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Evaluation of the safety profile of ethanolic leaf extracts of *B. ferruginea* and *L. uniflorus* on *Plasmodium berghei* infected albino rats

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Abstract
Introduction: Nigeria is blessed with dense vegetation and a myriad of medicinal plants which can be harnessed for the treatment of various ailments. There has been evidence of organ toxicity following prolonged intake of plant-derived drugs, hence this study evaluated the safety profile of ethanolic leaf extracts of *B. ferruginea* and *L. uniflorus*, used for the management of malaria.
Methodology: In phase 1 of acute toxicity, 12 male Wistar rats were divided into 3 groups of 4 rats each, treated with 10, 100 and 1000 mg/kg b. wt of the extracts and observed for the first 4 hours, then 7 days for signs of toxicity. In phase 2, higher doses of 1600, 2900 and 5000 mg/kg b. wt of both extracts were administered to another 3 groups of 4 fresh rats and monitored as in phase 1. In sub-acute toxicity, 28 male Wistar rats were divided into 7 groups of 4 rats each and treated for 14 days. Hematological parameters analyzed include hemoglobin, packed cell volume, mean corpuscular heamoglobin, red blood cell count, white blood cell counts, neutrophils, and lymphocytes. Liver function enzymes- alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and other biochemical parameters were accessed while liver sections were examined.
Results: No behavioural changes and mortality were recorded after administration of the stated doses. There were no significant ($p< 0.05$) differences between the serum biochemical and hematological parameters of treated and control animals. Relative organ weights (ROW) of treated animals did not differ significantly ($p< 0.05$) from control group and there were no remarkable histological changes in the liver sections of treated animals compared with control.
Conclusion: Since the extracts did not induce any chronic toxicity on the experimental animals, this study therefore justified the continuous use of these plants in the management of malaria.

Keywords: *Bridelia ferruginea*, *Lycopus uniflorus*, acute and sub-acute toxicity, safety evaluation, African medicinal plants

1. Introduction
Man is always in constant relationship with plants and his knowledge of plants and indegenous medicine dates back to as far as 1500 BC. Such knowledge were acquired from instincts, accidental discoveries, experiences or careful observations of the effects of particular plants on domestic animals and particular ailments, and thereafter passed from generation to another through tutelage, orally or other forms of communication (Sofowora, 2012) [30].
Plants have continued to be a source of medicine for several years and they play pivotal roles in rural health care delivery of more than 80% of the populace in under developed countries, particularly in remote areas (Enenebeaku *et al*., 2021b, Ezeigwe *et al*. 2020) [9, 11]. This is as a result of numerous phytochemical compounds present in these plants (Enenebeaku *et al*., 2022) [10]. The diverse nature of plants have contributed immensely to basic human needs such as clothing, food, feed and shelter, also as therapeutic remedies for man's well-being (Gurib-Fakim 2016) [14]. Many orthodox drugs owe their origin to plants, such as aspirin (*Salix alba*), quinine (*Cinchona officinalis*), digoxin (*Digitalis purpurea*) and morphine (*Papaver somniferum*) (Gurib-Fakim 2016) [14].
Owing to the increasing dependence on herbal remedies, using herbal medicines for the management of a variety of ailments has tremendously increased. Consequently, therapeutic plants could be referred to as nature's inestimable to man. Indegenous therapeutic plants and natural products are significant sources of new chemical substances with potential beneficial

values (Enenebeaku *et al*. 2021a) [8].

Traditional herbal medicine, equally known as 'Alternative or Complementary' medicine is an indigenous or local form of medicine (Ramos *et al*., 2015) [27]. It remains a crucial feature of our rich ancestral inheritance that has survived many generations. It involves the indigenous or local ways of preventing, diagnosing and managing ailments. Among the widely known traditional medicines is the Chinese traditional medicine (Li, 2020) [18], which accounts for about 40% of all health care delivery in China (Wu, 2005) [36]. Again, African Traditional Medicine, accounts for up to 80% of the African health care needs particularly for those in the rural areas (Gurib-Fakim, 2016) [14]. These lay credence to the global relevance, significance and rising recognition of traditional medicines as an important tool in addressing man's health care needs.

To accommodate the diversity of cultures and their indigenous mode and practices of traditional medicine, World Health Organization (WHO) has defined traditional medicine as the total sum of all the knowledge, skills, and practices which are based on theories, beliefs, experiences and testimonies which are indigenous to different cultures which could be explicable or not, used in maintaining the health, as well as in the preventing, diagnosing, improving or treating physical and mental ailments (WHO, 2005) [35]. Additionally, medicinal plants are defined as those plants from which herbal preparations are produced through subjection to extraction, fractionation, purification, concentration.

Although medicinal plants are usually regarded as safe, they may possibly not be entirely free from side effects or toxicity (Boukandou-Mounanga *et al*., 2015) [5]. Toxicity of these healing plants could vary with chemical composition of the particular plant. The toxicity of medicinal plants can occur from acute or chronic exposure to the extracts, even if the extracts have low toxicity. Exposure to plant extracts could also be mutagenic or carcinogenic (Ferreira-Machado *et al*., 2014) [13]. Although most medicinal plants are beneficial to mankind, some could pose a threat to the health of the users following potential harmful or side effects arising from improper usage, abuses, overdoses or toxic principles. Such could lead to cellular damages, acute toxicity or death of patients (Schultz *et al*., 2020) [28].

Usage of plants with medicinal values and herbal preparations may perhaps be connected to dysfunctions of vital organs such as liver and kidneys in humans. Studies have reported toxicity of some medicinal plants to different organs. Organ systems could be affected by toxic plants, while some plants with several toxic principles could affect multiple systems (Tamokou & Kuete, 2014) [33]. The medicinal plant *Aphania senegalensis* is hepatotoxic in rats (Fall *et al*., 2011) [12] while *Herniaria cinerea* is toxic for the gastrointestinal tract (Sokar *et al*., 2023) [31]. Although Nigerians and Africans widely patronize medicinal plants and herbal preparations, the toxicity profiles of these herbal medicines are largely unknown (Maroyi, 2017) [21].

Bridelia ferruginea (Benth) is a small scaly tree which grows to a height of about 15m (WAHP, 2013) [34]. The girth of the plant is up to 1.5m with twisted branches pointing downwards. The stem bark is darkish grey in colour, cracked, rough and slash thin with characteristic extended thin branches, sometimes with short spines. The leaves usually have slight wavy edges, small to medium, simple, alternate, spiral or distichous in arrangement. The fruits have a green pericarp when young, then blue-black colour at maturity (WAHP, 2013) [34]. This plant is widely used in traditional African medicine to treat a range of diseases. Studies have demonstrated that the plant has several properties, which justify its use in ethnomedicine. Akuodor *et al*., (2011) [2] studied the anti-inflamatory properties.

Arthritis, boils and diabetes, have been treated using *B. ferrugenia* extracts (Njamen *et al*., 2012) [23]. Dada and Akinyele, (2020) [6] reported ethanolic stem bark extracts' activity of this plant against *Salmonella typhi* justifying its traditional use in the treatment of enteric fever. In northern Nigeria, Abubakar *et al*., (2018) [1] reported its skin cancer proprties. Various pharmacological studies on different extracts of *B. ferruginea* justify usage of this herb for the management of diabetics in some West African Countries (Onyenibe & Udogadi, 2019) [24]. Reports have equally shown that *B. ferruginea* is rich in phytochemical compounds such as triterpenes, flavonoids, alkaloids, phenolics, cardiac glycosides, phytosterols, tannins and saponins (Abubakar *et al*., 2018) [1].

Lycopus uniflorus, known as bugleweed, is a plant which originated from Lebanon near freshwater wetland. In Lebanese folk medicine, it is used for the management of gastrointestinal disorders and inflammation. From available literature, the toxicity profiles of the leaf extracts of these medicinal plants have not been evaluated. Being common medicinal plants which are usually added to some traditional herbal mixtures, this present study is therefore aimed at evaluating the safety profile of ethanol extract of *B. ferruginea* and *L.uniflorus* leaves in albino rats. This will generate informed data on the level of safety of use of this medicinal plants in the management of malaria as well as other ailments.

Methods

Sample collection authentication of plant material

Fresh leaves of *B. ferruginea* and *Lycopus uniflorus* were collected from Avuvu Ikeduru, Imo State, located at latitude 50^0 35'N and longitude 70^o 12'E. The leaves were identified validated by a plant taxonomist and specimen with voucher numbers were deposited at the herbarium.

Sample preparation and extraction

The fresh leaves of *B. ferruginea* and *L. uniflorus* were air-dried for 2 weeks till a constant temperature (30 ± 0.5 °C). This was later crushed to powdered form using a mechanical blender (Kenwood-BL440A-UK). Preparation of ethanol extracts followed the description of Dejen *et al*. (2018) [7]. Using a soxhlet extractor, two hundred grams (200 g) of the crushed leaves were extracted with 1000 ml of 80% v/v ethanol. Extraction time was extended to 6 h to guarantee maximum extraction. Rotary evaporator was used to dry the filtrate and thereafter dried and stored in labeled bijou bottles which were kept at 4 °C prior to use.

Animal models

The 76 male Swiss wistar rats weighing 85-100 g were used for the study they were sourced from the Department of Veterinary medicine, University of Nigeria, Nsukka animal house. They were acclimatized under standard laboratory conditions of 12 h light/dark cycle, in properly aerated stainless cages for 14 days. Animals were given with free access to rat pellets and water *ad libitum*. Care and use of animals followed NIH guidelines and ethics for care and use of experimental animals.

Assessment of acute toxicity

Acute toxicity was evaluated by determining the LD_{50} of the extracts. The median lethal dose (dose at which 50% of experimental animals will die) of the crude ethanolic leaf extracts of *B. ferruginea* and *L. uniflorus* were carried out using modified method of Lorke (1983) [20] as described by Khan, Amupitan, Oyewale & Ndukwe (2015) [18], for the 2 phases, 24 male wistar rats were used. For both extracts, for the first phase, twelve albino rats were divided into 3 groups of 4 rats each (n=4) and were treated with10, 100 and 1000 mg/kg body weight of the extracts. Animals were observed for the first 4 hours, then for 7 days for signs like difficulty in respiration, loss of appetite, pains, weakness, paw licking, and death.

In phase 2, higher doses of 1600, 2900 and 5000 mg/kg b.wt of both extracts were administered to another 3 groups of 4 fresh rats through the same route. No sign of toxicity was observed as in phase 1.

Sub-acute toxicity study

The experimental animals were divided into 7 groups of 4 rats per group as follows:

Group 1 = Water + feed (normal control group).

Group 2 = No treatment (negative control).

Group 3 = 200 mg/kg b.wt of standard antimalarial drug (arthemeter and lumefantrine)

Group 4 = 200 mg/kg b.wt of ethanol leaf extract of *Bridelia ferruginea*

Group 5 = 1000 mg/kg b.wt of ethanol leaf extract of *Bridelia ferruginea*

Group 6 = 200mg/kg b.wt of ethanol leaf extract of *Lycopus uniflorus*

Group 7 = 1000 mg/kg b.wt of ethanol leaf extract of *Lycopus uniflorus*

Animals were treated once daily for 14 days. On the 15th day, animals were fasted overnight and were sacrificed and blood and liver samples were collected. Biochemical, haematological and histological parameters were accessed in all animals both treated and control groups.

Determination of relative organ weight

The liver, heart, and kidney of control and treated animals were collected, weighed and relative organ body weights (ROW) were calculated on the final day using the formula: Absolute organ weight (g) / Body weight of rats.

Evaluation of hematological parameters

EDTA bottles were used to collect blood samples for hematological parameters and analyzed using the hematological analyzer, Mindray Auto BC-5200, USA according to manufacturer's instructions. The analyzed parameters include hemoglobin (Hb), packed cell volume (PCV), red cell count (RBC), white cell count (WBC),average body hemoglobin(MCH), average body hemoglobin concentration (MCHC),neutrophils, and lymphocytes.

Evaluation of biochemical parameters

Blood samples were collected for biochemical parameters in simple bottles and centrifuged for 10 minutes at 3,000 rotations per minute (rpm), the resulting serum was analyzed with the help of a diagnostic kit from the University of London's Randox laboratory. The liver function enzymes alkaline phosphatase (ALP), aspartate aminotransferase, (AST) and alanine aminotransferase (ALT), were analysed using Yakubu *et al*. (2017) [37] method. Bilirubin, urea, serum protein, and albumin were also determined using Oyinloye *et al*. (2016)'s [26] method.

Histological examinations

From the sacrificed animals, liver samples were carefully excised and fixed in 4% formaldehyde. Afterwards, they were processed and embedded in paraffin wax. Samples of the tissue were prepared using the description of Slaoui & Fiette (2011) [29] and examined with an optical microscope. Tissue blocks were sectioned 5µm thick and Haematoxylin and Eosin (H & E) were used for staining before thorough examination.

Statistical analysis

Obtained data were analyzed using One Way Analysis of Variance (ANOVA) (SPSS version 20.0 software USA) and the results were presented as mean ± SEM. Duncan's multiple range test was used to distinguish between mean values at a significance level of $p< 0.05$.

Results

Acute toxicity test

No sign of behavioural change and death was observed in phase 1 of the test comprising 10, 100 and 1000 mg/kg b. wt of extracts. In the second phase, comprising higher doses of 1600, 2900, and 5000 mg/kg b. wt of extracts, there were also no changes in the behaviour of treated animals neither was any death recorded (Table 1). The oral LD_{50} of ethanol extracts of *B. ferruginea* and *Lycopus uniflorus* in rats were therefore ≥ 5000 mg/ kg b. wt.

Table 1: Effects of ethanol leaf extracts of *B. ferruginea* and *L. uniflorus* extracts on the behavioural changes and mortality of albino rats

Behavioural Changes	Treatment (mg/kg b.wt)					
	Phase 1			Phase 2		
	10	100	1000	1600	2900	5000
Abdominal pain	0/4	0/4	0/4	0/4	0/4	0/4
Agression	0/4	0/4	0/4	0/4	0/4	0/4
Diarrhoea	0/4	0/4	0/4	0/4	0/4	0/4
Hyperactivity	0/4	0/4	0/4	0/4	0/4	0/4
Appetite loss	0/4	0/4	0/4	0/4	0/4	0/4
Respiratory difficulties	0/4	0/4	0/4	0/4	0/4	0/4
Paw licking	0/4	0/4	0/4	0/4	0/4	0/4
Weakness	0/4	0/4	0/4	0/4	0/4	0/4
Coma	0/4	0/4	0/4	0/4	0/4	0/4
Death	0/4	0/4	0/4	0/4	0/4	0/4

Table 2: Effects of *Bridelia ferruginea* and *Lycopus uniflorus* ethanol leaf extracts on total and differential white blood cell count of male albino rats infected with *Plasmodium berghei*

Parameters	Stage	GP 1	GP 2	Gp3	GP 4	GP 5	GP6	GP 7
WBCx10^3	B.I	5.53 ± 0.42^{a,b}	-	6.40 ± 0.20^{a,b}	5.73 ± 0.46^{a,b}	6.20 ± 0.40^{a,e}	6.23 ± 0.74^{a,b}	5.27 ± 0.31^{a,e}
	A.I	5.73 ± 0.42^{a,c}	-	6.40 ± 0.20^{a,b}	6.53 ± 0.31^{a,c}	6.20 ± 0.35^{a,e}	6.37 ± 0.51^{a,b}	5.73 ± 0.31^{a,e}
	AT	5.80 ± 0.35^{a,b,c}	-	5.87 ± 0.50^{a,b}	6.07 ± 0.12^{a,b,c}	5.40 ± 0.53^{a,e}	5.73 ± 0.46^{a,b,c}	5.33 ± 0.32^{a,e}
Lym (%)	B.I	21.04 ± 0.90^{a,b}	-	21.34 ± 0.39^{a,b}	21.12 ± 0.29^{a,b}	20.30 ± 0.50^{a,b}	21.10 ± 0.87^{a,b}	20.43 ± 0.62^{a,e}
	A.I	21.52 ± 0.50^{a,b}	-	20.34 ± 0.23^{a}	20.54 ± 0.52^{a,b}	19.75 ± 0.66^{a,b}	20.59 ± 0.74^{a,b}	19.70 ± 0.38^{a,e}
	AT	21.92 ± 0.57^{a,b}	-	21.74 ± 0.35^{c}	21.27 ± 0.51^{a,c}	20.93 ± 0.10^{a,b}	21.89 ± 1.01^{a,c}	22.55 ± 1.49^{a,e}
Neut (%)	B.I	11.24 ± 0.22^{a,d}	-	11.10 ± 0.93^{c,d}	10.62 ± 0.57^{a,d}	11.52 ± 0.57^{a,b}	10.61 ± 0.56^{a,b}	12.21 ± 0.55^{a,b}
	A.I	11.60 ± 0.38^{a,d}	-	11.24 ± 0.22^{a,d}	10.27 ± 0.86^{a,d}	10.84 ± 0.56^{a,c}	10.63 ± 1.58^{a,b}	11.80 ± 0.53^{a,c}
	AT	12.23 ± 0.74^{a,d}	-	12.06 ± 0.24^{c,d}	11.19 ± 1.14^{a,d}	12.03 ± 0.58^{a,d}	11.88 ± 1.52^{a,c}	12.59 ± 1.61^{a,b}

Results are mean ± SD of 5 determinations. Mean values bearing different first letter superscript across rows are significantly different across groups (p<0.05), second and third superscript letters indicate significantly difference within group (p<0.05).

WBC = White blood cells, LYM= lymphocytes, NEUT= neutrophils
B.I = Before induction, A.I= After Induction, A.T =After treatment

Table 3: Effects of *Bridelia ferruginea* and *Lycopus uniflorus* ethanol leaf extracts on red blood cell indices of male albino rats infected with *Plasmodium berghei*

Parameters	Stage	Gp 1	Gp 2	Gp3	Gp 4	Gp5	Gp6	Gp 7
RBC x10^6/L	B.I	2.17 ± 0.21^{a,c}	-	2.13 ± 0.15^{a,d}	2.13 ± 0.06^{a,d}	1.93 ± 0.12^{a,d}	2.10 ± 0.17^{a,d}	2.23 ± 0.15^{a,d}
	A.I	2.10 ± 0.10^{a,c}	-	1.83 ± 0.15^{b,d}	2.03 ± 0.06^{a,c}	1.80 ± 0.20^{a,d}	1.87 ± 0.21^{a,e}	1.70 ± 0.26^{a,d,}
	A.T	2.27 ± 0.21^{a,c}	-	1.60 ± 0.10^{b,d}	2.30 ± 0.10^{a,d}	2.37 ± 0.06^{a,e}	2.37 ± 0.21^{a,d}	2.40 ± 0.20^{a,d,e}
HGB (g/dl)	B.I	13.80 ± 0.85^{a,b}	-	13.47 ± 0.76^{a,c}	13.47 ± 0.90^{a,b,c}	13.50 ± 0.78^{a,b}	13.93 ± 0.65^{a,b}	12.97 ± 0.50^{a,b}
	A.I	14.20 ± 0.87^{a,b}	-	11.80 ± 0.62^{a,b}	12.37 ± 0.45^{a,b}	12.10 ± 0.85^{a,c}	12.50 ± 0.46^{a,c}	11.40 ± 0.20^{a,c}
	A.T	14.40 ± 0.87^{a,b}	-	14.20 ± 0.61^{a,c}	14.13 ± 0.58^{a,c}	14.10 ± 0.44^{a,b,c}	13.97 ± 0.47^{a,b,d}	13.13 ± 0.71^{a,b,c}
PCV (%)	B.I	40.67 ± 2.52$^{a,\ b}$	-	39.67 ± 2.08^{a,b}	40.33 ± 3.21^{a,b}	39.67 ± 2.31^{a,b}	41.00 ± 2.00$^{a,\ b}$	40.00 ± 2.65^{a,b}
	A.I	41.67 ± 1.53^{a,b}	-	36.00 ± 2.65^{a,b}	30.33 ± 0.58^{a,c}	31.67 ± 1.53^{a,c}	36.67 ± 1.53^{a,c}	32.00 ± 2.00^{a,c}
	A.T	42.33 ± 2.08^{a,b}	-	44.00 ± 2.00^{a,b,c}	42.67 ± 3.21^{a,b}	42.33 ± 3.51^{a,b}	43.00 ± 1.00^{a,b}	42.67 ± 4.16^{a,b}
MCH (pg)	B.I	5.90 ± 0.02$^{a,\ b}$	-	5.56 ± 0.29^{a,b}	5.77 ± 0.19^{a,b}	5.20 ± 0.25^{a,b}	4.90 ± 0.11^{a,b,d}	4.87 ± 0.49^{a,b}
	A.I	5.78 ± 0.05$^{a,\ b}$	-	4.55 ± 0.61^{a,c}	4.60 ± 0.35^{a,b}	4.32 ± 0.64^{a,b}	3.73 ± 0.60^{a,b}	4.36 ± 0.32^{a,b}
	A.T	5.93 ± 0.06^{a,b}	-	5.60 ± 0.29^{a,b}	5.84 ± 0.09^{a,b}	5.52 ± 0.13^{a,b}	5.33 ± 0.23^{a,c,d}	5.51 ± 0.21^{a,b}
MCHC (g/dl)	B.I	15.59 ± 0.38^{a,b}	-	15.55 ± 0.90^{a,b}	15.07 ± 2.15^{a,b}	14.38 ± 1.49^{a,b,c}	16.10 ± 1.07^{a,b,c}	13.91 ± 1.43^{a,b}
	A.I	14.27 ± 0.83^{a,b}	-	14.18 ± 1.27^{a,b}	14.69 ± 2.29^{a,b}	13.91 ± 1.18^{a,b}	15.50 ± 1.39^{a,b}	13.47 ± 1.48^{a,b}
	AT	15.58 ± 0.64^{a,b}	-	15.84 ± 2.07^{a,b}	15.66 ± 2.04^{a,b}	15.14 ± 1.45^{a,c}	16.62 ± 1.11^{a,b,c}	14.36 ± 1.52^{a,b,c}

Results are mean ± SD of 5 determinations. Mean values bearing different first letter superscript across rows are significantly different across groups (p<0.05), second and third superscript letters indicate significantly difference within group (p<0.05).

RBC = redbloodcells, HGB = haemoglobin, PCV = Packed cell volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration
B.I = Before induction, A.I= After Induction, A.T =After treatment

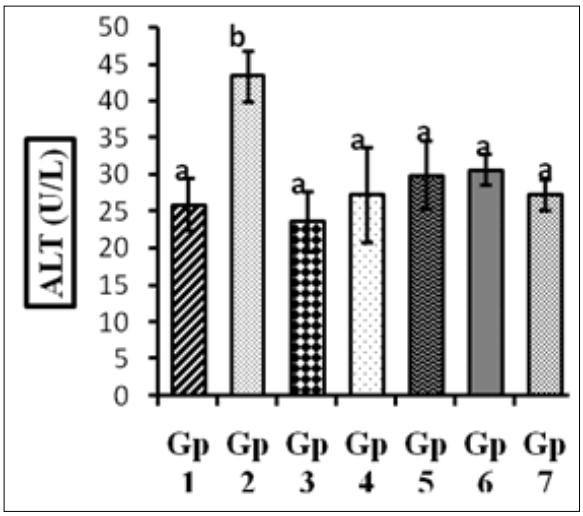

Fig 1: ALT activity

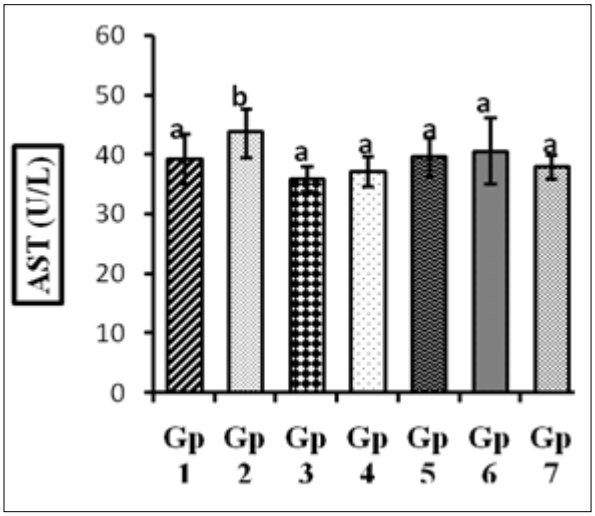

Fig 2: AST activity

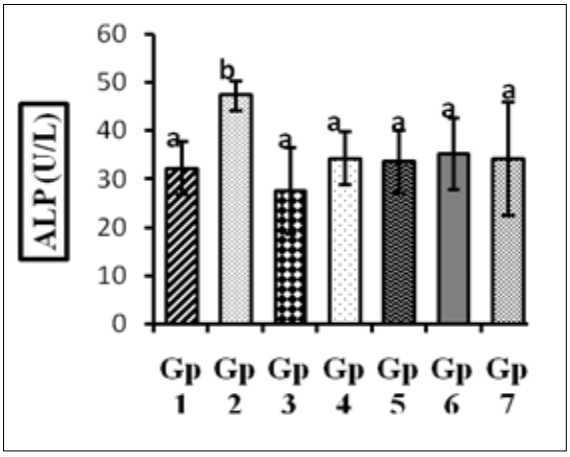

Fig 3: ALP activity

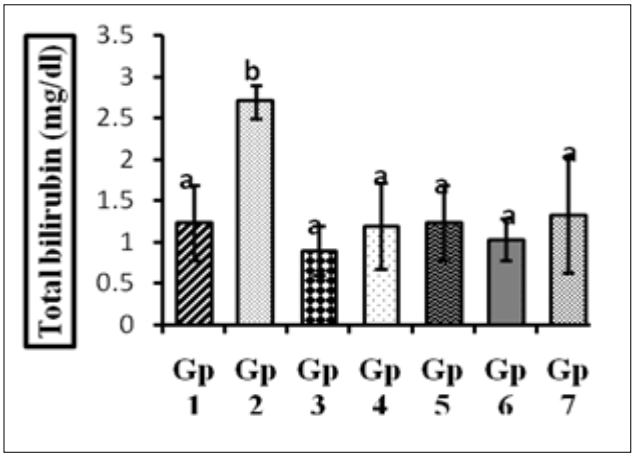

Fig 4: Total bilirubin concentration

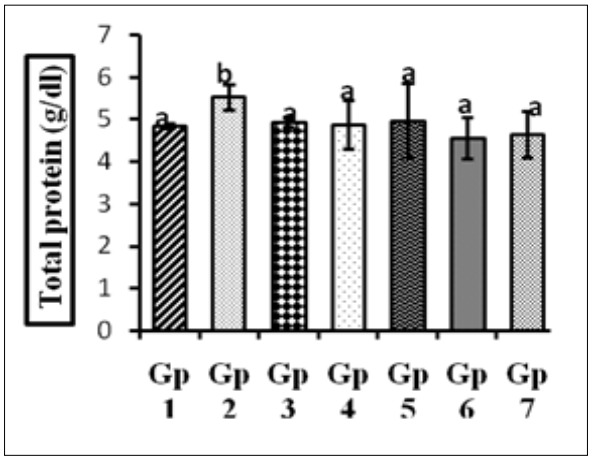

Fig 5: Total protein concentration

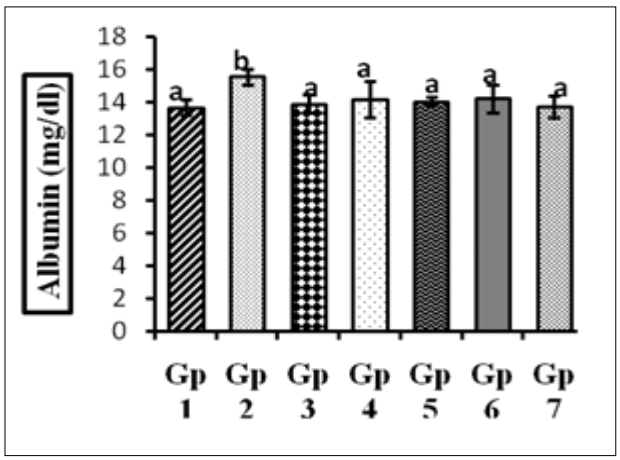

Fig 6: serum albumin concentration

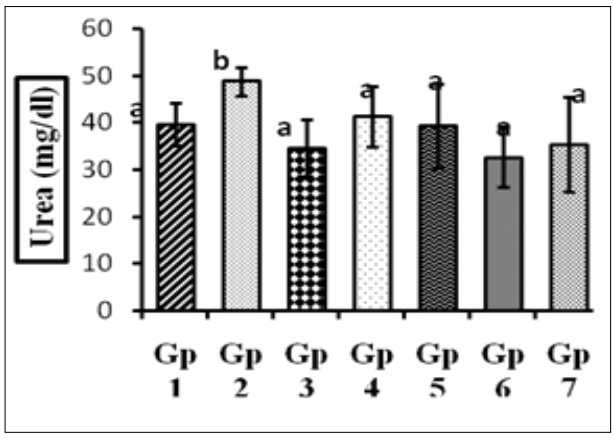

Fig 7: Serum urea concentration

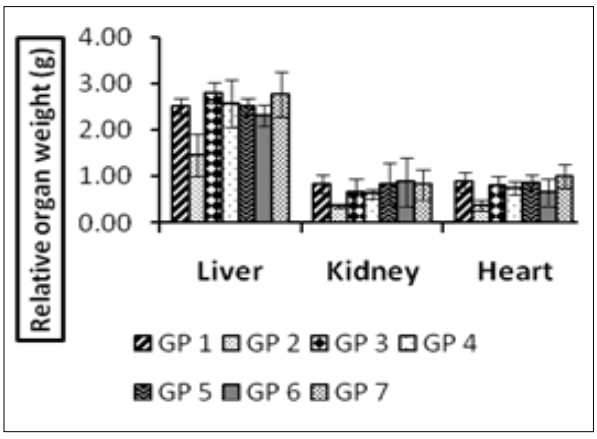

Fig 8: Relative organ weights of treated animals

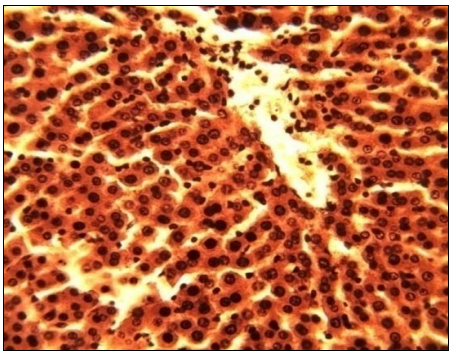

Plate 1: Photomicrograph of liver section from control group (Group I) showing hepatic portal vein with normal plates of hepatocytes with no remarkable histologic change H and E X400.

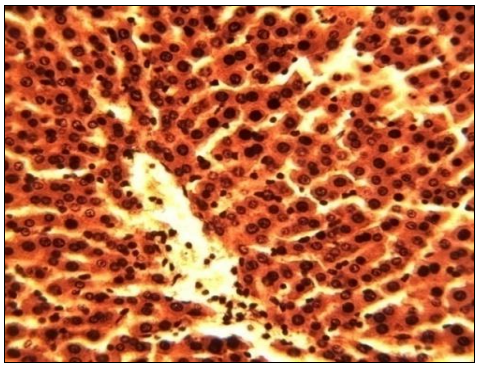

Plate 2: Photomicrograph of liver section from animals treated with 200mg/kg b.wt of artemether and lumefantrine tablet (ACT) showing perivascular edema of the central vein (CV). H and E X400

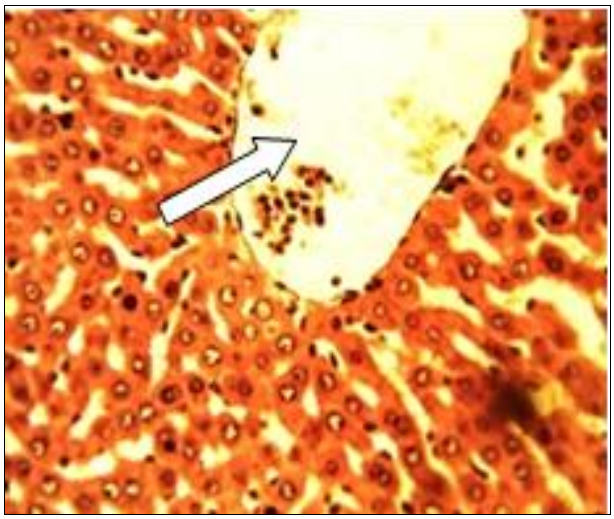

Plate 3: Photomicrograph of liver section from animals that received 200mg/kg body weight of ethanol leaf extract of *Bridelia ferruginea* showing nuclear vesiculation with mild venous congestion. H and E X400.

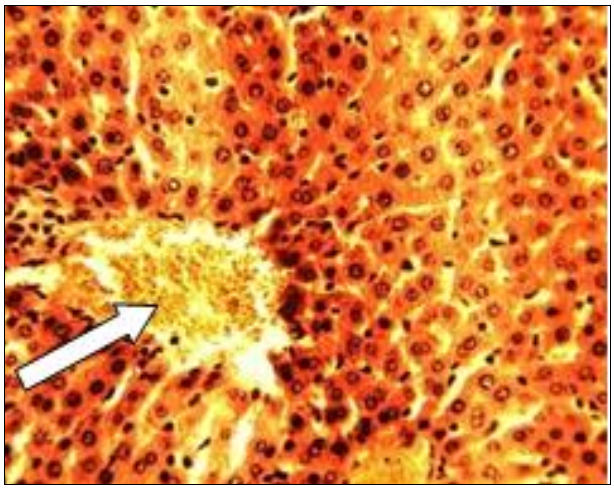

Plate 4: Photomicrograph of liver section from animals that received 1000 mg/kg body weight of ethanol leaf extract of *B. ferruginea* showing the central vein (CV) and sinusoids lined by kupffer cells with no remarkable histologic change. H and E X400.

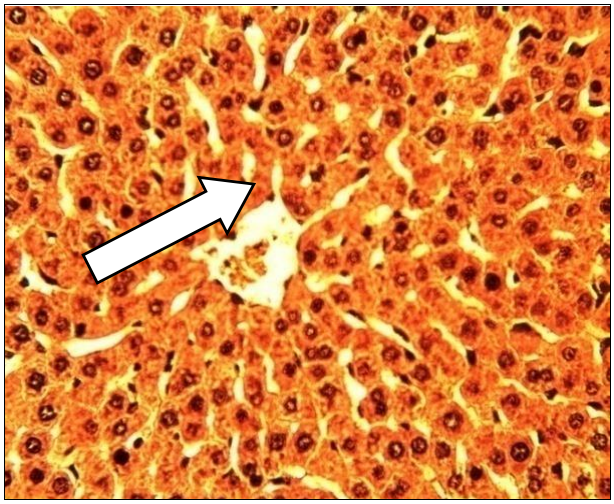

Plate 5: Photomicrograph of liver section of animals that received 200 mg/kg b.wt of ethanol leaves extract of *Lycopus uniflorus* showing the central vein (CV) and sinusoids lined by kupffer cells with no remarkable histologic change. H and E X400.

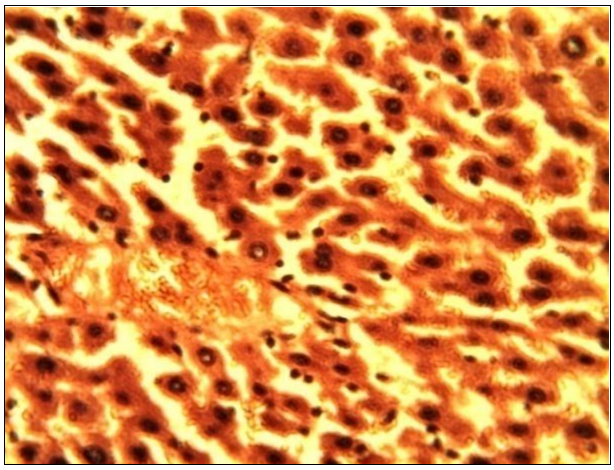

Plate 6: Photomicrograph of liver section of animals that received 1000 mg/kg b.wt of ethanol leaves extract of Lycopus uniflorus showing the central vein (CV) and sinusoids lined by kupffer cells with no remarkable histologic change. H and E X400.

Discussion

Nigeria is a country blessed with an array of medicinal plants. Natives acquire information about these medicinal plants, preserve them and pass to their generations (Enenebeaku *et al*., 2021a) [8]. Therapeutic properties of these medicinal plants are credited to the phytochemicals present in them (Asiwe *et al*., 2023) [3].

Adverse or toxicological effects of an ingested substance could be detected from behavioural, biochemical, and hematological changes in animal models. Medicinal plants used in the present study are not likely to elicit any harmful effect in human beings because substances with LD_{50} above 5000 mg/kg body weight are considered harmless (Onwusonye *et al*., 2020) [25]. The biochemical and hematological parameters examined in the present study are useful indicators for assessing the toxicity of plant extracts in animals. Biochemical and hematological parameters examined in this study are useful indicators in the evaluation of toxicity of plant extract in animals.

In this study, behavioural patterns of the experimental animals were similar to those of the control group. There was no significant (p<0.05) difference between the examined serum biochemical (Fig 1-7) and hematological (Tables 2 & 3) parameters of the treated and the control animals. This is because no adverse effects which could aggravate a change in the level of these parameters were recorded with the plant extracts used in the study.

There were also no remarkable histological changes in the liver sections of treated animals when compared with control animals and those that received the standard antimalarial drug (arthemether and lumefantrine) However, results obtained for the biochemical parameters of non-treated animals significantly differed from the control. This is probably due to the *Plasmodium* feeding on the blood cells of the animals resulting in functional irregularities of the kidney and liver owing to their involvement in metabolism (John-Africa, 2019) [17]. Mild venous congestion was also noticed in the liver photomicrograph of animals that received 200mg/kg b.wt of *B.ferruginea*, an indication that this particular dose may not be suitable for the treatment of the animals. The relative organ weights (ROW) of the treated animals that received the extracts did not differ significantly (p< 0.05) from the control and standard groups, while those of the untreated animals were significantly (p< 0.05) reduced as seen in Fig. 1. The significant decrease in the weight of untreated organs could

be due to the presence of malaria parasites in these untreated animals, which coincides with Sulaiman *et al*. (2016) [32].
Findings of this study suggest that ethanol extracts from the leaves of *B.ferriginea* and *L. uniflorus* had no negative effects on animal models, rather, they have protective effects on hematology and biochemical parameters which justifies their continuous use in ethnomedicine.

Conclusions
The extracts of *B. ferruginea* and *L. uniflorus* were not toxic due to the absence of serious changes in behavioural observations and the absence of mortality after administration of the stated doses in the sub-acute toxicity study. Therefore, this study provided evidence of unchanged hematological, histopatological and biochemical indicators due to the use of these extracts. This explains its continuous use in the management of malaria and other diseases**.**

List of abbreviations
Mg/kg: milligram/kilogram; b/wt: body weight; L_D: Lethal dose; NIH: National Institute of Health; *P.berghei*: *Plasmodium berghei*; RBC = redbloodcells, HGB = haemoglobin, PCV = Packed cell volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration
B.I = Before induction, A.I= After Induction, A.T =After treatment. H & E = Haematoxylin and Eosin
ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase

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