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Phytochemical and oral toxicity profile of the methanol leaf extract of *Haematostaphis Barteri* hook f. (Anacardiaceae)

Cyril Ogbiko[1*], Abubakar Sani Yelwa[2], Babagana Ali[3]
[1, 2] Department of Pure and Applied Chemistry, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria
[3] Department of Chemistry, Kashim Ibrahim College of Education Maiduguri, Borno State Nigeria

Abstract
Despite the increase use of herbal medicine, there are inadequate research evidences on their toxicity. *Haematostaphis barteri* is an important medicinal plant used for the treatment of anemia, hemorrhoid and malaria among others. The phytochemicals and acute oral toxicity profile of the leaves of the plant was investigated using established procedures. Forty albino rats were grouped into 6 groups of 5 rats each receiving 250, 500, 1000, 2000, 3000 and 4000 mg/kg respectively. The control groups received 1 ml of tween-80 the control vehicle. The phytochemical screening revealed the presence of important secondary metabolites, the results of the acute toxicity studies did not show any apparent pharmacotoxic signs. There was no body weight gain and that of vital organs during the observation period. The median lethal dose was found to be greater than 4000 mg/kg body weight. *Haematostaphis barteri* leaves extract is relatively safe when administered orally to rodents.

Keywords: *haematostaphis* barteri**,** phytochemicals, toxicity, methanol extract

1. Introduction
H. barteri have been used by traditional healers in northern Nigeria for the management of ailments such as, cancer (Kubmarawa *et al*., 2007) [1], stomach ache, and vomiting (Rabo and Sanusi, 2001) [2], anemia and hemorrhoid, malaria, hepatitis and sleeping sickness (Boampong *et al*., 2013) [3] among others. Plant based traditional medicine has been used all over the world for thousands of years (Tarkang *et al.*, 2012) [4]. The World Health Organization (WHO) estimated that 80% of the world's population relies on traditional healing modalities including herbal medicine for primary health care and wellness (WHO, 2002; Karunamoorthi *et al*., 2013) [5, 6, 1]. In spite of all the medicinal relevance of medicinal plants, the knowledge of the extent of adverse effects associated with their use has been a major challenge (Koduru *et al.*, 2006) [7]. Toxicity testing is important in evaluating the safety profile of drugs and herbs intended to be used by mankind for treatment of diverse ailments (Rajalakshmi *et al*, 2014) [8]. Hence this study is designed to provide information of the safety profile of the methanol leaf extract of the plant.

2. Materials and methods
2.1. Procurement of plant material and preparation of extract
The leaves of *Haematostaphis barteri* were collected from Jabo district in Tambuwal Local Government of Sokoto State Nigeria and authenticated by Abdulaziz Sani of Botany Unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto where a herbarium specimen was deposited. The leaves were washed with running tap water and shade dried until a constant weight was obtained. They were then crushed with the aid of a pestle and mortar to a powdered state after which 350 g of the powdered leaves was weighed and extracted with 2 L of absolute methanol via cold maceration for 72 hours. The crude methanol extract of *H. barteri* (CMHB) obtained was allowed to evaporate under open air at room temperature. The dried extract was weighed stored in an air tight glass container until use.

2.2. Phytochemical screening
Phytochemical screening was conducted on the methanol leaf extract of *H. barteri* to validate the presence or otherwise of secondary metabolites such as alkaloids, cardiac glycosides, saponins, tannins, streroids, anthraquinones among others using standard methods as described by Stahl [9] and Trease and Evans [10].

2.3. Animal's procurment
Wistar adult non-pregnant albino rats of both sexes were acquired from Animal House, Department of Biochemistry, Usmanu Danfodiyo University Sokoto. The weights of the rats were between 120-150g. The rats were kept under normal laboratory conditions with access to food and water. The animals were randomly selected, marked to allow individual identification and kept in their cages for 14 days prior to the experiment in order to allow for acclimatization to the laboratory conditions. The animals were housed in improvised plastic cages with sawdust litter at room temperature. Adequate lighting was maintained to ensure 12 hours of light and 12 hours of dark for each 24 hours experimental period. Each cage was identified by a card stating the cage number, number and weight of the animals, test substance code, administration route and dose per animal. The animals were fed with standard laboratory feeds and clean water during the experimental period. Animals were used in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

2.4. Acute Toxicity Study

Prior to the experiment, the animals were fasted overnight but had free access to water *ad libitum*. The CMHB extract was administered intra-peritoneally. The procedure was divided into 2 phases. The first phase had 15 rats sorted into groups A_1, A_2 and A_3 with 5 rats in each group which received 250, 500 and 1000 mg/kg body weight of the CMHB extract respectively. The second phase had 3 groups B_1, B_2 and B_3 with 5 rats in each group receiving 2000, 3000 and 4000 mg/kg body weight of the CMHB extract respectively. Groups A_4 and B_4 the control groups received 1 ml of tween-80 each the control vehicle for the extract preparation. The median lethal dose (LD_{50}) was calculated using the following formula:

$$LD_{50}=\sqrt{\text{Minimal lethal dose} \times \text{maximal survival dose}}$$

The animals were observed for clinical signs, morbidity and mortality at regular interval of 4 hours on the first day and thereafter daily for 14 days for any sign of delayed toxicity and the weight loss or gain was determined at the end of the 14th day (Lorke 1983) [11]. Body weights were taken before extract administration and after 14 days duration. Animals that survived were exposed to chloroform anesthesia via inhalation for extraction of the kidney, heart and liver.

2.5. Statistical Analysis

The SPSS (Statistical Package for Social Sciences) software packages version 16 were used for statistical analysis. Results were presented as mean and standard error (Mean ± S.E). The statistical significance between the control and each of the treated groups was determined by Dennett's t- test after one-way ANOVA. The level of significance was set at $P < 0.05$.

3. Results and Discussion

3.1 Percentage yield and phytochemical screening

The percentage yield of the crude methanol extract was found to be 4.77%. The phytochemical screening revealed the presence of important phytoconstituents presented in Table 1.

Table 1: Preliminary phytochemical screening of the leaves of *H. barteri*

Phytochemicals	Test	Observation	Inference
Tannins	Ferric chloride	Blue black colour appeared	+
	Lead sub acetate	Coloured precipitate appeared	+
Saponins	Frothing	Foam was formed	+
Alkaloids	Mayer's	Precipitate formed	+
	Wagner's	Precipitate formed	+
	Dragendroff's	Precipitate formed	+
Cardiac glycosides	Keller-Killiani's	Colour change was observed	+
Flavonoid	Shinoda's	Reddish colour was observed	+
	Alkaline reagents	Intense yellow colour was formed	+
Reducing sugar	Molich's	Violet ring was formed at the junction	+
	Fehling's	Red precipitate was formed	+

Key: (+) = Presence of phytochemical;

Phytochemical screening of methanol leaf extract of *H. barteri* revealed the presence of some important secondary metabolites which might be responsible for its divese pharmacological activities.

3.2. General animal behaviour and mortality

Daily administration of the methanol leaf extract of *H. barteri* for 14 days did not produce observable signs of toxicity as well as mortality. Similarly, there were no changes in the skin, fur and eyes colour as well as appetite during the test period food and water consumption, as well as organ weights as presented in tables 2 and 3.

Table 2: Effect of ethanol extracts of *H. barteri* on acute oral toxicity in rats during observation in first 6 hours

Response	Phase I			Phase II		
	250 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg	4000 mg/kg
Alertness	Yes	Yes	Yes	Yes	Yes	Yes
Grooming	Normal	Normal	Normal	Normal	Normal	Normal
Touch Response	Yes	Yes	Yes	Yes	Yes	Yes
Torch Response	Yes	Yes	Yes	Yes	Yes	Yes
Tremor	No	No	No	No	No	No
Convulsion	No	No	No	No	No	No
Gripping Strength	Normal	Normal	Normal	Normal	Normal	Normal
Response to Food	Yes	Yes	Yes	Yes	Yes	Yes
Pupils	Normal	Normal	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	No	No	No	No	No	No
Hyperactivity	Normal	Normal	Normal	Normal	Normal	Normal
Skin colour	Normal	Normal	Normal	Normal	Normal	Normal
Corneal Reflux	Normal	Normal	Normal	Normal	Normal	Normal
Pinna Reflux	Normal	Normal	Normal	Normal	Normal	Normal
Sound Response	Normal	Normal	Normal	Normal	Normal	Normal

Table 3: Body weight indices after the administration of varied concentration of methanol extract of *H. barteri* leaves to rats and organ weight after sacrifice

Group	Dose (mg/kg bwt)	Difference in body weight (g)	Liver Weight (g)	Kidney Weight (g)	Heart Weight (g)
A_1	250	0.04 ± 1.41	4.22 ± 0.25	1.61 ± 0.14	1.28 ± 0.11
A_2	500	0.39 ± 1.01	4.01 ± 0.11	1.67 ± 0.04	1.22 ± 0.13
A_3	1000	1.69 ± 0.40	4.54 ± 0.06	1.59 ± 0.10	1.27 ± 0.14
B_1	2000	1.51 ± 0.66	5.05 ± 0.01	1.77 ± 0.01	1.98 ± 0.04
B_2	3000	1.22 ± 0.25	4.99 ± 0.09	1.71 ± 0.13	1.97 ± 0.06
B_3	4000	0.22 ± 0.22	5.11 ± 0.10	1.68 ± 0.09	1.91 ± 0.12
A_4 (Control)	1 ml tween-80	0.84 ± 0.28	4.67 ± 0.86	1.69 ± 0.14	1.26 ± 0.08
B_4 (Control)	1 ml tween-80	1.11 ± 0.22	5.08 ± 0.19	1.64 ± 0.09	1.97 ± 0.11

The results are the means of 5 determinations ± S.D P-value significant at < 0.05

There were no apparent pharmacotoxic signs observed upon the oral administration of the methanol extract of *H. barteri***.** There was no significant weight gain or loss over the test period. A median lethal dose was found to be greater than 4000 mg/kg body weight was observed for the extract and hence may be considered to be safe hence do not exert acute toxicity in the treated animals.

4. Conclusions

The findings of this study suggest that the methanol leaf extract of *H. barteri* contains bioactive constituents that are attributed to its diverse pharmacological applications. It also demonstrated that the methanol extract of *H. barteri* may be relatively safe when administered orally at 4 g/kg. However, a further study is recommended on the toxicological effect of the extract when administered chronically.

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