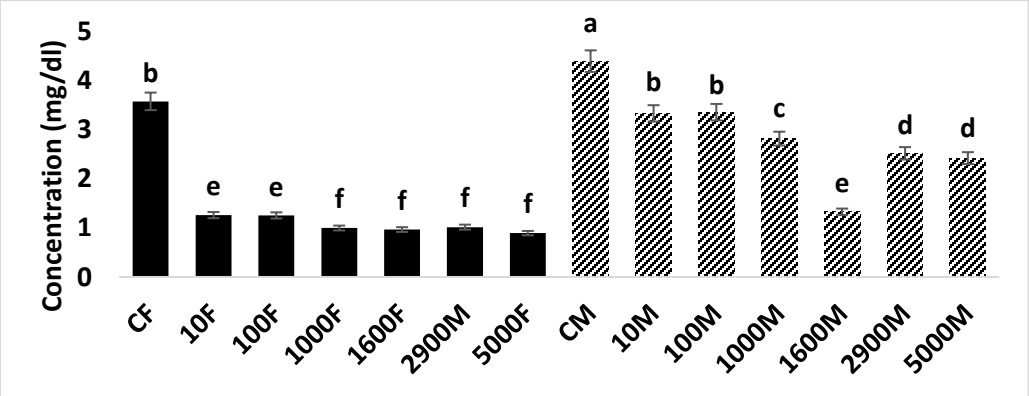

Figure 5: The concentration of creatinine (CREAT) in the blood of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

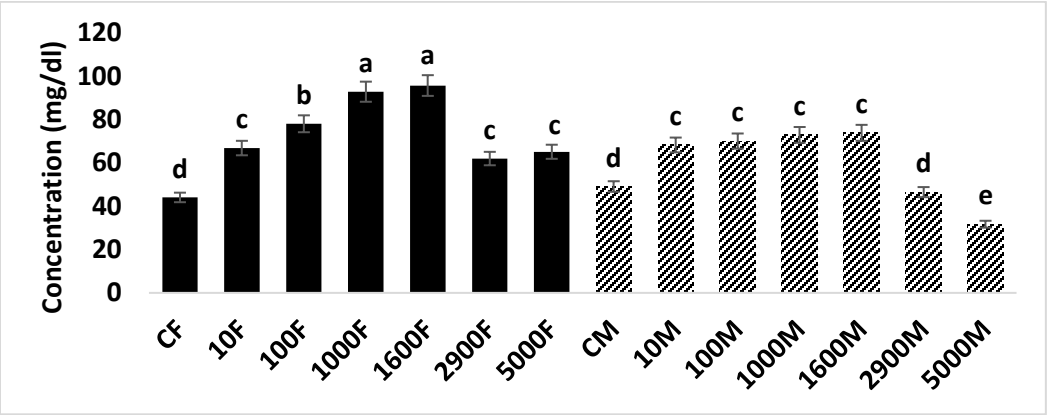

Figure 6: The concentration of total protein in the homogenates of livers of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

The effects of ELEOP administration on total protein of the liver (Fig. 6) and kidney (Fig. 7) homogenates in female and male rats were revealed. The effects of ELEOP on total protein concentration of liver homogenates of male and female rats revealed a significant increase in total protein concentration as the dose increased from 10 to 1600 mg/Kg BWT, but declined between 2900 and 5000 mg/kg BWT. The concentrations of total protein of the kidneys in the male and female rats increased with increase in the dose of ELEOP from 10 to 5000 mg/Kg BWT, however, the 5000 mg/kg BWT of ELEOP in male rats declined the concentration of total protein in the kidney.

MDA concentration in the livers and kidneys homogenates of female and male rats was revealed in Fig. 8 and Fig. 9. MDA concentrations in female and male rats decreased as the dose of ELEOP increased, there was sharp rise in concentration of MDA at 2900 and 5000 mg/kg BWT among female rats, the concentration of MDA decreased as dose increased from 10 to 5000 mg/kg BWT among male rats. The effects of ELEOP on the kidney of female rats was a dose dependent decrease in MDA from 10 to 1600 mg/kg BWT and a slight increase in MDA as the dose increased to 2900 and 5000 mg/kg BWT. A consistent decrease in kidney MDA concentration was obtained among male rats as the dose increased from 10 to 5000 mg/kg BWT.

The findings from the results suggest that the use of ELEOP for intended medical use could be very toxic with adverse physiological effects.

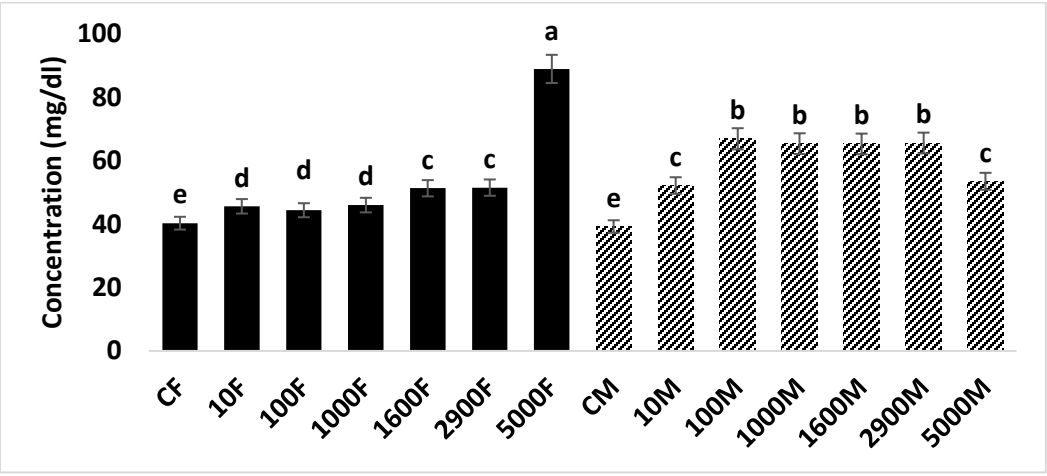

Figure 7: The concentration of total protein in the homogenates of kidneys of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

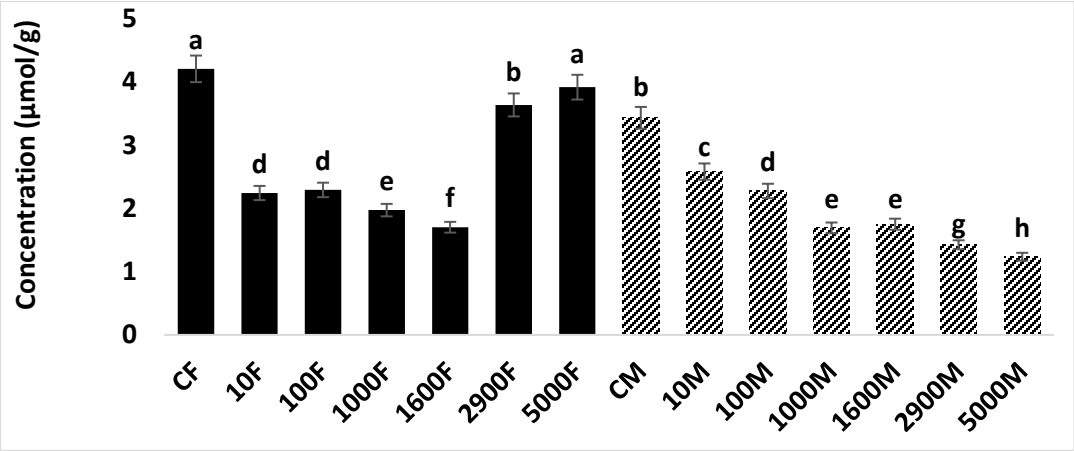

Figure 8: The concentration of MDA in the homogenates of livers of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

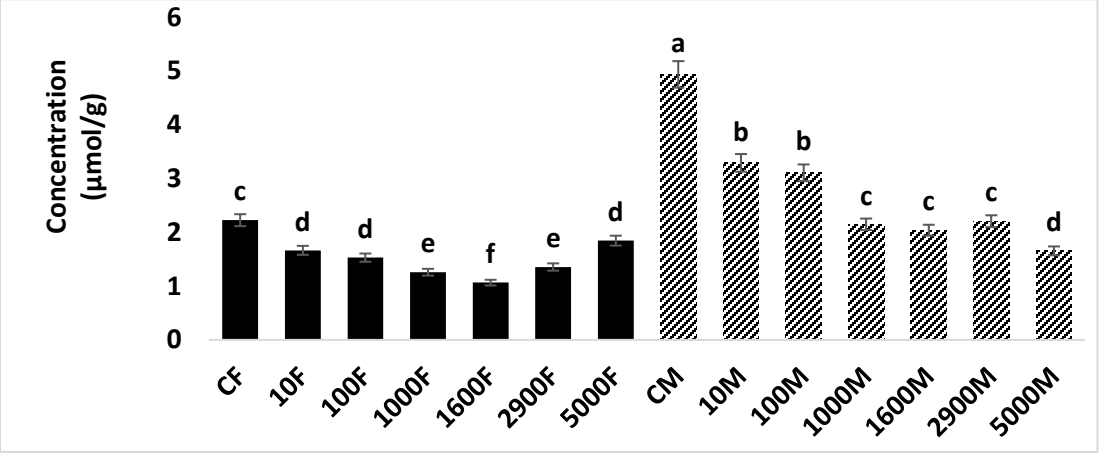

Figure 9: The concentration of MDA in the homogenates of kidney of male and female rats (n=5) administered doses of ethanolic leaf extract of *Abelmoschus esculentus Moench* (ELEOP). The values with different superscript are significantly different ($p \leq 0.05$).

Histology of the liver of female and male rats x100

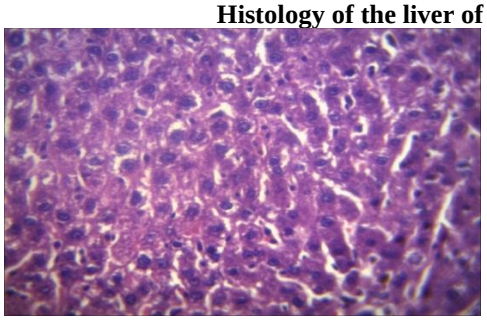

Plate 1: Female rats control (CF).

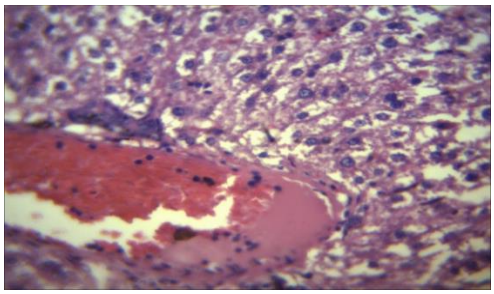

Plate 2: 10 mg/Kg BWT of ELEOP on female rats (10F)

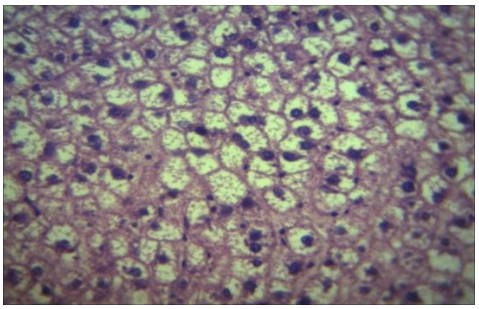

Plate 3: 100 mg/Kg BWT of ELEOP on female rats (100F)

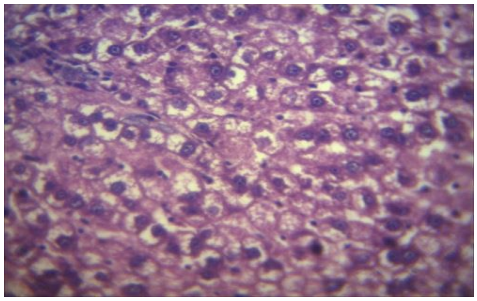

Plate 4: 1000 mg/Kg BWT of ELEOP on female rats (1000F)

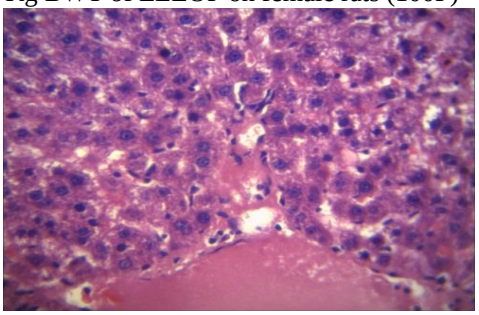

Plate 5:1600mg/Kg BWT of ELEOP on female rats (1600F).

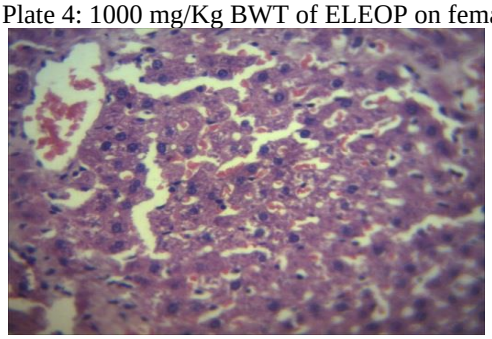

Plate 6: 2900 mg/Kg BWT of ELEOP on female rats (2900F)

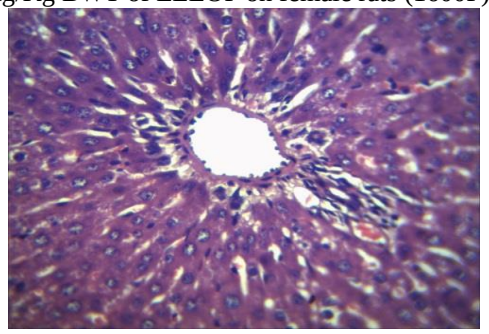

Plate 7: 5000 mg/Kg BWT of ELEOP on female rats (5000F).

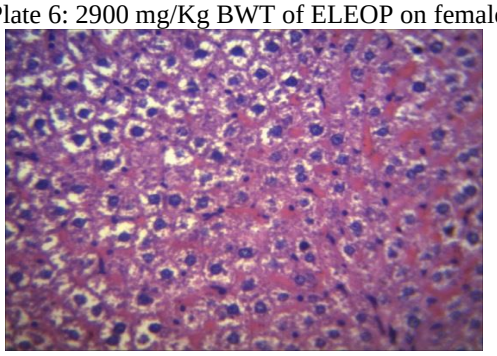

Plate 8: Male rats control (CM).

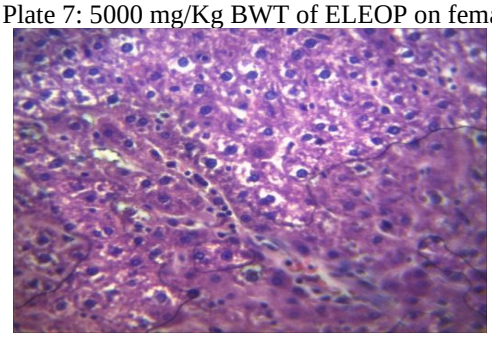

Plate 9: 10 mg/Kg BWT of ELEOP on male rats (10M)

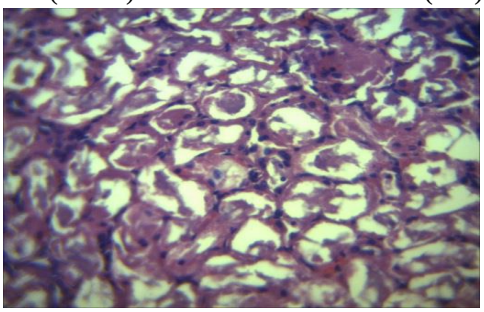

Plate 10: 100 mg/Kg BWT of ELEOP on male rats (100M)

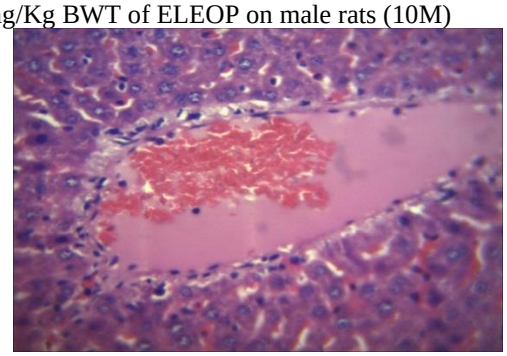

Plate 11: 1000 mg/Kg BWT of ELEOP on male rats (1000M).

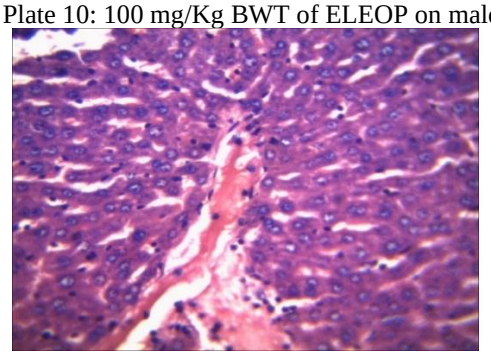

Plate 12: 1600 mg/Kg BWT of ELEOP on male rats (1600M)

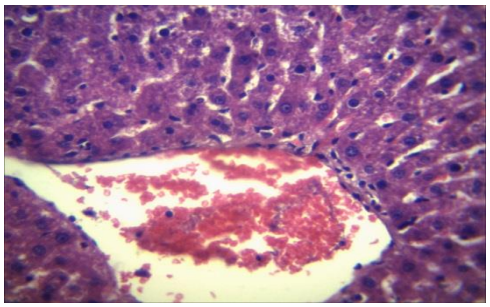
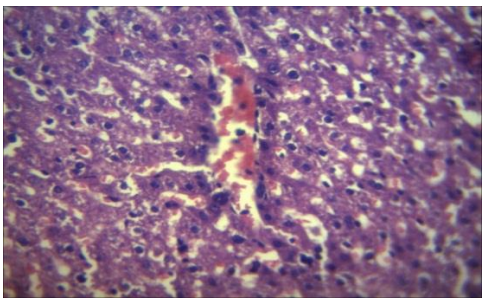

Plate 13: 2900 mg/Kg BWT of ELEOP on male rats (2900M). Plate 14: 5000 mg/Kg BWT of ELEOP on male rats (5000M)

Histology of the kidney of female and male rats x100

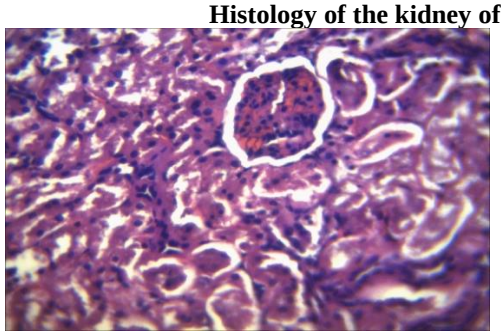
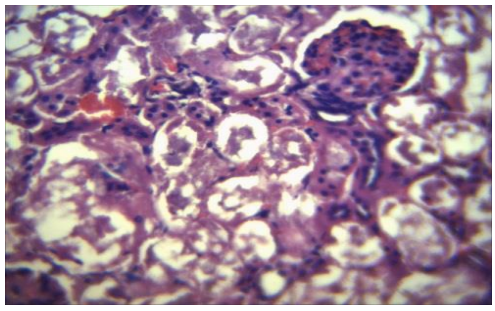

Plate 15: Female rats control (CF). Plate 16: 10 mg/Kg BWT of ELEOP on female rats (10F)

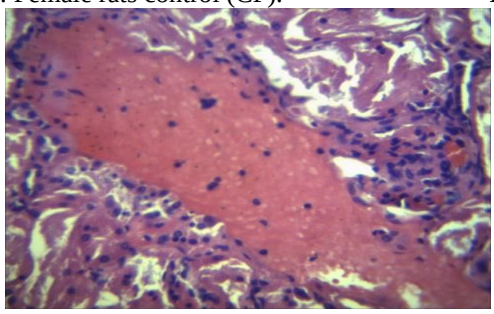
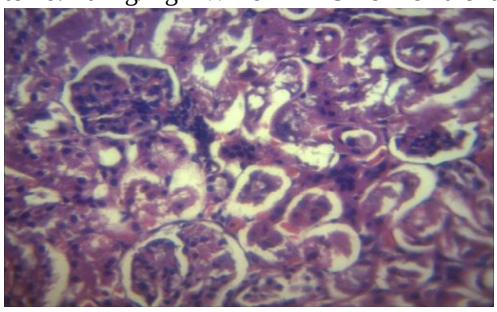

Plate 17: 100 mg/Kg BWT of ELEOP on female rats (100F) Plate 18: 1000 mg/Kg BWT of ELEOP on female rats (1000F)

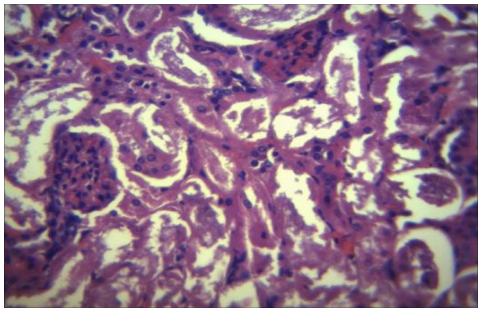
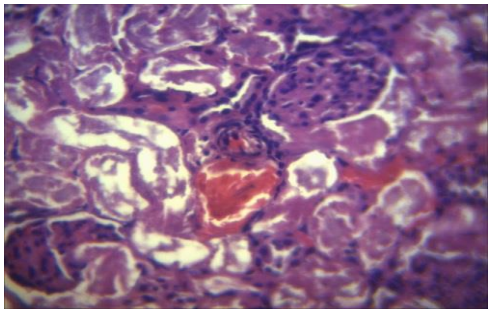

Plate 19: 1600 mg/Kg BWT of ELEOP on female rats (1600F). Plate 20: 2900 mg/Kg BWT of ELEOP on female rats (2900F)

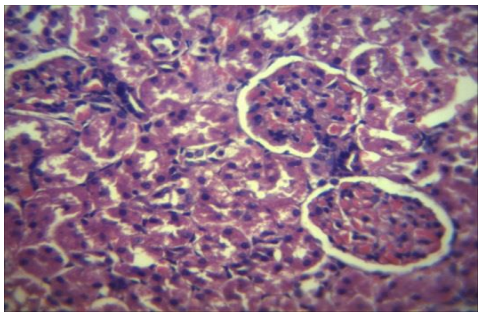
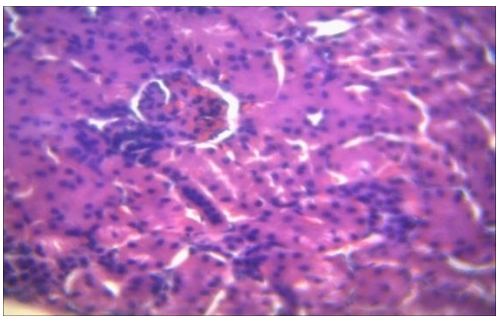

Plate 21: 5000 mg/Kg BWT of ELEOP on female rats (5000F). Plate 22: Male rats control (CF)

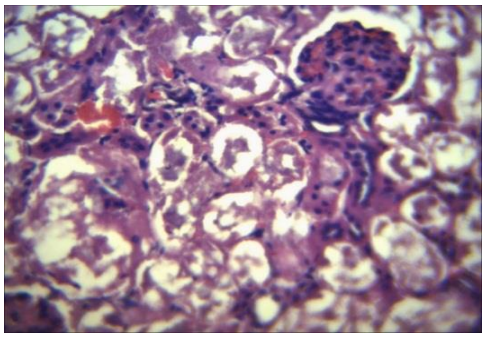

Plate 23: 10 mg/Kg BWT of ELEOP on male rats (10M)

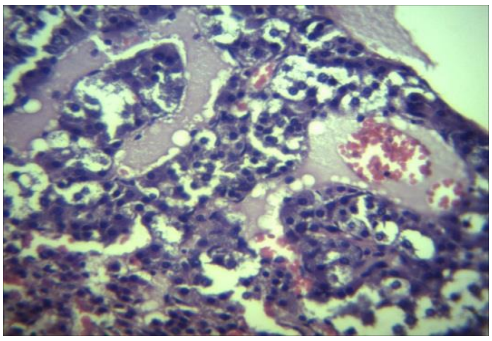

Plate 24: 100 mg/Kg BWT of ELEOP on male rats (100M)

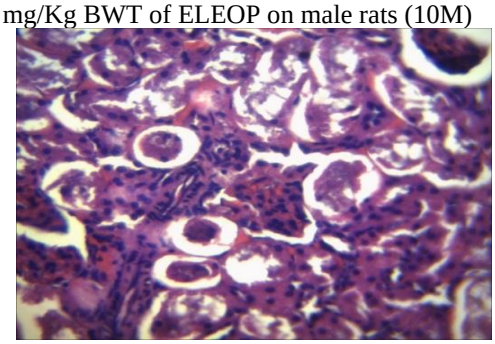

Plate 25: 1000 mg/Kg BWT of ELEOP on male rats (1000M).

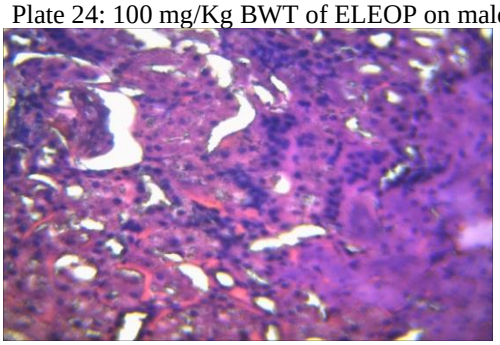

Plate 26: 1600 mg/Kg BWT of ELEOP on male rats (1600M)

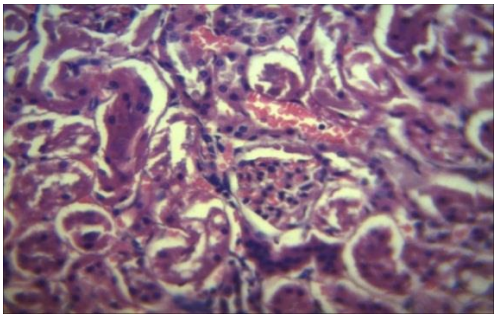

Plate 27: 2900 mg/Kg BWT of ELEOP on male rats (2900M).

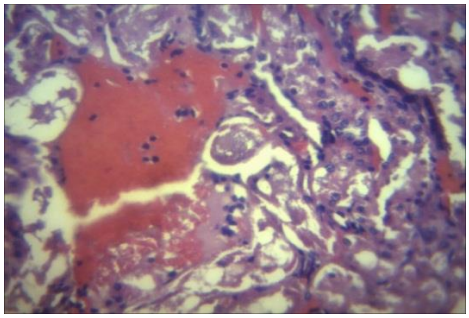

Plate 28: 5000 mg/Kg BWT of ELEOP on male rats (5000M)

Summary of histology

Plate codes	Histology of tissues female rats	Plate codes	Histology of tissues male rats
CF Liver	No visible lesions seen	**CM Liver**	There is a severe vacuolar degeneration of the hepatocytes. There are few foci of severe congestion of the sinusoids
10F Liver	There is a severe vacuolar degeneration of the hepatocytes. There is a moderate to severe portal congestion.	**10M Liver**	There is a moderate to severe vacuolar degeneration of the hepatocytes. There is a very mild cellular infiltration by mononuclear cells.
100F Liver	There is a severe diffuse vacuolar degeneration and necrosis of hepatocytes	**100M Liver**	There is a severe diffuse vacuolar degeneration of hepatocytes.
1000F Liver	There is a moderate to severe diffuse vacuolar degeneration and necrosis of hepatocytes	**1000M Liver**	There is a mild to moderate portal and central venous congestion
1600F Liver	There is a moderate portal and sinusoidal congestion. There is a mild diffuse vacuolar degeneration of hepatocytes	**1600M Liver**	There is a mild portal congestion, periportal cellular infiltration and proliferation of the connective tissue
2900F Liver	There is a severe vacuolar degeneration and necrosis of hepatocytes. The sinusoids are also congested	**2900M Liver**	There is a mild portal congestion and mild periportal cellular infiltration. There is a mild diffuse vacuolar degeneration of hepatocytes.
5000F	There is a mild congestion of the sinusoids,	**5000M**	There is a mild diffuse vacuolar degeneration of the

Liver	with a mild periportal cellular infiltration	**Liver**	hepatocytes
CF Kidney	No visible lesions seen	**CM Kidney**	No visible lesions seen
10F Kidney	There is a severe diffuse tubular degeneration and necrosis of the renal tubules. There are protein casts in their lumen. There is also a moderate to severe interstitial congestion	**10M Kidney**	There is a severe congestion of the renal interstitium. There are copious amount of proteinaceous material in the lumen of the renal tubules
100F Kidney	There is a mild to moderate congestion of the interstitium. There is a diffuse tubular degeneration with protein casts in the tubular lumen	**100M Kidney**	There is a severe diffuse tubular degeneration and necrosis of the renal tubules. There are protein casts in their lumen
1000F Kidney	There is a severe diffuse tubular degeneration and necrosis of the renal tubules. There are protein casts in their lumen	**1000M Kidney**	There is a mild to moderate congestion of the renal cortex. Several tubules have protein casts in their lumen.
1600F Kidney	Very many tubules have protein casts in their lumen.	**1600M Kidney**	There was a mild to moderate congestion of the renal cortex. The glomeruli appear shrunken
2900F Kidney	There is a mild congestion of the renal cortex. Many renal tubules have protein casts in the lumen	**2900M Kidney**	Many renal tubules have protein casts in the lumen
5000F Kidney	No visible lesions seen	**5000M Kidney**	There is a moderate congestion of the renal interstitium. Many tubules appear degenerate

Phytochemical characterization

Some of the important phytochemicals identified in the leaf included gallic acid, chlorogenic acid, caffeic acid, coumarin, rutin, quercetrin, quercetin and kaempferol. These had been reported as therapeutic agents delaying the progression of advance glycation end-products-mediated inflammatory diseases such as diabetes (Chandler *et al.*, 2010) and other ailments related to oxidative stress. Caffeic acid was identified as the major active component. The plant contains significant amounts of major active phytochemicals of therapeutic benefits. The correlations among the quantified phytochemicals therefore suggest the multifaceted uses of this plant in curing many types of diseases and ailments.

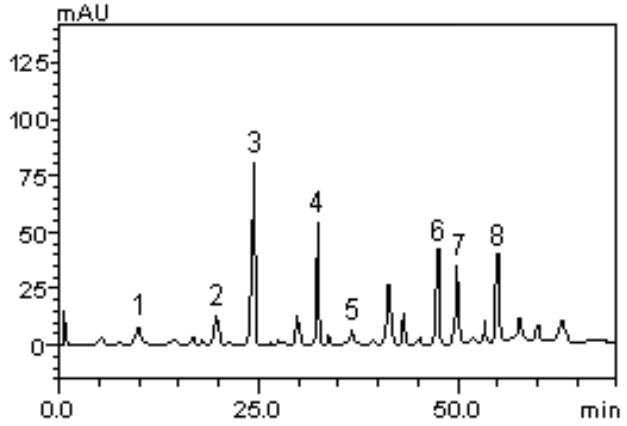

Figure 1: Representative high performance liquid chromatography profile of fb (1,2,3,4,5,6,7,8) extracts. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

Table 2: Phytochemical compositions determined and their respective amounts

Compounds	mg/g
Gallic acid	0.60 ± 0.02^{a}
Chlorogenic acid	0.97 ± 0.01^{b}
Caffeic acid	7.34 ± 0.03^{c}
Coumarin	5.98 ± 0.01^{d}
Rutin	0.53 ± 0.01^{a}
Quercitrin	4.08 ± 0.03^{e}
Quercetin	3.31 ± 0.01^{f}
Kaempferol	4.16 ± 0.02^{e}

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.01$.

4.0 Conclusion

Ethanolic extract of *Abelmoschus esculentus Moench* L leaf had an LD_{50} of 1250 mg/Kg BWT in female Wistar rats and 2333.33 mg/Kg BWT in male Wistar rats. This was evident from the effects it had on the indices of kidney and liver functions. The quantitative evaluation of phytochemical composition of the leaf extract revealed caffeic acid as the most predominant, other phytochemicals included coumarin, quercetrin, quercetin, kaempferol, gallic acid and chlorogenic acid. These are phytochemicals of antioxidative importance but that are required endogenously in limited amounts.

References

Aziz, N., Saleh, R.A., Sharma, R.K. *et al.*. (2004). Novel association betweensperm reactive oxygen species production, sperm morphologi-cal defects, and the sperm deformity index. *Fertil Steril*., 81(2):349-354

Bello, H.J., Maigoro, A.Y., Dalhat, M.H., Sheshe, S.M., Labbo, A.M., Abdulrahman, J. and Muazu, A. (2017). Antifertility Effect of Okra (Abelmoschus esculentus) in Men. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 2(4): 1-6.

Benie, T., Duval, J. and Thieulant, M.L. (2003). Effects of some traditional plant extract on rat oestrous cycle compared with clomid. *Phytotherapy Research*, 17(7):748-755.

Cao, L., Leers-Sucheta, S. and Azhar, S. (2004). Aging alters the functional expression of enzymatic and non-enzymatic antioxidant defense systems in testicular rat Leydig cells. *J Steroid Biochem Mol Biol*., 88(1):61-67

Chaemsawang, W., Prasongchean, W., Papadopoulos, K.I., Ritthidej, G., Sukrong, S. and Wattanaarsakit, P. (2019). The Effect of Okra (*Abelmoschus esculentus* (L.) Moench) Seed Extract on Human Cancer Cell Lines Delivered in Its Native Form and Loaded in Polymeric Micelles. *International Journal of Biomaterials*, Volume 2019, Article ID 9404383, 13 pages.

Daramola, O.I., Akindele, O.O., Bolarinwa, A.F. and Raji, Y. (2015). Effects of *Telfairia occidentalis* Seed Oil on Female Reproductive Functions in Wistar Rats. *Niger. J. Physiol. Sci.*, 30(2015) 095-101.

Emmanuele, M.A, Wezeman, F, and Emmanuele, N.V. (2002). Alcohol's effect on female reproductive function. *Alcohol Research and Health*, 26(4):274-281.

Gemede, H.F., Ratta, N., Haki, G.D., Ashagrie, Z. and Beyene, W.F. (2014). Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A Review. *Global Journal of Medical Research: K Interdisciplinary* 14(5):1.0

Jungwirth, A., Diemer, T., Dohle, G. and Giwercman, A. (2015). Guild Lines on Male Infertility.

Kumar, D.S., Tony, D.E., Praveen Kumar, A., Kumar, K.A., Srinivasa Rao, D.B., Nadendla, R. and Author, C. (2013). A Review on: *Abelmoschus esculentus* (Okra). *International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)* 2013;3(34).

Mehraban, Z., Ghaffari Novin, M., Golmohammadi, M. G., Sagha, M., Pouriran, K. and Nazarian, H. (2019). Protective effect of gallic acid on

apoptosis of sperm and in vitro fertilization in adult male mice treated with cyclophosphamide. *Journal of Cellular Biochemistry*. doi:10.1002/jcb.28987

Oladipo, G.O., Nlekerem C.M., Ibukun E.O., and Kolawole A.O. (2017). Quail (Cortunix japonica) Egg Yolk Bioactive Components Attenuate Streptozotocin-induced Testicular Damage and Oxidative Stress in Diabetic Rats. *Eur. J. of Nutr*., DOI 10.1007//s00394-017-1554-4.

Onyegeme-Okerenta, B.M. and Essien, E.B. (2015). Effect of Leaf Extract of Millettia aboensis on the Reproductive Hormones and Organs of Female Wistar Albino Rats. *International Journal of Biological Sciences and Applications* 2(5): 42-47.

Shafaei, A., Farsi, E., Ahamed, B.M.K., Siddiqui, M.J.A., Attitalla, I.H., Zhari, I. and Asmawi, M.Z. (2011). Evaluation of toxicological and standardization parameters and phytochemical investigation of *Ficus deltoidea* leaves *Am. J Biochem. Mol. Bio*. 1:237 -243.

Telefo, P. B., Moundipa, P. F., Tchana, A. N., Tchouanguep Dzickotze, C. and Mbiapo, F. T. (1998). Effects of an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera verticillata* on some physiological and biochemical parameters of reproduction in immature female rats. *Journal of Ethnopharmacology*, 63(3):193-200.