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Original Research Article

Acute oral toxicity study of ethanolic extract of *Hygrophila auriculata* and *Hygrophila polysperma* leaves using Wistar Albino rats

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ABSTRACT

Background: To find out toxicity and fix the safe dose of ethanolic extract of *Hygrophila auriculata* and *Hygrophila polysperma* leaves using female Wistar Albino rats.
Methods: Acute oral toxicity was assessed in wistar albino rat following organisation for economic co-operation and development guideline 423. The ethanolic extracts were administered orally at doses of 300 mg/kg and 2000 mg/kg body weight and animals were observed for 14 days for mortality, behavioural changes, body weight variation and gross pathological alterations.
Results: No mortality or significant signs of toxicity were observed in any treatment group. Body weight, food intake and organ morphology remained normal throughout the study period. The extracts were well tolerated at the tested doses.
Conclusions: The ethanolic leaf extracts of *H. auriculata* and *H. polysperma* were found to be safe up to 2000 mg/kg, indicating low acute toxicity and supporting their further pharmacological evaluation.

Keywords: Acute oral toxicity, *Hygrophila auriculata*, *Hygrophila polysperma*, OECD guidelines

INTRODUCTION

The use of herbal preparations in traditional folklore medicine has been prevalent in rural regions and these treatments continue to be commonly employed for a range of ailments.[1] Given their extensive use, current research is increasingly focused on evaluating the efficacy and safety profiles of medicinal plants.[2] Although therapeutic plants are often presumed to exhibit low toxicity because of their historical use in human populations, studies have demonstrated that numerous folkloric medicinal plants can produce adverse effects. Nevertheless, natural products remain the basis for a substantial proportion of contemporary pharmaceuticals.[3,4] According to the organization for economic cooperation and development (OECD), acute toxicity refers to effects that manifest shortly following the oral administration of either a single dose or multiple doses of a chemical within a 24-hour timeframe. Toxicological evaluations are categorized as acute, subacute or chronic, depending on the duration of chemical exposure in animal models. Acute toxicity studies specifically assess short-term toxicological outcomes, typically over a period of 1 to 2 weeks.[5]

Hygrophila auriculata (Schumach.) Heine, a member of the Acanthaceae family, is native to India and found throughout tropical and subtropical regions. Traditionally, it has been used to treat cancer and tubercular fistula. Its roots and seeds serve as a tonic and are used for asthma and dysentery. The leaves, roots and seeds are also employed in traditional medicine for inflammation, jaundice, hepatic obstruction, urinary infections, edema,

gout, diabetes and bacterial infections.[6] In Ayurvedic literature, the plant is known by several names, including Ikshura, Ikshugandha, Kokilaksha and Indian Cuckoo. Within the Ayurvedic system of medicine, it is further characterized by its properties, being referred to as Seethaveryam and Mathuravipaka.[7]

Table 1: Taxonomy of *H. auriculata*.[8]

Kingdom	Plantae
Class	Magnoliopsida
Subkingdom	Viridiplantae
Superorder	Asteranae
Infrakingdom	Streptophyta
Order	Lamiales
Super division	Embryophyta
Family	Acanthaceae
Division	Tracheophyta
Genus	Hygrophila R. Br.
Subdivision	Spermatophyta
Species	*H. auriculata*

Oppositely arranged, the leaves are ovoid or elliptic in outline and range from 6 to 12 cm long. They exhibit a mild green coloration with a slight luster under sunlight. Each leaf possesses a subtly serrated margin and pronounced venation, giving it an ear-like appearance.[8] Phytochemical investigations of *H. auriculata* indicate that the whole plant comprises phytosterols, tannins, carbohydrates, flavonoids, terpenoids and sterols. It analysed the fixed oil from seeds and reported the presence of uronic acid, palmitic acid, stearic acid, oleic acid and linoleic acid. Apigenin-7-O-glucuronide and apigenin-7-O-glucoside were isolated from the floral parts, whereas lupeol, betulin and stigmasterol were obtained from the plant. The roots were found to contain alkaloids, steroids, tannins, proteins, flavonoids, carbohydrates, fats and oils. In addition, the leaves demonstrated the presence of alkaloids, carbohydrates, proteins, steroids, glycosides, flavonoids, tannins, phenolic compounds, fats and oils.[7-9]

Table 2: Taxonomy of *H. polysperma*.[11]

Synonym	Justicia polysperma, Hemiadelphis polysperma, Adenosma polysperma
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Scrophulariales
Family	Acanthaceae
Genus	Hygrophila
Species	*H. polysperma*

Hygrophila polysperma (East Indian hygrophila) is an amphibious species occurring in both submersed and terrestrial habitats along littoral and riparian zones. It was first documented outside cultivation in Florida during the 1960s. The species exhibits both asexual vegetative propagation and sexual reproduction via seeds, although the ecological relevance of seed production remains unclear.

Despite its ornamental value in the aquarium trade, *H. polysperma* is recognized as an invasive species capable of displacing native flora, altering aquatic ecosystem structure and impeding hydrological flow and irrigation infrastructure.[10-12]

METHODS

In the present study female wistar albino rats served as an experimental animal.

Drugs and Chemicals

Ketamine HCl, ethanol, Petroleum ether was obtained from Naresh Scientific Company, Puducherry.

Study type

The study was in-vivo preclinical study

Study period

The study was conducted from January 2025 to February 2025.

Study place

The study was conducted in Animal House, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur.

Collection of plant material

Leaves of *H. auriculata* were collected from Tirunelveli District in the month of June 2024 to November 2024 and it was authenticated by Dr. S. Mutheeswaran, Scientist, St. Xaviers College, Tirunelveli (Specimen Number: XC17-40613). A fully grow submerged plant of Hygrophila polysperma stem was buying in Premium plants, Chennai. Each stem was cut into two small pieces and planting in a pot. The plant was authenticated by Dr. M. Syed Ali Fathima, Assistant Professor and Head, Sadakathullah Appa Arts and Science College, Tirunelveli. (Specimen Number: SAC/BOT/2024).

Extraction of plant material

The collected leaves of Hygrophila auriculata and Hygrophila polysperma were dried at room temperature and powdered the plant material. Then it was defatted with petroleum ether using Soxhlet apparatus followed extraction with ethanol. Finally, it was concentrated by vacuum evaporator.

Experimental animals

Female Wistar Albino rats (150-200 gm) were purchased from Mass Biotech, Chengalpat District, Tamil Nadu, India. Animals were maintained in polyacrylic cages under standard laboratory conditions with standard food and water ad libitum for one week before the start of experiment. The experimental protocol was approved by Institutional Animal Ethics Committee.

Study design[11,12]

Acute oral toxicity study was carried out according to OECD guidelines for testing of chemicals 423. Acute toxic class method OECD (2001) was followed to arrive the maximum safety dose of the extract. For animal welfare reasons the starting dose for plant extract used is 300 mg/kg body weight. Three Albino Wistar rats-single dose 300 mg/kg of the extract will be orally administrated to overnight fasted rats.

On subsequent dosing, animals will be examined individually for the first 30 min, particular focus for 4 hours, intermittently for 24 hours and further assessed for a period of 14 days. Mortality rate observed in 2 or 3 animals–considered as toxic dose. Mortality of one animal or no mortality observed-same dose is repeated again for confirmation. Further higher dose will be decided based on the results obtained. Absence or presence of mortality of the animals dosed at one step will be determined the next step (i.e.,). No further testing is needed. Dosing of three additional animals, with the same dose. Dosing of three additional animals at the higher or the next lower dose level. Ethanolic Extract of *H. auriculat*a (HA) 300 mg/kg–3 animals. Ethanolic Extract of *H. polysperma* (HP) 300 mg/kg–3 animals.

Mortality rate observed in 2 or 3 animals–considered as toxic dose. Mortality of one animal or no mortality observed, same dose is repeated again for confirmation (3 animals). Further higher dose (2000 mg/kg) will be given by using 3 animals. Mortality rate observed in 2 or 3 animals–considered as toxic dose. Mortality of one animal or no mortality observed, same dose is repeated again for confirmation (3 animals). After the end of the study, the blood was withdrawn through cardiac puncture under ketamine anaesthesia. The blood was placed into EDTA tubes hematological estimation and plain tubes for clinical biochemistry estimation

Evaluation parameters

The following clinical observation were made and recorded pre-terminal deaths, body weight, cage side observation, physical examination

Haematological analysis

Complete blood count (CBC), Haemoglobin content (Hb), erythrocyte sedimentation rate (ESR) and clotting time were recorded,

Biochemical analysis

Clinical biochemistry such as liver profile (total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST)), renal parameters such as urea and uric acid, cardiac profile includes creatinine kinase (CK), lactate dehydrogenase (LDH) and also includes blood glucose and lipid profile

Organ weight

The organs such as liver, kidney, lungs, brain, spleen and heart were weighed and histopathological examination includes liver and kidney.

Statistical analysis

All results were expressed as mean±SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. P values<0.05 were significant.

RESULTS

Preterminal deaths

The study does not result with any signs of toxic effect in the dose levels of 300 mg/kg and 2000 mg/kg. There are no morbidity and mortality in the period of study.

Body weight

There is no difference in the body weight in the period of study.

Cage side observation

There is absence of abnormal home cage activity, colour and consistency of the faeces, behaviour of the animal.

Physical examination

No abnormal in lacrimation, eye prominence, eye lid closure, corneal reflex, lighting reflex and mucus membrane and also absence of any signs of tremor, convulsion, salivation, lethargy, diarrhoea, sleep and coma noted. Tail elevation, static limb position, head position, righting reflex and pinna reflex.

Haematological and biochemical analysis

Histopathology of liver

HA 300 mg/kg shows intact hepatocytes arranged in normal cords around the central vein, with well-preserved cytoplasm and nuclei and HA 2000 mg/kg shows mild clustering of inflammatory cells around the portal region may be seen, but the overall hepatic structure remains largely normal.

HP 300 mg/kg shows mild aggregation of inflammatory cells is present near the portal tract, but without marked tissue destruction. HP 2000 mg/kg no clear evidence of cellular degeneration, necrosis, fibrosis or fatty infiltration. The portal triad structures also appear normal without significant inflammatory infiltration.

Figure 1: Leaf of (a) *H. auriculata* and (b) *H. polysperma*.

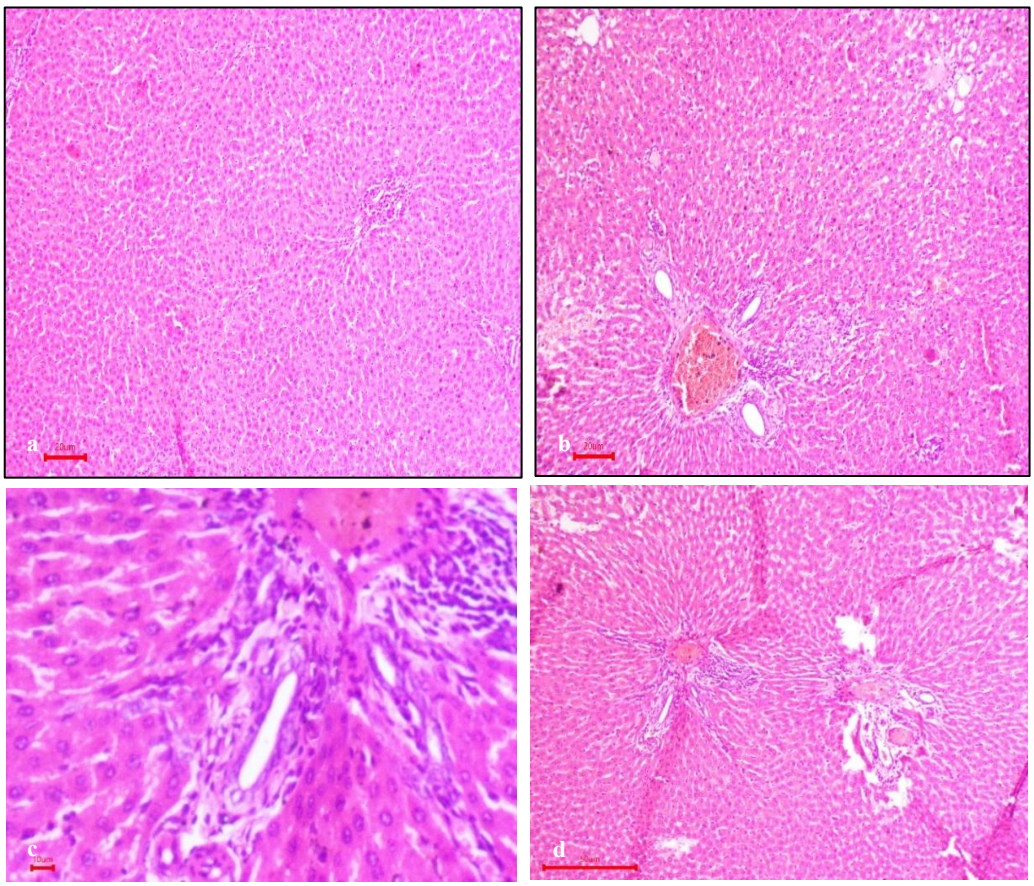

Figure 2: Histopathology of liver of (a) HA 300 mg/kg, (b) HA 2000 mg/kg, (c) HP 300 mg/kg and (d) HP 2000 mg/kg.

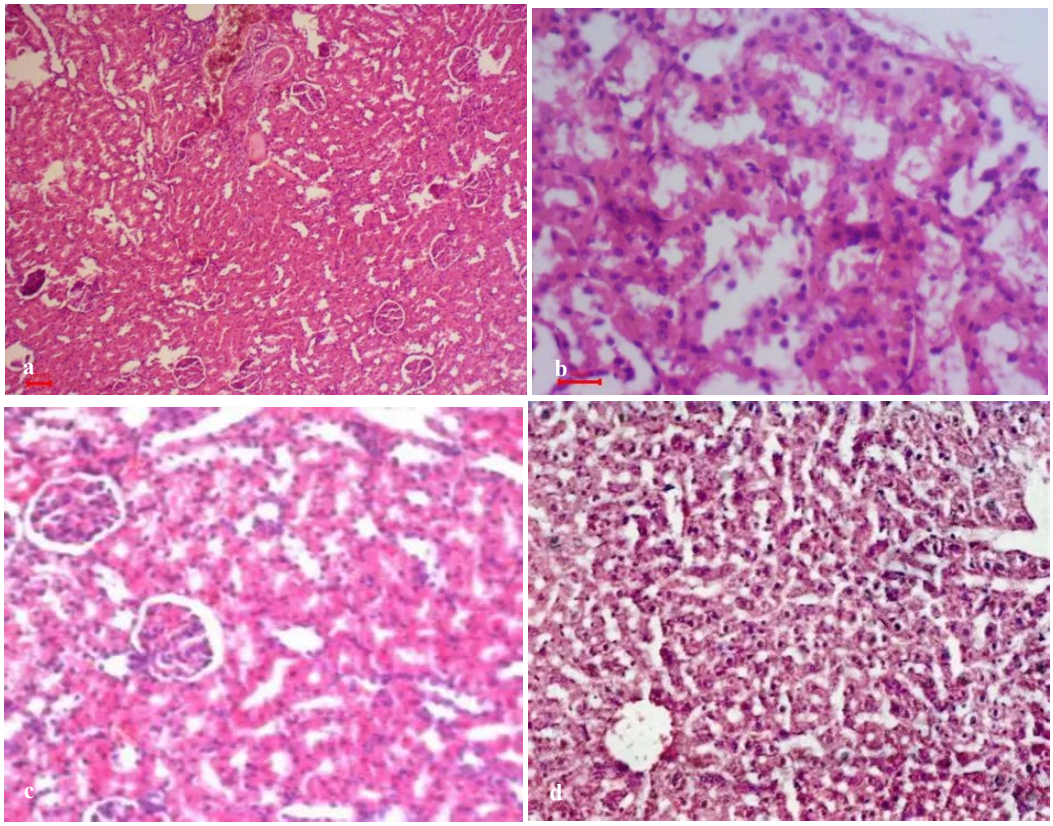

Figure 3: Histopathology of kidney cells of (a) HA 300 mg/kg, (b) HA 2000 mg/kg, (c) HP 300 mg/kg and (d) HP 2000 mg/kg.

Table 3: Haematological values of ethanolic extract of *H. auriculata* and *H. polysperma* leaves.

Treatment	RBC (10^6×µl)	WBC (10^3×µl)	Hb (gm%)	ESR (mm/1st hr)	Platelets (K/µl)	Clotting time (sec)
HA (300 mg/kg)	3.73±0.29	2.66±0.26	10.73±0.43	2.73±0.23	2.63±0.08	58.3±1.66
HA (2000 mg/kg)	6.86±0.17	4.2±0.34	4.26±0.29	13.2±0.2	4.66±0.29	56.6±1.66
HP (300 mg/kg)	4.53±0.17	3.33±0.14	2.73±0.03	11.2±0.23	2.86±0.17	33.3±3.33
HP (2000 mg/kg)	7±0.11	4.26±0.29	4.66±0.29	12.9±0.35	4.73±0.24	53.3±4.4

Values are expressed as mean±standard deviation (n=3).

Table 4: Renal and cardiac profile of ethanolic extract of *H. auriculata* and *H. polysperma* leaves.

Treatment	BUN (mg/dl)	Creatinine (mg/dl)	Urea (mmol/l)	Uric acid (mmol/l)	CK (U/l)	LDH (U/l)
HA (300 mg/kg)	22.80±1.4	0.42±0.06	4.25±0.21	3.36±0.28	1168±9.64	1669±12.9
HA (2000 mg/kg)	22.8±1.78	0.64±0.03	4.74±0.13	2.63±0.32	3294±26.8	1098±8.66
HP (300 mg/kg)	24.36±0.6	0.41±0.14	4.01±0.12	1.81±0.08	1460±24.7	956±23.3
HP (2000 mg/kg)	29.5±2.43	0.58±0.08	5.21±0.39	3.43±0.63	3444±22.6	1469±25.4

Values are expressed as mean±standard deviation (n=3).

Table 5: Organ weights of ethanolic extract of *H. auriculata* and *H. polysperma* leaves.

Treatment	Heart (gm)	Lungs (gm)	Spleen (gm)	Liver (gm)	Brain (gm)	Left kidney (gm)	Right kidney (gm)
HA (300 mg/kg)	0.679±0.03	1.07±0.05	0.59±0.04	5.31±0.21	0.26±0.02	0.78±0.04	0.7±0.02
HA (2000 mg/kg)	0.568±0.02	1.23±0.02	0.30±0.02	3.75±0.14	0.26±0.02	0.59±0.02	0.678±0.02
HP (300 mg/kg)	0.61±0.03	1.73±0.03	0.59±0.02	5.18±0.11	0.33±0.05	0.59±0.02	0.71±0.03
HP (2000 mg/kg)	0.64±0.05	1.2±0.1	0.75±0.04	6.05±0.04	0.32±0.03	0.84±0.07	0.9±0.01

Values are expressed as mean±standard deviation (n=3).

Table 6: Liver profile and blood glucose levels of ethanolic extract of *H. auriculata* and *H. polysperma* leaves.

Treatment	Bilirubin (mg/dl)	SGOT (U/l)	SGPT (U/l)	ALP (U/l)	T. Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A: G ratio	Blood glucose (mg/dl)
HA (300 mg/kg)	0.39±0.02	141.71±1.52	31.36±1.95	183.1±1.94	5.41±0.5	3.45±1.52	2.58±0.23	1.11±0.05	72.33±2.18
HA (2000 mg/kg)	0.47±0.03	136.01±6.74	54.44±3.25	230.94±2.34	7±0.27	3.53±0.27	4.08±0.11	0.67±0.02	75.66±1.45
HP (300 mg/kg)	0.37±0.02	109.44±1.41	56.56±1.48	262.67±14.9	6.01±0.4	3.15±0.17	2.68±0.11	1.16±0.02	74.66±2.18
HP (2000 mg/kg)	0.42±0.04	104.47±6.81	74.79±3.69	209.64±5.72	5.38±0.3	4.34±0.71	4.26±0.58	0.56±0.08	73.66±2.84

Values are expressed as mean±standard deviation (n=3).

Table 7: Lipid profile of ethanolic extract of *H. auriculata* and *H. polysperma* leaves.

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
HA (300 mg/kg)	86±3.05	108.66±4.91	45±1.73	28.33±3.75	24.66±3.38
HA (2000 mg/kg)	62.33±1.76	90±3.60	48.33±4.33	32.33±1.76	22.66±3.17
HP (300 mg/kg)	97±3.21	88.66±4.8	41.66±4.91	35.33±2.84	13.66±1.2
HP (2000 mg/kg)	90.66±5.36	83±2.88	67±2.33	38.33±3.17	21±2.51

Values are expressed as mean±standard deviation (n=3).

Histopathology of kidney cells

HA 300 mg/kg shows mild interstitial congestion may be present, but no major pathological lesions are evident. HA 2000 mg/kg, showing renal tubules and interstitial spaces. The tubular structures are largely preserved, with intact epithelial lining.

HP 300 mg/kg renal cortex section showing two glomeruli and surrounding tubules with no obvious abnormalities HP 2000 mg/kg showing glomeruli and tubules with no obvious abnormalities.

DISCUSSION

The acute oral toxicity study evaluated that the ethanolic leaf extracts of *H. auriculata* and *H. polysperma* did not produce any mortality or visible toxic signs in Wistar albino rats at the tested dose, suggesting a favourable safety profile. Organ weight analysis showed no significant changes in the relative weights of vital organs such as liver, kidney, heart, spleen and lungs compared to the control group. Since organ weight is a sensitive indicator of toxicity, the absence of abnormalities indicates that the extracts did not induce organ hypertrophy, atrophy or oedema. Similarly, haematological analysis revealed normal values for haemoglobin, RBC count, WBC count, platelet count, ESR and clotting time. This indicates that the extracts did not affect haematopoiesis or immune function.

Liver function profile assessment revealed no significant changes in biochemical markers such as serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic pyruvic transaminase (SGPT/ALT), Alkaline phosphatase (ALP), total bilirubin and total protein levels when compared to reference range. These enzymes are important indicators of hepatocellular integrity and liver function. The absence of significant elevation suggests that the ethanolic extracts of *H. auriculata* and *H. polysperma* did not produce hepatocellular damage or impair normal liver metabolism. This finding supports the hepato-safety of both extracts under acute exposure conditions. The renal profile parameters, including serum urea, creatinine and uric acid and BUN remained within normal physiological limits. This suggests that the extracts did not impair kidney function or cause nephrotoxicity. In the lipid profile, levels of total cholesterol, triglycerides, HDL, LDL and VLDL showed no significant alterations.

Maintenance of normal lipid levels indicates that the extracts did not adversely affect lipid metabolism and may suggest metabolic safety. Blood glucose analysis showed no significant variation in serum glucose levels between the treated and control groups. The maintained glucose concentration suggests that the ethanolic extracts of *H. auriculata* and *H. polysperma* did not interfere with carbohydrate metabolism or pancreatic function during the acute toxicity period. This indicates that the extracts are metabolically safe and do not induce hyperglycaemia or hypoglycaemia under the tested conditions.

The cardiac profile markers such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) are mild significant elevation, indicating the absence of mild cardiotoxic effects. The liver sections showed well-preserved hepatic architecture with normal arrangement of hepatocytes around the central vein and intact sinusoidal spaces. No significant pathological alterations such as necrosis, fatty degeneration, fibrosis or congestion were observed. Mild periportal inflammatory cell infiltration was noted in some sections, but this was minimal and not associated with tissue damage. Since the liver is the

primary organ for xenobiotic metabolism, the absence of major structural changes suggests that the extracts did not induce hepatotoxicity.

Similarly, the kidney sections exhibited normal renal histology with intact glomeruli, Bowman's capsules and tubular structures. No evidence of tubular necrosis, glomerular degeneration or severe inflammatory changes was observed. Mild tubular lumen dilation and slight vascular congestion were noticed in certain sections, but these changes were not severe enough to indicate renal impairment. These findings correlate with the normal renal biochemical parameters such as urea and creatinine. Overall, the findings indicate that both plant extracts are relatively safe in acute exposure and can be considered non-toxic at the tested dose. These results support their potential for further pharmacological and therapeutic investigations.

This study was limited to single-dose acute oral toxicity assessment in Wistar rats using ethanolic leaf extracts. Long-term toxicity, detailed organ histopathology, identification of active constituents, sex-wise toxicity differences and specialized toxicity studies such as genotoxicity and reproductive toxicity were not evaluated.

CONCLUSION

The acute oral toxicity study demonstrated that the ethanolic leaf extracts of Hygrophila auriculata and Hygrophila polysperma were well tolerated by Wistar albino rats following a single oral administration. No mortality, treatment-related clinical signs of toxicity or significant behavioural abnormalities were observed throughout the 14 days observation period. The animals exhibited normal body weight gain, feed and water intake and no significant alterations in biochemical or haematological parameters. Gross necropsy findings revealed no treatment-related abnormalities in the major organs. These findings indicate that both ethanolic leaf extracts possess a favourable acute safety profile under the conditions of the study and can be considered relatively safe at the tested dose. However, further subacute, subchronic and chronic toxicity studies are recommended to establish their long-term safety before therapeutic use.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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