The Effects of the Ethyl Acetate, Aqueous and N-Butanol Fractions of the Root of *Combretum Molle* (R.Br. Ex. G. Don) and Its Histology to Wistar Rats

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***Abstract*: - *Combretum molle* has been used by many traditional medicine practitioners in the treatment of microbial infections. This work was carried out with the aim of determining the acute toxicity and histology of the aqueous, n-butanol and the ethyl-acetate fractions of the root of *Combretum molle* to Wistar rats. Ethanol was used as solvent for extraction, after which differential fractionization was carried out using distilled water, ethyl acetate and *n*-butanol. The limittest at 5000 mg/kg of the Organization for Economic Cooperation and Development (OECD) guidelines were used for the study. In the acute toxicological investigation, there was no mortality in the experimental animals after orally administering the fractions of *C. molle* 5000 mg/kg indicating that the LD_{50} was above 5000 mg/kg. There was no histological alterations or changes at the extract dose of 5000 mg/kg body weight in the kidney organs of rats in the control group, but all the other organs from the fractions tested displayed certain observed alterations. Tubular vacuolation (TVN), Lymphocyte hyperplasia (LH), Glomerular necrosis (GN), Plaques formation (P), Tubular necrosis (TN) and Tubular distortions (TD) were observed in the kidneys. There was no histological alterations or changes at the extract dose of 5000 mg/kg body weight in the internal organs of rats in the control group and in the liver of group VI, but all the other organs from the fractions tested displayed certain observed alterations. Vascular congestion (VC), vascular congestion with slight necrosis (VCN) and hepatocellular necrosis (HN) were observed in the livers of all the groups administered the fractions.**

***Key words*: *Combretum molle*, ethanol, acute toxicity, Wistar rats.**

I. INTRODUCTION

People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. In many remote areas in African countries people consult the traditional healer of the village in case of illness (Wood *et al*., 1997). *Combretum molle* is a small, graceful, deciduous tree 3-13 m high; trunk crooked or leaning, occasionally swollen at the base, up to 30 cm in diameter. Bark grey and smooth when young, grey-brown to almost black, rough and flaking when older, twigs often with reddish hairs. It is deciduous and it yields a gum. Leaves opposite, simple, leathery, 5-17 cm long, 2.5-9 cm wide, narrowly elliptic, broadly ovate-elliptic to almost circular, with dense, grey, velvety hairs on both sides (Palmer, 1972). *Combretum molle* has been used in many traditional medicines for treatment of microbial infections (diarrhea, dysentery, fever) and several inflammatory conditions such as abdominal pain, headache, and toothache(Newman *et al*., 2003).

II. MATERIALS AND METHODS

Source and preparation of plant materials

The plant roots were collected from neighboring communities near ABU dam, in Samaru, Zaria (latitude 11.07° N, longitude 7.73° E and altitude 613meters), Nigeria. These were brought and identified by a Taxonomist with voucher number 900191 at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. The plant parts were air-dried for two weeks at room temperature (25°C) in the laboratory and then ground to powder.

Extraction procedures

The ground plant parts were extracted at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, following the methods of Sofowora (2006).

Preparation of Ethanol Extraction of C. molle

Approximately 400 g of the dried roots of *C. molle* were extracted with 10 litres of 80% (v/v) ethanol by maceration at (25°C) for 3days. The total mixture was strained and filtered. The filtrate was concentrated to dryness on a water bath at 100° C to obtain the dry extract after which was stored at -20ºC for further studies.

Differential Fractionation of the Ethanol Extract of C. molle in Different Solvents

The dried ethanol extract obtained from the roots of *C.molle*(50 g) were each suspended in 1 litre of distilled water and partitioned in sequence with ethyl acetate (1 litre), and *n*-butanol (1 litre). The different solvent fractions were concentrated on a water bath at 100° C to obtain the dry extract after which was stored at -20ºC.

Acute Oral Toxicological Evaluation of the fractions of C. molle

This study was carried out according to the Organisation for Economic Cooperation and Development (OECD) guidelines (OECD, 2000). Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. This is a measure of the interaction of induced substance with biomolecules after a single administration within fourteen days

Limit Test at 5000 mg/kg for the Experimental Rats study

The limit test is primarily used in situations where the experimenter has information indicating that the test plant is likely to be nontoxic. Since there was prior information on the use of the test plant (Wickens, 2000), therefore the limit test was used.

Animals (Wistar rats)

A total of 12 female albino rats of Wistar strain weighing about 230 – 280 g were obtained from the Animal house, Department of Pharmacology and Therapeutic, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were fed a standard rat pellet diet and water was provided and maintained under standard laboratory conditions (Temperature 21-24 °C, relative humidity 40 - 60%).

Grouping and Administration of plant fractions

A total of 12 female albino Wistar rats were used for the acute toxicity study. The test substance was administered orally, in a single dose by gavage intubation cannula. The animals were divided in to 4 groups of 3 each. Group I rats were given ethyl-acetate root fraction. Group II rats were given n-butanol root fraction. Group III rats were given aqueous root fraction. Group IV served as normal healthy control. The weight of experimental rats was measured and recorded on Days 1, 7 and 14 respectively. The histological parameters of the experimental animals were evaluated after 14 days. The Wistar rats were subjected to fasting (for food but not water over-night) prior to dosing. Following the period of fasting, they were weighed, and the test substance administered. After the fractions were administered, food was not given to the rats until after 3-4 hours. The plant fractions at fixed doses of 5000 mg/kg body weight were administered to 3 groups, each containing 3 rats which was done in two stages. A single rat each from the first 3 groups were administered the plant fractions (5000mg/kg) and observed for 24 hours, after which all of them survived. The 2 remaining rats were also administered the dose and were observed during the first 30 minutes, periodically for 4 hours, then hourly the first 24 hours and daily thereafter, for a total of 14 days. All observations were systematically recorded, with individual records being maintained for each animal.

Histological studies of the kidneys and livers

The experimental animals were sacrificed (chloroform as anesthesia) and the kidneys and livers were excised and taken to the Department of Human Anatomy, Faculty of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria for histological studies. The various organs were sliced and placed in embedded tissue baskets. Thereafter, they were fixed with 10 % formalin for 48 hours and afterwards dehydrated with methanol (70, 90 and 100 %) at different concentration in ascending concentration and different time to remove water from the tissues. Thereafter, clearing with toluene was done to remove alcohol and prepare the tissue for waxing. Embedding was done using paraffin wax by impregnating cassettes with molten wax at 60^{o} C for 3 h. Slicing was done at 5 microns using a Leica microtome (model no: RM2125RTS). The slide was dried for 20 min on hot plate. Afterwards, dewaxing and hydration were done using xylene and methanol (70, 90 and 100%) at different concentration in ascending concentration and different time to remove water from the tissues. Thereafter, staining was done with Cole's hematoxylin for 10 min to stain the nucleus after which eosin was used to stain the cytoplasm for 3 min. Dehydration was once again carried out in alcohol and alcohol cleared with xylene. A mounting medium, dibutyl phthalate xylene (DPX) was placed on the tissue section and they were viewed using the microscope.

III. RESULTS AND DISCUSSION

Table 1. Average body weight of the rats after oral administration of the fractions of *C. molle* measured in grams (g), (Mean ±SE)

GROUPS (fractions of extracts)	Day 0	Day 7	Day 14
I (ethyl-acetate root)	278±1.0	281±1.0	287±1.0
III (n-butanol root)	258±1.0	260±1.0	263±1.0
V (aqueous root)	278±1.0	283±1.0	288±1.0
VII (control)	229±1.0	234±1.0	236±1.0

Mean body weight ± SE

Acute toxicological evaluation

The body weights of all tested groups increased progressively throughout the duration of the experiment (Table 1). The effect of the extract in causing drowsiness in all the treated groups was observed for the first hour after administering the fractions of *C. molle*, compared with control which showed no drowsiness. No mortality was recorded for any treated groups throughout the duration of the experiment. Since the treatment did not result in latent toxicity, the LD_{50} was therefore estimated to be above 5000 mg/kg.

Histopathology of the kidneys of the animals

There was no histological alterations or changes at the extract dose of 5000 mg/kg body weight in the kidney organs of rats in the control group, but all the other organs from the fractions tested displayed certain observed alterations. Tubular vacuolation (TVN), Lymphocyte hyperplasia (LH),

Glomerular necrosis (GN), Plaques formation (P), Tubular necrosis (TN) and Tubular distortions (TD) were observed in the kidneys of groups I-III (Plates I---III).

Histopathology of the liver of the animals

There was no histological alterations or changes at the extract dose of 5000 mg/kg body weight in the internal organs of rats in the control group and in the liver of group III, but all the other organs from the fractions tested displayed certain observed alterations. Vascular congestion (VC), vascular congestion with slight necrosis (VCN) and hepatocellular necrosis (HN) were observed in the livers of groups I--III (Plates IV--VI).

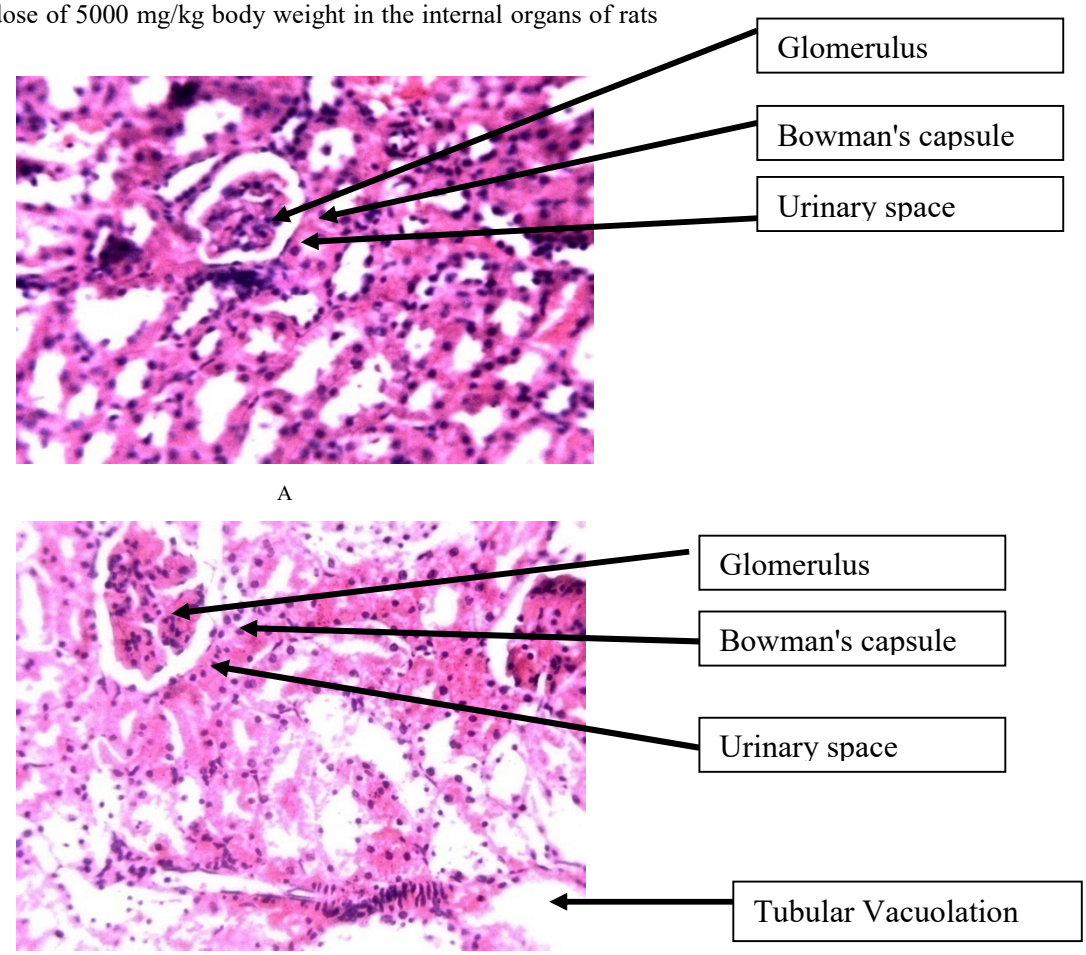
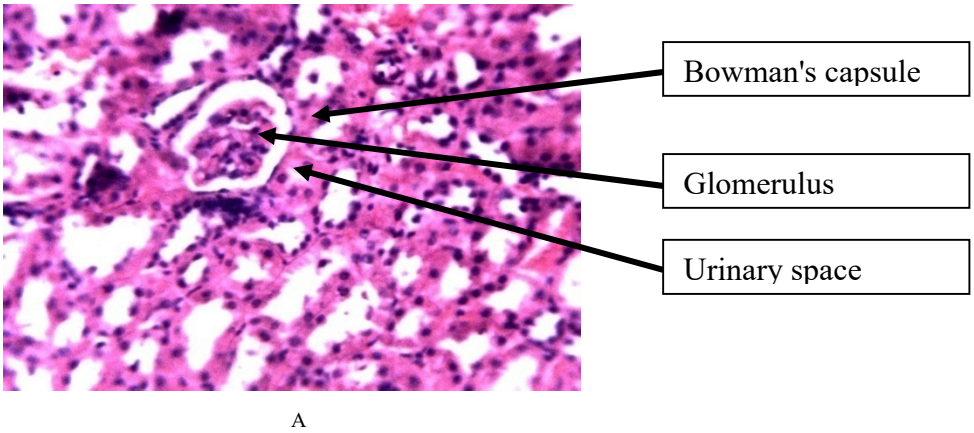


Plate I: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus, compared with; (B) Orally administered ethyl-acetate root fraction showing tubular vacuolation (TV). (Magnification, × 400).



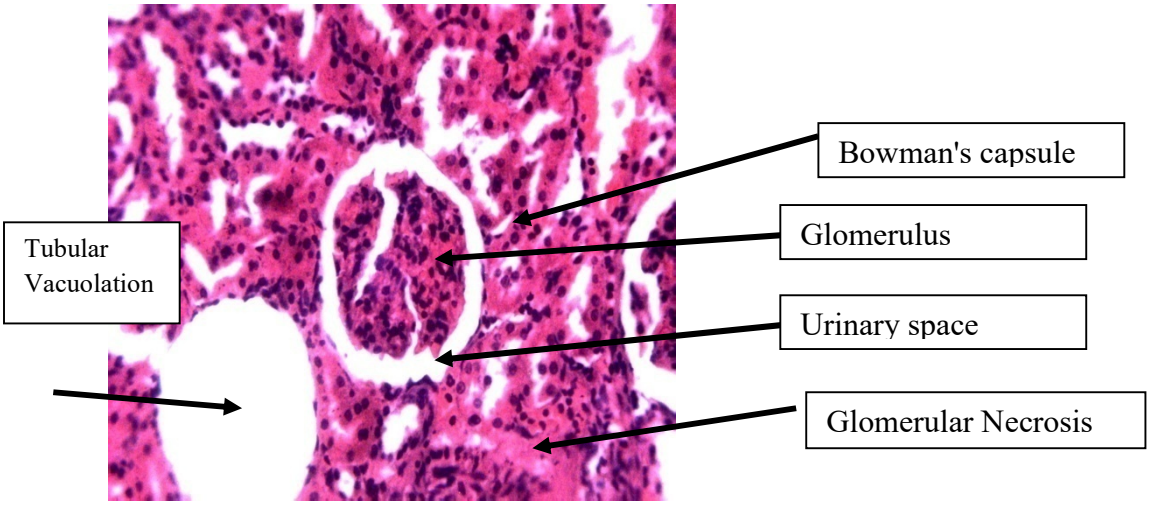


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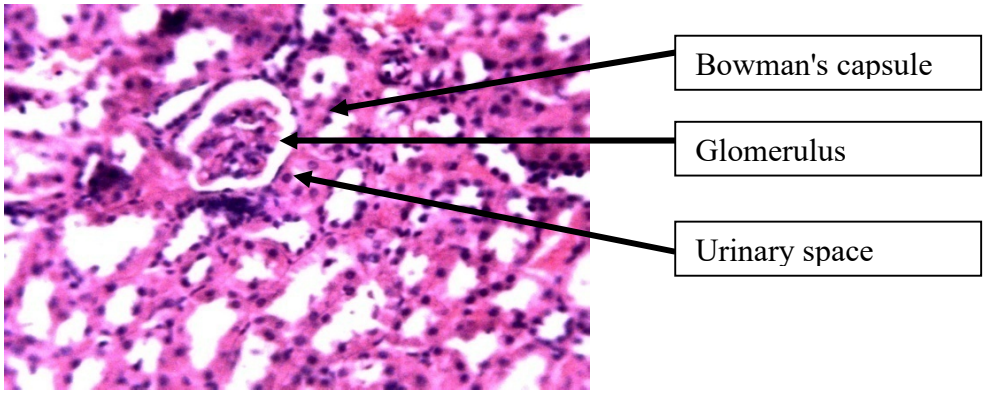
Plate II: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus, compared with; (B) Orally administered n-butanol root fraction above showing slight glomerular necrosis (GN) and tubular vacuolation (TV). (Magnification, × 400).



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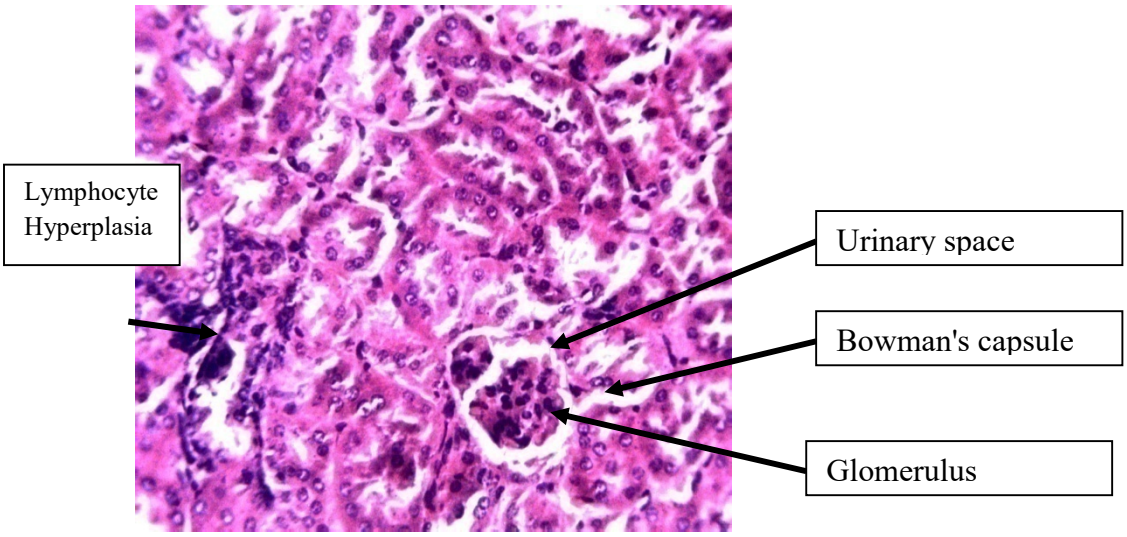

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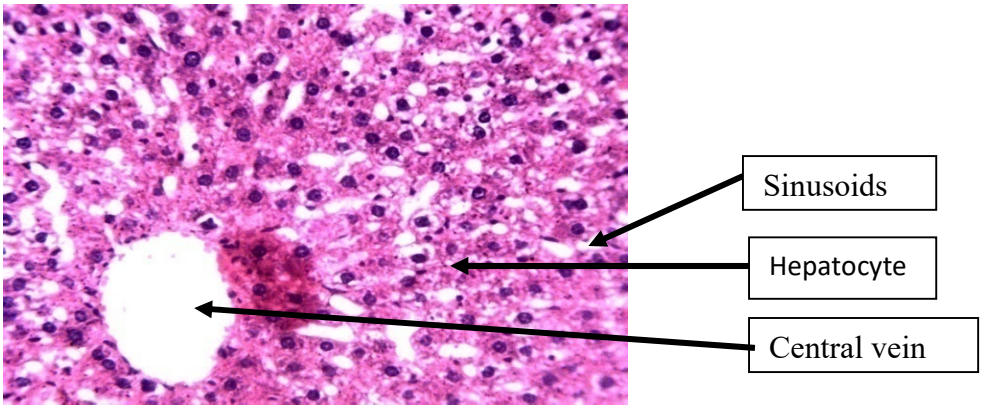
Plate III: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus, compared with; (B) Orally administered aqueous root fraction above showing moderate lymphocyte hyperplasia (LH). (Magnification, × 400).



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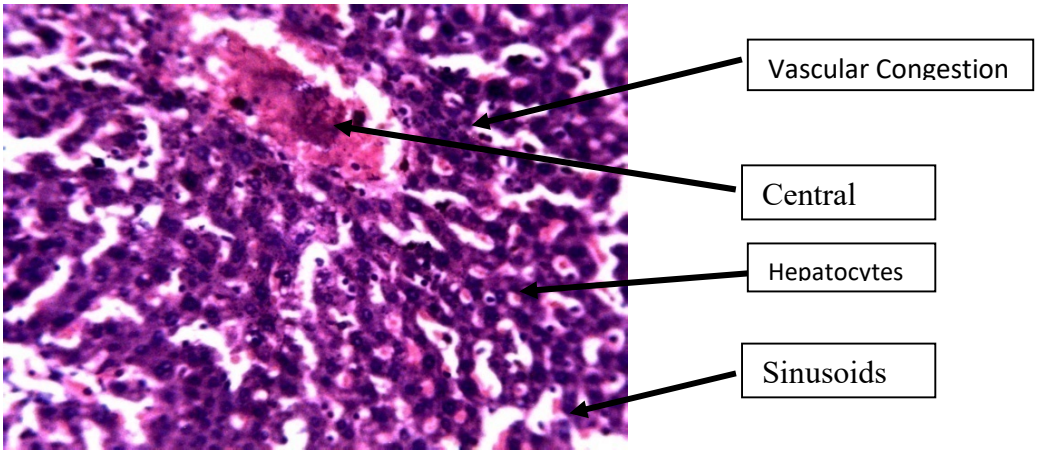

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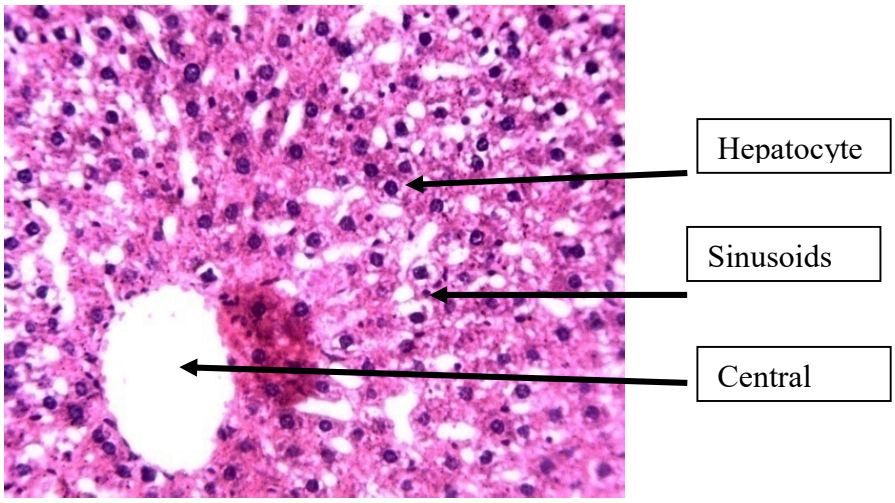
Plate IV: A) Photomicrograph of the Rat Liver Control showing normal hepatocytes, compared with; (B) Orally administered ethyl-acetate root fraction administered liver which showed vascular congestion (vc). (Magnification, × 400).



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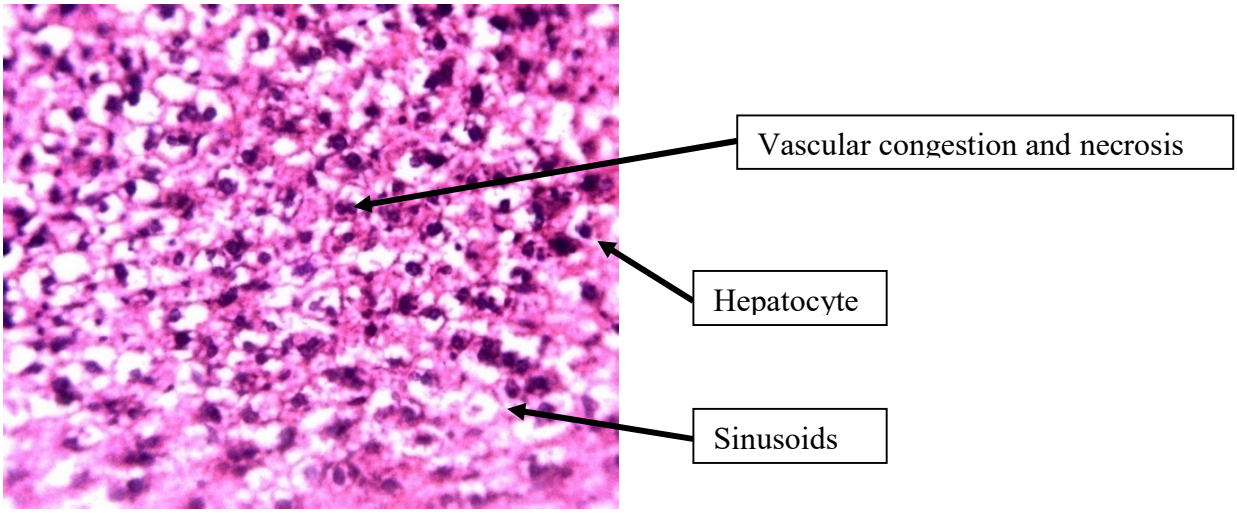

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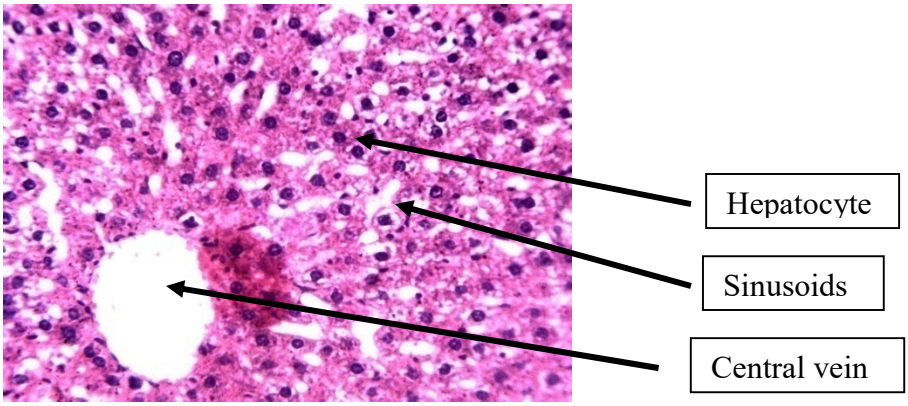
Plate V: (A) Photomicrograph of the Rat Liver of control showing normal hepatocytes, compared with; (B) Orally administered aqueous root fraction above shows slight vacoulation and necrosis(VCN). (Magnification, × 400).



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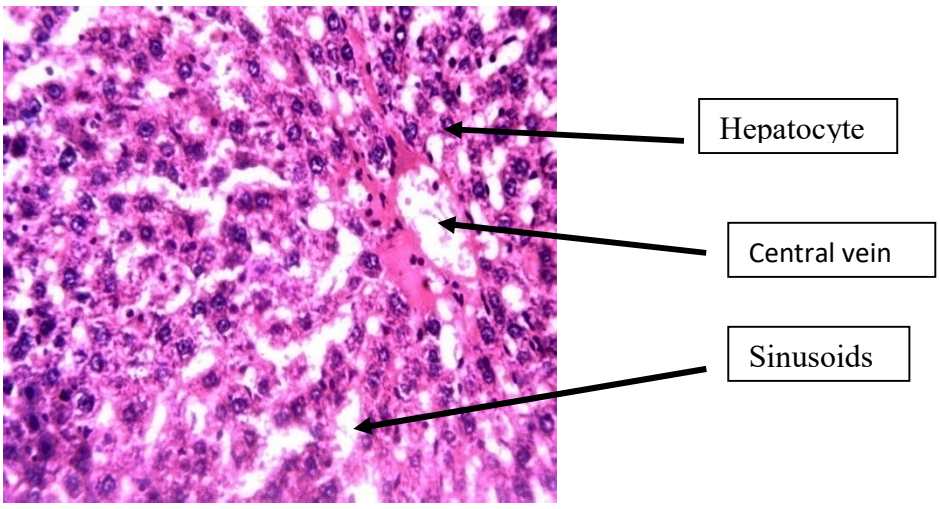

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Plate VI: (A) Photomicrograph of the Rat Liver of control showing normal hepatocytes, compared with; (B) Orally administered N-butanol root fraction above showing normal features. (Magnification, × 400).

There was a progressive increase in the body weight of all animals throughout the duration of the experiment and when compared to the control, there was no significant difference. The behaviors of all the tested animals revealed that, the animals were alert and responded to pain and touch. The animals showed no signs of depression or restlessness. Monitoring the body weight during treatment provides an index of the general health status of the animals; such information is important for gauging their health (Sharma *et al.*, 2009). According to Rhiouani*et al.* (2008) high doses of plant extracts induce stress in rats, thereby reducing their food and water intake which leads to a loss in weight. Thus, the increase in weight were considered normal. Similar researches are that of Dodehe*et al*. (2012) who carried out the acute and sub-acute toxic study of the aqueous leaf of *C. molle* and at dose of 5000mg/kg observed gradual increase in body weight. In addition, Zaza*et al*. (2016) investigated the oral toxicity of the X_{42} fractions of *Terminaliaivorensis* observed gradual increase in body weight.

There were some histopathological alterations in the cellular anatomy of the liver and kidney. This manifested as alterations in the biomarkers of toxicity and cellular damage to the liver and kidney. The kidneys administered ethyl-acetate, aqueous and n-butanol leaf fractions showed slight tubular distortions and plaque formations. Plaque is an interstitial medullary and papillary deposit of calcium phosphate in the form of apatite that begins in the basement membranes of the thin loops of Henle and finds its way into the interstitium and eventually down to the sub epithelial space (Evan *et al*., 2007). They are significant because renal stones are known to grow attached to renal papillae, and specifically to regions of papillae that contain Randall's plaque (interstitial apatite deposits), the mechanisms of stone overgrowth on plaque are not known (Evan *et al*., 2007). From the kidneys administered ethyl-acetate and n-butanol root fraction, slight tubular vacoulation was observed. Tubular vacoulation is said to occur when the vacuoles of the kidneys that were administered the fractions are larger than that of the control (Sharma *et al.*, 2009).There were some histopathological alterations (Slight vascular congestion) observed from the liver administered root ethyl-acetate and aqueous root fractions. This finding corroborates the finding of Sagar and Vidyasagar (2010) who observed slight changes in cellular architecture of the 202 livers of animals treated with 250 mg/kg body weight of an ethyl acetate extract of *Caesalpiniabonducella.*

IV. CONCLUSION

The fixed dose of *C. molle* at 5000 mg/kg body weight is not toxic to experimental animals. This is because no mortality was observed in all the animals tested. Based on the present assessments and investigations, the daily use of concentrated extracts of the root of *C. molle* should be carefully considered in view of the observed alterations recorded in the histological investigation. It is clear from this study that potential toxicity might arise from the continuous intake of plants such as *C. molle* at high concentrations.

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