Toxicological and Histological Studies of the Ethyl Acetate, Aqueous and N-Butanol Fractions of the Leaf of *Combretum Molle* (R.Br. Ex. G. Don) to Wistar Rats

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Abstract- 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 leaves 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 limit test 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.

Index Terms- Combretum molle, plant fractions, acute toxicity, Wistar rats,

I. INTRODUCTION

Thousands of secondary plant products have been identified and it is estimated that thousands of these compounds still exist (Koehn and Carter, 2005). Since secondary metabolites from plants have been elaborated within living systems, they are often perceived as showing more "potentials and are biologically non-toxic than totally synthetic molecules" making most of them, good candidates for further drug development (Koehn and Carter, 2005). According to Alaribe (2008) majority of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Existing data and contemporary researchers seem to authenticate the assumption for general health improvement of the masses by traditional healers. Ethnobotany is a preliminary method of research, suitable for gathering information on the use of plants. It has been proven, time and time again, that the medical knowledge handed down by the common people constitutes sources of information useful for scientific research and that many plants utilized exclusively in popular tradition, when exposed under scientific investigation, have been found to be useful for different sectors in the industry, therefore science and tradition have a strong connection between them. Science in fact often has traditional origins (Lentini, 2000). The use of plants in the tropical and subtropical regions is diversified and most of the uses are for medicine, source of food, clothing and shelter. However, the medicinal uses of plants are rapidly declining among the present generation of local people because of modernization and civilization (Cox, 2005).

II. MATERIALS AND METHODS

Source and preparation of plant materials

The plant leaves 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 leaves 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 -20°C for further studies.

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

The dried ethanol extract obtained from the leaves 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 100º 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 Organization 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 leaf fraction. Group II rats were given n-butanol leaf fraction. Group III rats were given aqueous leaf 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 substance was 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 60º 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 leaf)	247±1.0	253±1.0	261±1.0
II (n-butanol leaf)	235±1.0	237±1.0	241±1.0
III (aqueous leaf)	256±1.0	260±1.0	263±1.0
V (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).

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).

Histopathology of the liver of the animals

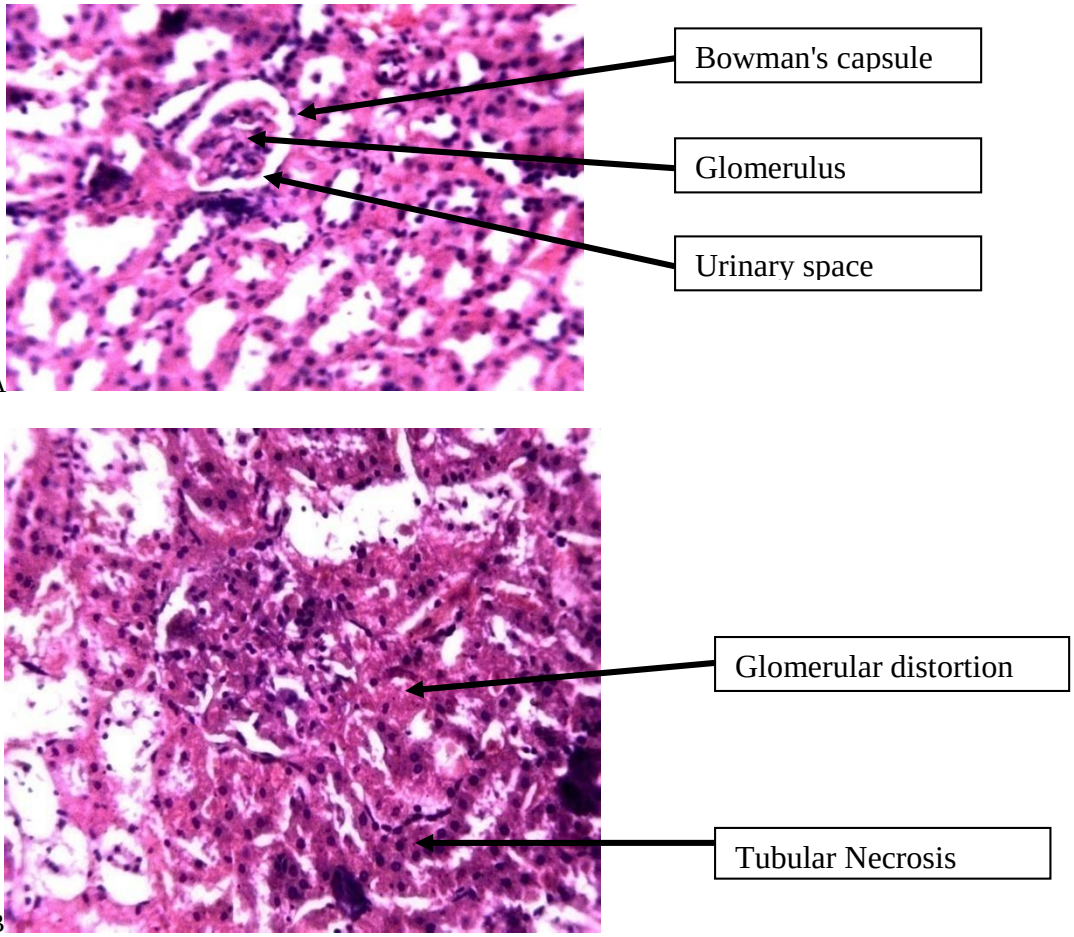


Plate I: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus, compared with; (B) Orally administered ethyl-acetate leaf fraction above showing moderate glomerular distortion (GD) and tubular necrosis (TN). (Magnification, × 400).

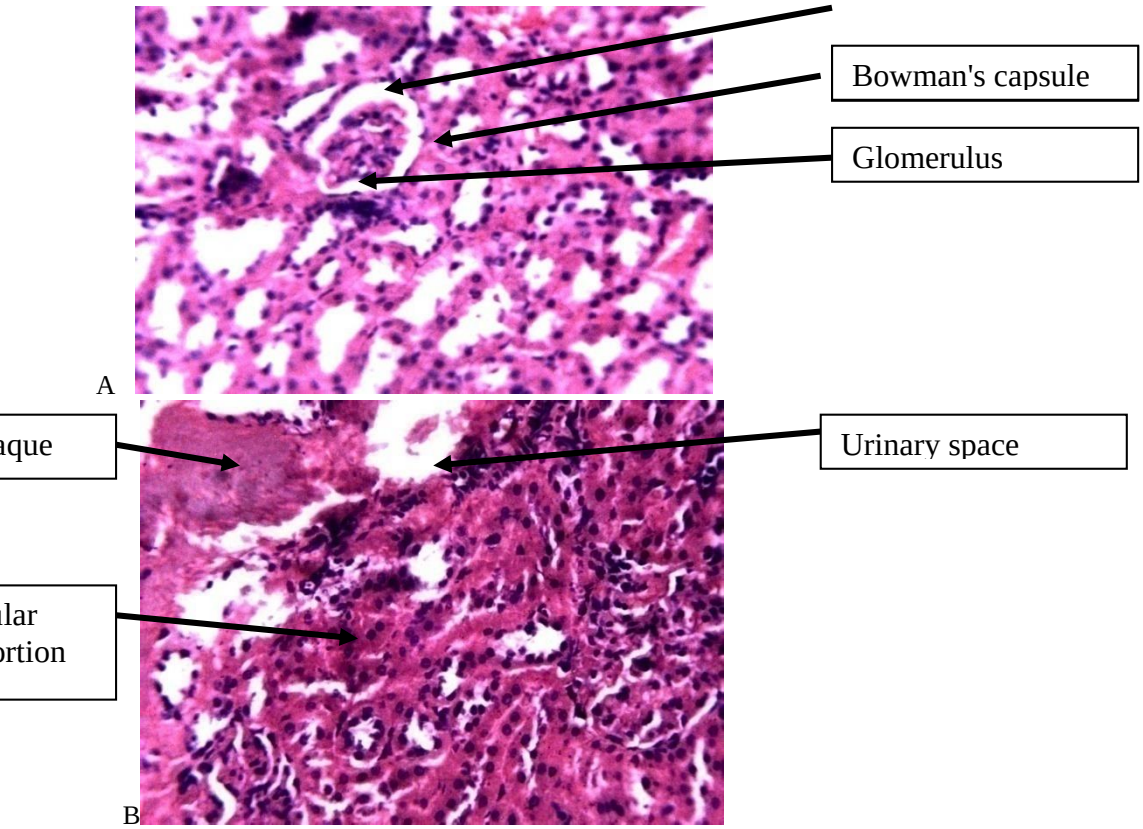
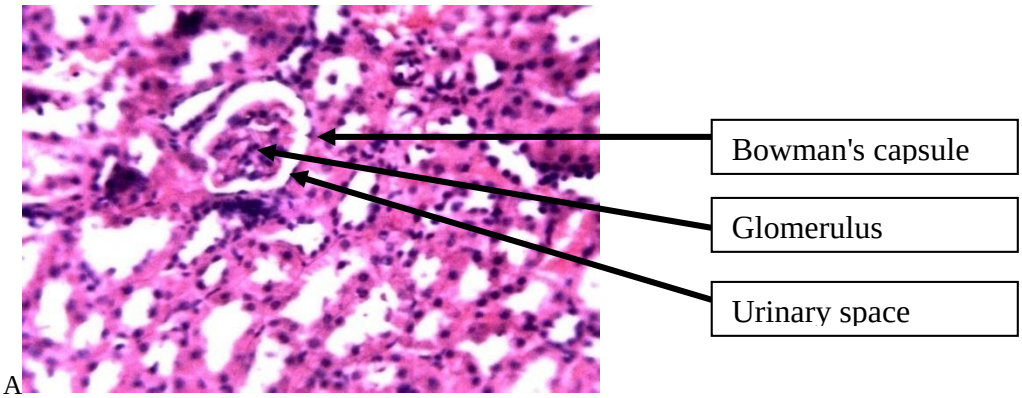
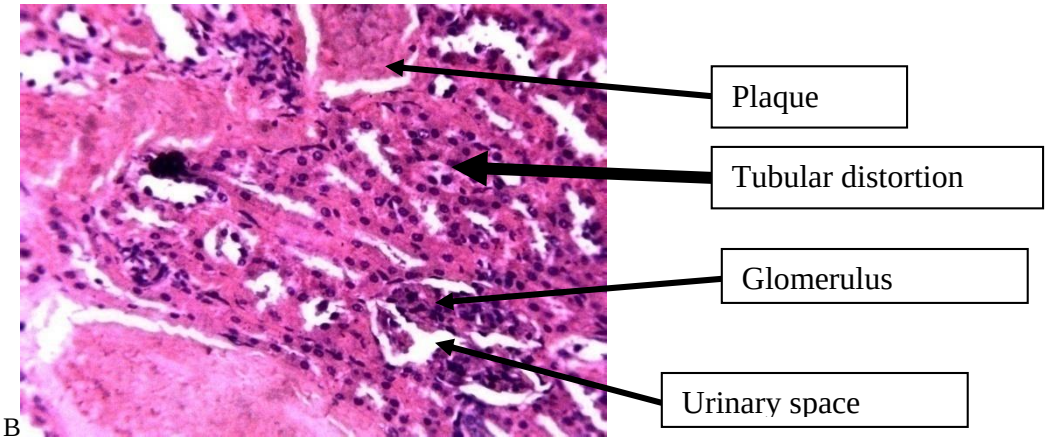


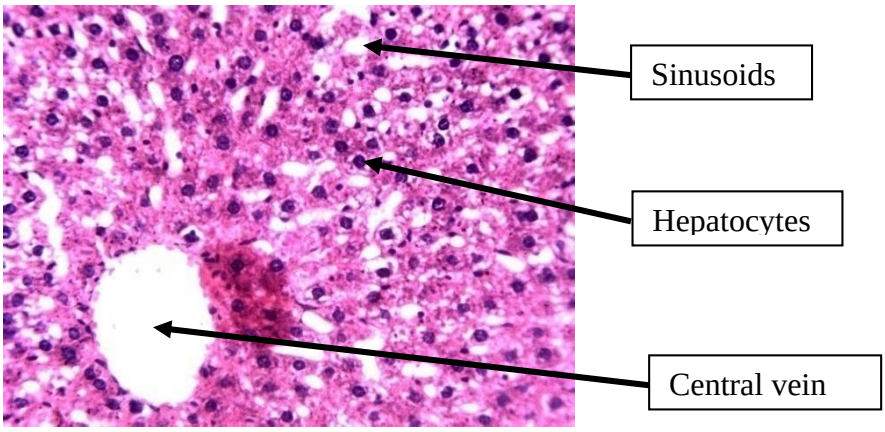
Plate II: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus, compared with; (B) Orally administered n-butanol leaf fraction above showing plaques formation (P) and slight tubular distortion (TD). (Magnification, × 400).



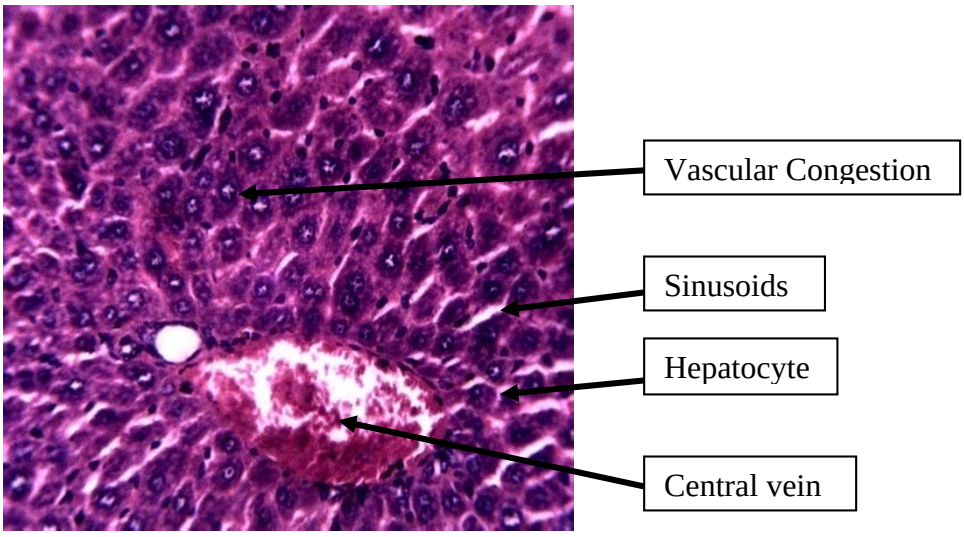


B

Plate III: (A) Photomicrograph of the Rat Kidney under control treatment showing normal tubules and glomerulus compared with; (B) Orally administered aqueous leaf fraction above showing plaque formation (P), with slight tubular distortion (TD). (Magnification, × 400).



A



B

Plate IV: (A) Photomicrograph of the Rat Liver of control showing normal hepatocytes, compared with; (B) Orally administered ethyl-acetate leaf fraction above shows vascular congestion (VC). (Magnification, × 400).

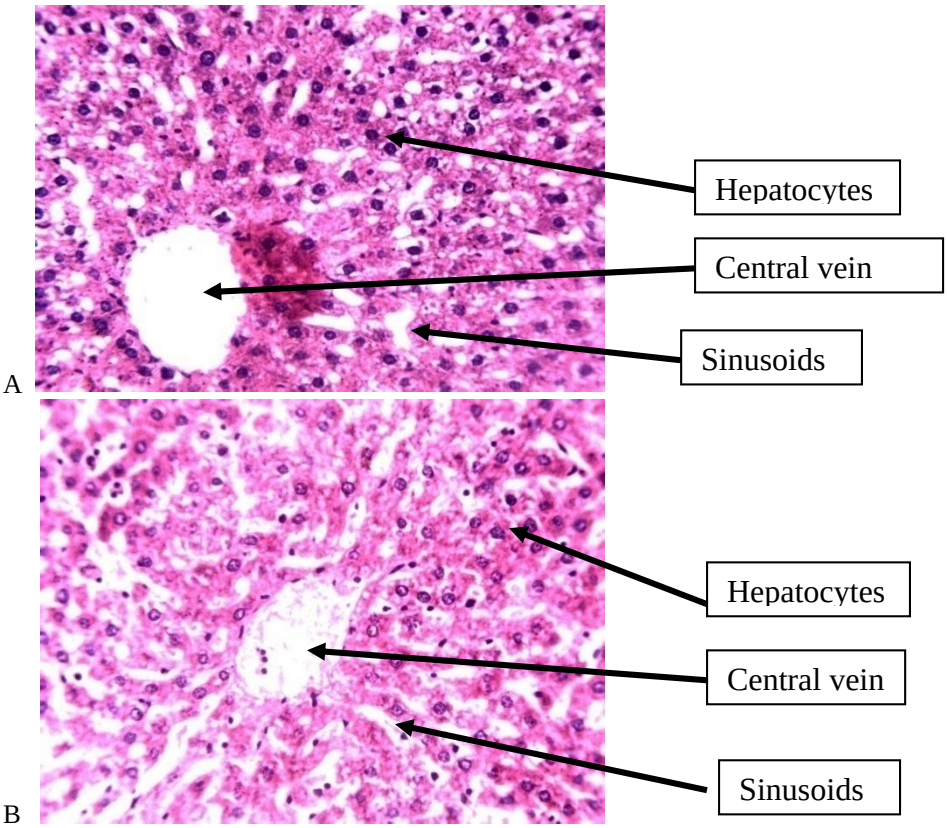
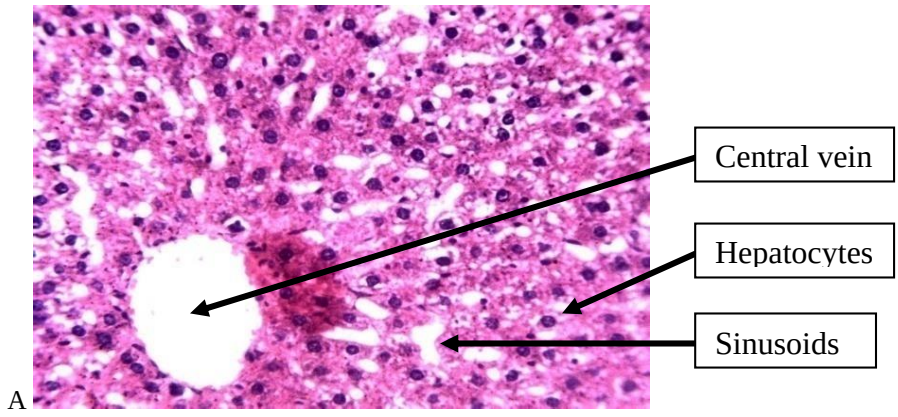
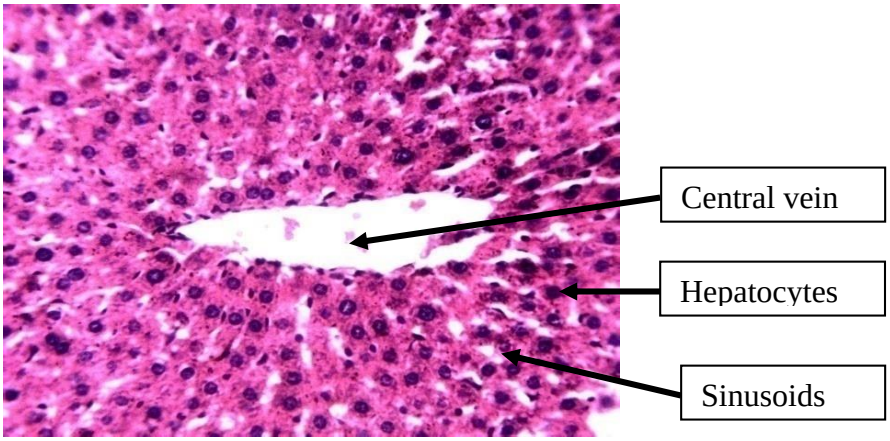


Plate V: (A) Photomicrograph of the Rat Liver of control showing normal hepatocytes compared with; (B) Orally administered N-butanol leaf fraction above also showing normal feature. (Magnification, × 400).





B

Plate VI: (A) Photomicrograph of the Rat Liver of control showing normal hepatocytes compared with; (B) Orally Administered 5000 mg/kg of aqueous leaf fraction also showing normal feature. (Magnification, × 400).

In the acute toxicological investigation, there was no mortality in the experimental animals at all treatment doses of 5000 mg/kg body weight. The acute toxicity did not result in any mortality therefore the mean lethal dose (LD_{50}) cannot be determined, rather estimated that it is above 5000 mg/kg. There were no signs of toxicity effects such as change in skin color, behavioral pattern nor diarrhea. This finding suggests that, the fractions at the limit dose tested are nontoxic and safe in oral formation. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, (2000) the fractions of *Combretum molle* were assigned class 5 status (LD_{50} > 5000 mg/kg) which is the lowest toxicity class. This is in line with the investigation of David *et al*. (2015) in the study of the toxicological evaluation of aqueous and acetone extracts of *Combretum molle* leaves in Wistar Rats. They observed that the limit dose of 2000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. Similarly, Kamo *et al*. (2015) studied the acute and subacute toxicity effects of hydro - alcoholic extract of *Terminalia mantaly*. They also observed that, the limit dose of 5000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. Similarly, Dodehe *et al*. (2012) investigated the acute and sub-acute toxic study of aqueous leaf extract of *Combretum Molle* in Wistar rats. They observed that the limit dose of 8000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. Also, Oyewo *et al*. (2012) investigated the effects of aqueous extract of *Citrullus lanatus* on the histology of the kidney of adult Wistar Rats. The aqueous extract of *Citrullus lanatus* was given once a day to the animals for 27 days of which there was no mortality recorded. The kidney administered leaf ethyl-acetate had slight hyperplasia, which is similar to the work of David *et al*. (2015) who studied the toxicological effects of aqueous and acetone extracts of *Combretum molle* twigs in Wistar rats. They observed hepatotoxicity and mesengial hyperplasia in the livers and kidneys. This is different from the result of Oyewo, *et al*. (2012) who did not record any histological distortion of the kidney after the administration of the extract. This could also be as a result of different concentrations used for the test. There were no histopathological alterations observed from the liver administered aqueous leaf and leaf n-butanol fractions. This is similar to the work of Zaza *et al*. (2016) investigated the oral toxicity of the X_{42} fractions of *Terminalia ivorensis*, observed no histological distortion of the liver.

IV. Conclusion

There was no mortality observed for all the leaf fractions tested on the Wistar rats. This finding suggests that, the fractions at the limit dose of 5000 mg/kg body weight tested are nontoxic and safe in oral formation. The histological investigation, however, showed certain alterations in the cellular anatomy of the liver and kidney. This alterations are biomarkers of toxicity and cellular damage to the liver and kidney. Caution therefore should be taken in the continuous intake of *C. molle* at high concentrations, since it may result to permanent damage of tissues.

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