



Original Research Article

Acute Toxicity Study of *Hygrophila auriculata* L. Leaves Methanolic Extract in Albino Rats

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ABSTRACT

The plant *Hygrophila auriculata* (K. Schum) Heine, has been traditionally used for the treatment of inflammation, pain, urinary infection, edema, gout and as a diuretic. However, toxicity study of the *Hygrophila auriculata* leaves extract is still lacking. The present study is to investigate the acute toxicity of methanol extract of leaves of *Hygrophila auriculata* L. on albino rats at a dose of 2000 mg/kg body weight (BW). The purpose of acute toxicity studies is to determine the LD₅₀ values which help in determining the safe dose range at which the drug can be used such that there is no harmful effect on the animal. Sighting study was conducted in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg BW. Based on the sighting study, we have selected a dose of 2000 mg/kg BW and animals were observed for 14 days. The single oral dose of the *Hygrophila auriculata* leaves extract did not produce mortality or significant changes in the body weight, food and water consumption. The internal organs weights of the control & test animals were normal. Apart from triglyceride, other biochemistry parameters demonstrated no significant changes as compared to the control.

Keyword: *Hygrophila auriculata* L.; methanolic extract; acute toxicity; albino rats.

INTRODUCTION

Hygrophila auriculata (L.), a generally occurring wild herb belonging to Acanthaceae family has been advocated for the treatment of variety of diseases including most commonly diabetes and dysentery [1-3]. As per our tradition, roots, seeds, and aerial parts of the plant has been

used in the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, urinary infection, gout and malaria [4]. The plant has been reported to contain flavonoids (apigenin 7-O-glucuronide, apigenin 7-O-glucoside) [5], alkaloids (asteracanthine and asteracanthicine)

[6], aliphatic esters (25-oxo-hentricontyl acetate, methyl-8-hexyltetracosanoate) [7], minerals (Fe, Cu, Co) [8], sterols (stigmasterol) [9], triterpenes (lupeol, hentricotane, betulin, luteolin, luteolin 7-O-rutinosides) [7,10]. Earlier scientific investigation showed that the crude extract of *Hygrophila auriculata* has anti-nociceptive [11], antitumor [12,13], antibacterial [14,15], antioxidant [16,17], hepatoprotective [18-20], hypoglycemic [21], haematinic [22], diuretic [23], anabolic and androgenic activities [24]. However, the toxicology study of *Hygrophila auriculata* L. has not been carried out. Therefore the present study is to investigate the acute toxicity of *Hygrophila auriculata* leaves extract on albino rats.

MATERIALS AND METHODS

Plant material

The plant leaves of *Hygrophila auriculata* L. were collected from Ratnagiri district, Maharashtra in the month of Oct-Nov 2011 and authenticated by Dr. S. G. Bhawe, HOD, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, College of forestry, Dapoli, Ratnagiri.

Preparation of plant extract

The dried powder of shade dried leaves was defatted with petroleum ether (60-80^o C) in a Soxhlet apparatus. The defatted powder materials thus obtained were further extracted with, chloroform, acetone, methanol and aqueous solvents successively. The solvent was removed by distillation under low pressure. Based on the preliminary phytochemical studies, methanolic extract was selected for toxicity studies.

Experimental Animals:

Wister albino female rats (150-200g) of approximate same age were procured from listed suppliers of National Institute of Bioscience, Pune, India. The animals were fed with standard pellet diet (Hindustan lever Ltd., Bangalore) and water ad libitum. All the animals

were housed in polypropylene cages. The animals were kept under alternate 12 hours dark/light cycle. The animals were acclimatized to the laboratory condition for one week before starting the experiment [25]. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocol was approved by Institutional Animal Ethics Committee (Reg.No.1092/ac/07/CPCSEA/02/2012).

Study design and selection of doses

Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001) [26]. The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study. The sighting study comprised of female rats dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. Starting with 5 mg/kg BW, the test article was administered orally to one rat. The rat was then observed for toxic effect for the first 30 min followed by hourly for 8 h for the first 24 h. If there is no signs of toxic effect or mortality observed on the rat within the 24 hours, then we dosed another rat with the next dose (50 mg/kg BW) and a similar procedure was carried out. A stepwise procedure was carried out until the highest dose, 2000 mg/kg BW is reached. If all the rats survived, they were monitored and observed once daily for the next 13 days. The sighting study showed that the rats dosed with 5, 50, 300 and 2000 mg/kg BW with the test article survived. Based on this observation we decided to use the highest dose 2000 mg/kg BW for the main acute toxicity study. The acute toxicity study comprised of two groups, one control and one treatment group that consisted of five female rats in each group. Female rats were chosen because it is the most sensitive gender to see the effect of treatment [27]. The treatment group received

methanolic extract of *Hygrophila auriculata* leaves at a dose 2000 mg/kg BW given orally once. The control group received water delivered in the same volume and same procedure as the treatment group. The experimental animals were observed for 30 min after treatment, followed by observation hourly for 8 h and once daily for the next 13 days.

Physical observation and mortality

Clinical observation were made once a day for mortality, moribund, ill health or reaction to treatment, such as changes in skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, diarrhea, sleep and coma.

Body weight, food and water consumption

The body weights (BW) of each rat were recorded once weekly. The differences of the BW were recorded. The amounts of food placed in the food tray were about 200 g and this amount was enough for a week. However, the amount of food left in the tray at the end of the week were calculated to obtain the amount of food consumed. The amounts of water placed in the bottle were 200 ml. The level of the water was measured weekly. The amounts of water consumed were calculated from this information.

Hematological and biochemical analysis

At the end of 14 days period blood was withdrawn through cardiac puncture from the posterior vena cava of all rats under ether anesthesia. The blood was placed into EDTA bottles for hematological assay and in plain bottle for clinical biochemistry determination. The relative blood indices white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), erythrocyte sedimentation rate (ESR), platelets and clotting time were determined using routine method.

The blood in the plain bottle was allowed to stand for a minimum of 3h for complete clotting. The serums were collected and transferred into another tubes to be centrifuged at 4000 rpm at 4°C for 10 min. The serums were then kept at -20°C until analysis for clinical biochemistry measurements using the Vita lab Selecta, E-series, Netherlands. Clinical Biochemistry values determination were for liver profile (total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Renal profile parameters measured were urea and uric acid. Creatinine kinase (CK) and lactate dehydrogenase (LDH) were the cardiac profile parameters and other biochemistries were glucose and HDL-Cholesterol, cholesterol and triglycerides for lipid profile.

Organ weight

All control and treatment rats were sacrificed using an overdose of ether on Day 15. A complete necropsy was performed. A comprehensive gross observation were carried out on the internal organs namely liver, kidneys, heart, lungs, spleen & brain. They were observed for any signs of abnormality and presence of lesions. The organs were then carefully dissected out, cleaned of any fats and weighed. Since liver and kidney are organs of metabolism and excretion, potentially toxic agents are likely to affect them, so portions of these organs were fixed in buffered 10% formalin and 5 µm thick paraffin sections were made and stained with haematoxylin and eosin for Histopathological study [28].

Statistical analysis

All findings such as body weight changes, food and water consumption, hematology and blood chemistry were tabulated and analyzed. The results were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by

Turkey multiple comparison tests. P values < 0.05 were considered as significant.

RESULTS

Sighting study

The sighting study did not result with any signs of toxic effect at all the dose level tested 5, 50, 300 and 2000 mg/kg BW. All the rats survived. Based on this observation we then selected a dose level of 2000 mg/kg BW for the main test (Acute Toxicity study).

Body weight, food and water consumption

The body weight of the treatment and control rats were as shown in Table 1. There were gradual increases in body weight of treatment and control rats. The body weight of the treatment rats were not significant different as compared to the control rats. The food and water consumption of the treatment rats were also not significantly different as compared to the control rats measured throughout the study (Table No. 1).

Hematological and clinical biochemistry

Hematology and clinical biochemistry data are presented in Tables No. 2, 3 and 4 respectively.

Hematological values measured showed a significant elevation of HGB level and RBC level with $p=0.035$ and $p=0.016$ respectively in treatment group. Other hematology values WBC, ESR, Platelets & clotting time were not significantly different as compared to the control rats and they remained within normal limits (control values). The clinical biochemistry values of total protein and triglycerides were elevated in the treatment rats as compared to the control rats, with significant value at $p=0.011$ and $p=0.02$ respectively (Table No. 3 & 4). Other clinical biochemistry parameters measured, albumin, ALP, AST, ALT, urea, uric acid, CK, LDH, HDL-cholesterol, cholesterol and glucose of the treatment rats were not significantly different as compared to the control.

Gross necropsy

Gross necropsy findings did not reveal changes in any of the organs examined. The organ weight recorded at the end of the study did not show any significant difference as compared with control (Table No. 5)

Table: 1 Body Weight (g), food consumption (g) & water intake (ml) by control & rats treated with methanolic extract of *Hygrophila auriculata* leaves recorded during acute toxicity study

Treatment	Body weight (g)		Food consumption (g)		Water intake (ml)	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
Control	185 ± 5.10	192 ± 4.31	79.2 ± 3.21	77.4 ± 1.69	211 ± 4.30	197.2 ± 2.08
Methanolic Ext. of HA 2000 mg/kg BW	187.4 ± 4.21	204.6 ± 3.75	81.4 ± 2.33	85.8 ± 2.37	225 ± 5.70	202 ± 2.55

Values are expressed as mean ± standard deviation, n = 5. *p value less than 0.05, (p < 0.05) significant value

Table: 2 Hematological values of control and rats treated with methanolic extract of *Hygrophila auriculata* leaves measured during the acute toxicity study

Treatment	RBC (10 ⁶ × μL)	Total WBC (10 ³ /μl)	Hb (gm %)	ESR (mm/1 st hr)	Platelets (K/μL)	Clotting time (Sec)
Control	6 ± 0.31	4.2 ± 0.37	12.6 ± 0.50	3.5 ± 0.37	4.2 ± 0.37	131 ± 1.00
Methanolic Ext. of HA 2000 mg/kg BW	7.2 ± 0.37*	3.8 ± 0.20	13.4 ± 0.50*	4.2 ± 0.20	3.8 ± 0.20	126.6 ± 1.40

Values are expressed as mean ± standard deviation, n = 5. Group given 2000 mg/kg BW of methanolic extract of *Hygrophila auriculata* leaves single dose orally and observed for 14 days. *p value less than 0.05, (p < 0.05): significant value

Table: 3 Clinical Biochemistry values of control & rat treated with methanolic extract of *Hygrophila auriculata* leaves measured during acute toxicity study

Treatment	Liver profile				Renal profile		
	Total protein (g/L)	Albumin (g/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	Urea (mmol/L)	Uric acid (μmol/L)
Control	152.6 ± 3.32	39.4 ± 1.07	258.6 ± 0.74	159.8 ± 1.31	47 ± 1.30	5.58 ± 0.14	161.8 ± 1.77
Methanolic Ext. of HA 2000 mg/kg BW	161.6 ± 2.54*	44 ± 1.14	262.2 ± 3.39	168 ± 1.58	51.6 ± 120	6.42 ± 0.17	202.2 ± 2.45

Values are expressed as mean ± standard deviation, n = 5. *p value less than 0.05, (p < 0.05) significant value

Table: 4 Clinical Biochemistry values of control & rat treated with methanolic extract of *Hygrophila auriculata* leaves measured during acute toxicity study

Treatment	Cardiac profile			Lipid profile		
	CK (U/L)	LDH (U/L)	HDL- Cholesterol (mmol/L)	Cholesterol (mmol/L)	Triglycerid es (mmol/L)	Glucose (mmol/L)
Control	1098.6 ± 40.81	2446.2 ± 23.61	0.68 ± 0.02	1.47 ± 0.03	0.78 ± 0.04	9.27 ± 0.19
Methanolic Ext. of HA 2000 mg/kg BW	1252.2 ± 23.42	2247.8 ± 15.13	0.79 ± 0.09	1.77 ± 0.04*	0.91 ± 0.06*	10.17 ± 0.11

Values are expressed as mean ± standard deviation, n = 5. *p value less than 0.05, (p < 0.05) significant value

Table: 5 Organ weights of control rats & rat treated with methanolic extract of *Hygrophila auriculata* leaves measured during acute toxicity study

Treatment	Liver (gm)	Kidney (gm)	Heart (gm)	Lungs (gm)	Spleen (gm)	Brain (gm)
Control	3.46 ± 0.12	1.17 ± 0.19	0.68 ± 0.03	1.25 ± 0.14	0.95 ± 0.07	1.65 ± 0.58
Methanolic Ext. of HA 2000 mg/kg BW	3.75 ± 0.10	1.13 ± 0.17	0.76 ± 0.04	1.15 ± 0.14	1.06 ± 0.06	1.69 ± 0.24

Values are expressed as mean ± standard deviation, n= 5.*p value less than 0.05, (p < 0.05) significant value

DISCUSSION

The use of herbal preparations as a treatment of diseases is very common. A survey of the published literature about *Hygrophila auriculata* showed that it is a popular remedy used by a variety of ethnic groups, vaidyas, Hakim and Ayurvedic practitioners to treat a variety of ailments. However, despite claims by traditional medicine practitioners, the acute toxicology study of *Hygrophila auriculata* (L.) has not been carried out. In our study it was observed that the animals fed with extract of *Hygrophila auriculata* leaves extract were healthy. There was no significant difference in the food and water consumption between the treatment and control groups. (Table No. 1) The *Hygrophila auriculata* leaves extract did not affect the body weight of the treatment rats when compared to the control rats. The increases in body weight were in line with the increase in food & water consumed by the rats. Gross pathological examination of treatment did not reveal any abnormalities, presence of lesions or changes in the color of the internal organs and the relative organ weight were not significant different to the control. For hematological parameter, HGB and RBC were found to be significantly increased as compared to the control rats. The HGB level was increased in treated rats may result from increased in red blood cell production and increased in production of growth factors [29]. This is also

supported by the findings of an increase of total protein in the treatment group which is another finding that indicate dehydration state [30]. The other hematology parameters as well as biochemical parameters (except triglycerides and total protein) were normal. The three most important and common liver enzymes like AST, ALT and ALP which were not affected by the administration of *Hygrophila auriculata* leaves extract (Table No.3). Renal profile such as urea, uric acid, CK and LDH were all normal as control group and indicated that there were no renal damage caused by *Hygrophila auriculata* leaves extract to the rats (Table No.3, 4).

CONCLUSION

It was concluded that the acute toxicity study of methanolic extract of *Hygrophila auriculata* leaves at 2000 mg/kg BW administered orally to albino rats did not caused any death or acute adverse effect on the clinical observation and mortality to the treatment rats. However, from the blood investigation, it showed that methanolic extract of *Hygrophila auriculata* leaves consumption may cause dehydration as demonstrated by increased in HGB and RBC as well as total protein level. Other parameters were normal. This finding will be monitored in the sub-acute toxicity study. The above results will be the beneficial for further pharmacological effects and independent activity of *Hygrophila auriculata* L. plant.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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