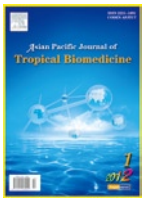
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Acute and subacute toxicity studies of *Lygodium flexuosum* extracts in rats

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ARTICLE INFO

Article history:
Received 5 January 2012
Received in revised form 12 January 2012
Accepted 25 February 2012
Available online 28 April 2012

Keywords:
Lygodium flexuosum
Lygodiaceae
Acute toxicity
Subacute toxicity

ABSTRACT

Objective: To investigate the acute and subacute toxicity of *Lygodium flexuosum* (*L. flexuosum*) extracts to substantiate the tribal or folk claims for its use as a safe hepatoprotective drug. **Methods:** The water, ethanol and *n*-hexane extracts of *L. flexuosum* were tested *in vivo* in Wistar rats. In acute toxicity model, rats received a single dose (5 g/kg) of water extract, ethanol extract, and *n*-hexane extract of *L. flexuosum* and kept under observation for 14 d. In subacute toxicity studies, animals were treated with daily doses (1 g/kg body weight) of water, ethanol and *n*-hexane extract respectively for 30 d. **Results:** In acute toxicity, the extracts administration up to a higher dose of 5 g/kg did not result in mortality or any change in behavior and biochemical parameters of the animals. Sub acute toxicity studies in rats showed that treatment with the extracts did not alter the serum biochemical and hematological parameters. **Conclusions:** The extracts were found to be devoid of any toxicity in acute (5 g/kg) and subacute (1 g/kg) toxicity evaluation in rats.

1. Introduction

Herbal medicines are generally complex mixtures of many bioactive compounds and compared to single-agent pharmaceuticals, phytomedicines may differ in the different mechanisms of action of bioactive constituents, in their dose-response relationships, and in the synergistic/combinatorial effects[1]. Pharmacological studies have established their growing relevance in search of more dependable herbal drugs free of any side effects[2]. Thus, toxicity testing in animals is carried out on new drugs to identify potential health hazards before the drugs are given to man, with doses well above the expected therapeutic range. Toxicity studies involve wide range of tests in different species with regular monitoring for physiological or biochemical abnormalities observed in long-term administration of the drug[3].

Lygodium flexuosum (*L. flexuosum*) (Lygodiaceae) is a climbing fern found all over the Western Ghat region of Kerala, India. Leaf paste is used to cure jaundice by Kadar tribes of Western Ghats of India[4]. The rhizome and root is used in indigenous medicine for the treatment of jaundice by Rabha, Oraon & Mech tribes of West Bengal, India[5]. Chemical characterization revealed the presence of saponins (27.6%), bitter principles (4.6%), sterols (2.0%) and triterpene alcohols (1.7%) in the *n*-hexane extract of *L. flexuosum*[6-7]. Activity of *L. flexuosum n*-hexane extract was further studied and showed efficacy in chronic disease models[8-10]. But the safety of the plant extract is not verified so far and the present work is aimed at to study the acute and sub-acute toxic effect of *L. flexuosum* in rats.

2. Materials and methods

2.1. Chemicals

LDH assay kit was from Sigma Chemical Co., St. Louis, MO, USA. AST, ALT, glucose, urea, triglycerides, cholesterol and creatinine assay kits were from Dialab, Austria. All other chemicals were of high purity grade.

2.2. Plant material and preparation of plant extracts

Plants were collected from its natural habitat at Thiruvananthapuram district during the month of November and authenticated. A voucher specimen (ETHNO.2) is maintained in the Institute. Plants were cleaned, dried

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under shade at room temperature and powdered. 10 g of dried powder of the whole plant of *L. flexuosum* was soxhlet extracted with 400 mL of *n*-hexane for 24 h. The step was repeated with fresh powder and solvent until the required quantity was achieved. The extract was concentrated in a rotary evaporator (yield 4.0%) and the concentrate was suspended in 5% Tween 80.

2.3. Experimental animals

Male Wistar rats (150-160 g) were used in the experiments. Animals were provided standard pellet diet and water ad libitum and maintained on a 12 h light/dark cycle. Animal studies were approved by the CPCSEA (No: IAEC/36/VVA/2006) and conducted humanely.

2.4. Acute toxicity evaluation in rats

Rats were divided into four groups. Group I was treated as normal control, Group II - IV received a single dose (5 g/kg) of water extract, ethanol extract, *n*-hexane extract of *L. flexuosum* and kept under observation for 14 d[11]. Initial and final body weights, water and food intake, state of faecal matter and body temperatures were monitored. Animals were sacrificed on 15th day. The serum levels were estimated for AST, ALT, LDH, glucose, urea, triglycerides, cholesterol and creatinine[12-19].

2.5. Sub-acute studies in rats

To study the subacute toxicity[11] in rats, four groups of animals were selected, Group I was normal control and Groups II-IV were treated with daily doses (1 g/kg body weight) of water, ethanol and *n*-hexane extract respectively for 30 d. The behavior of the animals was observed for 1 h (10-11 am) for 30 d. Initial and final body weights, water and food intake, state of faecal matter and body temperatures were monitored. The animals were sacrificed on the 31st day. Blood samples were collected from common carotid into heparinized and non-heparinized centrifuge tubes. The heparinized blood was used for hematological study that included leukocyte count, erythrocyte count and hemoglobin estimation. The serum was separated from the non-heparinized blood and was assayed for AST, ALT, LDH, creatinine, cholesterol, triglycerides, glucose and urea. Hematological and serum biochemical parameters were determined following standard methods[11].

2.6. Statistical analysis

Experiments were performed with 6 rats per group with values presented as Mean ± S.D. Statistical significance were determined by one way ANOVA followed by Tukey's post hoc analysis. *P*-values less than or equal to 0.05 were considered significant.

3. Results

3.1. Acute toxicity in rats

The extracts administration up to a high dose of 5 g/kg did not result in mortality or any change in behavior of the animals. Biochemical parameters such as AST, ALT, and LDH, creatinine, triglycerides, cholesterol, glucose and urea in the treated rats were comparable to those in the normal control (Table 1).

Table 1.
Biochemical parameters of rats treated with *L. flexuosum* extracts in acute toxicity studies (*n* = 6).

Treatment groups	AST(IU/L)	ALT(IU/L)	LDH(IU/L)	Urea(mg/dL)	Glucose(mg/dL)	Triglycerides(mg/dL)	Cholesterol(mg/dL)	reatinine(mg/dL)
Normal	158.7 ± 5.7	57.2 ± 5.3	165.1 ± 5.1	35.8 ± 1.4	175.2 ± 2.3	55.1 ± 3.7	75.7 ± 3.2	3.40 ± 0.13
Water extract(5 g/kg)	157.3 ± 4.9	58.2 ± 3.6	165.6 ± 3.6	35.4 ± 1.7	175.6 ± 2.2	55.6 ± 3.7	76.0 ± 3.2	3.40 ± 0.14
Ethanol extract(5 g/kg)	157.7 ± 5.1	57.7 ± 3.9	167.3 ± 3.7	36.1 ± 1.0	175.3 ± 2.2	57.4 ± 4.4	75.4 ± 3.6	3.50 ± 0.10
n-hexane extract(5 g/kg)	158.3 ± 5.6	58.8 ± 4.1	165.8 ± 4.0	35.6 ± 1.6	174.8 ± 2.4	55.9 ± 4.3	75.2 ± 3.5	3.40 ± 0.15

Values are Mean ± S.D; **P*<0.05 *vs.* normal control.

Table 2.
Biochemical parameters of rats treated with *L. flexuosum* extracts in sub acute toxicity (*n* = 6).

Treatment groups	AST(IU/L)	ALT(IU/L)	LDH(IU/L)	Glucose(mg/dL)	Urea(mg/dL)	Triglycerides(mg/dL)	Cholesterol(mg/dL)	reatinine(mg/dL)
Normal	161.8 ± 5.2	57.1 ± 4.5	158.4 ± 7.7	175.2 ± 1.9	36.7 ± 0.7	53.9 ± 1.2	74.8 ± 1.7	3.40 ± 0.10
Water extract(1 g/kg)	156.9 ± 5.1	57.7 ± 3.9	160.6 ± 6.1	174.1 ± 1.6	36.5 ± 1.3	56.7 ± 1.7	76.0 ± 1.6	3.30 ± 0.10
Ethanol extract(1 g/kg)	156.9 ± 5.8	58.4 ± 4.7	158.1 ± 6.1	173.8 ± 1.4	35.6 ± 0.9	55.8 ± 2.7	75.2 ± 1.7	3.50 ± 0.10
n-hexane extract(1 g/kg)	163.2 ± 4.0	60.4 ± 4.8	167.8 ± 6.1	169.2 ± 1.6	36.3 ± 1.1	58.6 ± 2.5	77.6 ± 2.7	3.50 ± 0.07

Values are Mean ± S.D; **P*<0.05 *vs.* normal control.

Table 3.
Hematological parameters of rats treated with *L. flexuosum* in sub acute toxicity (*n* = 6).

Treatment groups	Hemoglobin (g %)	WBC ($\times 10^3/\mu$ L)	Erythrocytes($\times 10^6/\mu$ L)
Normal	13.9 ± 0. 1	2.10 ± 0.07	10.0 ± 0.2
Water extract(1 g/kg)	14.0 ± 0.1	2.20 ± 0.09	10.0 ± 0.2
Ethanol extract(1 g/kg)	14.0 ± 0.1	2.10 ± 0.07	10.0 ± 0.2
n-hexane extract(1 g/kg)	14.2 ± 0.1	2.10 ± 0.05	10.6 ± 0.2

Values are Mean ± S.D; **P*<0.05 *vs.* normal control.

3.2. Sub acute toxicity in rats.

Sub acute toxicity studies in rats showed that treatment with the extract at a higher dose of 1 g/kg daily for 30 d did not alter the serum biochemical parameters like AST, ALT, LDH, creatinine, triglycerides, cholesterol, glucose and urea (Table 2). Hemoglobin content and the erythrocytes and leucocytes counts were also found to be normal in the treated rats when compared with the normal control group (Table 3). In sub acute toxicity evaluation, the extracts administration up to a high daily dose did not result in mortality or any change in behavior of the animals.

4. Discussion

Oral administration of the water, ethanol and n-hexane extracts of *L. flexuosum* in doses from 1 to 5 g/kg body weight did not produce significant changes in behavior, breathing, cutaneous effects, and gastrointestinal effects in rats. There are no adverse effects observed during the administration of *L. flexuosum* extracts, indicating that the median lethal dose (LD_{50}) is higher than 5 g/kg in rats. The doses used in this study were 10 to 25 times higher than those used in our previous *in vivo* pharmacological studies, such as: antifibrotic and antiangiogenic action of *L. flexuosum* extracts in Wistar rats[8-9]. The results show that these three extracts from *L. flexuosum* are safe in oral administration in rodents.

Hepatic function is well preserved by the administration of *L. flexuosum* extract in rats indicated by the serum enzyme levels that was comparable to normal control values. The investigation of the target organs (liver, lung, heart, and kidney) of the treated animals did not show prominent changes in color and texture when compared to the control group. Further, there were no treatment-related effects on the hematological parameters evaluated during the administration of extracts. In summary, the extracts at higher doses did not show any conspicuous toxicity indicating its potential use as a safe hepatoprotective herbal drug and also compromise the medicinal use of this plant in tribal or folk medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The work was supported by grants from Kerala Forest Department, Kerala, India. Financial assistance from Council of Scientific and Industrial Research (CSIR), New Delhi as Senior Research Fellowship to PJ Wills is thankfully acknowledged. The authors are grateful to Mrs. Ciji Varghese and Mr. K Sabulal for their technical assistance.

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