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PHARMACOLOGICAL SIGNIFICANCE OF *LEMNA MINOR* (DUCKWEED) IN KWASHIORKOR MANAGEMENT AND HEPATIC STEATOSIS

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Abstract

The present study was undertaken to evaluate the pharmacological significance and hepatoprotective activity of ethanolic extract of Lemna minor against D-GalN-induced hepatotoxicity in experimental rats. The plant material was extracted using ethanol and subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenols, and saponins. The percentage yield of ethanolic extract was found to be 8.25% w/w. Total phenolic and flavonoid contents were estimated as 0.57 mg/100 mg and 0.92 mg/100 mg respectively, indicating the antioxidant potential of the extract. Hepatoprotective activity was evaluated by assessing biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin levels in D-GalN-induced hepatotoxic rats. Administration of D-GalN significantly elevated liver enzyme and bilirubin levels while decreasing albumin concentration, indicating severe hepatic injury. Treatment with Lemna minor extract at doses of 100 mg/kg and 200 mg/kg significantly restored altered biochemical parameters toward normal values in a dose-dependent manner. The higher dose exhibited marked hepatoprotective activity comparable to the standard drug silymarin. The hepatoprotective effect of Lemna minor may be attributed to the presence of phenolic and flavonoid compounds possessing antioxidant and membrane stabilizing properties. The study concludes that Lemna minor possesses significant hepatoprotective potential and may serve as a promising natural therapeutic agent for the management of hepatic disorders.

Keywords: Lemna minor, Hepatoprotective activity, D-GalN, Hepatic steatosis, Flavonoids, Phenolic compounds, Silymarin, Liver enzymes, Antioxidant activity

Introduction

Hypertension, protein-energy malnutrition, and metabolic liver disorders remain major public health concerns worldwide, particularly in developing countries where nutritional deficiencies are highly prevalent (Awuchi et al., 2020).

Kwashiorkor is a severe form of protein malnutrition characterized by edema, fatty liver, growth retardation, muscle wasting, hypoalbuminemia, and impaired immune function (Michael et al., 2022).

One of the important pathological manifestations associated with kwashiorkor is hepatic steatosis, which occurs due to inadequate protein intake leading to impaired synthesis of lipoproteins and accumulation of fat within hepatocytes (Risi et al., 2021). Persistent hepatic lipid accumulation may further contribute to oxidative stress, inflammation, and liver dysfunction, thereby aggravating the disease condition (Conde de la Rosa et al., 2022). Therefore, identification of nutrient-rich natural sources with hepatoprotective and nutritional benefits has gained significant scientific interest for the management of kwashiorkor and associated hepatic complications.

Medicinal and aquatic plants have long been recognized as valuable sources of nutrients and therapeutic phytoconstituents. Among them, Lemna minor, commonly known as duckweed, has attracted considerable attention due to its exceptionally high protein content, rapid growth rate, and rich nutritional profile (Jaimes Prada et al., 2024).

Lemna minor belongs to the family Araceae and is a small free-floating aquatic plant widely distributed in ponds, lakes, and freshwater ecosystems. It contains substantial amounts of proteins, essential amino acids, vitamins, minerals, carotenoids, polyphenols, flavonoids, and antioxidants, which contribute to its nutritional and medicinal importance (Vulpe et al., 2025).

Previous studies have demonstrated that Lemna minor possesses several pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, immunomodulatory, and nutritional properties (Al-Snafi, 2019). The high protein and amino acid content of duckweed makes it a promising alternative nutritional supplement for combating protein-energy malnutrition. In addition, the presence of phenolic compounds and flavonoids may help reduce oxidative stress and lipid peroxidation associated with hepatic steatosis (Tarahi, 2024).

Antioxidant phytoconstituents present in Lemna minor are also reported to improve liver function by stabilizing hepatocellular membranes and enhancing endogenous antioxidant defense mechanisms (Seferli et al., 2024).

Hepatic steatosis is closely associated with oxidative damage, altered lipid metabolism, and inflammatory responses. Natural products rich in antioxidants and bioactive compounds may provide protective effects against fatty liver progression by reducing free radical generation and improving hepatic lipid utilization (Li et al., 2015).

Due to its rich nutritional composition and therapeutic potential, Lemna minor may serve as an effective natural agent for the management of kwashiorkor-associated hepatic steatosis. However, despite its nutritional importance, limited scientific studies are available regarding the pharmacological significance of Lemna minor in protein malnutrition and fatty liver disorders.

Therefore, the present study was undertaken to evaluate the pharmacological significance of Lemna minor in the management of kwashiorkor and hepatic steatosis using suitable experimental approaches. The study aims to investigate its nutritional, hepatoprotective, and antioxidant potential, along with its possible therapeutic role in improving protein deficiency and liver abnormalities associated with malnutrition.

Material and Methods

Material

Fresh whole plants of Lemna minor were collected and authenticated for the present study. Ethanol was used as extraction solvent for preparation of plant extract. D-Galactosamine (D-GalN) was used for induction of hepatotoxicity, while silymarin was used as standard hepatoprotective drug. Experimental animals used in the study were healthy Wistar rats maintained under standard laboratory conditions. Diagnostic kits for estimation of biochemical parameters such as ALT, AST, ALP, albumin, and bilirubin were procured from standard commercial suppliers. All chemicals and reagents used during the study were of analytical grade.

Methods

Extraction by maceration method

For extraction, 50 g of powdered plant materials was accurately weighed and transferred into a 250 mL airtight glass bottle. The material was subjected to maceration using an ethanol solvent system for 24 hours (Kokate, 1994). After completion of the extraction process, the liquid extract was collected in a pre-weighed conical flask. The solvent was removed by evaporation, and the obtained extract was dried to constant weight. Finally, the percentage yield of the extract was calculated on a weight-by-weight (% w/w) basis.

Determination of percentage yield

The percentage yields of extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of Leaves of *Lamna minor*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, proteins and amino acids (Audu *et al.,* 2007).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

Estimation of total phenol content Total phenol content of the extracts was evaluated with folin-ciocalteu method (Gaur Mishra *et al.,* 2017). Samples containing polyphenols are reduced by the folin-ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 2 ml aliquots of 5, 10, 15, 20, and 25 µg/ml gallic acid solutions were mixed with 1ml folin– ciocalteu reagent (diluted ten-fold) and 1 ml (7.5 g/L) sodium carbonate. After incubation at 25°C for 10 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer.

The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract (1000µg/ml) as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) mg per 100mg of extract.

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified (Gaur Mishra *et al.*, 2017). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 10 ml methanol and then diluted for 5-25 µg/ml. The diluted standard solutions (2 ml) were separately mixed with 1 ml of 2% aluminum chloride. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a UV spectrophotometer. Similarly, extracts solutions (1000 µg/ml) were reacted with aluminum chloride for determination of flavonoid content. Total flavonoid content was expressed as milligrams of Quercetin equivalent (QE) mg per 100mg of extract.

Pharmacological significance of *Lemna minor* (duckweed) in kwashiorkor management and hepatic steatosis

Animals

Wistar rats (150–200 g) were group-housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), India.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (Sewell *et al.*, 2024). Animals were kept fasting providing only water, *Lemna minor* (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioural changes for evaluation of a possible hepatoprotective.

Experimental designs

Group –I: Normal (Saline, p.o.)

Group –II: received D-GalN (d-Galactosamine, 400 mg/kg, i.p.)

Group –III: received Silymarin (100mg/kg, p.o.)

Group -IV: received *Lemna minor* (100mg/kg, p.o.)

Group –V: received *Lemna minor* (200mg/kg, p.o.)

Group I received normal saline for 14 days and served as normal control. Group II received normal saline (1 ml/kg, p.o.) for 14 days and served as a toxic control. Groups III and IV were treated with *Lemna minor* at a dose of 100 mg/kg p.o. and 200 mg/kg p.o each, respectively. On 15th day the groups II, III and IV, V received D-GalN (400 mg/kg, i.p.) (Long *et al.*, 2025). At the end of the experiments, rats blood was withdrawal via retroorbital plexus for biochemical parameters.

Biochemical Evaluation in Serum

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin levels were estimated using commercially available diagnostic kits according to the manufacturer's instructions. ALT and AST are important biochemical markers used for assessment of hepatic injury and liver function (Yadav et al., 2022; Youssef and Wu, 2024). ALP estimation was carried out to evaluate hepatobiliary dysfunction and membrane integrity (Salimi et al., 2022). Serum albumin levels were determined as an indicator of hepatic synthetic capacity and nutritional status (Jagdish et al., 2021), while bilirubin estimation was performed to assess hepatic clearance function and hepatocellular damage (Guerra Ruiz et al., 2021).

Results and Discussion

The ethanolic extract of *Lemna minor* showed a dark black solid appearance with a percentage yield of 8.25% w/w, indicating satisfactory extraction efficiency of phytoconstituents from the plant material (Table 1). Preliminary phytochemical screening revealed the presence of important bioactive constituents such as alkaloids, glycosides, carbohydrates, flavonoids, phenols, proteins and saponins in the ethanolic extract, whereas tannins, steroids, resins and diterpenes were absent (Table 2). The presence of these phytochemicals suggests that *Lemna minor* possesses significant medicinal and antioxidant potential, which may contribute to its hepatoprotective activity.

The total phenolic content and total flavonoid content of the ethanolic extract were found to be 0.57 mg/100 mg and 0.92 mg/100 mg respectively (Table 3 and Table 4). Phenolic compounds and flavonoids are well known for their antioxidant and free radical scavenging properties. These phytoconstituents may help protect hepatic cells against oxidative stress and cellular damage induced by hepatotoxic agents.

The hepatoprotective activity of *Lemna minor* was evaluated against D-GalN-induced hepatotoxicity by estimating various biochemical parameters including ALT, AST, ALP, albumin, and bilirubin levels. Administration of D-GalN significantly elevated serum ALT levels compared to the normal group, indicating severe hepatic injury. Treatment with *Lemna minor* extract at both dose levels significantly reduced ALT levels, with the 200 mg/kg dose showing greater protection and results comparable to the standard drug silymarin (Table 5). Similarly, AST levels were markedly increased in the toxic control group, whereas treatment with *Lemna minor* significantly restored AST levels toward normal values, indicating stabilization of hepatocellular membrane integrity (Table 6).

A significant elevation in ALP levels was observed in D-GalN-treated rats, reflecting hepatic dysfunction and biliary damage. Administration of Lemna minor extract significantly reduced ALP levels in a dose-dependent manner, suggesting improvement in liver function and reduction in hepatic injury (Table 7). Serum albumin levels were considerably decreased in the toxic control group due to impaired protein synthesis caused by liver damage. Treatment with *Lemna minor* significantly increased albumin levels, indicating improvement in hepatic synthetic capacity and restoration of normal liver function (Table 8).

Similarly, bilirubin levels were markedly elevated following D-GalN administration, indicating impaired hepatic clearance and hepatocellular damage. Treatment with *Lemna minor* extract significantly reduced bilirubin levels, especially at the higher dose of 200 mg/kg, which demonstrated marked hepatoprotective activity comparable to silymarin (Table 9). Overall, the findings suggest that *Lemna minor* possesses significant hepatoprotective potential, which may be attributed to the presence of phenolic and flavonoid compounds exhibiting antioxidant and membrane stabilizing properties.

Table 1: Extractive values obtained from *Lamna minor*

S. No.	Extract	Colour	% Yield (w/w)
1.	Ethanolic	Dark black solid	8.25

Table 2: Preliminary phytochemical screening of *Lamna minor*

S.N.	Phytoconstituents	Test Name	Ethanolic extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	**+ve**
		Hager's Test	-ve
2	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
		Legal Test	**+ve**
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	**+ve**
		Benedict's Test	**+ve**
4	Tannins	Vanillin- HCl Test	-ve
		Gelatin Test	**-ve**
5	Flavonoids	Lead acetate Test	**+ve**
		Alkaline Reagent Test	**+ve**
6	Resins	Turbidity Test	-ve
7	Steroids	Libermann- Bur chard Test	-ve
		Salkowski Reaction	-ve
8	Proteins & Amino acids	Biuret Test	**+ve**
		Precipitation test	**+ve**
9.	Phenols	Ferric chloride test	**+ve**
10.	Saponins	Froth Test	**+ve**
11.	Diterpenes	Copper acetate Test	**-ve**

[+ve = positive; -ve = negative]

Table 3: Total phenol content of Ethanolic extract of *Lamna minor*

S. No.	Extract	Total phenol content (mg/100mg)
1	Ethanolic extract	0.57

Table 4: Total flavonoid content of ethanolic extract of *Lamna minor*

S. No.	Extract	Total Flavonoid content (mg/100mg)
1	Ethanolic extract	0.92

Table 5: Effect of *Lemna minor* and Silymarin on ALT levels in D-GalN-induced hepatotoxicity in rats

Treatment	Dose	ALT(IU/L)
Normal	Saline	47.85 ± 4.8
D-GalN	400 mg/kg, i.p	162.74 ± 13.1#
Silymarin	100 mg/kg p.o.	51.26 ± 4.7***
Lemna minor	100 mg/kg p.o.	69.18 ± 5.6**
Lemna minor	200 mg/kg p.o	54.12 ± 5.3***

Table 6: Effect of *Lemna minor*and Silymarin on AST levels in D-GalN-induced hepatotoxicity in rats

Treatment	Dose	AST (IU/L)
Normal	Saline	34.12 ± 4.9
D-GalN	400 mg/kg, i.p	121.48 ± 10.2#
Silymarin	100 mg/kg p.o.	43.76 ± 4.2***
Lemna minor	100 mg/kg p.o.	61.45 ± 5.8**
Lemna minor	200 mg/kg p.o	47.93 ± 4.9***

Values expressed as mean ± SEM (n=6) ** P<0.01, *** P<0.001 as compared to

#P<0.001 D-GalN

Table 7: Effect of *Lemna minor*and Silymarin on ALP levels in D-GalN-induced hepatotoxicity in rats

Treatment	Dose	ALP(IU/L)
Normal	Saline	35.82 ± 4.8
D-GalN	400 mg/kg, i.p	104.36 ± 7.4#
Silymarin	100 mg/kg p.o.	39.15 ± 4.6***
Lemna minor	100 mg/kg p.o.	53.28 ± 5.0**
Lemna minor	200 mg/kg p.o	42.67 ± 4.3**

Values expressed as mean ± SEM (n=6) ** P<0.01, *** P<0.001 as compared to

#P<0.001 D-GalN

Table 8: Effect of *Lemna minor* and Silymarin on albumin levels in D-GalN-induced hepatotoxicity in rats

Treatment	Dose	Albumin (g/dL)
Normal	Saline	5.94 ± 0.05
D-GalN	400 mg/kg, i.p	3.28 ± 0.08#
Silymarin	100 mg/kg p.o.	5.88 ± 0.06***
Lemna minor	100 mg/kg p.o.	5.42 ± 0.05**
Lemna minor	200 mg/kg p.o	5.79 ± 0.06***

Values expressed as mean ± SEM (n=6) ** P<0.01, *** P<0.001 as compared to

#P<0.001 D-GalN

Table 9: Effect of *Lemna minor* and Silymarin on bilirubin levels in D-GalN-induced hepatotoxicity in rats

Treatment	Dose	Bilirubin (mg/dL)
Normal	Saline	0.28 ± 0.03
D-GalN	400 mg/kg, i.p	2.48 ± 0.07#
Silymarin	100 mg/kg p.o.	0.35 ± 0.03***
Lemna minor	100 mg/kg p.o.	0.74 ± 0.05**
Lemna minor	200 mg/kg p.o	0.52 ± 0.04***

Values expressed as mean ± SEM (n=6) ** P<0.01, *** P<0.001 as compared to

#P<0.001 D-GalN

Conclusion

The present study demonstrated that the ethanolic extract of *Lemna minor* possesses significant hepatoprotective activity against D-GalN-induced hepatotoxicity in rats. Phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, phenols, glycosides, protein and saponins, which may contribute to its therapeutic effects. Treatment with Lemna minor significantly restored altered biochemical parameters including ALT, AST, ALP, albumin, and bilirubin levels in a dose-dependent manner. The higher dose (200 mg/kg) showed pronounced protective activity comparable to silymarin. The study suggests that Lemna minor may serve as a promising natural source for the management of hepatic disorders and nutritional complications associated with liver dysfunction.

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