RESEARCH PAPER

In vivo evaluation of antioxidant and hepatoprotective potentials of *Ludwigia adscendens* (L.) H. Hara against arecoline-induced hepatotoxicity

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ABSTRACT
Background: Arecoline is the major areca nut pyridine alkaloid that causes severe hepatotoxicity primarily due to the disturbance of redox homeostasis and oxidative and nitrosative stress.
Objective: This study evaluated the antioxidant and hepatoprotective potentials of *Ludwigia adscendens* extract against arecoline-induced hepatotoxicity in mice.
Methods: Preliminary phytochemical analysis, total flavonoid and total phenolic content were performed. Antioxidant and anti-inflammatory assays were performed using DPPH, ABST, reducing power, and egg albumin denaturation assays. Arecoline was administered to induce toxicity in mice. *L. adscendens* extract was co-administered at different doses of 250mg/kg and 500mg/kg to evaluate the hepatoprotective potential. The oxidative and nitrosative damage were measured using LPO, GST, GSH, SOD, and NO. Hepatic damage and its mitigation were evaluated by measuring liver biomarkers, including TB, DB, SGOT, SGPT, ALP, and TP.
Results: Arecoline significantly increased oxidative and nitrosative stress leading to liver injury, demonstrated by the elevation of liver biomarkers. Meanwhile, treatment with *L. adscendens* extract (250mg/kg and 500mg/kg) successfully attenuated the liver injury.
Conclusion: *L. adscendens* extract possesses antioxidant, anti-nitrosative, and hepatoprotective properties. The extract contains various bioactive phytocompounds that actively scavenge free radicals, thereby preserving liver integrity and function.
Keywords: *Ludwigia adscendens*, phytocompounds, antioxidant, hepatoprotection
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INTRODUCTION

More than 600 million individuals consume areca nut, particularly in South and Southeast Asia, making it the fourth most commonly consumed psychoactive substance (Huang et al., 2024). Areca nut contains polyphenols, fats, alkaloids, crude fibres, and other metabolites. The International Agency for Research on Cancer identified areca nut as a Group 1 carcinogen (Ko et al., 2023). About 20 alkaloid secondary metabolites have been identified; which accounts for around 0.8% w/w of the mature fruit, is mainly responsible for many of the toxicological effects of areca nut (Huang et al., 2024; Wang et al., 2018). The primary sites for arecoline metabolism are the liver and kidneys (Cao et al., 2025). Prolonged exposure to arecoline leads to systemic toxicities, malignant diseases, oral submucosal fibrosis, cirrhosis, acute hepatic injury, and a higher risk of hepatocellular carcinoma, liver fibrosis, and hepatitis (Dasgupta et al., 2006; Huang et al., 2024; Jeng et al., 2014; Liu et al., 2016).

Arecoline-induced hepatotoxicity may be associated with excessive reactive oxygen species (ROS) generation. Arecoline induced cytochrome P450 enzymes, especially CYP2E1 (Run-mei et al., 2014). This CYP2E1-mediated metabolism is considered a major source of ROS in the liver. During xenobiotic metabolism, electron leakage in the CYP450 catalytic cycle produces superoxide anions and hydrogen peroxide, contributing oxidative stress (Lacour et al., 2005; Wang et al., 2018). This disrupts normal homeostasis, leading to accumulation of free radicals, lipid peroxidation, mitochondrial dysfunction, apoptosis, necrosis, and alterations in liver enzymes (Cao et al., 2025; Sun et al., 2023; Dasgupta et al., 2006). Furthermore, lipid peroxidation products formed by adducts with proteins and DNA contribute to cell dysfunction (Cianci et al., 2024; Lacour et al., 2005). Oxidative stress upregulated pro-inflammatory signalling cascades, increasing inflammatory cytokines and chemokines expression thereby recruiting immune cells, and enhancing liver damage (Ali et al., 2025; Gur et al., 2022; Lacour et al., 2005). The upregulation of nitric oxide (NO) induces protein nitration, causing irreversible damage and apoptotic cell death (Villanueva-Paz et al., 2021).

Ludwigia adscendens (L.) H. Hara is a semi-aquatic medicinal plant of Onagraceae family. In Manipur, it is commonly known as “Eshing kundo,” and it is distributed in tropical and subtropical regions, thriving in waterlogged areas, marshes, paddy fields, wetlands, and riverbanks. *L.*

adscendens is ethnomedicinally used for treating inflammatory conditions, infections, respiratory illnesses, skin disease, and metabolic disorders (Bhuiyan et al., 2023). It is considered an invasive weed due to its uncontrolled growth. However, it shows ecological importance by helping remove pollutants, such as heavy metals, from contaminated water (Ritu et al., 2024). This study aims to explore the antioxidant and hepatoprotective potentials of *L. adscendens.* This may help in understanding the therapeutic potential of the plant against liver injury.

Materials and methods

Collection of plant material

L. adscendens were collected from Manipur, India. The plant was deposited and identified by the Botanical Survey of India, Shillong (BSI/ERC/Tech/2023-24/1941). The whole plants were used for the study.

Preparation of plant extract

The whole plants were washed, dried, and pulverized into powder, then mixed with methanol at a 1:10 (w/v) ratio and stored for 72 hours. Thereafter, the combined methanolic crude extract was filtered, concentrated to dryness in a water bath (35°C), and the dry extract was stored in an airtight container (Basumatary, S., & Narzary, H., 2017).

Qualitative analysis

The plant extract was screened for preliminary qualitative analysis to check for the presence of terpenoids, steroids, cardiac glycosides, phenols, saponins, amino acids, carbohydrates, oils, and flavonoids (Shaikh & Patil, 2020).

Total phenolic content

The Folin-Ciocalteu (FC) method was used for total phenolic content (TPC) determination. 2 mL of gallic acid at different concentrations and a single dose of the plant extract were prepared. 1 mL of FC-phenol reagent was mixed with the mixture. 5 minutes later, 5mL of a 7.5% Na_2CO_3 solution was added. Incubated for 45 minutes at room temperature. Absorbance was measured at 760nm against the blank and was expressed as mg gallic acid equivalent (GAE) (Rao et al., 2016).

Total flavonoid content

The aluminium chloride colorimetric assay is used to evaluate phenolic content. 10% (w/v) AlCl3 was dissolved in double-distilled water. 9.8g of potassium acetate was dissolved in 100mL of water to form a 1 M potassium acetate solution, which was filtered. 3.2g of quercetin was dissolved in methanol to prepare different standard solutions. 2 mL of the different standards and plant extracts were mixed with 100 µL of $AlCl_3$ and 100 µL of potassium acetate (1 M). Then 2.8mL of distilled water was added. The blank was prepared by replacing the $AlCl_3$ with distilled water. Then incubated for 30mins at room temperature, and absorbance was taken at 415nm. Quercetin as the standard for flavonoid determination content, TPC of extract expresses as mg QE/g of extract (Rao et al., 2016)

Antioxidant activity

DPPH assay

8 mg of DPPH was dissolved in 100mL methanol. 2mL of DPPH was mixed with different grades of standard (ascorbic acid) and plant extracts at different concentrations ranging from 20-120µL/mL. Absorbance was measured at 517 after 30 minutes (Chima et al., 2014).

ABST assay

ABTS (7 mM) was mixed with APS (2.45 mM) and diluted 100-fold for making working reagent. Standards (Ascorbic acid) and samples (100 µL) at various concentrations were mixed with 2mL of ABTS. Incubated without light at room temperature for 10 min. Absorbance was taken at 750 nm (Alam et al., 2013).

Reducing power

Sample and standard at different concentrations were combined with 2.5ml potassium ferricyanide (1%) and 2.5mL phosphate buffer (pH 6.6). 20min incubation at 50ºC. Added 2.5 ml TCA (10%) to terminate the reaction. 2.5ml of supernatant were collected after centrifuged at 3000rpm for 10min then diluted with 2.5ml of water and 0.5ml of $FeCl_3$ (0.1%). Measured the absorbance at 700nm (Alam et al., 2013).

Animals

The 4-6 weeks-old mice weighing 25.0 ± 2.0g were housed in the animal house of Assam University, Silchar, with 12-h light/12-h light cycle with unrestricted access to standard feed and water. After 10 days of acclimatization, the mice were allocated randomly into five experimental groups with six each group. Group I assigned as control and administered 0.5 mL of normal saline by oral gavage. Groups II, III, IV, and V administered 20mg/kg of arecoline orally. Groups III, IV, and V administered 500mg/kg and 250mg/kg of methanolic extract of *L.adscendens* and 100mg/kg of silymarin, subsequently after arecoline, by oral gavage. The animals were treated daily for 28 consecutive days.

Blood sampling and tissue homogenate

Following the last treatment, the mice were fasted for 12 hrs and sacrificed to collect blood samples. Centrifugation at 3000rpm for 15 minutes let to separation of serum (Zhang et al., 2023, Yumnam et al., 2026). Mice were perforated using saline and livers were collected. 10% w/v liver tissue homogenate in ice-cold saline were prepared and centrifuged at 3000rpm for 10min (Rehman, 1984) for Lipid peroxidation assay (LPO), superoxide dismutase (SOD), and Glutathione S-transferase (GST).

Biochemical analysis

The biochemical serum parameters, such as TB (total bilirubin), DB (direct bilirubin), alkaline phosphatase (ALP), SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), and TP (total protein),

were estimated using chemical analyzer with the commercially available diagnostic kits. The manufacturer's instructions were used for the analysis.

Estimation of lipid peroxidation levels:

1 ml of the tissue homogenate supernatant was under incubation at 37°C for 2 h followed by addition of 1 ml of trichloroacetic acid (TCA, 10%, w/v). The mixture was centrifuged at 2000 rpm for 5min. 1 ml of the reaction mixture was mixed with 1mL of thiobarbituric acid (TBA) (0.67% w/v) and bathed at 100-110°C for 10min. Add 1 ml of distilled water after cooling. Optical density (OD) was recorded at 535nm (Rehman, 1984).

Estimation of superoxide dismutase activity:

100 µl supernatants of tissue homogenate were mixed with 2900 µl of Tris-HCl buffer (pH 8.5). 25 µl of pyrogallol (24 mM) was added to the solution followed by 1 min incubation. Absorbance was recorded at 420 nm (Marklund & Marklund (1974).

Estimation of reduced glutathione S transferase:

2.7 mL of phosphate buffer (0.1 M, pH 6.5) was mixed with 100 µL of reduced glutathione to form a final concentration of 1 mM. 100 µl of 1-chloro-2,4-dinitrobenzene (CDNB) (25nm) and the tissue homogenate supernatant of 100 µl was added. The absorbance was taken at 340nm (Habig et al. 1974).

Estimation of reduced glutathione:

Liver tissues were homogenized in 5-8 mL of EDTA (0.02 M), then ice-cold distilled water of 2 ml was added, followed by 1 mL TCA (50% w/v), followed by centrifuging at 6000 rpm for 15min. 1 mL of the supernatant was combined with 2 mL of 0.4M Tris buffer (pH 8.9) followed by addition of 100 µL of 0.1M DTNB. Optical density was recorded at 410nm (Sedlak & Lindsay, 1968).

Estimation of NO:

Griess method was used for NOx estimation. Each serum sample undergoes deproteinization by adding ethanol at a 1:2 (v/v) ratio and mixing thoroughly. Centrifuging the mixture for 10 min at 4°C for 10000 x g. 100 µL of supernatant from deproteinized serum samples was mixed with 100 µL of 8 mg/mL $VaCl_3$ for the reduction of nitrate (NO_3^-) to nitrite (NO_2^-). Sulfanilamide (2% prepared in 5% HCl) was mixed at a volume of 50 µL followed by equal volume of 0.1% N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD). Following the reaction to proceed for 30 minutes at 37°C, and absorbance was taken at 540nm. To determine nitrite concentration, the absorbance of a linear standard curve prepared from sodium nitrite was used (Ghasemi et al., 2007; Yucel et al., 2012).

Statistical Analysis

Statistics were estimated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for comparison of different parameters. The data were analyzed using SPSS version 27 and Microsoft Excel; p-values ≤ 0.05 and ≤ 0.01 were considered statistically significant.

Result

Qualitative phytochemical analysis of the methanolic extract of L. adscendens confirmed the presence of cardiac glycosides, alkaloids, flavonoids, saponins, phenolic compounds, tannins, quinones, carbohydrates, amino acids, carboxylic acids, and terpenoids (Table 1)

Table 1. Preliminary phytochemical screening of the methanolic extract of *L. adscendens*

Sl. no	Screening outcome	Phytoconstituents identified	Corresponding assays
1	Strongly present (++)	Cardiac glycosides; Flavonoids; Saponins	Keller–Killiani; Ferric chloride; $NaHCO_3$
2	Present (+)	Alkaloids; Carbohydrates; Amino acids; Phenolic compounds; Tannins; Quinones; Terpenoids; Carboxylic acids	Hager's; Molisch's; Ninhydrin; Lead acetate; Braymer's; Concentrated HCl; Salkowski's; Effervescence

Table 2. Total phenolic, flavonoid content, and antioxidant capacity of *L. adscendens*

Sample	**Total flavonoids (mg of QE/10g of dry extract)**	**Total phenolic content (mg GA/100g of dry extract)**
L. adscendens	15.88 ±0. 26	9.67 ± 0.03

The TPC and TFC of methanolic extract of *L. adscendens* were found to be 9.67 ± 0.03 mg GA/100g of dry extract and 15.88 ±0. 26 mg of QE/10g of dry extract, respectively (Table 2).

Table 3. Antioxidant activity of *L. adscendens*

Treatment	**DPPH IC_{50} (µg/mL)**	**Reducing power IC_{50} (µg/mL)**	**ABST (mg/mL)**
Standard (Ascorbic acid)	54.843 ± 3.01	162.24 ± 3.24	61.8 ± 3.2
L. adscendens	80.048 ± 5.38	101.57 ± 3.34	75.5 ± 4.2

the DPPH assay, the extract demonstrated an IC_{50} of 80.048 ± 5.38 µg/mL. The ABST assay showed an IC_{50} of 75.5 ± 4.2 mg/mL. In the reducing power assay, the extract showed an IC_{50} of 101.57 ± 3.34 µg/mL, against the standard, ascorbic acid, IC_{50} values of 54.843 ± 3.01 µg/mL, 162.24 ± 3.24 µg/mL, and 61.8 ± 3.2 µg/mL for DPPH, reducing power, and ABST (Table 3).

Table 4. Anti-inflammatory activity of *L. adscendens*

Concentration (µg/ml)	**Acetyl salicylic acid (ASA)**	***Ludwigia adscendens* L.**
200	69.43 ±0.20	29.24 ± 0.47
400	71.49 ± 0.32	41.01 ± 0.46
600	91.92 ± 0.26	55.29 ± 0.51
800	94.32 ±0.48	61.69 ± 0.54
1000	95.44 ±0.91	70.63 ± 0.71

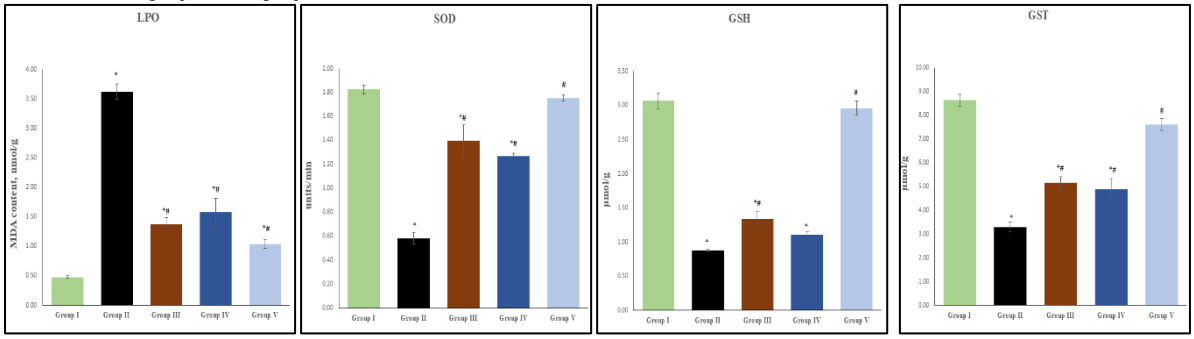
The anti-inflammatory potential of *L. adscendens* methanolic extract was assessed using an anti-inflammatory assay. The extract demonstrated concentration-dependent anti-inflammatory effects. The egg

albumin anti-inflammatory assay displayed minimum inhibition at 42.67 ± 0.71 and maximum inhibition of 79.20 ± 0.43 (Table 4).

Table 5. Effect of *L. adscendens* on oxidative stress in different mice groups

Groups	**LPO (MDA content, nmol/g)**	**SOD (units/min)**	**GSH (µmol/g)**	**GST (µmol/g)**
Group I	0.48 ± 0.02	1.82 ± 0.04	3.06 ± 0.12	8.64 ± 0.24
Group II	3.62 ± 0.13*	0.58 ± 0.05*	0.88 ± 0.02*	3.29 ± 0.21*
Group III	1.37 ± 0.11*#	1.40 ± 0.13*#	1.34 ± 0.11*#	5.15 ± 0.26*#
Group IV	1.58 ± 0.23*#	1.27 ± 0.02*#	1.11 ± 0.04*	4.90 ± 0.42*#
Group V	1.04 ± 0.08*#	1.75 ± 0.02#	2.96 ± 0.10#	7.61 ± 0.24#

Values = mean ± SEM (6 mice per group); * indicates significance at p≤0.01 compared with control and the remaining groups. # is the significance at p≤0.01 compared with the arecoline-treated group and other groups.



1 1 (b) 1 1 (d)

Fig 1 Effects of methanolic extract of *L. adscendens* plant extract on serum levels of (a) LPO, (b) SOD, (c) GSH and (d) GST in different groups.

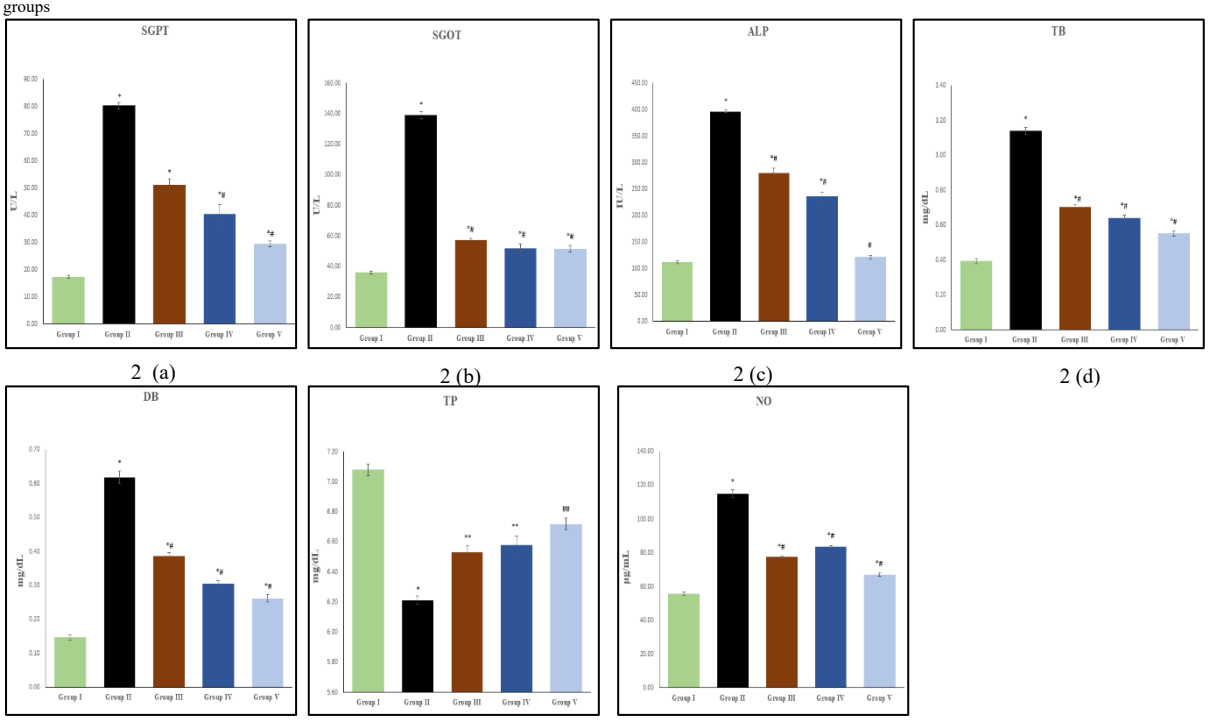
Administration of arecoline increased LPO content of liver in the arecoline-treated group (3.62 ± 0.13 MDA content, nmol/g) compared to the normal control group (1.04 ± 0.08 MDA content, nmol/g), p≤ 0.01. When treated with *L. adscendens* methanolic extract, a significantly lower MDA content was observed at doses of 250mg/kg (1.58 ± 0.23 MDA content, nmol/g) and 500mg/kg (1.37 ± 0.11 MDA content, nmol/g) relative to the arecoline group, p≤ 0.01 (Table 5 & Fig. 1a).

SOD was significantly reduced to 0.58 ± 0.05 units/min in arecoline-treated group compared with the control (1.82 ± 0.04 units/min), p ≤ 0.01. However, treatment with the *L. adscendens* extract at 500mg/kg and 250mg/kg elevated SOD levels to 1.40 ± 0.13 units/min and 1.27 ± 0.02 units/min, respectively, significant difference was observed relative to arecoline exposed group (p≤0.01; Table 5 & Fig 1 b). GSH activity was significantly reduced in the arecoline-treated group (p≤ 0.01). However, simultaneous treatment of arecoline with *L. adscendens* at 500mg/kg and 250mg/kg boosted the GSH levels at 1.34 ± 0.11 µmol/g and 1.11 ± 0.04 µmol/g, respectively, as compared to the arecoline-treated group (0.88 ± 0.02 µmol/g), p≤0.01 (Table 5 & Fig 1 c). Marked decreased in liver GST concentration was observed following arecoline exposure (3.29 ± 0.21 µmol/g) to control (8.64 ± 0.24 µmol/g, p≤0.01). Treatment with 250mg/kg of methanolic extract of *L. adscendens* significantly increased GST levels to 4.90 ± 0.42 µmol/g and 500mg/kg to 5.15 ± 0.26 µmol/g relative to the arecoline-treated group at p≤0.01 (Table 5 & Fig. 1d).

Table 6. Effect of *L. adscendens* on serum liver function biomarkers in different mice groups

Groups	TB (mg/dL)	DB (mg/dL)	SGPT (U/L)	SGOT (U/L)	ALP (IU/L)	TP (mg/dL)	NO (µg/mL)
Group I	0.39 ± 0.01	0.15 ± 0.01	17.30 ± 0.54	36.07 ± 0.91	111.87 ± 2.80	7.08 ± 0.04	55.76 ± 1.05
Group II	1.14 ± 0.02*	0.62 ± 0.02*	80.33 ± 1.27*	139.04 ± 2.34*	396.63 ± 2.28*	6.21 ± 0.03*	115.05 ± 2.27*
Group III	0.64 ± 0.01*#	0.31 ± 0.01*#	40.35 ± 3.48*#	51.88 ± 2.87*#	235.92 ± 8.04*#	6.58 ± 0.06**	83.70 ± 0.51*#
Group IV	0.70 ± 0.02*#	0.39 ± 0.01*#	51.20 ± 2.28*#	57.47 ± 0.79*#	280.37 ± 9.39*#	6.53 ± 0.04**	77.56 ± 0.55*#
Group V	0.55 ± 0.01*#	0.26 ± 0.01*#	29.43 ± 1.05*#	51.60 ± 2.08*#	121.37 ± 3.50#	6.72 ± 0.04##	67.03 ± 0.89*#

Values = mean ± SEM (6 mice per group); * indicates significance at p≤0.01 compared with control. * * is the significance at p≤0.05, compared with the control, # is the significance at p≤0.01, compared with the arecoline-treated group with other groups, ## is the significance at p≤0.05, compared with the arecoline-treated group with other groups



2 (a) 2 (b) 2 (c) 2 (d)

2 (e) 2 (f) 2 (g)

Fig 2 Effects of methanolic extract of *L. adscendens* plant extract on serum levels of (a) SGPT, (b) SGOT, (c) ALP, (d) TB, (e) DB, (f) TP and (g) NO in different groups. Data were analyzed by one-way ANOVA followed by Tukey post hoc test. *p<0.01, **p<0.05 vs. Group I, #p<0.01, ##p<0.05 vs. Group II

The study demonstrated a significant rise in SGPT concentration in the arecoline exposed group and in those administered the methanolic plant extract (250 mg/kg and 500 mg/kg) vs with the control ($p \leq 0.01$). There was a notable decrease in the serum SGOT concentration of mice when treated with the methanolic extract of *L. adscendens* (250 mg/kg and 500 mg/kg), with $p \leq 0.01$ compared with the arecoline group (Table 6 & Fig. 2 (a-b)).

The experiment also indicated a considerable increase in ALT (396.63 ± 2.28 IU/L), TB (1.14 ± 0.02 mg/dL), and DB (0.62 ± 0.02 mg/dL) levels in serum of the arecoline exposed group compared to those with control ($p \leq 0.01$) (Table 6 & Fig 2 (c-e)). The study also showed a significant decrease in ALT, TB, and DB concentrations in the treated groups with methanolic extract of *L. adscendens* at 250 mg/kg and 500 mg/kg ($p \leq 0.01$) compared with the arecoline-treated group. Regarding the total protein concentration, this study showed that there was a significant decreased among the arecoline treated mice (6.21 ± 0.03 mg/dL) and the control (7.08 ± 0.04 mg/dL) ($p \leq 0.01$) (Table 6 & Fig 2 f) and those treated with methanolic extract of *L. adscendens* at 250 mg/kg (6.53 ± 0.04 mg/dL) and 500 mg/kg (6.58 ± 0.06 mg/dL), $p \leq 0.05$, when compared to those with control mice group. However, no significant difference was observed in total protein concentration between the arecoline-treated mice and the methanolic plant extract-treated mice. Moreover, the total protein concentration between the arecoline-treated and silymarin-treated groups 6.72 ± 0.04 mg/dL ($p \leq 0.05$; Table 6 & Fig. 2f) showed differences.

The study showed a significant increase in serum NO concentration in arecoline-treated mice ($p \leq 0.01$) compared with normal controls. Moreover, those mice treated with the methanolic extract of *L. adscendens* at 250mg/kg and 500mg/kg ($p \leq 0.01$) showed a decline in serum NO concentration compared to the control group (Table 6 & Fig. 2g).

Discussion

Phytochemical profiling of *L. adscendens* revealed the presence of phenols, flavonoids, cardiac glycosides, quinones, saponins, and alkaloids. These phytochemicals exhibit a wide range of pharmacological and therapeutic potential against various diseases by modulating different pathways involved in these diseases (M. Sharma et al., 2022). The study reported that the total phenolic content was 9.67 ± 0.03 mg GA/100g of dry extract, and the flavonoid content was 15.88 ± 0.26 mg QE/10g (Table 2). Natural plant-derived compounds, mainly phenols and flavonoids, might be promising candidates for antioxidant and anti-inflammatory properties (Ali et al., 2025). Antioxidant activities were measured by the plant extract's ability to scavenge DPPH and ABTS and by its reducing power (Table 3). Antioxidants have free radical-scavenging properties that can prevent the oxidation of biomolecules by blocking the propagation of oxidative chain reactions (Ali et al., 2025). *L. adscendens* extract showed lower antioxidant potential than the reference compound, ascorbic acid. The presence of diverse phytochemicals and the significant antioxidant capacity may help protect the body against oxidative stress and liver damage. It has also been reported that flavonoids and phenolic compounds exhibit anti-inflammatory, anticlastogenic, immunomodulatory, antioxidant, hepatoprotective, and antitumor properties (Ali et al., 2025).

Continuous exposure to toxic substances is strongly associated with severe systemic dysfunction and profound liver abnormalities (Sun et al., 2023). Hepatotoxicity is characterized by loss of hepatocellular membrane integrity, leading to leakage of cytosolic enzymes into the systemic circulation (Nipanikar et al., 2017). Arecoline-induced hepatotoxicity progresses gradually by inducing oxidative damage, activating inflammatory pathways, impairing protein synthesis, disrupting membrane integrity, promoting apoptosis, and disrupting metabolic pathways, including cholestatic disturbances. The study demonstrated the hepatotoxic effect of arecoline, aligning with the previous studies (Cao et al., 2025).

Hepatic lipid peroxidation remarkably elevated in the arecoline-treated group. The marked increase in MDA levels indicated the initiation of peroxidative membrane degradation and functional integrity, leading to membrane disruption (Jeng et al., 2014; Lacour et al., 2005). Reduction in SOD levels in the arecoline-treated group indicated increased mitochondrial superoxide radical accumulation (Karkucinska-Wieckowska et al., 2022). The plant extract upregulated the enzyme-catalyzed conversion of superoxide radicals into hydrogen peroxide, which is further detoxified by other enzymes (Karkucinska-Wieckowska et al., 2022). GSH levels decreased in the arecoline-treated group due to depletion of intracellular thiol reserves (Villanueva-Paz et al., 2021). GST activity in the arecoline-treated group was significantly reduced, suggesting the impaired detoxification and conjugation of glutathione with reactive metabolites of arecoline upon long-term exposure (Donmez et al., 2025; Singh et al., 2024). Depletion of antioxidant enzymes like GSH, GST and SOD rendered hepatocytes more susceptible to oxidative damage, leading to cellular impairments, mitochondrial dysfunction, reduced detoxification capacity, and necrosis (Cao et al., 2025; Zhou et al., 2014). The restoration of antioxidant enzyme levels in *L. adscendens*-treated groups was dose-dependent. Methanolic extract of *L. adscendens* enhanced the capacity of conjugate formation and elimination of toxic metabolites. The antioxidant

system protects the body against free radicals and oxidative (Dasgupta et al., 2006). The plant extract attenuated hepatic injury by limiting free radicals' accumulation and enhancing the expression of endogenous antioxidant enzyme expression (Ali et al., 2025).

NO is usually produced in large amounts when there is inflammation, infection, and drug-induced liver injury (Senevirathna et al., 2023). Treatment with arecoline upregulates NO production, suggesting the potential for hepatic injury and inflammation (N. Sharma et al., 2014). Excess NO reacts with superoxide radicals to form peroxynitrite, causing nitrosative stress (Adnan et al., 2020). In the present study, L. adscendens-treated groups (III & IV) significantly attenuated arecoline-induced hepatotoxicity by suppressing NO overproduction, thereby reducing nitrosative and other oxidative stress.

Arecoline-induced hepatotoxicity is evident by increases in enzymes such as SGOT and SGPT (Table 6). This is caused by the accumulation of highly reactive metabolites, which initiate lipid peroxidation, disrupt hepatocellular membrane integrity, and lead to parenchymal necrosis, thereby allowing intracellular aminotransferase to escape into the bloodstream (Burra et al., 2025; Chakrabarty et al., 2022). SGOT indicates cytosolic and mitochondrial damage, as well as hepatocyte necrosis (Chakrabarty et al., 2022). Additionally, elevated bilirubin levels indicate ineffective bilirubin excretion, which may be due to biliary obstruction or hepatocyte destruction (Villanueva-Paz et al., 2021). Elevated TB and DB suggested improper bilirubin conjugation, indicating a broader failure of hepatic function (Thakur et al., 2024; Yang et al., 2017). Also, ALP is involved in biliary dysfunction and canalicular dysfunction (Seen, 2021). This suggested that arecoline induced hepatotoxicity in mice by altering membrane structure and bile-associated dysfunction. Hepatocellular dysfunction reduces the synthesis of blood plasma proteins (Nipanikar et al., 2017; Pandey et al., 2024). Moreover, arecoline downregulates total protein content, suggesting it induces liver injury.

The antihepatotoxic effects *L. adscendens* were compared with those of silymarin. In this study, oral administration of arecoline altered antioxidant enzyme levels, increased liver biomarker enzymes, and decreased total protein content. A significant decrease in liver biomarkers was observed following administration of a methanolic extract of *L. adscendens*. This could be due to differences in the phytochemicals with antioxidant potential. The terpenoids, phenols, tannins, flavonoids, and other secondary metabolites actively reduce lipid peroxidation and oxidative stress by scavenging ROS. *L. adscendens* extract restored the endogenous antioxidant and stabilized the membrane integrity, exhibiting dose-responsive effect. The plant extract also significantly restored total protein content to levels similar to those of the control. This indicated that the phytocompounds have the potential to restore the liver's synthetic function to produce proteins and maintain metabolic homeostasis. The high dose (500mg/kg) showed greater hepatoprotective activity than the 250mg/kg dose.

Conclusion

The present study demonstrated that prolonged exposure to arecoline caused hepatotoxicity. Arecoline exposure stimulated lipid peroxidation and NO production, causing oxidative and nitrosative stress, DNA damage, and hepatocyte apoptosis. This caused liver injury, parenchymal necrosis, and metabolic dysfunction. This study demonstrated the *L. adscendens* methanolic extract had strong potential to neutralize oxidative and nitrosative stress which stabilize the hepatocellular membrane, thereby stabilizing the lipid bilayer and preventing leakage of serum liver biomarkers. Simultaneously, the methanolic *L. adscendens* extract significantly restored normal liver function. In addition, the plant extract upregulated the liver's biosynthetic capacity. These effects may result from bioactive molecules such as flavonoids, phenols, terpenoids, and quinones. These findings highlight the hepatoprotective potential of the methanolic extract of *L. adscendens* against arecoline-induced liver damage, primarily through antioxidant mechanisms and the restoration of redox homeostasis. The extract suppressed lipid peroxidation and nitrosative damage, restored endogenous antioxidant potential, thereby preventing hepatic dysfunction and normalizing liver function. The plant demonstrated promise as a future candidate for hepatoprotection, potentially combating drug-induced hepatotoxicity and liver-related diseases.

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Ethical Approval: The handling of mice was approved under AUS/IAEC/PY-R(a)/2023-1/16 by Institutional Animal Ethics Committee, Assam University, Silchar.

Data Availability: The data that support the findings of this study are original and provided within the manuscript and supplementary file.

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