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Effects of subchronic administration of *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae) root extract on histopathological indices of rats

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Abstract

Background: *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae), is a medicinal plant used routinely by the indigenes of Nigeria's Niger Delta to treat malaria, hemorrhoids, urethritis, inflammation, diabetes, fevers and various pains (Udobang, Okokon and Etuk, 2016) [1]. The locals use this plant to treat various ailments, so it becomes necessary to investigate the effect of *Setaria megaphylla* ethanol extract on various organs in the body

Objectives: This work was therefore designed to investigate the effect of *Setaria megaphylla* root extract on histopathological indices of rats during sub chronic administration.

Methodology: *Setaria megaphylla* ethanol root extract (150, 300, 450 mg/kg) was investigated for histopathological effects in rats organs using standard procedures.

Results: There was non-significant reductions in the weight of rats and organs and negligible and non-significant toxic effect on the organs analysed.

Conclusions: The findings of this study revealed that *S. megaphylla* ethanol root extract exhibited negligible toxic issue effects.

Keywords: Histopathological, *Setaria megaphylla*, medicinal plant

1. Introduction

Setaria megaphylla is a perennial broad-leafed bristle grass, with robust roots 30 cm diameter at the base (Bromilow, 1995) [2]. Its leaves are soft, bluish grey green in colour and 1 m long and 10 cm broad. Its edges are glabrous and scarbrid with compressed and more or less keeled leaf sheaths (Bromilow, 1995) [2]. It is usually found besides rivers in low lying areas or forests and in areas with a lot of moisture, like tropical and subtropical areas of Africa, America (Van Oudtshoorn, 1999) [3].

A leaf-decoction has sedative effect on cough, and is also used to treat oedema (Burkill, 1985) [4]. Ijo in South East Nigeria rub leaves crushed with salt on the forehead for headache, and squeeze the sap on to a sore after it has been cleaned. The grass has a reputation for beneficial action on urino-genital troubles. Pressed juice of *Setaria megaphylla* leaves is used to treat anuria. The plant has anodyne and analgesic properties. Zulus in South Africa apply crushed leaves to bruises. In Republic of the Congo, sap is massaged into areas of pain. For more vigorous action the affected part may be scarified by rubbing with the rough leaf, and ash of the calcined plant applied (Burkill, 1985) [4].

2. Materials

2.1 Collection and Identification of Plant Sample

The roots of *Setaria megaphylla* were collected from Anwa forest in Uruan Local Government area of Akwa Ibom State, Nigeria. They were identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (FPHUU 221) was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2 Animal Stock

Adult albino rats and mice were obtained from the Animal House of the University of Uyo, Uyo, Akwa Ibom State and were maintained in the University of Uyo Animal House and fed with growers pellet feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum*.

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3. Methods

3.1 Extraction

The roots were washed and dried under shade until a constant weight was gotten. The dried roots were then cut into small pieces and grinded into powder with a blender. The powdered roots were macerated in 70 % ethanol for 72 hours, and the liquid filtrate concentrated and evaporated to dryness in a water bath at 60 °C. The yield was then weighed and stored in a refrigerator at -4 °C until used for the proposed experiments.

3.2 Toxicological Studies

Acute toxicity studies were carried out to determine the median lethal dose (LD₅₀) using the Miller and Tainter (1944)^[5] method as reported by Udobang, Okokon and Etebong (2017)^[6]. The mice were treated with various doses (1000 - 5000 mg) of the ethanol extract and observed for 24 hours. Physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and deaths were recorded.

Subchronic studies were carried out with emphasis on the histology of liver, kidney, brain, spleen, heart, lungs and ovary.

3.3 Evaluation of Subchronic Toxic Effect of Extract in Rats

Adult albino rats were weighed and randomized into four groups of six animals each. Group 1 received 10 ml/kg of distilled water orally, and served as control. Groups 2 - 4 received the ethanol extract at 150 - 450 mg/kg orally respectively. Drugs were administered daily for 28 days at 09.00 a.m. Mortality was monitored daily and weight changes of animals was recorded weekly. On day 29, after an overnight fast, the animals were anaesthetized with light chloroform.

The brain, heart, liver, spleen, kidney, lungs and ovaries were weighed immediately after removal. Samples of these organs were fixed in 10 % formalin and kept in that solution for further histopathological examination. (Nongporn, Wantana, Chatchai and Ruthaiwan, 2010; Diallo, Eklou-Gadegleku, Aklikokou, Creepie and Gbeassor, 2010)^[7, 8].

3.4 Tissue Processing for Histology

The fixed tissues were dehydrated through changes of graded alcohols to remove inherent excess water as follows; two changes of 70 % and 95 % alcohol for a period of two hours each, two changes of 100 % (absolute alcohol) for a period of two hours (2 hours). Dehydrated tissues were cleared using xylene. Tissues were impregnated with two changes of paraffin wax in the oven at the temperature of 60 °C at one hour thirty minutes (1 hour 30 mins) each to enable embedding and were transferred from the final wax bath to moulds filled with molten wax, allowed to solidify and thereafter, properly oriented for sectioning. The paraffin block was sectioned at five micron metres (5 µm) after cooling the surface of the tissues using ice bar. Ribbons were gently picked with Carmel brush and dropped in a water bath containing water at 60 °C to enable ribbons float, expand and flatten out. Slides were rubbed with thymol containing egg albumen, and gently dipped into the bath to pick up the flattened out tissue ribbons (Bancroft and Gamble, 2002)^[9].

3.5 Haematoxylin and Eosin Technique

Tissue sections were taken to water by deparaffinising in two changes of xylene and hydrated through graded series of alcohols in descending order, rinse in water and stain in

Haematoxylin for ten minutes (10 mins), sections were rinsed and differentiated in one percent (1 %) acid alcohol and blue in running water using saturated lithium carbonate solution until sections appear sky blue. The blued section was counterstained in eosin solution, for three minutes (3 mins). Tissues were washed well in water and dehydrated in ascending grade alcohol, cleared in xylene and mounted in dipolycysteine xylene (DPX) covered with coverslips and observed under microscope. Photomicrographs of the tissue sections were obtained using Olympus Binocular microscope (CX21) in the Department of Anatomical Pathology, University of Uyo Teaching Hospital.

3.6 Statistical Analysis

The data was analysed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison post-test. Differences between means were considered significant at 5 % and 1 % level of significance ie $p \leq 0.05$ and 0.01.

4. Results

4.1 Effect of extract on weight of organs

There was no significant or dose dependent effect by the extract (150 - 450 mg/kg) throughout the experiment. There were non-significant reductions in the weight of the kidneys, brain and lungs and in the low and high doses of the spleen, heart and low dose of the ovary. There was also a non-significant and non-dose-dependent increase in the weight of the liver (Table 1).

4.2 Effect extract on body weight of rats

During the period of the subchronic investigations, there was no significant increase in weight of the animals used in the experiment (Table 2)

4.3 Histology

Liver: The extract (150 mg/kg) group showed negligible toxic tissue effect as seen by absence of steatosis (fatty changes), necrosis and fibrosis, and mild portal inflammation observed here is taken as negligible toxic tissue effect. Doses 300 - 450 mg/kg were associated with mild toxic tissue effect due to the presence of thrombosed to congested portal blood vessels and inflammation, with absence of strong indices for hepatotoxicity such as steatosis (fatty changes), necrosis, fibrosis and bile spillage (Plates 1 -4). The extract may have some hepatotoxicity in the following increasing order: (150 mg/kg) < (300- 450 mg/kg).

Lungs: The extract (150 mg/kg) group showed negligible toxic tissue effect as seen by absence of intra-alveolar edema (pulmonary edema), exudates, hemorrhage, hemosiderin laden macrophages, necrosis and fibrosis. However, doses 300 - 450 mg/kg were associated with mild toxic tissue effect as seen by the presence of thrombosed to congested blood vessels within the inter-alveolar septae and the absence of strong indices for pulmonary damage such as alveolar edema (pulmonary edema), exudates, hemorrhage, haemosiderin laden macrophages, necrosis and fibrosis (Plates 5 -8). The extract may have negligible to sparing pulmonary damage in the following increasing order: (150 mg/kg) < (300- 450 mg/kg).

Kidneys: The extract (150 mg/kg) showed negligible toxic tissue effect as seen by absence of glomerular necrosis / thickening and tubular necrosis. However doses 300 - 450 mg/kg were associated with mild toxic tissue effect owing to

the presence of thrombosed to congested interstitial blood vessels despite absence of strong indices for renal damage such as glomerular necrosis / thickening, and tubular necrosis (Plates 11 -12). Thus, the graded doses extract may have negligible to sparing nephrotoxicity in the following increasing order: low dose (150 mg/kg) < middle and high doses (300- 450 mg/kg).

Spleen: Histologic sections of spleen show preserved normal architecture with variably sized lymphoid follicles and areas of hemorrhage (Plates 9 – 10). However, necrosis is not seen.

Brain: Histologic sections show the three layers of the cerebellar cortex consisting of the granular layer, the Purkinje cell layer and the molecular layer with unremarkable features. In some areas, the cerebrum show normal histologic findings

(Plates 13 -14).

Ovaries/Oviduct/Uterus: Histologic sections of ovarian tissues show preserved architecture. It is composed of dense fibrotic stroma of the medulla and the cortex with follicles at different stages of maturation (a primordial follicle, a primary unilaminar follicle, a secondary multilaminar follicle, and a corpus luteum). Histologic sections of oviduct show mucosa thrown into plicae and fibromuscular wall with unremarkable features. Histologic sections of uterus show endometrium and myometrium with unremarkable features (Plates 17- 20).

Heart: Histologic sections of the heart show striated muscle fibers admixed with areas of scanty cytoplasm (Plates 15 -16). However, neither inflammation nor necrosis is seen.

Table 1: Effect of extract on weight of organs of rats after subchronic administration.

Treatment (mg/kg)	Weights of organs (grams)						
	Kidney	Liver	Heart	Ovary	Lungs	Spleen	Brain
Distilled water	0.89 ± 0.03	5.77 ± 0.18	0.46 ± 0.03	0.18 ± 0.04	1.00 ± 0.04	0.65 ± 0.14	1.61 ± 0.08
Extract 150	0.81 ± 0.02	6.88 ± 0.18	0.45 ± 0.04	0.16 ± 0.02	0.98 ± 0.09	0.45 ± 0.05	1.52 ± 0.04
Extract 300	0.85 ± 0.02	6.16 ± 0.20	0.69 ± 0.03	0.18 ± 0.01	0.83 ± 0.01	0.68 ± 0.05	1.57 ± 0.03
Extract 450	0.82 ± 0.02	6.67 ± 0.34	0.45 ± 0.01	0.20 ± 0.01	0.83 ± 0.04	0.58 ± 0.03	1.53 ± 0.03

Data are expressed as mean ± SEM. n = 6.

Table 2: Effect of sub chronic toxicity of extract on body weight of rats.

Treatment (mg/kg)	Week 1	Week 2	Week 3	Week 4
Control (10ml/kg)	99.8 ± 1.46	115.2 ± 4.71	130 ± 3.16	142.4 ± 3.50
Extract 150	106.2 ± 2.31	116.2 ± 2.15	134 ± 3.27	139.8 ± 3.90
Extract 300	106.8 ± 2.47	117.2 ± 1.58	128.6 ± 6.07	132.6 ± 6.02
Extract 450	101.1 ± 1.58	108 ± 3.58	128.2 ± 5.25	134 ± 3.53

Data are expressed as mean ± SEM n = 6.

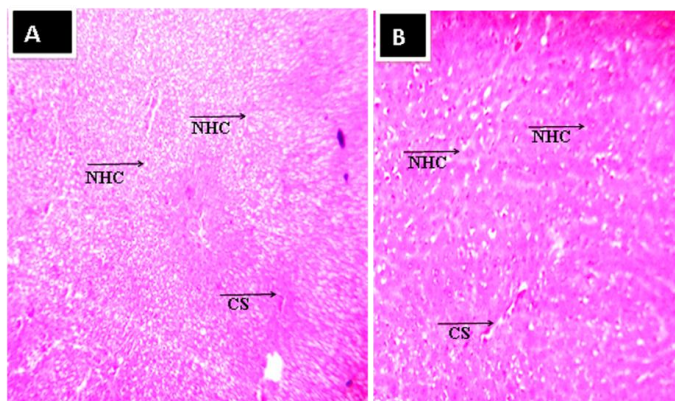


Plate 1: Photomicrograph of rats' liver administered with distilled water showed normal architecture with normal hepatocytes (NHC), and congested sinusoid (CS), H & E (A) and (B) x 40 magnification

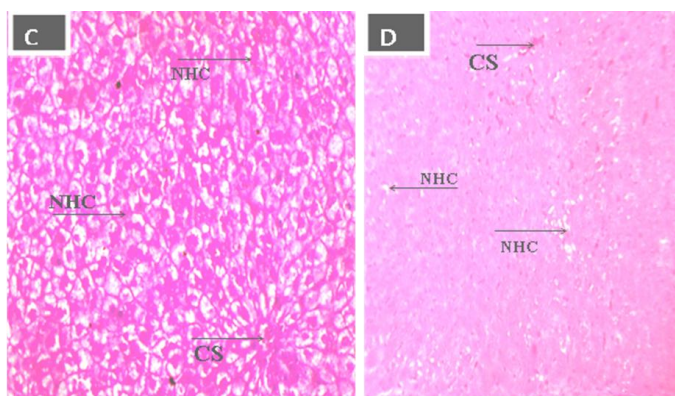


Plate 2: Photomicrograph of rats' liver administered with 150 mg/kg extract showed normal architecture with normal hepatocytes (NHC), and congested sinusoids (CS), H & E (C) x 40 and (D) x 10 magnification.

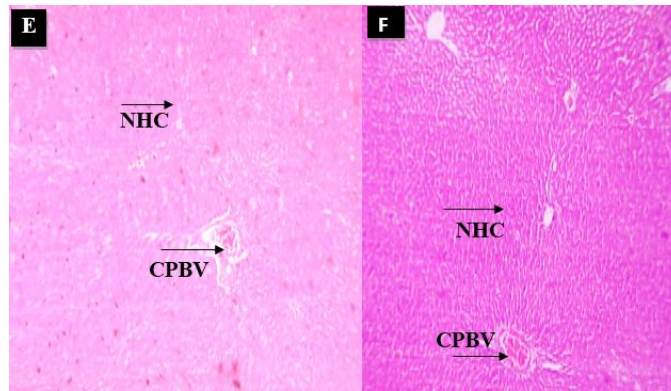


Fig 3: Photomicrograph of rats' liver administered with 300 mg/kg extract showed normal architecture with normal hepatocytes (NHC), and congested portal tract blood vessels (CPBV), H & E x 10 (E) and x 40 (F) Magnification.

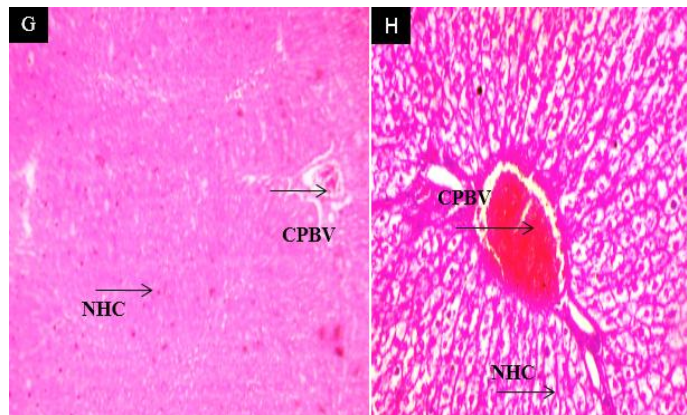


Plate 4: Photomicrograph of rats' liver administered with 450 mg/kg extract showed preserved architecture with normal hepatocytes (NHC), and congested portal tract blood vessels (CPBV), H & E, x 10 (G) and x 40 (H) magnification.

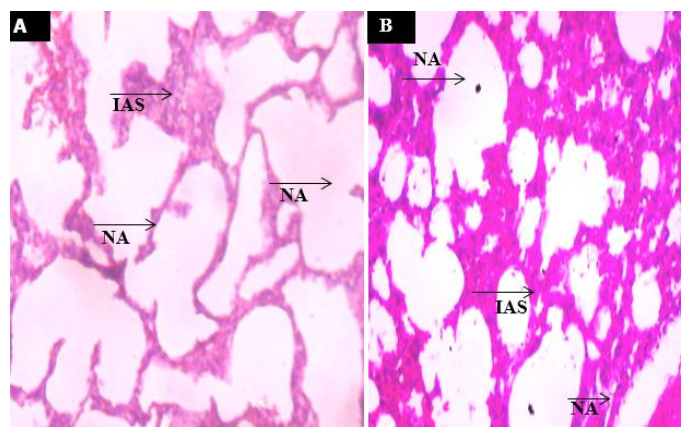


Plate 5: Photomicrograph of rats' lungs administered with distilled water showed normal lacy- like architecture with normal alveoli (NA) and inter-alveolar septae (IAS), H & E x 10 (A) and x 40 (B) magnification.

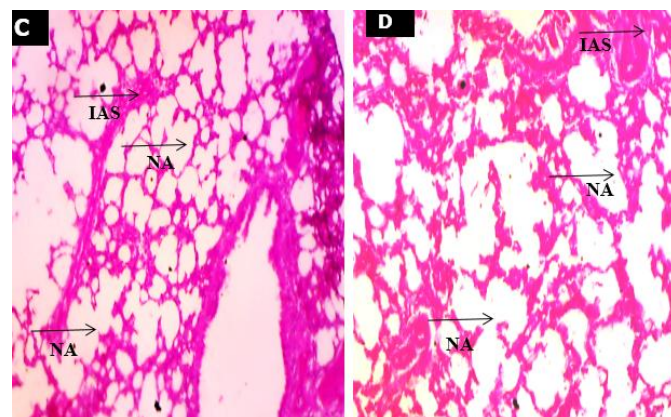


Plate 6: Photomicrograph of rats' lungs administered with 150 mg/kg extract showed normal lacy-like architecture with normal alveoli (NA) and inter-alveolar septae (IAS), H & E x 10 (C) and x 40 (D) magnification

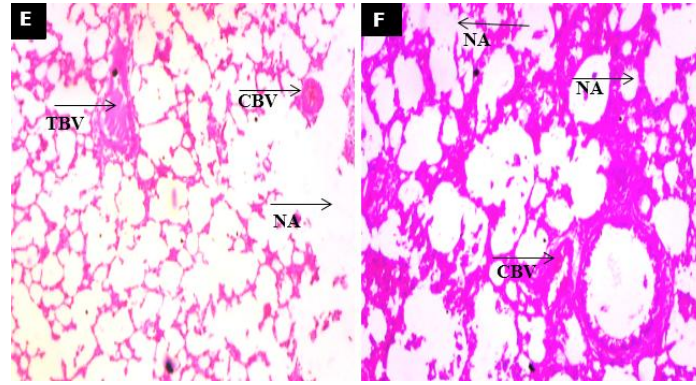


Plate 7: Photomicrograph of rats' lungs administered with 300 mg/kg extract showed normal lacy-like architecture with normal alveoli (NA), thrombosed inter-alveolar blood vessels (TBV) and congested inter-alveolar blood vessels (CBV), H & E x 10 (E) and x 40 (F) magnification

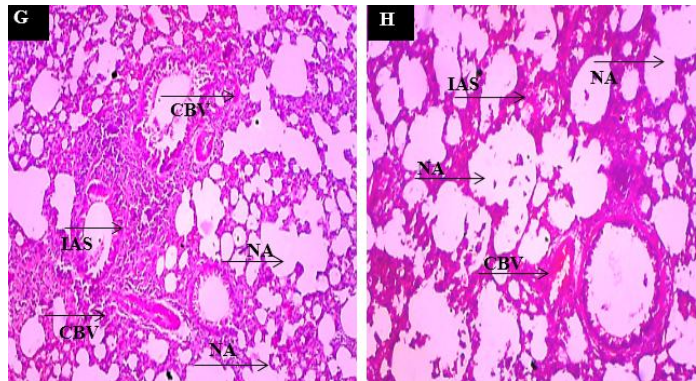


Fig 8: Photomicrograph of rats' lungs administered with 450 mg/kg extract showed normal lacy-like architecture with normal alveoli (NA) and inter-alveolar septae (IAS), and congested blood vessels (CBV). H & E x 10 (G) and x 40 (H) magnification.

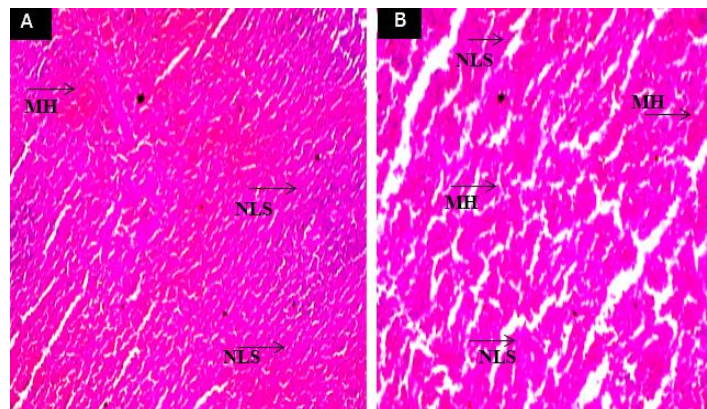


Plate 9: Photomicrograph of rats' spleen administered with distilled water (A) and 150 mg/kg extract (B) showed preserved architecture with normal lymphoid stroma (NLS), and mild haemorrhage (MH), H & E x 10 (A) and x 40 (B) magnification.

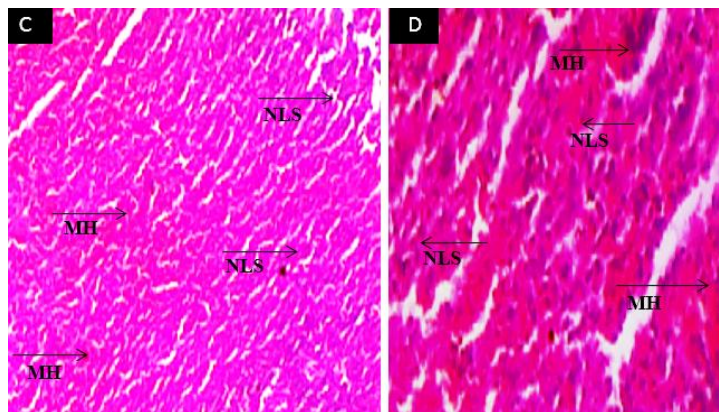


Plate 10: Photomicrograph of rats' spleen administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with normal lymphoid stroma (NLS), and moderate haemorrhage (MH), H & E x 10 (C) and x 40 (D) magnification

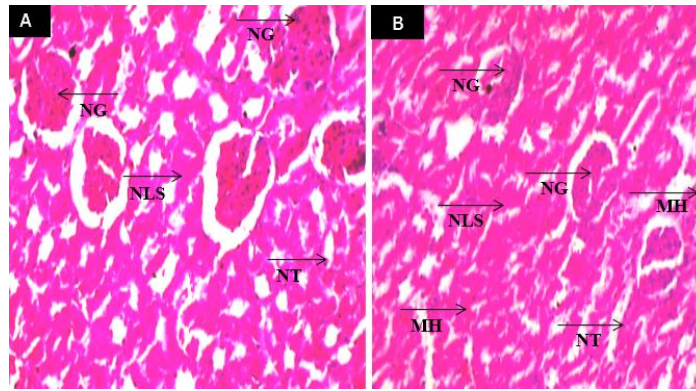


Plate 11: Photomicrograph of rats' kidney administered with distilled water (A) and 150 mg/kg extract (B) showed preserved architecture with normal glomeruli (NG), and normal tubules (NT), normal lymphoid stroma (NLS), and moderate haemorrhage (MH), H & E x 40 magnification.

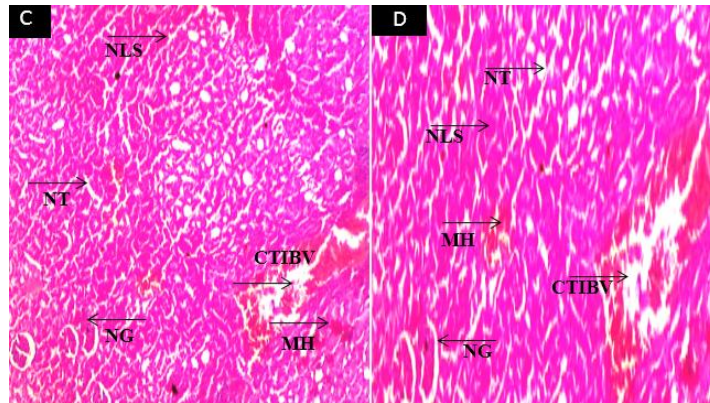


Plate 12: Photomicrograph of rats' kidney administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with normal glomeruli (NG), normal tubules (NT) and congested to thrombotic interstitial blood vessels (CTIBV), H & E x 10 (C) and x 40 (D) magnification

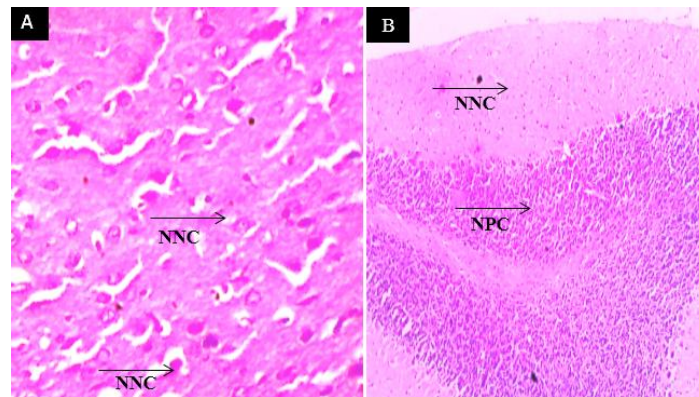


Plate 13: Photomicrograph of rats' brain administered with distilled water (A) and 150 mg/kg extract (B) showed preserved architecture with normal neuronal cells (NNC), and normal Purkinje cells (NPC), H & E x 40 magnification.

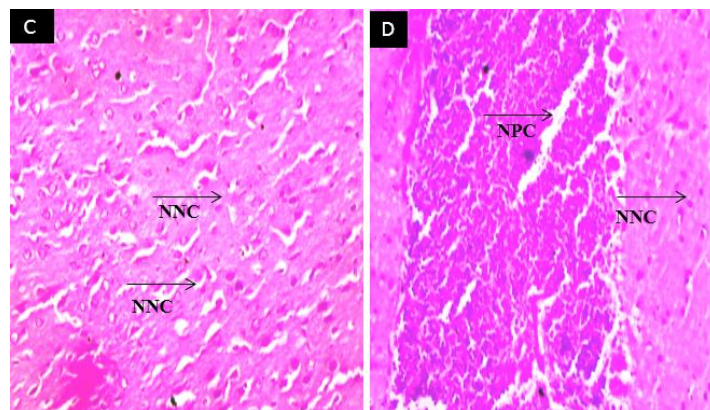


Plate 14: Photomicrograph of rats' brain administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with normal neuronal cells (NNC), and normal Purkinje cells (NPC), H & E x 40 magnification

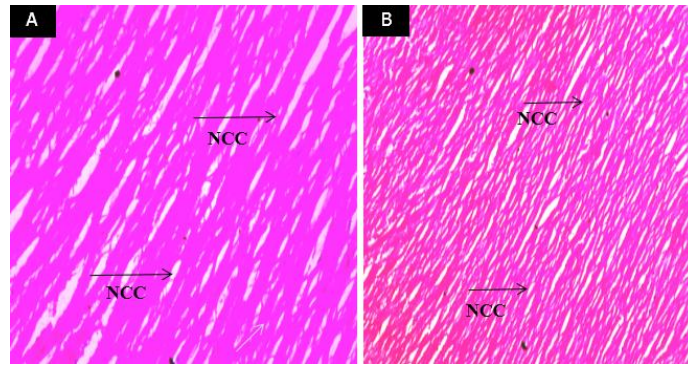


Plate 15: Photomicrograph of rats' heart administered with distilled water (A) and 150 mg/kg extract (B) showed preserved architecture with normal cardiac cells (NCC), H & E x 40 magnification.

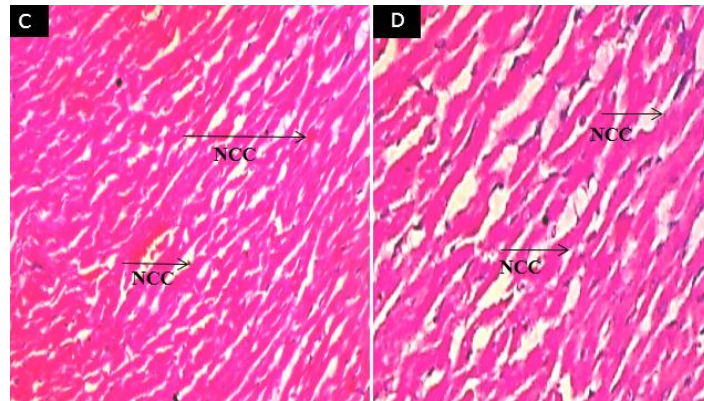


Plate 16: Photomicrograph of rats' heart administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with normal cardiac cells (NCC), H & E x 10 (C) and x 40 (D) magnification

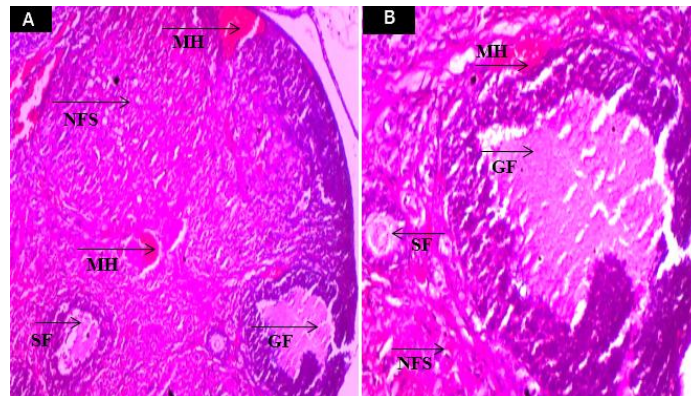


Plate 17: Photomicrograph of rats' ovary administered with distilled water (A) and 150 mg/kg (B) extract showed preserved architecture with secondary follicle (SF), Graafian follicle (GF), normal fibrotic stroma (NFS), and moderate hemorrhage (MH), H & E x 10 (A) and x 40 (B) magnification.

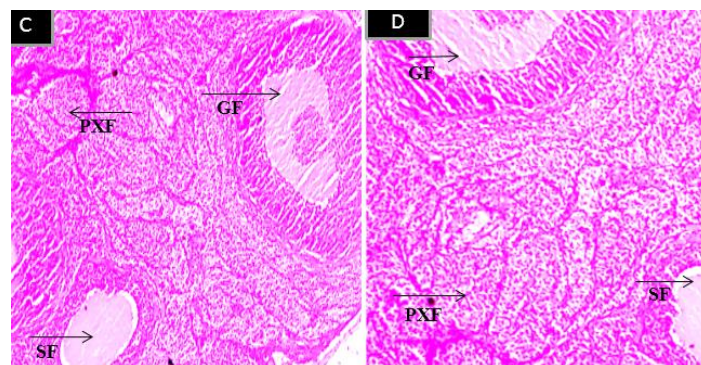


Plate 18: Photomicrograph of rats' ovary administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with primordial follicle (PXF), secondary follicle (SF), and Graafian follicle (GF), H & E x 10 (C) and x 40 (D) magnification.

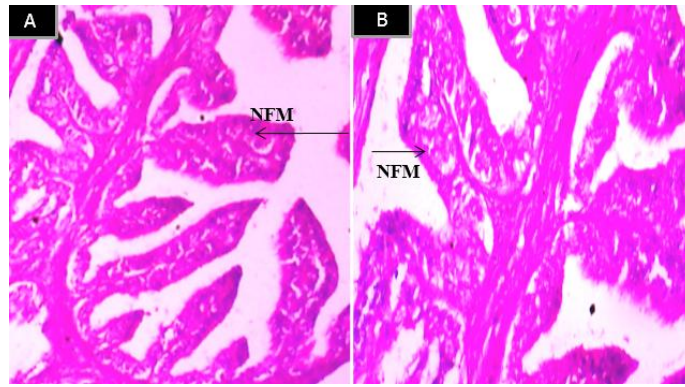


Plate 19: Photomicrograph of rats' oviduct administered with distilled water (A) and 150 mg/kg extract (B) showed preserved architecture with normal finger-like mucosa (NFM), H & E x 40 magnification.

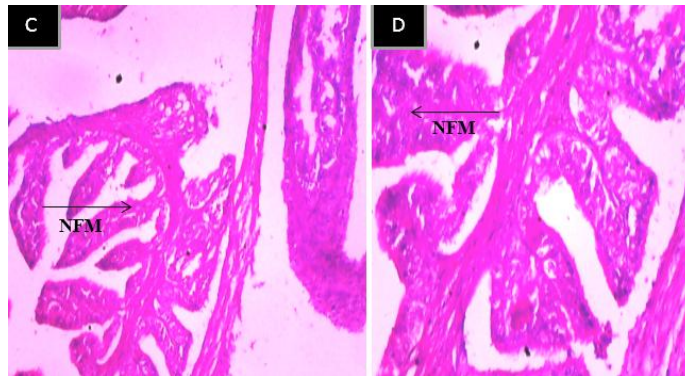


Plate 20: Photomicrograph of rats' oviduct administered with extract 300 mg/kg (C) and 450 mg/kg (D) showed preserved architecture with normal finger-like mucosa (NFM), H & E x 40 magnification.

5. Discussion

In the sub chronic toxicity investigations, there was no significant increase in the weight of the animals or the organs. This was corroborated by a non-significant effect in the level of total proteins and albumin (Udobang and Okokon, 2017) [12]

Increase in body weight of animals is usually attributed to the presence of active metabolites such as saponins and alkaloids (Joo, Cho and Kwon, 1978; Eteng, Etarrh and Owu, 2003) [10, 11] which though present in this extract were not identified in the most active fractions (Udobang, Bassey and Okokon, 2017) [12].

The liver filters and processes blood as it circulates through the body, metabolizes nutrients, detoxifies harmful substances and produces blood clotting proteins and bile among others. Damage to liver cells results in leakage of enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) into the blood, while blood levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) rise when bile flow is slow or blocked. Serum aminotransferase levels—ALT and AST—are two of the most useful measures of liver parenchymal cell injury. AST is raised in acute liver damage, but is also present in RBCs, cardiac and skeletal muscle, so is not specific to the liver, while ALT is almost exclusively found in the liver (Nyblom *et al*, 2006) [19]. Elevated levels of ALT and AST indicate liver damage but are not good measures of liver function since they do not reliably reflect the synthetic ability of the liver and they may come from tissues other than liver such as muscle. The extract produced a non-significant increase in AST that could have resulted from extra-hepatic sources as well (Udobang and Okokon, 2017) [12]. There was a significant increase in ALT in the low and middle dose of the extract indicating mild hepatic damage (Udobang and

Okokon, 2017) [12]. This corroborated the histologic findings of mild inflammation and could have resulted from the constituent of the extract menthofuran which is known to be hepatotoxic.

Biliary tract disease produces relatively greater increases in ALP than increases in ALT, AST, or lactose dehydrogenase (LD). ALP is associated with the plasma membrane of hepatocytes adjacent to the biliary canaliculus. Accordingly, diseases that predominately affect hepatocyte secretion due to obstruction or inflammation of the biliary tract will be accompanied by elevations of ALP levels. Bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis are some examples in which elevated ALP levels are often predominant over transaminase level elevation. Similar to ALT and AST, ALP is not specific for biliary tract disease since it is also released by osteoblasts, the ileum, and the placenta (Feldman, Friedman and Brandt, 2010) [20].

Elevations of the AST level may also be seen in acute injury to cardiac or skeletal muscle or to a lesser degree after vigorous exercise. Diseases that primarily affect hepatocytes, such as viral hepatitis, will cause disproportionate elevations of the AST and ALT levels compared with ALP level. Common causes of mild increases in AST and ALT levels include non-alcoholic fatty liver disease, hepatitis C, alcoholic fatty liver disease, and medication effect (e.g. due to statins). Total ALP elevation due to the hepatic fraction is a sensitive indicator of intra or extra hepatic cholestasis. Marked ALP elevation is usually noted with extra-hepatic biliary obstruction, primary biliary cirrhosis, and infiltrative processes such as neoplasm. Less dramatic ALP elevation can be seen in infectious mononucleosis, bile duct obstruction, hepatitis, heavy alcohol consumption and fatty liver. The significant increase in ALP in the middle dose of the extract

could indicate the limited inflammation reported in the histology but not cholestasis.

The kidneys function to filter waste products of metabolism, such as urea from protein metabolism and uric acid from DNA breakdown from the body, regulate levels of electrolytes (eg sodium, potassium and phosphate), acid-base balance of the body and blood pressure and produce erythropoietin which stimulates the bone marrow to produce red blood cells. The two waste products usually are measured are blood urea nitrogen (BUN), and creatinine. Creatinine, a waste product of meat protein in the diet and also of the normal wear and tear on muscles is usually completely filtered from the blood by the kidneys. (Oh, 2011) ^[23]. Normal Serum creatinine level range is 0.6 - 1.2 mg/dl and this can vary depending on age, race and body size. A creatinine level higher than 1.2 for women and 1.4 for men is a sign of a kidney problem. As kidney disease progresses, the level of creatinine in the blood increases. Urea is a breakdown product of food protein. A normal blood urea nitrogen (BUN) level is 7 - 20 mg/dl. As kidney function decreases, the BUN level increases. Common medications, including large doses of aspirin and some antibiotics, can also increase BUN, however BUN and creatinine will not be raised above the normal range until 60% of total kidney function is lost. Hence, the more accurate glomerular filtration rate (GFR) or its approximation of the creatinine clearance is measured whenever renal disease is suspected. (Pincus and Abraham, 2011) ^[24]. The extract produced no significant effect on the levels of Na⁺, CL⁻, K⁺, HCO₃⁻, and creatinine, but there was a significant (p < 0.001) increase of urea in the low dose of extract (Udobang and Okokon, 2017) ^[12]. The fact that there was no significant effect on creatinine, the most common indicator of renal injury that rises with marked damage to functioning nephrons and the rise in urea only in the low dose of the extract corroborates the negligible toxic effect seen on histology.

6. Conflict of interest statement

We declare that we have no conflict of interest.

7. Acknowledgments

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