Toxicity Studies of *Striga hermonthica* (Delile) Benth Leaf Extract in Rats

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ABSTRACT

Striga hermonthica is a ubiquitous hemi-parasitic plant commonly known as witch-weed and is used in West Africa as a traditional herbal medicine for the treatment of an array of diseases. In this study, methanol leaf extract of *S. hermonthica* was used to investigate the acute and sub-acute toxicity effects in male Wistar rats. In the acute toxicity studies, Wistar rats were divided into six groups comprising of negative control and extract treated groups (250, 500, 1000, 1500 and 2000 mg/kg of extract orally). The rats were observed for 72 hours while in the sub-acute oral toxicity studies, the rats were divided into 4 groups consisting of 5 rats per group. The extract was administered orally at doses of 25, 50 and 100 mg/kg daily for 28 days to groups II, III and IV respectively while group I (negative control) received 2 ml of distilled water. The dose of 2000 mg/kg did not cause any mortality or signs of toxicity in the treated rats during the acute and sub-acute toxicity studies did not show any treatment-related abnormalities in the hematological (RBC, Hb, WBC, Lymphocytes) and biochemical (AST, ALT, ALP, TB, Glucose, HDL, LDL, Total protein, Albumin) parameters while the liver revealed lesions in the histopathology studies, there were no treatment-related lesions observed in the heart, lungs, pancreas and kidney whereas, the weight of rats did not show significant difference ($p > 0.05$) between the control and the treated groups. The study showed that *S. hermonthica* caused hepatotoxicity and could be potentially harmful for use. A more comprehensive research is recommended to investigate on its safe use and mode of action.

Keywords: *Striga hermonthica*; toxicity, histopathology, crude extract.

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INTRODUCTION

Striga hermonthica, commonly known as witch-weed is a flowering, hemi-parasitic plant which belongs to the family Orobanchaceae (ex. Scrophulariaceae). It is deemed to be one of the most ubiquitous parasitic weed of rice (*Oryza sativa*), millet (*Pennisetum glaucum*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) [1].

Striga hermonthica is an erect annual plant, wide spread in the tropics and subtropics of Gambia, Ghana, Mali, Nigeria, Niger Republic, Senegal, and Sudan [2]. *Striga hermonthica* has many vernacular names in different countries for example in Sudan, it is named as 'Al Budda' and in West Africa, for example in Nigeria it is known as 'Kudiji', 'Makasar dawa' (Killer of guinea corn), 'Dodon dawa' and 'Wuta wuta' in Hausa [2].

Beside its parasitic devastating impacts on crops in some parts of Africa and India, *S. hermonthica* is well-known and used as a medicinal plant for the treatment of leprosy and leprosy ulcers while infusion or decoctions of its roots are administered orally as an abortifacient and in the treatment of pneumonia [3] and as anti-diabetic in Sudan [4]. The antimalarial [5] and *in vitro* trypanocidal effects [6] have also been reported. Concoctions of *Striga hermonthica* is used as folkloric medicine in the treatment of leprosy, jaundice, icterus by the *Gbagyi* and *Fulani* people of Gwagwalada in Abuja, Nigeria [3]. They also claim that infusions from the root, stem and leaves are effective in the treatment of bloat in ruminants, jaundice, and dermatitis in humans [4]. The aim of this research therefore is to evaluate the toxicity of *S. hermonthica* extract in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Forty (40) adult male Wistar rats weighing between 138 g and 317 g were procured from the Laboratory Animal House of the Faculty of Veterinary Medicine, University of Abuja,

Nigeria. The rats were housed in metallic cages in a well-ventilated environment with 12 hours light and dark cycle and ambient temperature. They were acclimatized for two weeks prior to the commencement of the experiment and were fed with standard pelleted poultry feed (Vital feed®) and potable water provided *ad libitum*.

Plant preparation and extraction

Fresh leaves of *S. hermonthica* were collected in farmlands within the Main Campus of the University of Abuja in the month of December. The plants were identified by a Taxonomist from the Department of Botany, University of Abuja and a voucher specimen was kept in the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja for reference purposes.

The air-dried leaves were pulverized to a coarse powder to obtain a total of 264.34g of the plant material. This was soaked in 2 liters of 80 % methanol and shaken at regular for 18 hours. The extract was collected and concentrated *in vacuo* using a rotary evaporator at 40°C to dryness and stored at 4°C until used.

The percentage yield was calculated using

$$\frac{\text{Weight of extract}}{\text{Weight of pulverized plant material}} \times \frac{100}{1}$$

Phytochemicals analysis

The presence of steroids, tannins, cardiac glycosides, phenols, flavonoids, saponins, terpenoids, resins, anthraquinones, were appraised using standard protocols [7, 8].

Acute toxicity study

The acute toxicity of the extract was conducted by the modified method of Lorke [9]. The rats were fasted for 12 h and then randomly placed into one of six groups of either the extract treated or vehicle treated; each group comprised six rats. *Striga hermonthica* extract (250, 500, 1000, 1500 and 2000 mg/kg) was separately administered orally to the rats in each group. The rats were observed for 72 hours for mortality and signs of toxicity.

Sub-acute toxicity study

This study was conducted in accordance with the OECD guideline [10]. Rats were randomly divided into 4 groups of 5 rats per group. The extract dissolved in distilled water was administered orally at doses of 25, 50 and 100 mg/kg for 28 days to groups II, III and IV respectively while group I received 2ml of distilled water. Rats were sacrificed after the 28 day treatment to assess organ weight and carry out histopathology.

Collection of blood samples and estimation of biochemical parameters

At the end of the 28-day experimental period, rats were sacrificed by cervical decapitation; blood samples were collected from the jugular vein into tubes with EDTA for haematological analysis for PCV, RBC, Hb concentration and WBC. Blood samples were allowed to coagulate in a tilted test tube, centrifuged at 3000 r/min for 15 min at 4 °C to separate serum. Sera were divided into aliquots and stored at -80 °C for biochemical assay. Standardized diagnostic kits (Randox Laboratory Ltd,) were used for Spectrophotometric determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine kinase (ALK), glucose, total protein, HDL and LDL.

Relative body weight

The weekly body weight of each rat was assessed using digital scale during the acclimatization period, before commencement of dosing, weekly during the dosing period and on the 29th day just before sacrifice.

Relative organ weight

The liver, kidney, heart, testes were excised observed for any gross abnormality in color and shape. The relative organ weight calculated as:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{weight of rat on sacrifice day (g)}} \times 100$$

Histopathology

Small pieces (5-8 mm) of tissue from the liver, kidney, lungs, pancreas and heart in all groups

were excised and were immediately fixed in 10% formal saline. Fixed samples were trimmed and processed for paraffin embedding. Sections (5-7 µm) were cut and picked up on clean saline-coated glass slides. After de-waxing and rehydration through descending concentrations of ethanol, the sections were stained with haematoxylin and eosin and examined microscopically.

Statistical analysis

All values are expressed as mean $\pm$ SEM. Statistical analysis was performed by One-way analysis of variance followed by Tukey's multiple comparison tests. The results were considered statistically significant at $P < 0.05$.

RESULTS

Extraction of the plant material

The extract of *S. hermonthica* leaves was dark, pasty, and had a pungent odor. The percentage yield of the extract was 17.57 w/w

Phytochemicals analysis

Phytochemical analysis of the leaves of *S. hermonthica* identified a number of secondary metabolites such as tannins, glycosides and phenols as shown in Table 1.

Table 1 : Phytochemical analysis

Phytochemical	Status
Alkaloid	-
Tannins	+
Glycosides	+
Cardiac glycosides	+
Steroids	-
Phenols	+
Flavonoids	-
Resins	-
Anthraquinones	-
Terpenoids	-
Saponins	-

+ = Present, - = Absent

Acute toxicity study

Striga hermonthica extract was apparently safe up to a dose of 2000 mg/kg orally. Behavior of the animals was observed for the first 8 h of treatment then at an interval of every 4 h during the next 72 h, the extract did not produce behavioral changes in the animals nor mortality. The LD₅₀ of the extract was therefore said to be about 2000 mg/kg

Body weights

Rats showed significant dose dependent

increase ($P < 0.05$) in body weight compared to their initial values only on day 14 of the treatment (Table 2). However, there was no significant difference ($P > 0.05$) between the different treatment groups and the control on days 0, 7, 21 and 28.

Relative organ weights

The relative organ weights recorded did not show any significant differences in the treatment and the control group (Table 3).

Table 2: Changes in body weight of rats during 28-day treatment with *S. hermonthica* extract at 25, 50 and 100 mg/kg orally.

Treatment groups	Days				
(mg/kg) orally	0	7	14	21	28
Control	218.08±24.30	201.34±18.53	193.80±29.46	222.90±17.79	238.20±17.13
25	219.82±28.49 ^a	201.10±24.24 ^a	199.80±31.82 ^a	208.90±30.58 ^a	221.60±32.70 ^a
50	216.00±22.42 ^b	197.06±19.48 ^b	207.50±21.23 ^b	216.80±24.10 ^b	209.10±21.67 ^b
100	210.60±23.65 ^c	215.40±19.48 ^c	239.75±16.50 ^c	230.38±18.51 ^c	238.27±15.33 ^c

^{a, b, c} = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.

Table 3: Relative organ weight of rats treated with *Striga hermonthica* leaf extract at 25, 50 and 100 mg/kg orally for 28 days

Treatment groups	Organ weight (g)					
(mg/kg)	Heart	Liver	Lungs	Pancreas	Kidney	Testis
Control	1.25 ± 0.25	8.0 ± 0.82	1.25 ± 0.25	1.0 ± 0.00	2.00 ± 0.0	2.75 ± 0.23
25	0.90 ± 0.11 ^a	7.0 ± 1.00 ^a	1.50 ± 0.29 ^a	0.9 ± 0.11 ^a	2.00 ± 0.0	2.80 ± 0.55 ^a
50	1.00 ± 0.00 ^b	6.2 ± 0.55 ^b	1.40 ± 0.27 ^a	0.8 ± 0.41 ^a	2.00± 0.0	2.40 ± 0.57 ^a
100	1.00 ± 0.00 ^c	6.8 ± 0.74 ^c	1.40 ± 0.20 ^a	0.8 ± 0.14 ^a	2.00 ± 0.0	2.80 ± 0.22 ^c

^{a, b, c} = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.

Hematological Parameters

There were no significant changes ($P>0.05$) observed in hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), lymphocytes, neutrophils, monocytes, eosinophils and basophils when compared with the control group (Table 4),

Serum Biochemical parameters

There was no significant change ($P>0.05$) in the serum biochemical parameters in all the treated groups as compared to respective control groups except for the total protein where a significant increase was observed (Table 5).

Table 4: Effects of a 28 -day oral administration 25, 50 and 100 mg/kg of *Striga hermonthica* leaf extract on hematological parameters of rats.

Treat ment (mg/ kg)	Hb (g/d L)	RB C (10 ⁶ ...)	WB C (10 ³ /μL)	PC V (%)	MCV (fl)	MC H (pg)	MCH C (g/dL)	LYM (%)	NEU (%)	MO N (%)	EOS (%)	BAS (%)
Cont rol	...	8.2± 0.8	7.4± 2.9	40± 1.2	48.78 ±4.2	14.88 ±1.8	20.50 ±0.80	75.00 ±4.80	14.70 ±2.40	5.20± 0.80	2.30± 1.50	2.80 ±0.6 0
25	11.3 ±0.8 ^a	8.0± 1.5	7.1± 0.8	38± 0.9 ^a	47.50 ±2.3 ^a	14.12 ±2.1	21.05 ±0.60 ^a	76.50 ±3.20	13.40 ±1.85 ^a	6.10± 2.14 ^a	1.50± 1.60 ^a	2.50 ±0.1 8
50	12.8 ±2.0	7.9± 2.2 ^a	7.5± 2.2	40± 1.4	50.63 ±1.6 ^b	15.10 ±2.8 ^a	19.75 ±1.1 ^b	74.90 ±2.30	13.80 ±0.90 ^a	6.20± 1.80 ^a	2.30± 0.25	2.80 ±1.0 2
100	12.0 ±1.8	8.1± 1.8 ^c	7.3± 2.0	42± 1.5 ^b	50.80 ±1.1 ^b	14.81 ±1.6	19.28 ±0.1 ^b	75.40 ±3..23	14.00 ±4.11	5.80± 1.64	2.30± 2.14 ^c	2.50 ±0.1 8

^{a, b, c} = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.

Key :Hb=haemoglobin, RBC=red blood cells, WBC=white blood cells, PCV=packed cell volume, MCV=mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration, Lym=Lymphocytes, Neu = Neutrophils, Mon=Monocytes, Eos=Eosinophils, Bas=Basophils.

Table 5: Effects of a 28 -day oral administration of 25, 50 and 100 mg/kg of *Striga hermonthica* leaf extract on biochemical parameters of rats.

Treatment group (mg/kg)	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	T.B (μmol/L)	D.B (μmol/L)	Gluc. (Mmol/L)	HDL (mg/dL)	LDL (mg/dL)	T. prot. (mg/dL)	Albumin (g/L)
Control	68±1.31	60±1.60	235±1.20	1.71±0.60	1.21±0.21	145±2.2	0.96±0.01	2.59±0.20	48±0.20	19.0±0.20
25	66±1.22 ^a	62±2.20 ^a	225±1.60 ^a	1.75±0.50	1.30±0.20	142±2.8	0.92±0.02	2.57±0.20	42±0.43 ^a	21±0.84 ^a
50	69±2.10 ^b	59±1.80 ^b	215±1.20 ^b	1.75±0.50	1.25±0.30	141±1.2	1.05±0.10	2.28±0.43	42±0.43 ^a	19±0.65
100	65±1.82 ^a	61±1.18 ^a	226±2.30 ^a	1.60±0.45	1.30±0.20	148±2.1	1.02±0.81	2.24±0.46	39±0.62 ^b	19±0.65

^{a, b, c} = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean ± SEM of 5 animals per group.

Key: AST=Aspartate alanine transferase, ALT=Alanine transferase, ALP= Alanine amino phosphate, T.B=Total bilirubin, D.B=Direct bilirubin, Gluc=Glucose, HDL=high density lipoprotein, LDL=Low density lipoprotein, T.prot=Total protein, Albumin

Histopathology of organs

Histopathological effects on organs such as heart, lungs, liver, kidney and pancreas of rats that were exposed to chronic treatment with *S. hermonthica* leaf extract at different doses (25, 50 and 100 mg/kg) orally is presented in Plates 1, 2, 3, 4 and 5 respectively. There were no observable treatment-related lesions in the

sections of the heart, pancreas and kidney. However, the lungs in all the groups presented with mild interstitial mononuclear inflammatory cells infiltration and alveolar wall breakage, while in the liver there was bile duct proliferation and extensive necrosis of tissue in the inter and intra lobular areas.

Histopathology results

Plate 1: Photomicrograph of rat liver at day 28 for control (A), 25 mg/kg extract (B) shows centrilobular congestion, 50 mg/kg (C) and, 100 mg/kg extract (D) showing bile duct proliferation and extensive necrosis of tissue in the inter and intra lobular areas (arrows) (H&E x 400).

Plate 2: Photomicrograph of cardiac muscles from experimental rats of control (A), 25 mg/kg extract (B) 50 mg/kg (C) and, 100 mg/kg extract (D) at day 28 with no observable lesions. See the nuclei of cardiac muscle cells (arrow). H and E x 400

Plate 3: Photomicrograph of kidney sections from experimental rats of control (A), 25 mg/kg extract (B) 50 mg/kg (C) and, 100 mg/kg extract (D) at day 28 with no observable lesions. H and E x 400

Plate 4: Photomicrograph of sections of the lung from experimental rats of control (A), 25 mg/kg extract (B) 50 mg/kg (C) and, 100 mg/kg extract (D) at day 28 with mild interstitial mononuclear inflammatory cells infiltration and alveolar wall breakage (arrow). H and E x 400

Plate 5: Photomicrograph of the pancreas from experimental rats of control (A), 25 mg/kg extract (B) 50 mg/kg (C) and, 100 mg/kg extract (D) at day 28 showing the pancreatic islet cells (PIC) with no observable pathologic change. H and E x 400

DISCUSSION

The strategy for establishment of safety of a test item depends on demonstration of its toxicity or non-toxicity under the conditions of exposure to its high doses to the experimental animals [11]. In determination of the LD_{50} value of *S. hermonthica* leaf extract in rats orally, the extract at a dose of 250, 500, 1000, 1500 and 2000 mg/kg presented no signs of toxicity nor mortality throughout the period of 72 hour study. The LD_{50} of 2000 mg/kg in the present study is in agreement with the findings of Kiendrebeogo [11] who evaluated the intraperitoneal LD_{50} of acetone extract of *S.*

hermonthica and found it to be 1753 mg/kg in mice. According to the toxicity scale of Hodge and Sterner [12], the crude methanol extract of *S. hermonthica* which exhibited an LD_{50} value of 2000 mg/kg could be qualified as relatively nontoxic to rats when administrated orally.

There was no significant ($P > 0.05$) increase in weight of the rats in the control group in comparison with the entire treatment group except for day 14 of treatment. This is an indication of the non-toxic nature of the extract [11].

The haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological or pathological state. The blood profile also gives vital information on the response of the body to injury or stress [13]. The daily administration of *S. hermonthica* leaf extract orally for 28 days did not produce any significant change in haematological parameters of the treated rats at all the doses used when compared to the control group. This suggests that the extract may have no toxic effects on the haematopoietic system. Bilirubin is a breakdown product of haemoglobin and is associated with symptoms of hepatic diseases like jaundice and ineffective erythropoiesis and an increased bilirubin level reflect the depth of jaundice [14]. In this study there was no significant difference in the levels of serum bilirubin and albumin of both the treated and control rats. This suggests that the extract had no toxic effect on the erythropoietic system; this is in agreement with earlier reports [14]. Liver enzyme results indicated, further alludes to the nontoxic nature of the extract since there was no significant difference in the values of the treated groups as compared with the control.

Mild interstitial mononuclear inflammatory cell infiltration and alveolar wall breakage was observed in the lungs of all the groups including the control; this may be attributed to inhalation of saw dust which was used as the bedding material. In the liver however, there was bile duct proliferation and extensive necrosis of tissue in the inter and intra lobular areas.

Conclusion

The present study, administration of leaf extract of *Striga hermonthica* at the dosage of 250, 500, 1000 mg/kg and limit dose of 2000 mg/kg may present hepatotoxicity and hence could be potentially harmful for use. Consequently more comprehensive research is required to conclude thoroughly on the use, mode of action and safety of this plant.

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