Original Research Article

An Assessment of Hepato-protective activity of *Hygrophila auriculata* root extract against Hepatic injured Rodent Model

Abstract:

Liver is a crucial organ that is susceptible to several hepatotoxins causing hepato-toxicities. These days, medicinal plants have been considered to be more promising compared with the conventionally utilized medications for hepatic treatments. In light of the dire circumstances affecting worldwide currently, *Hygrophila auriculata* is a vital plant with an abundance of health advantages and hepatoprotective properties. In this experiment, carbon tetrachloride (CCl_4) was utilized to cause hepatotoxicity in rats and this plant's anti-hepatotoxic activity was evaluated through assessing the outcomes of several parameters (Liver functioning tests, kidney functioning tests and lipid profiles). Subsequent research revealed that the plant may, to varying degrees and in a dependent way, successfully restore the disrupted pathological state to healthy status. To interpret statistical analysis, the "One-way Anova test" was conducted using the SPSS 16 software. In the case of SGPT, the medium and high dose produced statistically significant ($p<0.05$) outcomes as compared to the negative control group and for SGOT decrease in all three groups were non-significant ($p>0.05$). Then for creatinine levels, the outcome for medium, and high dosages were statistically significant ($p<0.05$) whereas in case of urea level only the high dose imparted significant ($p<0.05$) decrease. However, none of the dosages for total cholesterol and triglyceride level produced statistically significant ($p<0.05$) effects. Only in the medium and high doses, HDL levels improved statistically significantly ($p<0.05$) whereas only the high dosages reduced reduce the LDL quantity in the blood significantly ($p<0.05$). As a result, *Hygrophila auriculata* is thought to possess a significant capacity for liver protection and may serve as a more secure and effective substitute for the commercially available synthetic medication, silymarin. To sum up, additional research is required in the future before this appealing plant could get utilized in the arena of hepatotoxic treatments.

Keywords: *Hygrophila auriculata*, SGPT, SGOT, Medicinal plants, Creatinine, Cholesterol.

Introduction:

The liver is an important organ that has a crucial function in metabolizing and eliminating substances. Hence, it remains vulnerable to the harmful effects of various substances. These substances are called hepatotoxins that induce hepatotoxicity. Some medications, such as acetaminophen and halothane, can cause organ damage when used in excessive amounts or even within recommended doses. Certain chemical substances, including those commonly used in laboratory and industrial settings, as well as natural compounds such as alpha-amanitin, and herbal treatments can potentially cause liver damage (Friedman et al., 2003). The majority of hepatotoxic substances heavily affect liver cells by triggering peroxidation of lipids and other forms of oxidative injury ((Asha Tukappa et al., 2015; Dianzani et al., 1991).At this point, it is estimated that hepatotoxicity will impact around 71 million people globally. Annually, this results in about 1.4 million fatalities and is accountable for 4% of the global mortality rate and almost two-thirds of all liver-related mortality. Thus, the current status of people is quite concerning and has even turned into a worldwide issue (Devarbhavi et al., 2023; Jefferies et al., 2018).

Some of the most promising and newly discovered chemicals in medicine for the treatment of hepatic disorders are Silymarin, Metadoxine, Curcumin, Bicyclol and Glutathione. These medications primarily act as free radical scavengers and antioxidants, reducing oxidative stress in the liver. However, the existing synthetic medicines for liver therapy have significant limitations, including nephrotoxicity, jaundice, stability, inadequate absorption and specificity. Furthermore, they are mostly subject to experimental observation. Because conventional therapies have doubtful efficacy, there is a growing interest in alternate and supplemental therapies for the treatment of liver disease. As a result, medicinal herbs have an excellent possibility of replacing them through genetic modification that incorporates a variety of biotechnological technologies (M. Ali et al., 2018; Li et al., 2021; Muriel & Rivera-Espinoza, 2008).

Medical herbs have long been used to treat hepatic diseases; over eighty percent of people worldwide depend on using herbal treatments. About 160 phytoconstituents from 101 different plants have been shown to have liver-protective qualities. Because of their broad variety of

pharmacological effects, particularly antioxidant and hepatoprotective ones, recently sterols, flavonoids, terpenoids, polysaccharides, plant lipids, essential oils and various other natural chemicals produced from medicinal plants have drawn lots of interest (Ekor, 2014; Fitoterapia & 1986, n.d.).

A few naturally occurring plants with the potential to be anti-hepatotoxic include *Salvia miltiorrhiza, Hygrophila auriculata, Phyllanthus amarus, Glycyrrhiza glabra, and Silybum marianum., Astragalus membranaceus, Amole tuber, Cochlospermum vitifolium, Heterotheca inuloides, Sabdariffa, Hibiscus Leucophyllum frutescens, Psidium Guajava, Rosmarinus Officinalis, Verbena Carolin, American centaurea, Juglans mollis; Krameria ramossisima; Turnera diffususum, Spinosa spinosa, cichorium intybus, Solansum nigrum, Sapindus mukorossi Gaertn. Ginkgo biloba; Woodfordia fruticosa; Vitex trifolia; Schisandra chinensis; x; Lycium barbarum; Angelica sinensis; Diels; Litsea coreana, Hosta plantaginea; Ligusticum chuanxiong, Daniela oliveri, Mangostana García,* Solanum melongena, Vaccinium myrtillus, *Picrorhiza kurroa, Citrus medica, Glycyrrhiza glabra* etc. have been widely used commonly as well as efficiently for the treatment of hepatic conditions. (S. A. Ali et al., 2019; Asadi-Samani et al., 2015; Thilagavathi et al., 2023; Torres-González et al., 2014).

Out of these medicinal plants, *Hygrophila auriculata* holds enormous Ayurvedic importance. It is a species of the Indian Acanthaceae family. It is found in subtropical to tropical regions of India, Sri Lanka, Burma, Malaysia, and Nepal. This plant is not associated with any documented toxicity or adverse effects yet. Terpenoids, butelin, lupeol, fatty acids, flavonoids, and polyphenolic chemicals are some of this plant's major components. The plant's roots, seeds, and ashes are widely utilized in conventional healthcare to treat a range of conditions including jaundice, hepatic blockage, arthritis, discomfort, inflammation, urinary tract infections, edema, gout, dysentery, and diabetes.The plant possesses anticancer, hypoglycemic, antimicrobial, free radical scavenging and lipid peroxidation-associated hepatoprotective properties (Dhanalakshmi et al., 2020; Manya Mboni et al., 2023; Sarvananda & Premarathna, 2018; Sd & As, 2017; Shanmugasundaram & Venkataraman, 2006; Sultana et al., 2018).

Therefore, the purpose of this research is to determine whether the roots of *Hygrophila auriculata* exhibit hepatoprotective properties against rats that have had liver damage caused by CCl4.

Materials and Methods:

Plant Collection and Extract Preparation

The roots of *Hygrophila auriculata* were obtained from a local market in Dhaka. The Department of Pharmacy, University of Dhaka acknowledged the content. The *Hygrophila auriculata* roots were dried in the air and then crushed extensively. The pulverized root was eventually treated to a 15-day extraction process using a 50% ethanol solution. The extract went through filtration every three days. Then using a rotary evaporator under regulated temperature and pressure conditions, the derived material was dried. Ultimately, the necessary pharmacological examination was performed with the crude remnants.

Drugs and Chemicals

Carbon tetrachloride (CCl4), an established hepatotoxicity producing chemical, was obtained from the Sigma corporation in the United States. The silymarin medicine, known for its antioxidant properties, was acquired under the brand name Livasil 140 mg from Incepta Pharmaceuticals Ltd.

Experimental Animal Procurement, Nursing, and Grouping

All the male rats weighing 120-150 grams were procured from Jahangirnagar University in Savar, Dhaka. Each was maintained in a climate-controlled facility with temperature of 25±3°C, relative humidity of 55±5%, and a 12-hour light/dark cycle at the Institute of Nutrition & Food Science (INFS) of the University of Dhaka. They were allowed to consume regular food and clean water. All of them were kept in this environment for minimum one week before the experiment to allow for adaptation. All research methods met the Institutional Animals Ethics Committee's (IEAC) guidelines.

Animal Model Sample Size Detection

The number of rats was determined to be 9.78 by applying the formula for sample size, which is 2 SD2 (Zα/2 + Zβ) 2/d2. Furthermore, our laboratory's prior hepatoprotective investigations have demonstrated that the research is facilitated by the use of ten rats per group. Therefore, all rats were randomly divided into nine groups and each group contain 10 rats. In all investigations, rats

were randomly allocated to each group to improve the study's reliability. During the breeding period, constant surveillance was maintained on the rat all day. Both positive and negative control groups were included in our research.

Dose Selection and Route of Administration for Respective Study

Carbon tetrachloride (CCL4) is a popular chemical agent used in labs to research a spectrum of liver ailments, both chronic and acute in nature. The CYP2E1 isozyme-produced CCL4 metabolite known as trichloromethyl free radical (CCL3) reacts with proteins and lipids in cells to produce trichloromethyl per-oxy radical, which damages lipids on the outer layer of the endoplasmic reticulum more quickly than trichloromethyl free radical and results in lobular necrosis and lipid peroxidation. In all animal groups except the typical control group, a single oral administration of CCl4 combined with olive oil as the carrier in the ratio of 1:1 (3 ml/kg of rat body weight per group) resulted in liver damage. Animals suffering from liver damage were given *Hygrophila auriculata* extracts as a post-treatment. The extract had been administered orally in varied doses.

Evaluation of Hepato–Protective Activity

For this experiment, all rats were randomly picked and equally divided into nine groups (Table 1).

Table 1: Application of treatment efficacy

Group Number	**Group Specification**	**Treatment species**	**Dose treatment species (mg/kg)**	**Abbreviation of Groups**
1	Negative Control	Physiological saline	10 ml/kg	N
2	CCl_4 Control	N/A	N/A	A
3	CCl_4 + Silymarin	Silymarin	80	A+ S_{80}
4	CCl_4 + *Hygrophila*	*Hygrophila*	300	A + HA_{300}

	auriculata	*auriculata*		
5	CCl_4 + *Hygrophila auriculata*	*Hygrophila auriculata*	600	A + HA_{600}
6	CCl_4 + *Hygrophila auriculata*	*Hygrophila auriculata*	1200	A + HA_{1200}
7	*Hygrophila auriculata*	*Hygrophila auriculata*	300	HA_{300}
8	*Hygrophila auriculata*	*Hygrophila auriculata*	600	HA_{600}
9	*Hygrophila auriculata*	*Hygrophila auriculata*	1200	HA_{1200}

Statistical analysis:

We used Microsoft Excel to record and evaluate all of our numerical parameter findings on a broadsheet. Then descriptive statistics were implemented to analyze the collected data, and the results were presented as the mean standard deviation. The "One-way Anova test" of the SPSS 16 software was employed to interpret heterogenicity among groups in terms of multiple biological variables in order to assess statistical significance. The incidences are regarded as statistically significant due to the fact that the 'p' value was lower than 0.05 ($p<0.5$).

Results and discussion:

Table 2: Liver functioning tests (SGPT and SGOT) of rat after administration of drug and extract of *Hygrophila auriculata*

Group	**Abbreviation of Groups**	**SGPT**	**SGOT**

Number			
1	N	35.24 ± 3.46	42.63 ±5.21
2	A	112.43 ±12.24	119.25± 13.53
3	A+ S_{80}	60.45 ± 8.63	68.67 ± 10.21
4	A + HA_{300}	109.23 ± 10.42	116.29 ± 8.54
5	A + HA_{600}	104.37 ± 9.89*****	114.20 ± 7.71
6	A + HA_{1200}	99.24 ± 8.48*****	110.28 ± 5.59
7	HA_{300}	37.22 ± 4.63	44.20 ± 6.39
8	HA_{600}	38.29 ± 5.57	46.80 ± 5.29
9	HA_{1200}	39.27 ± 6.60	42.42 ± 5.13

The study results indicate that the concentrations of SGPT and SGOT exhibited a dose-dependent reduction in all three groups (low, medium, and high). Regarding SGPT, the administration of medium and high doses of Hygrophila auriculata extract results in significant variation ($p<0.05$), whereas the low dose demonstrates a non-significant ($p>0.05$) reduction in SGPT levels. In case of SGOT level, decline in all three groups revealed non-significant ($p>0.05$) effect.

Table 3: Kidney functioning tests (Creatinine and Urea) of rat after administration of drug and extract of *Hygrophila auriculata*

Group Number	Abbreviation of Groups	Creatinine	Urea
1	N	0.54 ± 0.23	35.36 ± 4,61
2	A	2.88 ± 0.95	111.23 ± 13.39

3	A+ S_{80}	1.43 ± 0.65	55.67 ± 11.23
4	A + HA_{300}	2.66 ± 0.87	108.21 ± 9.69
5	A + HA_{600}	2.23 ± 0.54*****	105.45 ± 10.62
6	A + HA_{1200}	1.87 ± 0.63*****	100.23 ± 6.87*****
7	HA_{300}	0.63 ± 0.83	37.58 ± 5.67
8	HA_{600}	0.68 ± 0.77	35.29 ± 6.82
9	HA_{1200}	0.73 ± 0.83	37.21 ± 5.73

In medium, and high doses, creatinine levels dropped in statistically significant ($p<0.05$) way. In case of low dose, the level reduced although in a non-significant ($p>0.05$) way. However, in case of urea level, only high dosage imparts significant ($p<0.05$) fall whereas low and medium doses exhibit non-significant ($p>0.05$) decline.

Table 4: Lipid profile (Total Cholesterol, HDL, LDL, Triglyceride) of rat after administration of drug and extract of *Hygrophila auriculata*

Group Number	**Abbreviation of Groups**	**Total Cholesterol**	**HDL**	**LDL**	**Triglyceride**
1	N	115.24 ± 9.39	89.46 ± 6.29	42.52 ±3.52	55.37 ± 2.19
2	A	205.31 ±16.31	44.57 ± 6.15	150.47 ± 5.16	114.24 ± 4.16
3	A+ S_{80}	150.47 ± 15.31	63.82 ± 8.26	85.85 ± 8.23	75.71 ± 5.58
4	A + HA_{300}	203.70 ± 10.12	47.56 ± 5.19	146.24 ± 4.83	113.31 ± 7.21

5	A + HA_{600}	199.68 ± 12.61	50.29 ± 6.39*****	143.70 ± 6.25	110.23 ± 6.75
6	A + HA_{1200}	196.93 ± 11.29	54.71 ±7.21*****	139.28 ± 10.12*****	107.99 ± 6.21
7	HA_{300}	118.89 ±8.22	87.26 ± 5.38	86.25 ± 6.25	57.52 ± 3.24
8	HA_{600}	114.97 ± 7.63	90.80 ± 6.35	87.19 ±5.21	52.99 ± 5.20
9	HA_{1200}	114.83 ± 6.23	87.80 ± 6.23	90.70 ± 4.91	55.20 ± 2.30

It has been noticed that the abnormally raised total cholesterol and triglyceride levels were drop however in non-significant ($p>0.05$) way for all low, medium and high dosages. At medium and high dosages HDL level increases statistically substantially ($p<0.05$) while at low doses it raised in non-significant ($p>0.05$) manner. When compared to the diseased control group, LDL levels reduced considerably ($p<0.05$) at high dosages and non-significantly ($p>0.05$) at low and medium doses.

Conclusion:

The experiment revealed that the ethanolic extract of *Hygrophila auriculata* exhibited hepatoprotective properties and was essential in rectifying the disrupted clinical condition. This demands further investigation to discover the exact component from the total extract that genuinely delivers the hepato-protective effect by a screening technique and to consider *Hygrophila auriculata* as an effective choice in hepatotoxic therapies.

Ethical approval:

Animal Research Ethical Approval Committee of Khulna Central Hospital approved our research protocol under the Section of Experimental Pharmacology and sub section of Preclinical Study. Our Approval Id- KCH/EP/PS/009/24.

Disclaimer (Artificial intelligence)

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Details of the AI usage are given below:

1.

2.

3.

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