Algerian Journal of Biosciences
ISSN: 2716-9375
Journal homepage: https://www.journal.acse.science/index.php/ajb/index

Original Article

Toxicological assessment of the aqueous leaves extract of *Combretum platypterum* (Welw) Hutch & Dalziel

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ARTICLE INFOR

Article history:
Received 28 July 2022
Revised 25 December 2022
Accepted 30 December 2022

keywords:
Combretum platypterum;
Leaf;
Toxicological;
Herbal medicine;
Safety.

ABSTRACT

Combretum platypterum belongs to the family of Combretaceae. The leaf is used to treat fever, conjunctivitis, febrifuge, coughs, sexually transmitted diseases, diarrhea, and as a tonic. Despite it being used in ethnomedicine to treat various sicknesses, the safety profile of the leaf extract has not been reported. This study was aimed at testing the acute and sub-acute toxicological assessments of the aqueous leaf extract of *Combretum platypterum*.The acute study was carried out using mice and rats. In sub-acute experiments, the animals received 0.5, 1, and 2.5 g/kg of the plant extract orally per day for 28 days. The first weight and the last weight were taken. The liver, spleen, kidney, heart, lungs, and stomach were obtained, weighed, and fixed. Blood was obtained for haematology and biochemical assays. The LD50 of the root extract was indeterminable since there was no death in the mice and rats used. The leaf extract significantly increases ($p<0.05$) the body weight at the dose of 2.5g/kg compared to control. The body weight index was not affected compared to control ($p>0.05$). At 0.5 and 1 g/kg, the extract significantly increased the level of monocytes ($p < 0.05$) compared to the control. haematological parameters were not affected. Upon bichemical analysis of the leaf extract, 2.5 g/kg significantly ($P<0.05$) increased the level of HDL and 0.5 and 1 g/kg significantly ($P<0.05$) increased the level of TRI compared to control. Other parameters were not affected. This study shows that aqueous root extract is safe.

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1. Introduction

Herbal medicine is a naturally occurring material derived from plants that is used to treat, prevent, and manage disease [11]. Plants have long been used in medicine, either as active principles or in traditional preparations. Medicinal plants differ from conventional drugs in that they have a larger therapeutic spectrum and can be used for long periods of time. They are more readily available and less expensive than synthetic medications [5, 11]. Herbal remedies that mend, fortify, and aid in destroying irritating germs with few or no adverse effects are becoming increasingly popular around the world [15]. The necessity to assess the safety of plants used in ethnomedicine has arisen as a result of the adoption of herbal medicine. Combretum platypterum is a member of the Combretaceae family. It can be found in rain forests, secondary forests, scrub savannas, and swampy areas. It is known as mmanyanza in Igbo, Ogan dudu in Yoruba, and 'ove', 'oven', or "ovben-' in Binis [1, 4]. The infusion of the leaves is used to cure fevers and as a tonic. To treat eye issues, including blindness, Combretum platypterum is powdered, mixed with palm oil, and licked [3]. Leaf decoction is used as a tonic and febrifuge in Lagos, while leaf sap is used as eye drops to cure conjunctivitis in the Central African Republic. o treat coughs in Gabon, leaf maceration is drunk or leaf pulp is dried,

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Peer review under responsibility of University of El Oued.
DOI : https://doi.org/ 10.57056/ajb.v3i2.62

combined with salt, and eaten [3]. In the Democratic Republic of Congo, a macerate of crushed leaves is heated in the sun and then sipped twice a day to treat sexually transmitted infections and helminthiasis. To control post-partum bleeding, apply leaf sap in hot water as a bath [13]. To treat diarrhea, the leaf or proof powder is added to food or an infusion is drunk. The young leaves of Seierra leon are sometimes used in soups. Children suck flowers, and sunbirds visit the flowers to drink the nectar. The flowers and fruits are a brilliant red, and the shrub is well worth cultivating. Despite its use in ethnomedicine to treat a variety of ailments, the leaf extract's safety profile has not been published. The goal of this study was to evaluate the toxicological profile of Combretum platypterum aqueous leaf extract.

2. Materials and Methods

2.1. Collection of Plant Material

Fresh leaves of Combretum platypterum were collected between January to June, 2016 from Igbanke, Orhionmwon Local Government Area, Edo State, Nigeria. Dr. H. Akinnibosun of the Department of Plant and Biotechnology, Faculty of Life Sciences, University of Benin City, Edo State, Nigeria, identified and authenticated the plant. The plant was given the number UBHc063 and deposited in the herbarium of the University of Benin.

2.2. Preparation of Plant Material and Aqueous Extracts

Leaves were cleaned and air dried for two weeks at the University of Benin's Department of Plant and Biotechnology, Faculty of Life Sciences, Benin City. Using an impact mill, the leaves were ground into powder (200g). Pulverized leaves were macerated for 24 hours in distilled water (5L). The extracts were filtered and the filtrate concentrated over a water bath. Before usage, the concentrated extracts were placed in universal bottles that were labeled and kept at 4 °C [18].

2.3. Experimental Animals

Male and female albino rats weighing 100-220 g and mice weighing 20-35 g were obtained from a commercial farm in Benin City and maintained in the animal facility of the University of Benin's Department of Biochemistry. The mice were given a two-week acclimation period before being kept in a conventional laboratory environment with a 12-hour light/dark cycle. They were given ad libitum feedings of rodent pellets and water. To ensure hygiene and maximum comfort for the animals, the litter in the cages was changed three times a week. The animals were handled in accordance with laboratory animal standard protocols (National Institute of Health USA: Public Health Service policy on humane care and use of laboratory animals, 2002). Approval (LS20945) for the experimental protocols was obtained from the Ethics Committee of Faculty of Life Sciences, University of Benin.

2.4. Acute and sub-acute toxicity studies

2.4.1. Acute Toxicological Assessment using mice

Updated Miller and Tainter's graphical technique was used for the acute study [14, 17, 19, 20]. This experiment used five group of six mice, each weighing 20 to 30 grams. The first group was given distilled water (10ml/kg p. o.). Using an orogatsric tube, groups 2 to 5 were given leaf extract (1g/kg, 2g/kg, 6g/kg, and 8g/kg p. o.) For 72 hours, and then for 14 days, mice were monitored during these days for poisoning symptoms and death and to detect potentially lethal long-term doses.

2.4.2. Acute Toxicological Assessment using rats

The rats in this study were divided into five groups of six, each weighing between 150 and 200 g. Group 1 received distilled water (2ml/kg p. o.). Using an orogatsric tube, groups 2 to 5 were given leaf extract (1 g/kg, 2 g/kg, 6 g/kg, and 8 g/kg p. o.). For 72 hours, and then for 14 days where mice were observed during the treatment days for behavioral signs of toxicity and to find out a potentially lethal dose in the long term.

2.4.3. Sub-acute Toxicological Assessment using rats

The study utilized four groups of six rats of both sexes weighing 120 to 200 grams. Distilled water (2ml/kg p. o.) was given to Group 1. Groups 2–4 are given leaf extract (0.5mg/kg, 1g/kg, and 2.5mg/kg p. o.) via an orogastric tube for 28 days. On day 29, the animals were slaughtered using chloroform as an anesthetic. Blood was taken for haematology and biochemical testing. Tissue was extracted, weighed, and fixed in 10% formal saline for histological tissues (heart, liver, kidney, lungs, and spleen).

2.5. Haematology parameter

An automated equipment model PCE-2100, JAPAN, was utilized to examine the blood in EDTA containers for haematology.

2.6. Biochemical analysis

The blood was taken and placed in lithium heparin sample vials, which were centrifuged at 3000 revolutions per minute (rpm) to separate the plasma

into transparent, labeled bottles. The divided samples were kept at -20°C in a deep freezer until the assays were completed. The randox test kit was used to conduct the biochemical analysis. The amount of albumin in the blood was determined by measuring serum albumin [8]. The International Federation of Clinical Chemistry and Laboratory Medicine technique was used to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [22]. The Principle of Analysis of Alkaline Phosphatase technique was used to measure alkaline phosphate [21]. The Biuret method was used to determine total protein [6]. The Jendrassik Grof technique was used to examine bilirubin [7].

2.7. Histological assessment

Before being sectioned using a microtome, the fixed tissue specimens were dehydrated in an ascending series of alcohol, treated in xylene, and then embedded in paraffin wax. Hematoxylin and Eosin were used to stain tissue sections (5 μm thick) after being deparaffinised with xylene and hydrated in alcohol. A histopathologist who was unfamiliar with the experimental grouping and protocols examined all sections under a Leica® light microscope (Model DM500) at x100 magnification.

2.8. Statistical Analysis

The data was presented as a mean standard error (S. E. M.). One-way analysis of variance (ANOVA) was used to assess the data, followed by Tukey's post hoc test. GraphPad Prism V.6.01 was used for statistical analysis, and $p<0.05$ was considered significant.

3. Results and Discussion

In acute and sub-acute toxicity studies, no toxicity signs and lethal effects were noted on rats and mice.

The LD50 of the leaf extract is unknown because the mice and rats utilized did not die. Regular paw licking, grooming, increased motility, erratic movement, and relaxation are all symptoms of the leaf extracts (Table 1 and 2).

In sub-acute research, the leaves significantly increased ($p<0.05$) body weight at a dose of 2.5g/kg compared to the control after 28 days of daily extract administration (Table 3). Table 7 show that the extract of the leaves had no effect on the body weight index when compared to the control ($p>0.05$). At doses of 0.5 and 1g/kg, the leaf extract significantly enhanced monocyte levels ($p<0.05$) as compared to control. Other haematological variables were unaffected (Table 6). In a bichemical examination, the leaf extract at 2500 mg/kg considerably ($p<0.05$) increased HDL levels, and the 500 and 1000 mg/kg significantly ($p<0.05$) increased TRI levels compared to the control. Other variables were unaffected (Table 4 and 5). Histological effects of leaf extract on the heart, lungs, kidney, liver, and stomach are shown in Figures 1 to 24

Table1: Result of acute toxicological test of aqueous leaves extract of *Combretum platypterum* on mice.

Dose (g/kg)	Number of Deaths/ Number of mice	Mortality (%)	Symptoms
0	0/6	0	None
1	0/6	0	Circular movements and calmness
2	0/6	0	Increase locomotion and calmness
4	0/6	0	Increase locomotion and calmness
6	0/6	0	Increase locomotion for few minute calmness followed by irregular movements
8 licking	0/6	0	Increase locomotion activity regular paw and calmness

The animals were observed for 72hrs and then for additional 11 days after extract administration

Table 2: Result of acute toxicological test of aqueous leaves extract of *Combretum platypterum* on rats.

Dose (g/kg)	Number of Deaths/ Number of mice	Mortality (%)	Symptoms
0	0/6	0	None
1	0/6	0	Calmness
2	0/6	0	Calmness
4	0/6	0	Calmness
6	0/6	0	Grooming, increase locomotion activities and calmness
8	0/6	0	Grooming, increase locomotion activity, regular paw licking and calmness

The animals were observed for 72hrs and then for additional 11 days after extract administration

Table 3: Percentage weight change following 28 days daily oral administration of aqueous leaves extract of *Combretum platypterum*.

Dose(mg/kg)	weight change (%)						
	1	2	3	4	5	6	7
Control	18.18±7.92	15.38±7.93	15.58±6.18	17.00±5.29	17.81±4.93	21.40±7.47	7.42±6.41
500	1.66±2.09	15.38±7.93	6.92±3.93	20.46±4.99	13.82±5.80	28.36±6.06	28.44±6.53
1000	5.42±1.06	7.68±1.20	7.00±1.48	7.68±1.20	6.80±1.81	11.84±2.47	9.00±2.45
2500	20.06±5.51	23.50±5.78	27.82±1.48	28.06±3.29	36.12±2.30	40.12±5.06	45.34±5.62*

2.5g/kg of the extract affect the weight of the rat (*p<0.05) compared to control. Values are represented as Mean±SEM, n= 5.

Table 4: Biochemical indices following 28 days daily oral administration of aqueous leaf extract of *Combretum platypterum*

GROUP (mg/kg)	ALT (U/L)	AST (U/L)	HDL (mg/dl)	CHOL (mg/dl)	TRI (mg/dl)	T.P (g/dl)	ALB (g/dl)	D.B (mg/dl)
CON	43.60±3.39	44.36±2.65	52.20±2.56	63.49±25.80	48.58±13.35	5.73±0.42	2.676±0.16	0.046±0.02
0.5	48.00±2.03	75.96±14.1	54.78±1.40	66.33 ±11.2	100.9±13.2*	6.314±0.19	2.180±0.55	0.053±0.01
1	42.80±1.88	58.50±4.75	57.62±1.40	74.44±9.42	89.00±5.72*	5.678±0.75	2.856±0.12	0.072±0.02
2.5	46.00±2.07	46.98±4.84	65.29±5.12*	79.92±26.23	36.60±2.50	7.730±0.77	2.880±0.40	0.047±0.01

ALT: Alanin aminotransferase, AST: Aspartate aminotransferase, ALB: Albumin, T.P: Total protein. CHOL: Total cholesterol, HDL: High density lipoprotein, TRIG: Triglyceride Values were express as Mean± Standard error of mean, n = 5 per group.

Table 5: Biochemical indices following 28 days daily oral administration of aqueous leaf extract of *Combretum platypterum*

GROUP (mg/kg)	T.B (mg/dl)	ALP (UI/L)	Na^+ (mmole/l)	K^+ (mmole/l)	Cl^- (mmole/l)	$HCO_3^=$ (mmole/l)
CON	0.60±0.28	79.17±2.38	135.2±3.30	7.46±0.58	93.00±2.280	14.20±1.72
0.5	0.14±0.39	73.81±9.53	131.6±3.64	7.680±1.45	94.40±3.234	17.60±1.12
1	0.21±0.06	71.27±3.41	128.2±3.20	7.42±1.12	90.40±4.534	15.00±1.52
2.5	0.21±0.07	76.35±5.71	132.2±1.02	7.460±1.63	93.60±2.786	17.20±1.16

ALP: Alkaline phosphatase T.B: Total bilirubin, Na: Sodium, K: Potassium, Cl: Chloride HCO3: Bicarbonate. Values were express as Mean± Standard error of mean, n = 5 per group.

Table 6: Hematological indices following 28 days daily oral administration of aqueous leaf extract of *Combretum platypterum*.

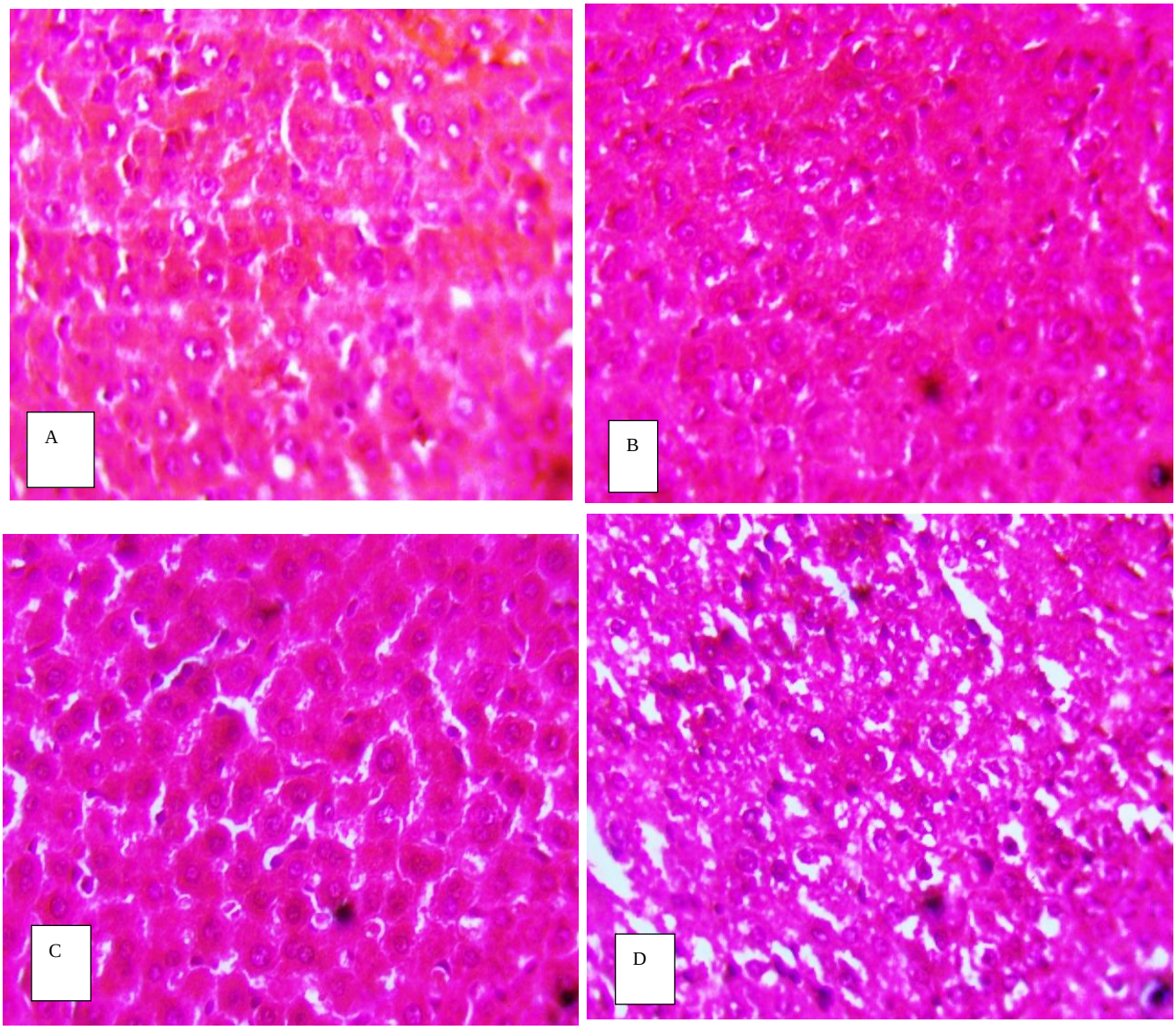
	WBC (x 10^3/µl)	LY (%)	MO (%)	GR (%)	PLT (x 10^{-3}/µl)	PCV (%)	Hb (g/dl)	RBC (x 10^3/µl)
Control	13.94± 1.34	57.98 ± 1.81	7.94 ± 0.34	35.70 ± 1.92	1115.0 ± 56.1	43.90 ± 1.41	16.24 ± 0.51	7.34±0.23
0.5 g/kg	9.38 ± 2.57	61.66 ± 6.83	12.64 ± 0.85**	25.70 ± 7.18	1023.0 ± 61.1	46.24 ± 1.97	13.42 ± 0.39	8.42±0.39
1g/kg	11.88 ± 3.70	50.88 ± 4.41	11.90 ± 1.11*	37.22 ± 5.31	1140.0 ±216.8	43.56 ± 2.08	16.74 ± 2.90	7.94±0.46
2.5 g/kg	10.30 ± 1.31	70.30 ± 2.42	7.20 ± 0.53	22.50 ± 2.06	1115.0 ± 229.2	41.86 ± 1.43	14.76 ± 0.59	6.91±0.34

The level of monocyte was significantly increase (**p<0.01, *p<0.05) at the dose of 0.5 and 1g/kg compared to control. WBC, white blood cell count; LY, lymphocytes; MO, monocytes; GR, granulocytes; PLT, platelets: PCV, packed cell volume (hematocrit); and Hb, hemoglobin. n = 5 per group.

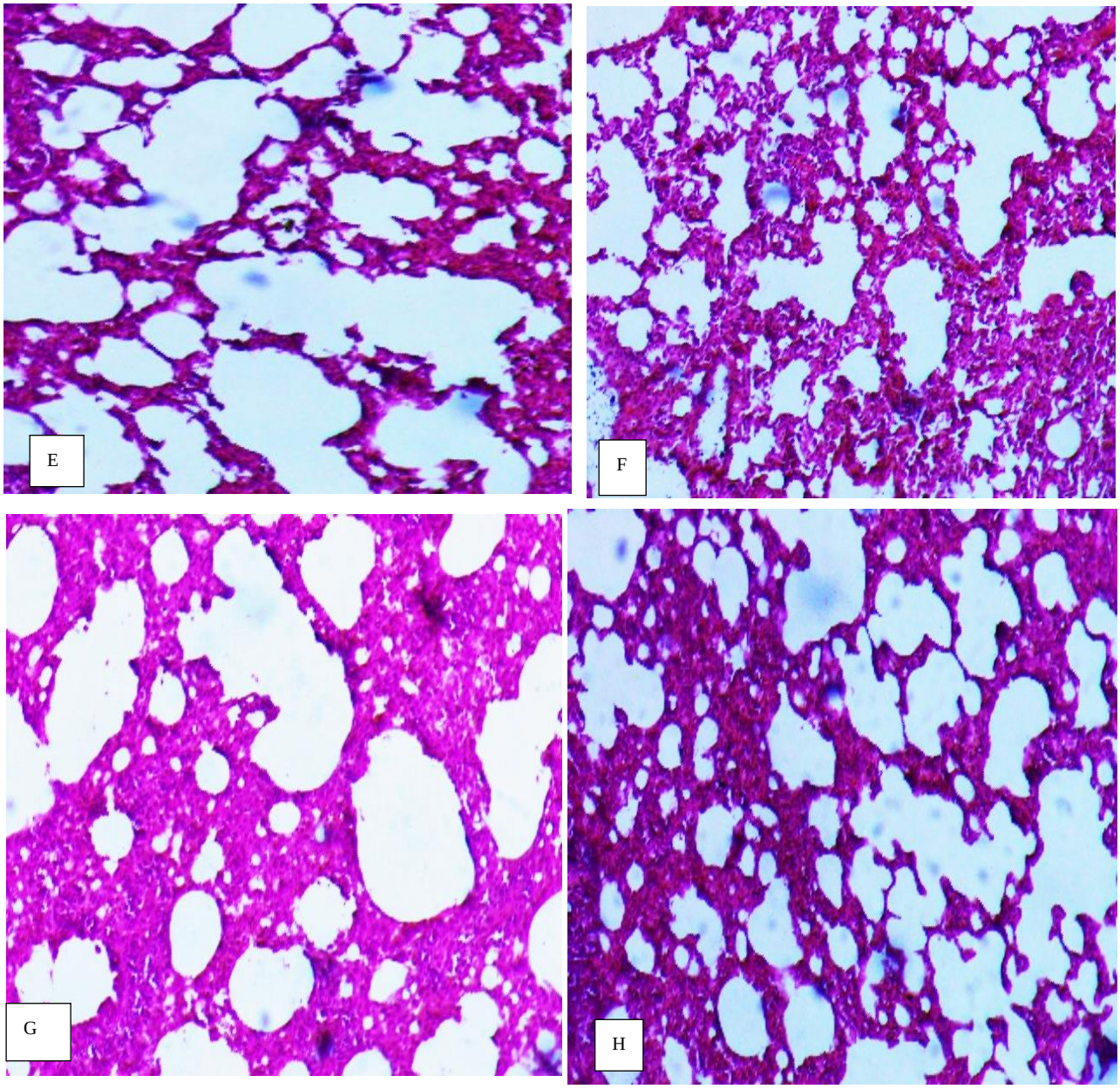
Table 7: Organ to body weight ratio following 28 days daily oral administration of aqueous leaves extract of *Combretum platypterum*.

Dose (g/kg)	L:BW (10^{-2})	S:BW (10^{-3})	Lu:BW (10^{-3})	K:BW (10^{-3})	H:BW (10^{-3})
Control	3.70±0.21	3.78±0.37	9.02±0.77	6.00±0.45	4.16±0.68
0.5	3.58±0.14	2.20±0.20	7.80±0.37	5.60±0.24	3.20±0.20
1	3.30±0.19	2.06±0.60	7.38±1.60	6.58±0.24	3.00±0.00
2.5	3.84±0.34	3.20±0.38	8.26±1.15	5.54±0.48	4.66±0.83

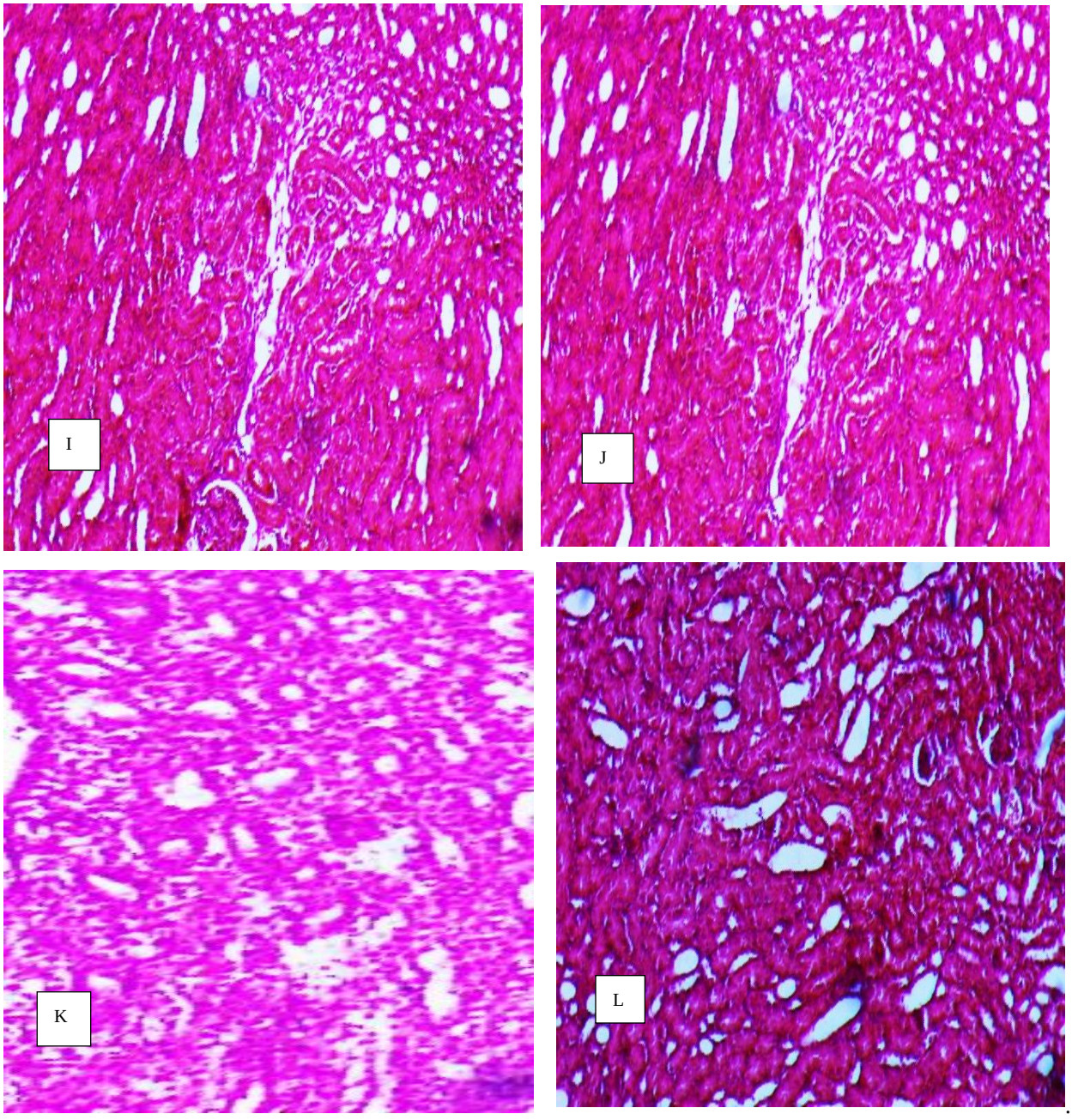
Organ-to-body weight ratios are not significantly different from controls ($p>0.05$). L: BW: Liver to Body weight ratio, S: BW: Spleen to Body weight ratio, Lu: BW: Lungs to Body weight ratio, K: BW: Kidney to Body weight ratio, H: BW: Heart to Body weight ratio. Values are represented as Mean±SEM, n = 5 per group.



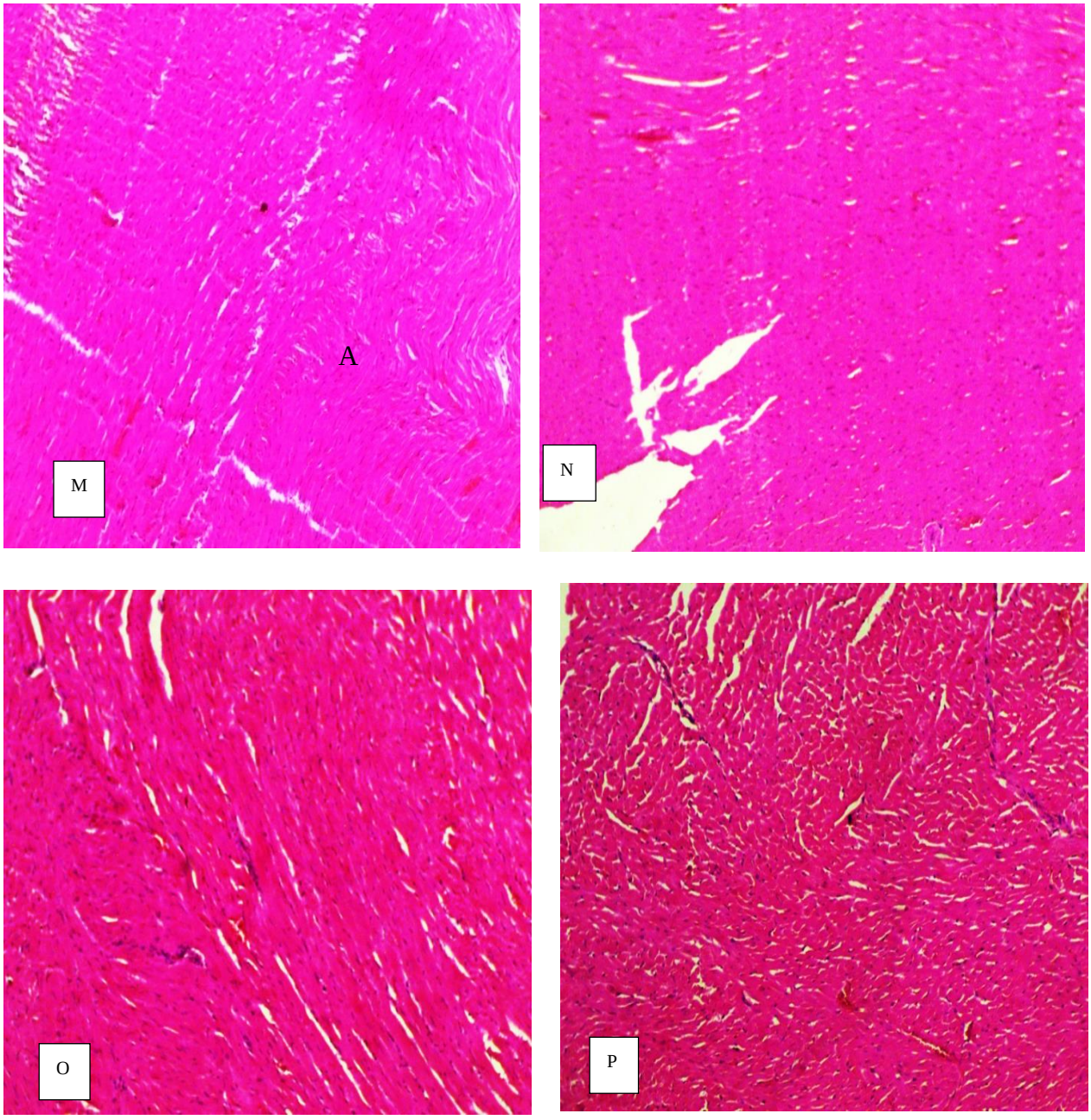
A. Control: Histological slide of the Liver showing normal hepatocyte plates
B. 0.5 g/kg leaf extract: Histological slide of the Liver showing bile plug within the bile canaliculi **A**
C. 1 g/kg leaf extract: Histological slide of the liver showing the bile canaliculi
D. 2.5 g/kg leaf extract: Histological slide of the liver showing hepatocyte necrosis H/E X400



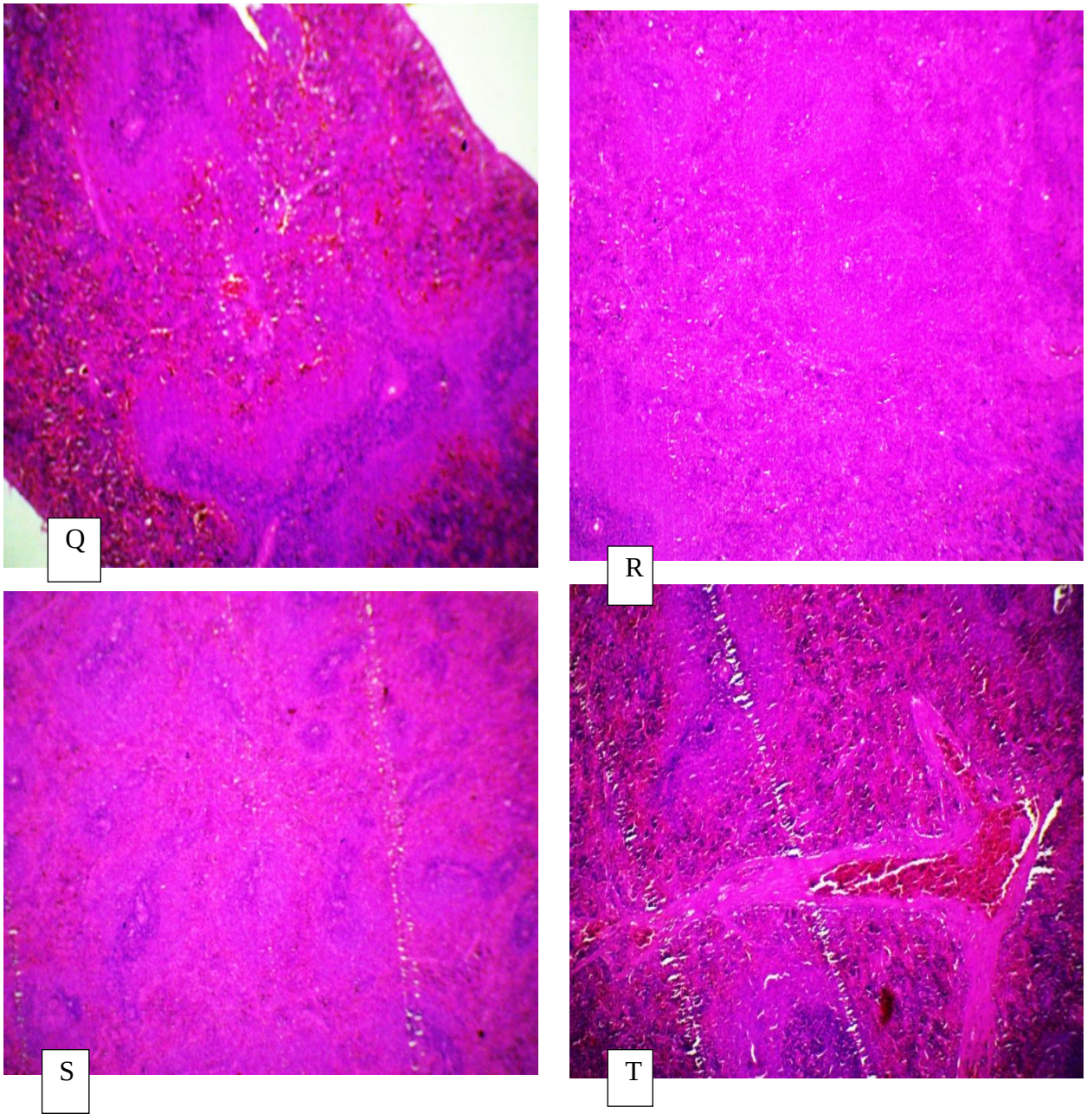
E. Control: Histological slide of the lungs showing normal lungs
F. 0.5 g/kg leaf extract: Histological slide of the lungs showing interstitial infiltration
G. 1 g/kg leaf extract:Histological slide of the lungs showing dense interstitial infiltration by mixed inflammatory cells with spill into air spaces
H. 2.5 g/kg leaf extract: Histological slide of the lungs showing interstitial inflammation with focal spill into the air spaces. H/E X100



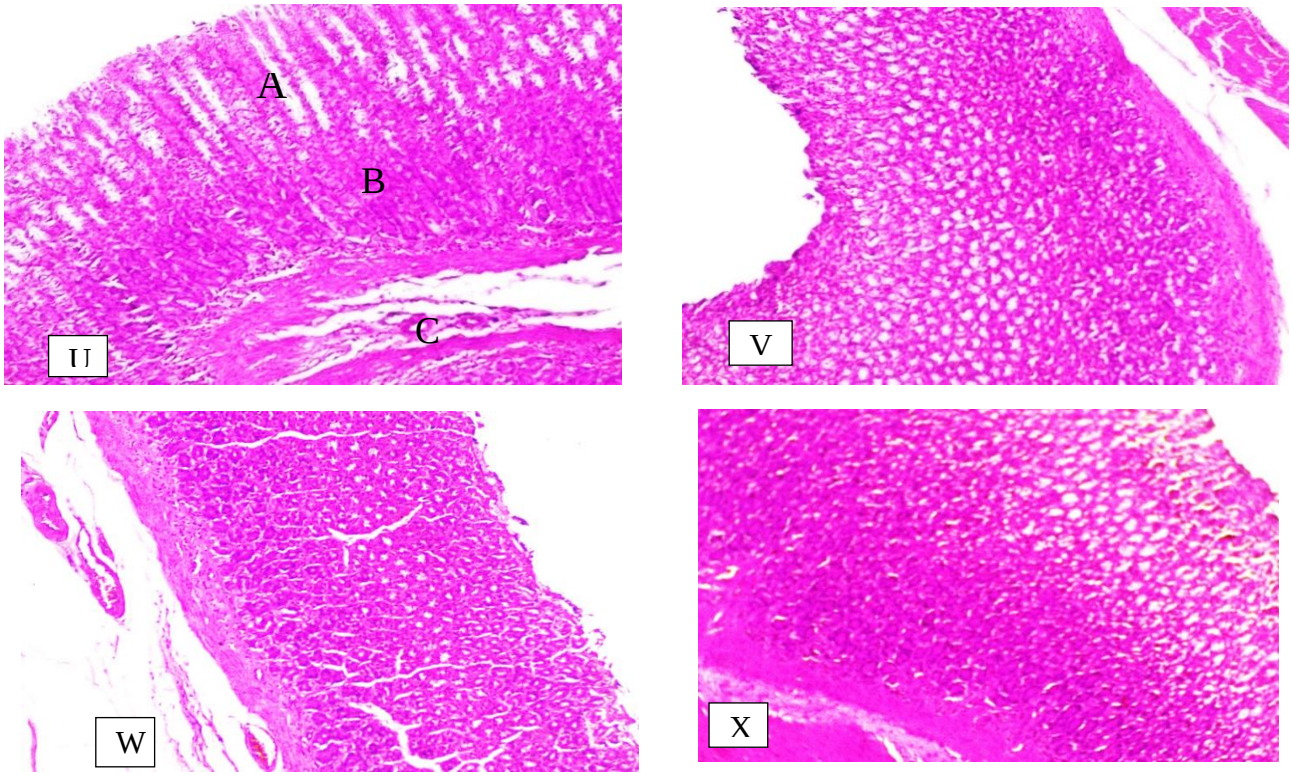
I. Control: Histological slide of the kidney showing normal kidney,
J. 0.5 g/kg leaf extract: Histological slide of the kidney showing normal tubules and glomeruli
K. 1 g/kg leaf extract:Histological slide of the Kidney showing normal tubules and glomeruli
L. 2.5 g/kg leaf extract: Histological slide of the Kidney showing normal tubules and glomeruli, H/E X100 , a- normal tubules, b- normal glomeruli



M. Control: Histological slide of the spleen showing normal red and white pulp
N. 0.5 g/kg leaf extract: Histological slide of the spleen showing normal red and white pulp
O. 1 g/kg leaf extract:Histological slide of the spleen showing normal red and white pulp
P. 2.5 g/kg leaf extract: Histological slide of the spleen showing a dilated and congested sinus. A- dilated and congested sinus. H/E X400



A. Control: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa, A- Mucosa, B- Muscular layer, C- Seros
B. 0.5 g/kg leaf extract: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa,
C. 1 g/kg leaf extract:Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa,
D. 2.5 g/kg leaf extract: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa. H/E X100



J. Control: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa, A- Mucosa, B- Muscular layer, C- Seros
K. 0.5 g/kg leaf extract: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa,
L. 1 g/kg leaf extract:Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa,
M. 2.5 g/kg leaf extract: Histological slide of the stomach showing a normal mucosa containing glands, muscular layer and serosa. H/E X100.

Discussion

Despite its flaws, the LD50 determination has remained a helpful tool in determining the safety of substances [20]. Acute testing of the aqueous leaf of *Conmbretum platypterum* revealed that the LD50 is unknown, as no death was observed in the mice and rats utilized (Table 1 and 2). The capacity of the tested drug to kill or decrease half of the tested population to half, or LD50, is defined as the ability of the tested substance to kill or reduce half of the tested population to half, or 50%. Because no death occurred after an oral dose of 8 g/kg of *Conmbretum platypterum* leaf extract was given to mice and rats, this shows that the leaf extract of *Conmbretum platypterum* is safe to take orally [20]. The use of significantly greater doses in toxicological tests gives an indication of the extract's safety margin [19]. Using the Hodye and Sterner scale for toxicity categorization of substances [9, 10] cited in Ozolua et al [20] the LD50 of *Conmbretum platypterum* leaf was greater than 5 g/kg, classifying it as a non-toxic sub-stance[20]. Regular paw licking, grooming, increased motility, erratic movement, and relaxation are toxicological symptoms of leaf extract (Table 1 and 2). In the lack of definitive LD50 values to guide subacute dosage selection, fractions of the maximum permissible limit of LD50 value (5 g/kg) have been utilized in subacute investigations [2, 20].

Following 28 days of daily administration of the extracts at a dose of 2.5 g/kg, the leaf extract increases body weight compared to control in a sub-acute study (Table 3). The body-to-weight ratio, on the other hand, was unaffected (Table 7). Blood parameter analysis is important for risk assessment because changes in the predictive value for human toxicity are important [16]. The leaf extract of *Conmbretum platypterum* had no effect on red blood cells (RBC), white blood cells (WBC), platelets (PLT), lymphocytes, or hemoglobin (Hgb) in this

investigation. The leaf extract increased monocyte levels when given at doses of 0.5 and 1g/kg (Table 6). ALT, AST, and ALP levels are routinely used to determine the extent of underlying cellular injury [12, 23]. Increases frequently indicate non-specific damage to internal organs such the liver, kidneys, and lungs. The levels of the enzymes AST, ALT, and ALP were not significantly affected by leaf extract of *Conmbretum platypterum* in this investigation (Table 4). At 2.5g/kg, the leaf extract had a considerable impact. raise HDL levels and dramatically increase TRI levels at doses of 0.5 and 1 g/kg The leaf extract had no effect on albumin, total protein, total cholesterol, total bilirubin, direct bilirubin, sodium, potassium, chloride, and bicarbonate (Table 4 and 5). Normal histology of the liver, kidney, stomach, heart, and spleen treated with the leaf extract was found to be repudiated. At a dose of 2.5g/kg of the leaf extract, the liver shows hepatocyte necrosis, bile plugs, and piecemeal necrosis. At doses of 0.5 and 1g/kg of leaf extract, interstitial infiltration was observed in the lungs. Interstitial inflammation with focused leak into the air passages in the lungs and dilated and clogged sinuses in the spleen are seen in 2.5 g/kg of leaves. The aqueous leaf extract was found to be safe based on acute, biochemical, and haematological results. However, a histopathological investigation suggests that when utilized for a prolonged period of time, vigilance is required.

Conclusion

This research suggests that aqueous leaf extract is safe to use. When utilized for a longer duration, however, prudence is advised. To confirm the degree of safety, additional toxicological tests beyond 28 days must be conducted and using other solvants.

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Recommended Citation

Dickson, O. U. &MacDonald, I. Toxicological assessment of the aqueous leaves extract of *Combretum platypterum* (Welw) Hutch & Dalziel. *Alger. j. biosciences.* 2022, 03(02):104-115.