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Acute Toxicity, *In-vitro* Urolithiatic & Diuretic Evaluation of Methanolic Extract of *Hygrophila salicifolia*, Whole herb in Rat

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ABSTRACT

Hygrophila salicifolia (Family: Acanthaceae) is an erect herb, nearly glabrous, mainly whole herb used as raw material in medicine. The aim of present investigation was to evaluate acute toxicity, *in vitro* urolithiatic and diuretic activity of methanolic extract of *Hygrophila salicifolia*, whole herb. Methanolic extract of *Hygrophila salicifolia* was administered to experimental rats orally at 2000 mg/kg *p.o*. The acute toxicity of the extract was evaluated as per OECD guideline 420. The LD_{50} of the extract was found to be between 2000-5000 mg/kg *p.o*. Urolithiatic activity was determined for all extracts using Conductometric titration and crystal growth inhibition. Methanolic extract of *Hygrophila salicifolia* was administered to experimental rats orally at doses 300 & 500 mg/kg *p.o*. Furosemide (10 mg/kg) was used as positive control. The diuretic potential of the extracts was evaluated by measuring urine volume, Diuretic index, Lipchitz value, Saliuretic index, Na^+/K^+ ratio and excretion of sodium-potassium content. The present study provided quantitative and qualitative basis for explaining non toxicity, urolithiatic and diuretic potential of methanolic extract of *Hygrophila salicifolia*

Keywords: *Hygrophila salicifolia,* Acute toxicity, Urolithiatic, Diuretic activity, Methanolic extract.

INTRODUCTION

Herbal drugs have been used since ancient times to treat various diseases of mankind. When we apply any new compounds to the biological system of the body, it produces various drug interactions and dose related responses. In many cases these interactions are good, advantageous but sometimes it shows other effects which are toxic. So toxicity tests are conducted in the development of new drugs. These tests include acute, sub-acute and chronic toxicity. Acute toxicity study gives us LD_{50} value. Oral acute toxicity study provides us primary screening test to evaluate the toxicity of new developed drug. Acute toxicity study is single dose study, dose up to 2000 mg/kg given at once or successively. Drug-induced diuresis is advantageous in many life frightening ailments such as hypertension, congestive cardiac failure, pregnancy, kidney failure and liver dysfunctioning. [1] Most of diuretic drugs show side effects such as weakness, impotence and fatigue. [2] Hence there is urgent necessity to search safe diuretic drug which is potassium sparing and causes excretion of sodium. [1-3] Now-a-days 80% of world population relies on natural remedies to heal various diseases. But major drawback in case of herbal drugs is that they are devoid of clinical and scientific data to prove their safety & efficacy as therapeutic agent. *Hygrophila salicifolia* has been advocated for the treatment of various diseases including jaundice, urinary infection, gouts, hepatic disorder, rheumatism, impotence, anti-bacterial and inflammation. [4] It is an

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erect or ascending herb belongs to the family Acantheceae. It is bestowed with many medicinal uses in traditional systems of medicine including ayurveda. Seeds and leaves used as poultice on inflammatory swellings. Leaves are strongly diuretic. The plant is found in moist and marshy places throughout the greater part of India. Stems up to 3 ft. long, more or less quadrangular, rooting at the lower nodes, leaves sub sessile, linear-lanceolate; flowers pale purple in dense axillary whorls, capsule oblong, seeds many, ovoid, compressed, mucilaginous, hairy. The leaves are eaten as pot- herb. They contain 8% ash rich in potassium and are strongly diuretic. In Malaya, the leaves are used as poulticing swellings. The seeds swell into a gelatinous shining mass with water and used in java, in poultices for headaches and fevers. They yield 25% of a fatty oil and contain traces of unidentified alkaloid, a bitter substance and 4% ash consisting chiefly of calcium phosphate and potassium chloride. [4-5] The plant has been reported to contain chlorophyll, pigments, gums, glucose, starch, fat, various minerals, alkaloids, flavanoids, tannins and sterols. The toxicology, diuretic & urolithiatic study of *Hygrophila salicifolia* has not been carried out. Therefore an effort was made in this investigation to evaluate the acute toxicity, urolithiatic activity and diuretic potential of methanolic extract of *Hygrophila salicifolia*.

MATERIAL AND METHODS

Procurement of chemicals

Furosemide was obtained from khandelwal laboratories, Mumbai. The solvents used were of laboratory grade obtained from Emerck Ltd. Mumbai.

Plant material & extract

Whole herb was obtained from the local areas in and around Gujarat. The herb was identified and authenticated as *H. salicifolia* in the Bioscience department, Vallabh Vidyanagar, Gujarat. The plant was shade dried and ground to a fine powder in grinder to produce a coarse powder. Plant extracts were then obtained using soxhlet's apparatus with successive solvents. The extracts were concentrated using vacuum evaporator. It was dried in desiccators. [6-8]

Animals

Wistar albino rats weighing between (150-200 g) were selected for the work. They were kept in environmental conditions and fed with standard rodent diet and water *ad libitum*. All animal experimental procedures were done accordingly CPCSEA guidelines and approved by the IAEC. (Reg. No. ARL/PT/005/2014 & ARL/PT/024/2015 for diuretic activity & acute toxicity study respectively).

Acute toxicity

Acute oral toxicity test was carried out according to the organization for Economic co-operation and development (OECD) guideline 420. The investigation was started with sighting study aimed to determine the dose for the acute toxicity study. The study consisted of female rats which were given highest single dose 2000 mg/kg of methanolic extract of *Hygrophila salicifolia*. Immediately after dosing the animals were observed for their behavior continuously for the first four hours. They were kept under observation up to 14 days after extract injection to check the mortality & body weight. [9-12]

In vitro urolithiatic activity

Double diffusion gel study

The crystallization apparatus used was glass U-tubes with 2.5 cm internal diameter, 16 cm limb lengths and the separation between two limbs approximately 12 cm. The neutral gel was prepared by mixing hydrated sodium meta-silicate solution of specific gravity 1.06 with 3M acetic acid solution, so that the pH of mixture was maintained at 5.5. The mixture was transferred into the U-tubes for setting the gel. After the gel was perfectly set, 1M oxalic acid solution was poured into the left limb and the same volume of mixture containing 1M $CaCl_2$ and 1M magnesium acetate solutions in equal amounts was poured into the right limb. The crystals were grown at the gel–liquid interface in the left limb part of the U-tubes. [13] The following solutions were selected to be poured into the right limb of the U tubes. Solution A: is having 10 ml, 1M calcium chloride and 10 ml, 1M magnesium acetate solutions. Solution B: is having 5 ml, 1M calcium chloride, 5 ml, 1M magnesium acetate and 10 ml Successive extracts of *Hygrophila salicifolia* (4% w/v). Apparent length of Crystal was used as evaluation parameter.

Conductometry Titration

10 ml 0.05M $CaCl_2$ and 0.05 M $Na_2C_2O_4$ were taken in beaker and burette respectively. Conductance was measured in the absence and presence of different extracts of test drugs. [14] The curve of conductance (mmhos) vs. Volume of titrate (ml) was drawn to find out the urolithiatic activity.

Evaluation of Diuretic activity

Male wistar rats weighing 150-200g deprived of food and water for an overnight period. They were divided into four groups. Each consisted of six rats. Lipchitz *et al*., method was used for the evaluation of diuretic activity. Before treatment, all animals were hydrated with (0.9% NaCl) physiological saline. Group-I (control) was received with normal saline solution (25 ml/kg, *p.o*). Group-II (Standard, Positive control) was received Furosemide (20 mg/kg, *p.o*) in saline. Group-III & Group-IV received the methanolic extract at doses 300 mg/kg and 500 mg/kg respectively. Doses of extract were based on acute toxicity studies. Immediately after oral administration, the rats were paired in metabolic cages. Urinary output was collected in graduated cylinder and recorded at the end of 5 h. Evaluation parameters such as total urine volume and concentrations of Na+, K+ and Cl- in the urine were noted. Concentration of Na^+, K^+ were determined using flame photometer and Cl^- concentration was estimated titrimetrically using (N/50) $AgNO_3$ with few drops of

5% potassium chromate solution as indicator. [15-22] Diuretic index, Saliuretic index, Lipchitz's value and Na^+/K^+ ratio were determined.

Statistical Analysis

The data were expressed as mean ± S.E.M and analyzed using one way ANOVA followed by Dunnett's multiple comparison test. $P< 0.05$ was considered as statistically significant.

RESULTS & DISCUSSION

In the acute toxicity study, the single administration of the extract up to 2000 mg/kg body weight did not produce adverse reaction or mortality. There were no significant changes in body weight and feed consumption after administration of test article in animals (Table 1, Figure 1).

Table 1: Evaluation Parameter: Body Weight (g)

Mark	Change in Body weight (g) at specific Day		
	0	7	14
H	492	490	499
B	486	463	468
T	457	503	503
BT	453	443	462
HT	460	455	473
U	432	434	436
AVG	471.5	483	485.5
Sd	20.50609665	28.28427125	24.74873734
SEM	11.83920042	16.32993162	14.28869017

Table 2: Evaluation Parameter: Detailed clinical observation

		Dose: 2000 mg/kg								
Animal Mark	Observation Parameter	Change at Specific Time interval (h)								
		0	0-30	1	2	3	4	4-12	12-24	After 24
H	Fur	White	White	White	White	White	White	White	White	White
	Tremor	NO	NO	NO	NO	NO	NO	NO	NO	NO
	Salivation	NO	NO	NO	NO	NO	NO	NO	NO	NO
	Diarrhoea	NO	NO	NO	NO	NO	NO	YES	NO	NO
	Lethargy	NO	NO	NO	NO	NO	NO	YES	NO	NO
	Sleep	NO	NO	NO	NO	NO	NO	NO	NO	NO
	Respiratory	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	Behaviour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	Mortalirty	NO	NO	NO	NO	NO	NO	NO	NO	NO

Table 3: Evaluation Parameter: Organ Weight

Animal Mark	Organ weights				
	liver	Kidney	brain	heart	lungs
HT	14.89	2.77	2.01	1.62	2.52
BT	13.76	2.43	2.12	1.41	2.31
U	14.62	3.04	2.11	1.39	2.45

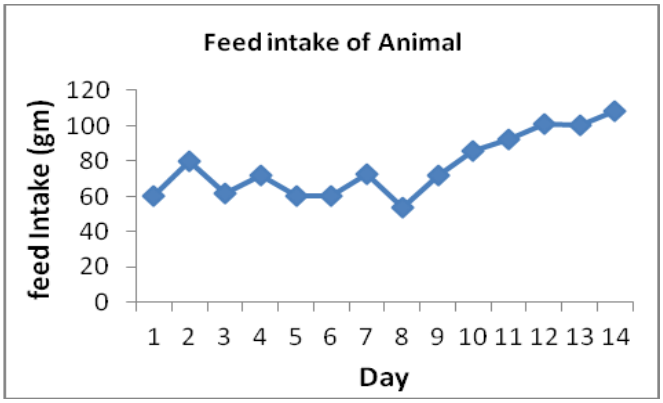

Fig. 1: Evaluation Parameter: Feed Intake (g)

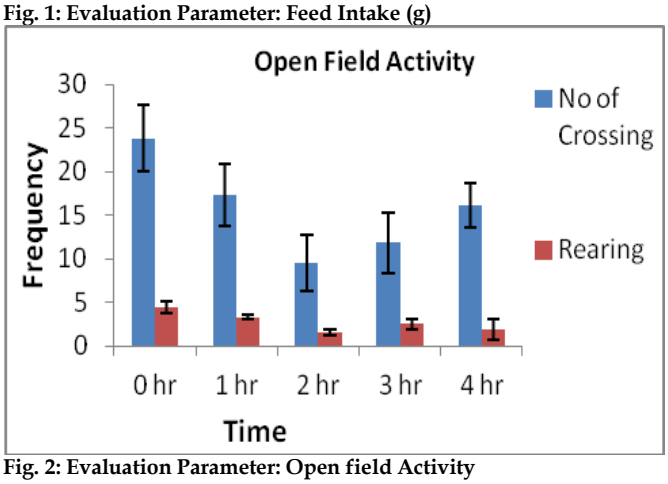

Fig. 2: Evaluation Parameter: Open field Activity

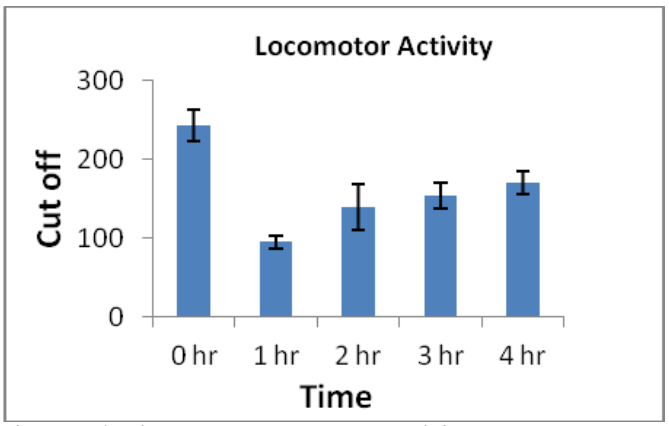

Fig. 3: Evaluation Parameter: Locomotor activity

There was decreased in Locomotors activity up to four hours after dose administration in Open Field test and Actophotometer test (Figure 2 & 3). After dose administration, no sign of skin and/or eye irritation, tremors, convulsion, Salivation, Diarrhea, Lethargy, Sleep and coma were observed (Table 2). After 14 days gross necropsy and histology of major organ like, brain, lungs, heart, liver and kidney was performed and no sign of toxicity were observed (Table 3, Figure 4).

It was found from double diffusion gel method that average apparent length of crystals decreased significantly with time compared to control in methanolic extract than other successive extracts of *Hygrophila salicifolia* (Figure 5-9). Conductometric titration of $CaCl_2$ with $Na_2C_2O_4$ was carried out in the absence and presence of different extracts of *Hygrophila salicifolia*. Conductometric titration curves showed shift

in end point towards lower side in a conc. dependent manner due to reduction in free Ca^{2+} Content (Table 4). It was found that the methanolic extract of *Hygrophila salicifolia* at high dose showed equipotent diuretic activity as that of the standard drug (furosemide). As compared to control, the methanolic extract of *Hygrophila salicifolia* caused increase in Na^+, K^+ and Cl^- in dose dependent manner. Urine volume of Methanolic extract of *Hygrophila salicifolia* at the dose of 300 mg/kg body weight and 500 mg/kg body weight was 8.38 ± 0.25 and 13.15 ± 0.12 ($p < 0.05$) respectively, compared to the control group which was 3.02 ± 0.20 (Table 4 & 5). However urinary electrolyte excretions of both doses were less when compared with standard drug furosemide. So it was revealed from the result that methanolic extract of the plant showed significant diuretic action (Table 4 & 5). As emphasized diuretic property of methanolic extract of *Hygrophila salicifolia* might be due to its phytoconstituents such as alkaloid, flavanoid and tannins.

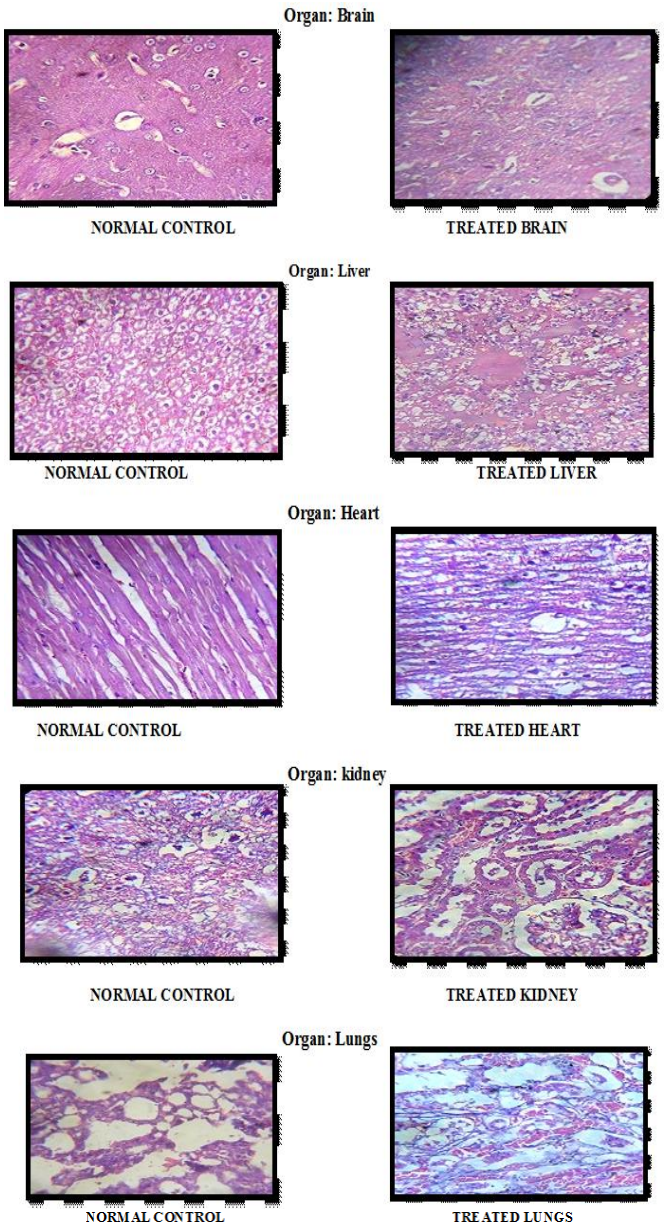

Fig. 4: After 14 days gross necropsy and histology of major organ

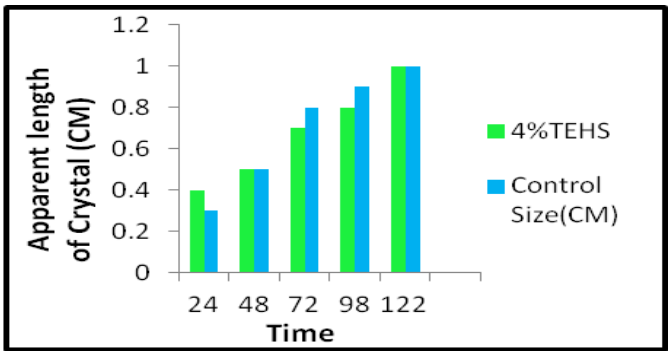

Fig. 5: Effect of growth Control VsToluene Extract

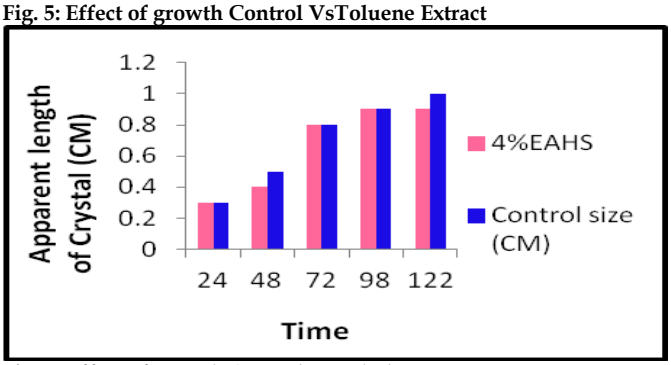

Fig. 6: Effect of growth Control Vs Ethyl acetate Extract

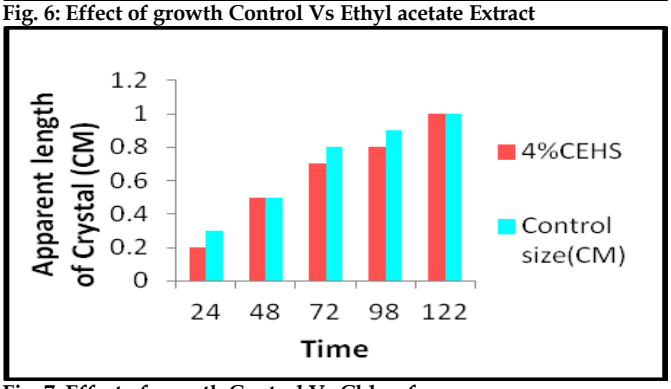

Fig. 7: Effect of growth Control Vs Chloroform

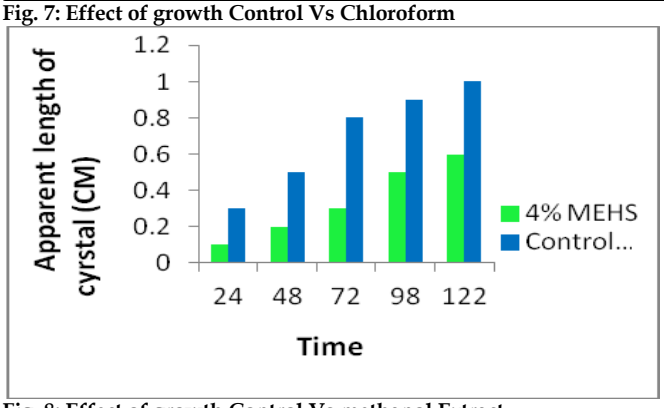

Fig. 8: Effect of growth Control Vs methanol Extract

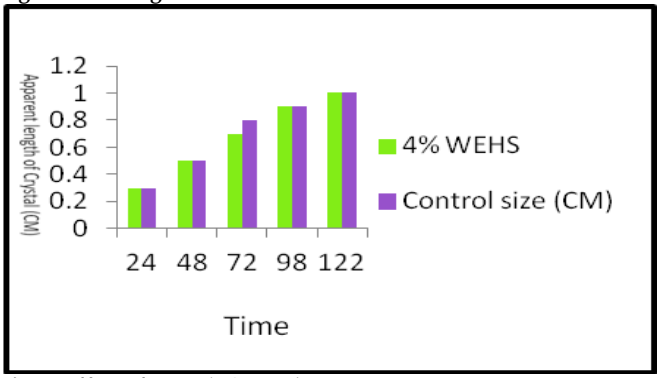

Fig. 9: Effect of growth Control Vs water Extract

Table 4: Conductometric titration: in absence and presence of various extracts of *Hygrophila salicifolia*

Control	4% TEHS	4% CEHS	4% EAEHS	4% MEHS	4% WEHS	Volume of $Na_2C_2O_4$ (ml)
1.2	1.25	1.22	1.18	1.8	1.23	0
1.15	1.2	1.18	1.3	1.23	1.19	1
0.86	0.93	0.92	0.82	1.12	0.81	2
0.61	0.9	0.88	0.78	0.73	0.6	3
0.6	0.91	0.85	0.75	0.62	0.59	4
0.53	0.88	0.82	0.69	0.78	0.54	5
0.5	0.84	0.79	0.62	0.82	0.52	6
0.49	0.82	0.76	0.59	0.89	0.49	7
0.39	0.75	0.7	0.54	0.93	0.44	8
0.35	0.85	0.89	0.78	0.98	0.83	9
0.86	0.88	0.85	0.84	1.13	0.89	10
0.99	0.97	0.98	0.91	1.23	0.95	11
1.13	1.1	1.15	1.15	1.28	1.15	12
1.3	1.5	1.8	1.6	1.3	1.4	13
1.44	1.49	1.48	1.39	1.49	1.47	14
1.58	1.5	1.61	1.54	1.6	1.55	15
1.76	1.73	1.78	1.72	1.82	1.78	16
1.91	1.89	1.93	1.88	1.95	1.88	17
2.07	2.09	2.04	2.1	2.5	2.08	18

Table 5: Effect of methanolic extract of *Hygrophila salicifolia* on urine volume

Sr. No.	Treatment	Mean urine volume (ml)	Lipchitz value	Diuretic index
1	Control	3.02 ± 0.20	---	---
2	Standard	14.63 ± 0.15	---	4.85
3	Methanolic extract- 300 mg/kg	8.38 ± 0.25	0.57	2.86
4	Methanolic extract- 500 mg/kg	13.15 ± 0.12	0.89	4.47

Table 6: Effect of methanolic extracts of *Hygrophila salicifolia* on electrolyte concentration

Sr. No.	Treatment	Saliuretic index	Concentration Electrolyte Na^+	Concentration Electrolyte K^+	Na^+/K^+ ratio	Concentration Electrolyte Cl^-
1	Control	----	4.51 ± 0.17	9.68 ± 0.16	0.47	13.57 ± 0.17
2	Standard	1.65	12.70 ± 0.11	15.54 ± 0.08	0.82	18.54 ± 0.01
3	Methanolic extract- 300 mg/kg	1.31	9.39 ± 0.07	12.62 ± 0.16	0.74	14.41 ± 0.14
4	Methanolic extract- 500 mg/kg	1.55	11.45 ± 0.12	13.90 ± 0.21	0.82	17.63 ± 0.34

A single oral dose of 2000 mg/kg of Methanolic extract administration in animal followed by continues observation up to four hour and then daily two time observation was carried out. In a period of four hours after dosing, locomotor activity of animal was decreased. The bodyweight and feed consumption of those animals were normal. There was no significant difference in gross necropsy as well as histology of major organ like brain, lungs, heart, liver and kidney. As per OECD guideline 420, The LD_{50} of Methanolic extract of *Hygrophila salicifolia* was found to be 2000-5000mg/kg, orally and it was safe, non toxic. It was found from *in vitro* study that methanolic extract of plant showed urolithiatic activity than other successive extracts of the plant. Methanolic extract of *Hygrophila salicifolia* was found to be strong diuretic agent.

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