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Acute and sub-acute oral toxicity studies of crude aqueous and ethanolic extracts of *Tapinanthus preussii* leaf in normal adult male wistar albino rats

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Abstract
Tapinanthus preussii **(**African mistletoe), is widely used in ethno medicine for the prevention and management of numerous diseases. Hence this study was aimed at evaluating the acute and sub-acute toxicological effects of its crude aqueous and ethanolic leaf extracts in Wistar albino rats. Acute toxicity tests were conducted in two phases by a single oral administration of both extracts at increasing dosages: 10, 100, 1000, 1600 2,900 and 5000 mg/kg body weight of the rats. This resulted in no clinical signs or symptoms of toxicity within 24h, 72h and 14 days revealing that both extracts were safe up to 5000 mg/kg. In the sub-acute toxicity study, the rats received increasing dosages of both aqueous and ethanolic extracts (200, 350 and 500 mg/kg body weight) p. o. daily for 28 days. The absolute bodyweight, liver function markers (serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), bilirubin, albumin and total proteins) and kidney function indices (urea, creatinine and electrolytes) were not significantly (p>0.05) different in all the extracts treated groups compared to the control. This indicates that both extracts were safe since they were neither hepatotoxic nor nephrotoxic. Histopathological studies revealed no lesions in the liver and kidney of extract treated groups further confirming the safety of both extracts. Therefore these results validate the safety of this medicinal plant use indigenously to alleviate various diseases, optimize health and useful in pharmacological studies.

Keywords: *Tapinanthus preussii*, African mistletoe, ethno medicine, hepatotoxicity, nephrotoxicity, acute and sub-acute toxicity

1. Introduction
There is growing global interest in ethno medicine research and therapeutic applications for home remedies as well as their commercial production which cannot be over emphasized. Developmental organizations like Heifer Project International (HPI) assist in commercial or background cultivation, preparation and storage of medicinal plants for wider efforts in promoting biodiversity and conservations (Pei *et al*., 2020) [1]. Developing countries like Nigeria have a thriving ethno medicinal industry and are gaining more recognition as demonstrated in their extensive phytochemical research programmes and trade in medicinal products annually (Adodo and Iwu, 2022) [2]. Herbal medicine aids the skin, lungs, liver, bowels, heart and kidneys to eliminate, as well as promote the proper functioning of these organs. Economic self-reliance has been greatly boosted in developing countries because plants are used in traditional medicine hence substituting drug importation.
Medicinal plants refers to one or more of its parts like the leaves, flowers, roots, stems, bark and fruits that contains a varied phytochemical substances like flavonoids, phenolic acids, flavonoids, alkaloids, terpenes, sterols, saponins and tannins used for therapeutic purposes or as precursors for new pharmaceutical chemicals (Kafaru, 1994; Cai *et al*., 2004) [3.4]. It is assumed that in humans they initiate, activate or catalyze some curative reactions (Farnsworth, 1989) [5], like broad spectrum antimicrobial agents, antioxidants, anti-inflammatory and anticarcinogens (Kalemba and Kunica, 2003) [6]. Phytochemists now scout for medicinal plants, isolate and characterize the active components aimed at synthesizing varied analogues with different biological effects or better actions for future drug development (Cline, 1985) [7].

Mistletoes are ever-green plant parasites that uses the haustoria to anchor and invade the internal tissues of several deciduous host trees like cocoa, coffee, custard, apple, guava, hevea, shea and citrus fruits all year round extracting water and minerals (Griggs, 1991)[8]. Chlorophyll is present in their leaves and used for photosynthesis like all green plants. The phytoconstituents present in these plants are host specific (especially alkaloids) with observed variations in the same species occurring on different hosts in the same locality. This variation in metabolites had been observed in *Agelanthus dodoneifolius* on eleven different hosts (Deeni and Sadiq, 2002) [9]. In Pharmacognosy the different host trees are as important as the mistletoe plant due to these differences in the phytochemical constituents (Burkill, 1995) [10] and reason why their use in the treatment of an ailment is usually host dependent (Ibrahim *et al*., 2009; Adesina *et al*., 2013) [11, 12].

Globally, over 1,500 mistletoe plants species are known (O'Neill and Rana, 2019) [13]. Mistletoe are parasitic on numerous host trees and refers to genera such as Viscum (60 species), Loranthus, Tapinanthus etc. *Loranthus* the largest genus of the family, is predominantly African referred to as African mistletoe with about 75 genera and over 900 species (Judd *et al*., 2002) [14]. It is assumed to have originated from Eastern Asia or on the Gondwanan land mass spreading south into the Malaysian and Australian regions (Barlow, 1990) [15]. The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites (Wilson and Calvin 2006) [16]. Angiosperms and gymnosperms are host to most Loranthacean mistletoes which are commonly tropical (Dembele *et al*., 1994) [17]. *Phragmanthera* Tieghem, *Tapinanthus* [Blume] Reichb, *Loranthus* L., *Globimetula* Tieghem, *Agelanthus* Tieghem *and Englerina* Tieghem are the Six major genera found in Nigeria. *Tapinanthus* is far more widespread in the Nigerian Savanna (Omolaja and Gamaye, 1998) [18]. The taxa infests many wild and domesticated tree and shrub species of ethnobotanical and economic value, causing various degrees of structural and economic damage (Bako *et al*., 2001) [19]. The African mistletoes have a characteristic regional distribution e.g. Loranthus *bengwensis* Linn. (Northern Nigerian species), *Tapinanthus olefolius and Tapinanthus vittatus* (Southern African species), *Loranthus micranthus* Linn. (Eastern Nigerian species) and *Erianthemum ulugurense* (Kenyan species). *Tapinanthus Globiferus* (*A. Rich) Tieghem*, *Tapinanthus dodoneifolius (DC.)* Danser are Savannah species while *Tapinanthus bengwensis* (Engl. and Krause) Danser are found in the Bamenda highlands, Cameroon. Other African mistletoes include the East African mistletoes such as *Viscum engleri*, *Viscuin Fischeri* and *Phragmanthera dschallensi* (Wiens and Barlow, 1975; Gill, 1973), *Tapinanthus globferus (A. Rich.) Danser and Tapinanthus ophioides (Sprague) Danser* found in Burkina Faso and parasitic on shea trees (Boussim *et al*., 1991) [20].

Tapinanthus preussii aqueous and ethanolic crude leaf extracts are commonly used indigenously as traditional remedies for the treatment and management of varied disorders like hypertension, diabetes mellitus, chronic cramp, epilepsy, headache, menopausal symptoms, infertility, arthritis, rheumatism, convulsive distemper, haemorrhages, contraceptive (berries), anticancer, nervous system disorders, stroke, indigestion, palpitation of the heart, breathing difficulties, hot flushes, as well as an antispasmodic, emetic, narcotic tonic or nervine without adequate information on its oral toxicity profile. Hence, this present study aims at investigating the acute and sub-acute toxicity effects of both the aqueous and ethanolic crude leaf extracts of *T. preussii*, in adult male wistar albino rats.

2. Materials and Methods

2.1 Plant material collection, authentication and extraction

Tapinanthus preussii leaves parasitic on *Theobroma cacao* (cocoa plant) were harvested from a private cocoa plantation in Kumba, South west Region of Cameroon. Taxonomic identification and authentication were carried out by a Botanist named Litonga N. Elias in the Limbe Botanic Garden, South West Region of Cameroon. A voucher specimen SCA 5533 of the plant was deposited in the herbarium of the same department for future reference.

The harvested leaves were washed thoroughly with clean water to remove debris, air dried in the shade for four days at room temperature and further oven dried at 40 ºC for a constant weight, and then powdered using a blender. The aqueous and ethanolic extracts were obtained by weighing about 250g into two portions of the powdered leaves, cold macerated in 1300 ml of distilled water and 95% ethanol respectively. The mixtures were intermittently shaken through a 72 hour period then the supernatants filtered using a sieve and then cotton wool (Sofowara, 1993; Williams and Omoh, 1996; Harbourne, 1998) [21, 22, 23]. The aqueous and ethanolic crude extracts were lyophilized to obtain a constant weight, then eventually recovered and reconstituted to appropriate concentrations before administration orally via gavage to the experimental animals.

2.2 Experimental Animals

Adult male Wistar albino rats weighing between 200-250 g obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, University of Benin were used for this study. They were acclimatized under animal house conditions for two weeks at room temperature (25-28 ºC), with naturally illuminated conditions of 12:12 hours light and dark cycle and allowed free access to water and standard rat feed obtained from Bendel Feed Flour Mill, Ewu, Benin City, Edo State. All the rats were fasted for 12hrs prior to commencement of all the experiments.

2.3 Acute toxicity (LD_{50}) studies in normal rats

Oral acute toxicity studies were conducted in two phases using the method of Lorke (1983) [24] as follows: In phase one, eighteen (18) normal adult male Wistar albino rats were divided into six groups of three animals each. Groups 1, 2 and 3 were orally administered 10, 100 and 1000 mg of *T. preussii* crude aqueous extracts per kg body weight of the rats respectively. While groups 4, 5 and 6 were also orally administered 10, 100 and 1000 mg of *T. preussii* crude ethanolic extracts per kg body weight of the rats respectively. The rats were observed through 24hrs for clinical signs and symptoms of toxicity (changes in skin, hair, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, motor activity, convulsion, tremors, salivation, diarrhoea, lethargy, or sleep) and mortality according to the specifications of the OECD (2001) [25]. Based on the results obtained in this phase, further specific doses were administered in the second phase in order to determine the LD_{50}. In phase 2, six albino Wistar rats were divided into six groups of one rat

each. Groups 1, 2 and 3 were treated with 1,600, 2,900 and 5,000 mg of the aqueous extract per kg body weight of the rats respectively. While groups 4, 5 and 6 were also treated with 1,600, 2,900 and 5,000 mg of the ethanolic extract per kg body weight of the rats respectively. The animals were observed through 24hrs for any behavioural as well as neurological changes and symptoms of toxicity and mortality. They were further monitored for 14 days for the long-term possible lethal outcome before terminating the experiment.

2.4 Sub-acute toxicity studies in normal rats

Thirty five (35) normal male Wistar albino rats were distributed in to seven groups of 5 rats each and allotted seven different treatments through 28 days as follows: Group 1 rats served as normal control and were administered 1ml of normal saline, Groups 2, 3 and 4 were administered 200, 350 and 500 mg of crude aqueous extracts per kg body weight of the rats respectively. While groups 5, 6 and 7 were administered 200, 350 and 500 mg of crude ethanolic extracts per kg body weight of the rats respectively. During the study period the body weight of the animals were obtained on days 7, 14, 21 and 28. After 28 days the rats were fasted overnight (12hours) and anaesthetized in a container saturated with chloroform vapour. Blood samples were immediately taken from the heart after sacrifice and carefully placed in plain bottles to prevent hemolysis and allowed to clot at room temperature. The samples were centrifuged at 4000 rpm for 10 minutes and the serum collected and stored at -20 ^{o}c until required for further biochemical analysis.

2.5 Parameters investigated

2.5.1 Measurement of Percentage change in Body weight

The animals were weighed weekly and the percentage (%) change in body weight was calculated using the following equation:

Percentage change in Body weight = Wf-Wi/Wix100

Where W_f = final weight; W_i = initial weight.

2.5.2 Liver Function Test

Total Serum Protein was determined using the Biuret method as described by Henry *et al*., (1957) [26], serum albumin was measured using the method of Doumas and Biggs (1972) [27]; serum AST and ALT enzyme activities were estimated by the colorimetric method of Reitman and Frankel (1957) [28] using Randox diagnostic kits; Serum Alkaline Phosphatase (ALP) activity was estimated by an optimized standard method according to the Duetsche Gesellschaft fur Klinische Chemie (1972) [29]; serum conjugated and Total Bilirubin determination was based on the method of Mallory and Evelyn as modified by Meites and Cheng, (1982) [30];

2.5.3 Kidney Function Tests

Serum Urea was determined photometrically by the Berthelot's reaction (Weatherburn, 1967) [31]; Serum Creatinine was determined by the colorimetric method of Henry *et al*. (1974) [32]; Serum Potassium was determined by the method of Terri and Sesin, (1958) [33]; Serum Sodium concentration was determined by the method of Maruna (1958) [34]; Serum Chloride concentration was determined by a direct method based on a modification of the colorimetric method described by Skeggs and Hochestrasser (1964) [35] using Teco diagnostic kit.

2.5.4 Histopathological studies

The liver and kidney were excised after the animals were sacrificed, preserved in 10% formal saline and eventually processed for histopathological studies as described by Humason (1962) [36]. All tissues stained were examined microscopically using 100 times magnification and assessed using the following criteria: Nuclear and cytoplasm staining and changes if any in the histopathological architecture. Finally all the prepared tissue slides were photographed using Leica stereomicroscope for documentation of all findings.

2.6 Statistical Analysis

Data collected were subjected to statistical analysis using SPSS version 20. Analysis of variance (ANOVA) was also used to compare the means of some of the parameters measured and where significant differences were observed, at 95% confidence level, $p<0.05$ for all treatments carried out compared to the control, Duncan's New Multiple Range test (IBM Corp., 2013) [37] was used to separate the means.

3. Results

3.1 Acute toxicity (LD_{50}) Studies

In both phase 1 and phase 2 of this study, *Tapinanthus preussii* crude aqueous and ethanolic leaf extract treated rats, showed no overt alteration in their neurobehavioral patterns, clinical signs and symptoms of toxicity or mortality at all dose levels (10, 100, 1,000, 1,600, 2,900 and 5,000 mg of extract per kg body weight of the rats. The LD_{50} was found to be above 5,000 mg/kg body weight of the rats.

3.2 The effect of crude aqueous and ethanolic extracts of *Tapinanthus preussii* leaf on the body weight (g) of normal Wistar albino rats

The mean body weight changes of the rats upon treatment with increasing dosages (200, 350 and 500mg/kg body weight) of both the aqueous and ethanolic *Tapinanthus preussii* leaf extracts over a period of 28 days are represented in Table 1. It was generally observed that there was a statistically significant ($p<0.05$) increase with a positive effect of the extracts on the body weight in all the dosages of the aqueous as well as ethanolic extract treated rats when compared to the normal control. However various levels of increases were observed in the body weight of all the normal rats administered aqueous extract as well as ethanolic extract evidenced by their percentage body weight changes i.e. 13.2%, 10.3%, 19.3%, 9.9%, 7.6% and 8.7% respectively compared to the normal control (5.9%) with the highest body weight attained on day 28.

Table 1: Mean Body weight (g) Changes of normal albino rats treated with *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts

Treatment (p.o) mg/kg	Before treatment	Body weight (g)				Body weight Changes	% Body weight changes
	Day 0	Day 7	Day 14	Day 21	Day 28		
NS (1ml)	128.1±11.1^{a}	130.1±8.3^{a}	133.5±6.3^{a}	133.9±7.2^{a}	135.7±6.6^{ab}	7.6	5.9
AE 200	152.5±15.4^{b}	148.1±17.4^{b}	152.6±20.6^{b}	164.5±22.1^{ab}	172.7±20.4^{a}	20.2	13.2
AE 350	171.4±12.1^{b}	175.8±14.6^{a}	181.8±14.1^{a}	182.5±15.4^{a}	189.1±19.5^{a}	17.7	10.3
AE 500	132.4±9.4^{b}	133.7±6.1^{b}	134.6±6.1^{b}	139.7±8.1a^{b}	158.0±5.9^{a}	25.6	19.3
EE 200	174.4±11.0^{b}	176.0±14.3^{b}	177.5±12.9^{b}	179.5±15.5^{b}	191.6±16.4^{a}	17.2	9.9
EE 350	184.5±9.6^{b}	191.9±10.6^{a}	190.2±9.9^{a}	193.3±11.4^{a}	198.6±8.9^{a}	14.1	7.6
EE 500	184.4±11.1^{b}	187.2±10.8^{a}	190.1±12.3^{a}	192.3±14.0^{a}	200.4±17.3^{a}	16.0	8.7

Values= Mean ±Standard error of mean (SEM). NS = Normal saline, AE = Aqueous extract, EE = Ethanolic extract. Means of the same row followed by different lettered superscripts differ significantly (p<0.05).

3.3 Effects of *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts on Serum Liver Function parameters in normal rats

Table 2 shows a summary of the mean values of serum liver function parameters obtained after administration of aqueous and ethanolic crude leaf extracts of *Tapinanthus preussii*. Statistical evaluation of the serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities showed no significant differences (p>0.05) in all the extracts treated groups when compared with the normal control (normal saline treated group). The serum level of total protein, albumin, total bilirubin and conjugated bilirubin showed statistical similarities in all the extract treated groups compared to the normal control groups.

Table 2: Serum Liver function indices, of normal albino rats administered aqueous and ethanolic leaf extracts of *Tapinanthus preussii*

Treatment/ Dosage (p.o)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (mg/dl)	Albumin (mg/dl)	Total Bilirubin (µmol/l)	Conjugated Bilirubin (µmol/l)
NS (1ml)	99.40±24.0^{ab}	38.00±2.5^{bc}	125.76±6.0^{b}	8.02±0.5^{a}	3.47±0.7^{ab}	0.62±0.04^{ab}	0.32±0.02^{a}
AE 200	120.72±12.3^{a}	45.60±1.0^{bc}	125.06±2.7^{b}	7.89±0.6^{a}	4.13±0.3^{ab}	0.62±0.05^{ab}	0.36±0.02^{a}
AE 350	99.00±15.8^{ab}	62.0 ±6.7^{ab}	119.65±12.1^{b}	7.46±0.7^{a}	3.99±0.3^{ab}	0.50±0.09^{b}	0.34±0.04^{a}
AE 500	118.60±18.5^{a}	37.20±3.1^{bc}	119.62±6.5^{b}	8.33±0.8^{a}	5.12±0.2^{a}	0.66±0.02^{a}	0.36±0.02^{a}
EE 200	125.20±24.4^{a}	41.20±4.4^{bc}	117.96±4.9^{b}	6.88±0.1^{ab}	4.02±0.3^{ab}	0.66±0.02^{a}	0.36±0.02^{a}
EE 350	84.40±5.2^{ab}	51.40±6.2^{ab}	113.66±2.2^{b}	8.22±1.2^{a}	4.72±0.6^{ab}	0.60±0.05^{ab}	0.36±0.02^{a}
EE 500	80.20±8.9^{ab}	44.80±3.7^{bc}	142.83±2.3^{ab}	7.87±0.9^{a}	4.45±0.6^{ab}	0.70±0.01^{a}	0.28±0.02^{a}

Values= Mean ±Standard error of mean (SEM); n=5; NS = Normal saline, AE = Aqueous extract, EE = Ethanolic extract. Means of the same column followed by different lettered superscripts differ significantly (p<0.05).

3.4 Effects of crude aqueous and ethanolic leaf extracts of *Tapinanthus preussii* on some Serum Kidney Function indices in normal albino rats

The effect of crude aqueous and ethanolic leaf *Tapinanthus preussii* extracts on serum kidney function indices in normal albino rats was investigated and the results presented below on Table 3. The kidney function status was assessed by estimating the serum urea, creatinine and electrolytes (Na^{+}, Cl^{-}, K^{+}, Ca^{2+} and HCO_3^{-}) levels. It was generally observed that there were no significant differences (p>0.05) in of all these listed indices when compared to the normal control.

Table 3: Serum Kidney function indices in Normal albino Rats administered crude leaf extracts of *Tapinanthus preussii*

Treatment/Dosage (p.o)	Creatinine (mg/dl)	Urea (mg/dl)	Sodium (mmol/l)	Chloride (mmol/l)	Potassium (mmol/l)	Bicarbonate (mmol/l)	Calcium (mmol/l)
NS (1ml)	0.77±0.05^{ab}	35.15±3.5^{a}	148.60±3.0^{a}	107.0±1.5^{ab}	6.38±1.2^{a}	18.0±1.3^{a}	4.72±0.1^{a}
AE 200	0.84±0.03^{a}	32.48±4.8^{a}	142.20±1.7^{a}	107.60±1.7^{ab}	6.36±1.4^{a}	16.40±2.1^{a}	5.22±0.2^{a}
AE 350	0.66±0.05^{b}	32.54±1.5^{a}	147.20±3.4^{a}	108.00±1.7^{ab}	5.76±1.6^{a}	16.60±1.9^{a}	5.70±0.7^{a}
AE 500	0.84±0.04^{a}	31.52±3.2^{a}	143.60±0.9^{a}	106.00±0.6^{ab}	5.50±0.8^{a}	17.80±1.4^{a}	5.58±0.9^{a}
EE 200	0.68±0.09^{ab}	33.72±2.8^{a}	147.40±3.0^{a}	107.80±1.1^{ab}	4.30±0.2^{a}	19.20±0.7^{a}	5.28±0.5^{a}
EE 350	0.80±0.03^{ab}	26.97±3.2^{a}	147.20±2.0^{a}	109.80±0.7^{a}	4.34±0.3^{a}	17.40±1.4^{a}	5.48±0.7^{a}
EE 500	0.74±0.06^{ab}	36.37±1.6^{a}	148.60±3.8^{a}	104.20±1.4^{ab}	7.20±1.4^{a}	16.80±1.1^{a}	6.02±0.6^{a}

Values represent mean ±standard error of mean (SEM); NS = Normal saline, AE = Aqueous extract, EE = Ethanolic extract. Means of the same column followed by different lettered superscripts differ significantly (p<0.05). Means of the same column followed by the same lettered superscripts are similar or differ insignificantly (p>0.05).

3.5 Histopathological evaluation of some organs in normal Wistar albino rats administered *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts

The effects of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts on the liver and kidney were examined microscopically. The results are shown in Plates 1 and 2.

3.5.1 Effects of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts on the liver of normal Wistar albino rats.

Plate 1 shows the photomicrograph sections of liver tissues of normal albino rats treated with aqueous and ethanolic crude leaf extracts of *Tapinanthus preussii*. It was generally observed that the section of the liver in all the extract treated as well as the normal control (administered normal saline) groups showed normal architecture composed of portal tract, central vein and hepatocytes separated by sinusoids. There was however no evidence of any vacuolation or degeneration.

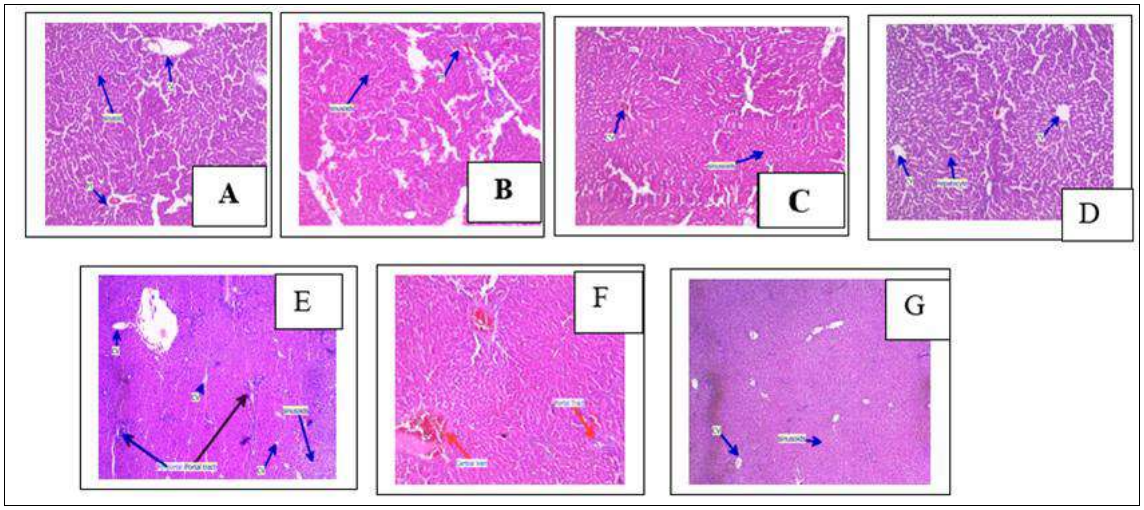

Plate 1: Photomicrograph of liver sections in normal Wistar albino rats treated with aqueous and ethanolic crude extracts of *Tapinanthus preussii* leaves (H&E X 100).

A. Normal control group. The microanatomy section of the liver showing normal architecture composed of a regular central vein (CV), portal tracts (PT) and plates of hepatocytes that are separated by sinusoids. B. 200 mg/kg b.wt aqueous extract group. Section of the liver appears essentially normal with regular central vein (CV) and sinusoids. C. 350 mg/kg aqueous extract group. Section of the liver appears essentially normal with regular portal tracts (PT) and plates of hepatocytes separated by sinusoids. D. 500 mg/kg b. wt. aqueous extract group. The microanatomy section shows normal liver in which there is a regular central vein (CV) and plates of hepatocytes that are separated by sinusoids. E. 200 mg/kg b. wt. ethanolic extract group. Section shows normal liver with regular central vein (CV), portal tracts and plates of hepatocytes separated by sinusoids. F. 350 mg/kg b. wt. ethanolic extract group. Section of the liver appears essentially normal with a regular central vein (CV) and portal tracts (PT). G. 500 mg/kg b. wt. ethanol extract group. Section shows normal liver with regular central vein (CV), portal tracts and plates of hepatocytes separated by sinusoids.

3.5.2 Effects of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts on the kidney of normal Wistar albino rats.

Plates 2 shows the photomicrograph sections of renal tissues of normal albino rats treated with *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts. It was generally observed that the section of the renal tissues in all the extract treated as well as the normal control (administered normal saline) groups showed normal architecture composed of normal glomerulus, bowman's space, tubules and interstitium. There was however no evidence of degeneration, inflammatory cells, necrosis or malignancy.

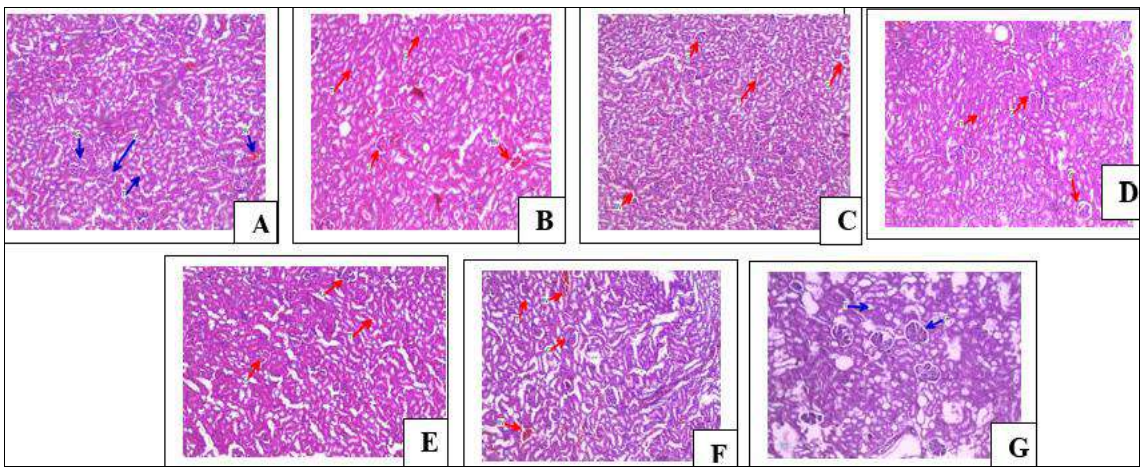

Plate 2: Photomicrograph of kidney sections in normal Wistar albino rats treated with aqueous and ethanolic crude extracts of *Tapinanthus preussii* leaves (H&E X 100).

A. Normal control group. The microanatomy section is showing renal tissue whose architecture is maintained. The glomeruli (G), interstitium, blood vessels (BV) and tubules (T) that are lined by regular cuboidal epithelium are seen. B. 200 mg/kg b.wt aqueous extract group. Section of the renal tissue composed of the cortex and the medulla is essentially normal and similar to the control. C. 350 mg/kg aqueous extract group. Section of the renal tissue composed of the cortex and the medulla. The glomeruli (G), interstitial spaces, tubules (T) and blood vessels (BV) are all normal with no malignancy detected. D. 500 mg/kg b. wt. aqueous extract group. The microanatomy section shows normal renal tissues like the glomeruli (G), interstitial spaces and tubules (T). E. 200 mg/kg b. wt. ethanolic extract group. Section shows normal renal tissues similar to the control. F. 350mg/kg b. wt. ethanolic extract group. Section of the renal tissues appears essentially normal with glomeruli (G), interstitial spaces and tubules (T). G. 500 mg/kg b. wt. ethanol extract group. Section shows normal renal tissues similar to the control.

4. Discussion

For many years herbal products for medicinal benefits have played a vital role in nearly every culture on earth. Njoya *et al*. (2018) [38] scientifically justified that water and locally distilled alcohol were good solvents in the extraction of crude leaf extracts of *Tapinanthus preussii* (African mistletoe) evidenced by the presence of clinically relevant phytoconstituents (phenolics, flavonoids, alkaloids, tannins, carotenoids, cardiac glycosides, anthraquinones and saponins), inorganic minerals (Fe^{2+}, Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, and PO_4^{2-}) and proximate components (carbohydrates, lipids and proteins) that are nutritionally essential in varied significant concentrations. The occurrence of these numerous phytoconstituents may be related to the use of this species of mistletoe especially in folkloric medicine.

The first step in ascertaining the safety profile of an unknown plant extract is acute toxicity studies (Lorke, 1983) [24] and its index is LD_{50}. Consequently, it is best recognized as providing, a ballpark estimate of human lethality (Zbinden and Flury, 1981) [39], seeks to determine its possible collateral effect and prevent acute intoxication due to overdose of any substance which may interfere with the results of experiments. The current results of the oral acute toxicity in the albino rats used established that the LD_{50} of both the aqueous and ethanolic crude leaf extracts of *T. preussii* was beyond 5000mg/kg body weight of the rats since the experimental rats tolerated the extracts without any symptoms of acute toxicity e.g. no mortality, physical skin and fur, eyes and mucous membrane (nasal) changes, aggressiveness, diarrhea, restiveness, seizures, dizziness, weakness or withdrawal from either food or water. Thus the respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) were normal. The doses were safe enough to give 100% survivors of the animals without any changes in the behavioural, neurological and anatomical response for two weeks implying that these extracts were practically non-toxic and regarded as being safe.

United States Environmental Protection Agency's 4-category hazard classification (Gadaleta *et al*., 2019) [40]: Category I ($LD_{50} \leq 50$ mg/kg) is the highest toxicity category. Category II (moderately toxic) includes chemicals with $50 < LD_{50} \leq 500$ mg/kg. Category III (slightly toxic) includes chemicals with $500 < LD_{50} \leq 5000$ mg/kg. Safe chemicals (LD50 > 5000 mg/kg) are included in Category IV. Thus, the lower the LD_{50} the more toxic the extract tested whereas, LD_{50} beyond 5000mg/kg is of no experimental significance (Lorke, 1983) [24]. Since the LD_{50} of both the aqueous and ethanolic crude leaf extracts of *T. preussii* was found to be above 5,000 mg/kg body weight of the rats; therefore the extracts are suggested to be safe for both consumption as well as pharmacological studies.

The liver is uniquely responsible for the interconversions of diet derived metabolic precursors, excretion of toxic substances from the systemic circulation and the hepatic portal blood (Tahmasebi *et al*., 2018) [41]. Many so called liver function tests are in fact reflecting damage to hepatocytes or biliary epithelial cells rather than their function. Thus a combination of the levels of the enzymes: serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin (total and conjugated) and the proteins are regularly analyzed to ascertain the effectiveness of the liver (Towseef' *et al*., 2019) [42]. In this study, the results (Table 1), showed no significant changes ($p>0.05$) in the serum levels of AST, ALT and ALP in all the extracts treated groups relative to the normal control. The enzymes studied are liver markers whose serum concentrations above the homeostatic limits could be associated with cirrhosis, lesions or other damages to the liver cellular integrity by agents considered unsafe. The results of this investigation suggested that the indigenous use of both the aqueous and ethanolic extracts of *Tapinanthus preussii* in the management or prevention of any pathologic conditions in some parts of the world especially West and Central Africa may be considered safe. This is against the back drop of the results obtained where no generalized significant elevations of those enzymes in the serum were established since it did not compromise the integrity of the liver at the dosages used. Therefore, it could be concluded that both the aqueous and ethanolic crude leaf extracts of *Tapinanthus preussii* at all the dosages used probably did not contain toxic chemical substances capable of causing severe lesions to the liver of normal experimental rats. The estimation of the levels of total protein, albumin, total bilirubin and conjugated bilirubin is used to determine the synthetic and excretory function respectively of the liver hepatocytes (Towseef' *et al*., 2019) [42]. In this study there were no significant changes in the total protein, total bilirubin and conjugated bilirubin levels in all the treatment groups compared to the normal control. These observed results may imply that the crude aqueous and ethanolic leaf extracts of *Tapinanthus preussii* did not interfere with the excretory and synthetic functional status of the liver due to the absence of toxic substances which otherwise would have resulted in either high or low levels of the parameters assayed.

Renal function parameters are usually required to assess the normal functioning capacity of different parts of the nephron (Ogbe *et al*., 2020) [43]. No significant changes were observed in the serum urea and creatinine level of all the extract treated groups when compared to the normal control suggesting that the extracts were not toxic to the kidneys evidenced by the sustenance of good renal clearance. Both extracellular and intracellular fluids contain large quantities of inorganic electrolytes which can readily dissociate into

their constituent ions or radicals, capable of compromising their transfer across the biological membrane (Ogbe *et al*., 2020) [43]. Serum electrolyte values were not significantly different from the extract treated rats relative to the control group which implies that it did not lead to nephrotoxicity.

The sub-acute effect of administering varied dosages of crude aqueous and ethanolic extracts *Tapinanthus preussii* leaf on the histoarchitecture of some vital organs like the liver and kidney in normal Wistar albino rats were evaluated. There was a normal histoarchitecture for all the control and extract treated groups with no abnormalities detected. This implies that *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts may not have led to impaired venous outflow in these tissues. The liver being the primary organ of detoxification and distribution of drugs, it is usually the first organ that encounters all absorbed materials from the gastrointestinal tract whereas the kidney is the major excretory organ. Both organs could be assessed to establish the safety of a substance (Gupta *et al*., 1994) [44]. Therefore, the liver and kidney are bound to manifest toxicological injury in multiple ways like cellular degeneration, necrosis and fibrosis as well as bile duct hyperplasia (Ogbe *et al*., 2020) [43]. The preserved architecture of the liver and kidney in normal control as well as the extract treated rats explains its protective detoxification property. Hence no hepatocyte morphological lesion, micro-vesicular steatosis, tubular necrosis and glomerular degeneration were seen. This is therefore an indication that the extract was not toxic to the hepatocytes and nephrons or did not cause liver inflammation or kidney dysfunction at the doses and duration utilized in this study.

5. Conclusion

The findings of this study show that both the aqueous and ethanolic crude extract of *T. preussii* leaf may not contain any toxic phytoconstituents to cause adverse effects in wistar albino rats. Therefore, this validates the safety and relevant use of this plant extract in traditional medicine for the prevention and treatment of diseases in humans.

6. Recommendations

Further studies may be conducted to isolate, characterize the bioactive phytochemicals responsible for the pharmacological activities of this plant extracts and to investigate its mechanism of action in the respective disease conditions where it is efficacious.

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8. References

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