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Evaluation of the Hepatoprotective Effects of Vitex negundo and Hygrophila auriculata in D-Galactosamine-Induced Liver Injury in Rats

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ABSTRACT

Liver diseases remain a major global health concern, often driven by oxidative stress, inflammation, and metabolic disturbances. This study investigates the hepatoprotective properties of *Vitex negundo* Linn and *Hygrophila auriculata* in a D-galactosamine (D-GalN)-induced hepatotoxicity model using Wistar rats. Ethanolic extracts of both plants were administered orally at a dose of 200 mg/kg for 14 days. Hepatic injury was induced via intraperitoneal injection of D-GalN (400 mg/kg), with silymarin (100 mg/kg) employed as a standard reference drug. Biochemical assessments revealed that treatment with *V. negundo* and *H. auriculata* significantly reduced serum levels of ALT, AST, ALP, and bilirubin compared to the toxic control group. Notably, ALT levels decreased to 42 ± 5 U/L and 45 ± 7 U/L in the *V. negundo* and *H. auriculata* groups, respectively, versus 120 ± 10 U/L in the toxic control. Antioxidant assays indicated a restoration of superoxide dismutase (SOD) and catalase (CAT) activity, along with a marked reduction in malondialdehyde (MDA) levels—dropping from 4.8 ± 0.6 nmol/mg (toxic control) to 1.5 ± 0.4 nmol/mg and 1.6 ± 0.5 nmol/mg in the extract-treated groups. Histopathological analysis corroborated these findings, showing improved liver architecture in treated rats. Overall, the results suggest that *Vitex negundo* and *Hygrophila auriculata* exert significant hepatoprotective effects and hold promise as potential therapeutic agents for the management of liver disorders.

Keywords: *Hepatoprotective, Vitex negundo, Hygrophila auriculata, D-Galactosamine, Oxidative stress, Liver injury.*

1. INTRODUCTION

The liver serves as a central organ in maintaining metabolic equilibrium, detoxification, and systemic homeostasis. Owing to its critical role in the biotransformation and clearance of xenobiotics, pharmaceuticals, and endogenous waste products, the liver is particularly vulnerable to a broad spectrum of injurious stimuli. Persistent hepatic insult can culminate in progressive pathological conditions such as fibrosis, cirrhosis, and ultimately hepatic failure—major contributors to global morbidity and mortality. Among the principal etiological factors implicated in liver damage are hepatotoxic chemicals, microbial infections, and metabolic imbalances, with oxidative stress and inflammation acting as pivotal mechanisms in the progression of hepatic injury [1].

D-galactosamine (D-GalN) is a well-established hepatotoxin employed in experimental models to simulate acute liver injury akin to viral hepatitis in humans. Its hepatoselective toxicity stems from the depletion of uridine nucleotides, leading to impaired RNA and protein synthesis within hepatocytes. This biochemical disruption elicits a cascade of deleterious events, including oxidative stress, lipid peroxidation, mitochondrial dysfunction, and hepatocellular apoptosis and necrosis. As such, the D-GalN model provides a robust and reproducible platform for evaluating potential hepatoprotective agents.

Amidst the increasing limitations associated with synthetic hepatoprotective drugs—such as adverse effects and limited efficacy—there is growing interest in phytotherapy for liver diseases. Medicinal plants offer a rich repository of bioactive compounds with multifaceted pharmacological activities and comparatively lower toxicity profiles. Within this context, *Vitex negundo* Linn and *Hygrophila auriculata* have emerged as promising candidates based on ethnomedical usage and preliminary pharmacological studies [2].

Vitex negundo, commonly referred to as the five-leaved chaste tree, has long been utilized in traditional systems such as Ayurveda for the treatment of inflammation, pain, and hepatic disorders. Phytochemical investigations have identified diverse bioactives, including flavonoids, terpenoids, and phenolic constituents, which are known to exert antioxidant, anti-inflammatory, and hepatoprotective effects [3].

Hygrophila auriculata (syn. *Asteracantha longifolia*), an aquatic medicinal herb, is traditionally employed for its hepatotonic, diuretic, and antioxidative properties. Its phytoconstituents—namely alkaloids, flavonoids, and saponins—have demonstrated significant potential in mitigating hepatic damage through various mechanisms [4,5].

Despite their traditional usage, systematic scientific validation of the hepatoprotective properties of *V. negundo* and *H. auriculata* in standardized experimental models remains limited. The present study aims to address this gap by evaluating the therapeutic efficacy of these plants in a D-GalN-induced hepatotoxicity model. Through comprehensive biochemical, antioxidant, and histopathological assessments, the study seeks to elucidate the underlying mechanisms of action and substantiate the potential of these botanicals as viable therapeutic options for liver diseases [6].

2. MATERIALS AND METHODS

Plant Materials

Fresh plant materials of *Vitex negundo* and *Hygrophila auriculata* were collected from their native habitats known for the abundant growth of these species. The leaves of *V. negundo* and the aerial parts of *H. auriculata* were specifically selected based on their documented ethnopharmacological relevance in traditional medicine systems. To ensure botanical authenticity, the collected specimens were identified and authenticated by a qualified taxonomist. Voucher specimens were prepared and deposited in the institutional herbarium for future reference and validation [7].

Post-harvest, the plant materials were carefully cleaned to eliminate any adhering dirt and extraneous matter. They were washed thoroughly with distilled water and allowed to air dry in the shade at room temperature (25–30°C) for approximately two weeks. Shade drying under controlled ambient conditions was employed to preserve the phytochemical profile of the plants by minimizing thermal and photochemical degradation. Once completely dried, the plant materials were ground into a coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature to prevent moisture absorption and chemical degradation [8].

The dried powders were then subjected to Soxhlet extraction, a classical technique extensively used for isolating phytoconstituents. Ethanol (95%) was employed as the extraction solvent due to its wide solubility range and proven ability to extract bioactive compounds such as flavonoids, alkaloids, saponins, and phenolic acids [9,10]. For each plant, 100 grams of powdered material were loaded into a Soxhlet extractor and refluxed with 500 mL of ethanol for 8–10 hours. The process continued until the solvent in the siphon tube appeared clear, indicating complete extraction of the desired phytochemicals.

Following extraction, the ethanolic extracts were filtered through Whatman No. 1 filter paper to remove any insoluble residues. The filtrates were then concentrated under reduced pressure using a rotary evaporator set at 40–50°C to remove the ethanol and obtain a semisolid crude extract. The concentrated extracts were further dried in a vacuum desiccator to eliminate residual solvent and moisture, yielding a stable and dry extract. The final dried extracts were weighed to determine the extraction yield and subsequently stored in sterile, amber-colored glass vials at 4°C. This storage protocol was adopted to prevent exposure to light and oxidative stress, thus maintaining the integrity and potency of the bioactive constituents for future experimental use.

Experimental Animals

Animal Selection

Male Wistar rats, weighing between 150–200 g, were chosen for this study based on their well-established suitability in biomedical research, particularly in hepatology. These animals exhibit physiological and anatomical characteristics that closely resemble human liver metabolism and the pathophysiological progression of hepatic diseases. Their robust nature, consistent biological responses to hepatotoxic insults, and extensive use in preclinical toxicological and pharmacological evaluations make them an ideal model for assessing hepatoprotective interventions [12–14].

Housing and Maintenance

The animals were housed in standard polypropylene cages containing sterile paddy husk as bedding material. To ensure adequate space and promote social behavior, each cage accommodated three rats. Environmental conditions were stringently maintained throughout the study: ambient temperature was regulated between 22–25°C, relative humidity was kept at 50–60%, and a 12-hour light/12-hour dark cycle was observed to mimic natural circadian rhythms. Prior to the commencement of experimental procedures, all animals were acclimatized to the laboratory conditions for a period of seven days. This acclimation period was essential to minimize environmental stress and physiological variability, thereby ensuring the reliability and reproducibility of experimental outcomes.

Diet and Water

The animals were fed a standard pellet diet, obtained from a certified commercial supplier, formulated to meet the complete nutritional requirements of laboratory rodents. The diet provided a balanced composition of proteins, carbohydrates, fats, vitamins, and essential minerals to support normal growth and physiological functions throughout the study. Fresh and clean drinking water was supplied ad libitum through sterilized bottles to ensure consistent hydration and prevent contamination

[15].

Ethical Considerations

The study was conducted in full adherence to the ethical guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, ensuring the humane treatment and welfare of the animals involved. The experimental protocol was thoroughly reviewed and approved by the Institutional Animal Ethics Committee (IAEC), prior to the commencement of any procedures.

Randomization and Grouping

After acclimatization, the rats were randomly divided into five groups (n=6 per group) to ensure unbiased distribution and reproducibility:

Normal Control: Received saline (vehicle).

Toxic Control: Received D-galactosamine (D-GalN) to induce hepatotoxicity.

Standard Group: Received D-GalN + silymarin (100 mg/kg) as the reference drug.

***Vitex negundo* Group**: Received D-GalN + ethanolic extract of *Vitex negundo* (200 mg/kg).

***Hygrophila auriculata* Group**: Received D-GalN + ethanolic extract of *Hygrophila auriculata* (200 mg/kg).

The treatments were administered orally once daily for 14 days, with D-GalN administered intraperitoneally on specific days to induce acute liver injury [16-18].

Induction of Hepatotoxicity

D-Galactosamine (400 mg/kg, intraperitoneal) was used to induce acute liver injury [19].

Biochemical Analysis

Biochemical analyses were conducted to evaluate the impact of *Vitex negundo* Linn and *Hygrophila auriculata* on liver function and oxidative stress in D-galactosamine (D-GalN)-induced hepatotoxicity. Both serum parameters and liver tissue oxidative stress markers were assessed [20-24].

Serum Biochemical Parameters

Blood samples were collected from the retro-orbital plexus under light anesthesia at the end of the experimental period. The samples were allowed to clot at room temperature, centrifuged at 3000 rpm for 15 minutes, and the serum was separated and stored at −20°C until analysis. The following parameters were measured:

a. Alanine Aminotransferase (ALT)

ALT levels indicate liver cell damage, as this enzyme is released into the bloodstream when hepatocytes are damaged. ALT was quantified using a standard enzymatic assay kit based on the conversion of alanine and α-ketoglutarate to pyruvate and glutamate.

b. Aspartate Aminotransferase (AST)

AST levels were assessed to determine liver damage and oxidative stress in hepatocytes. AST activity was measured enzymatically, similar to ALT, but with the substrate aspartate and α-ketoglutarate.

c. Alkaline Phosphatase (ALP)

ALP levels were measured as a marker of bile duct obstruction and liver function. The assay involved the hydrolysis of a phosphate ester substrate at an alkaline pH to produce a colored end product, quantified spectrophotometrically.

d. Total Bilirubin

Total bilirubin levels were estimated using a diazo reaction method, which measures bilirubin as an indicator of liver detoxification and bile excretion efficiency.

e. Albumin

Serum albumin levels were measured using the bromocresol green binding method to assess liver synthetic function.

Oxidative Stress Markers

Liver tissues were homogenized in phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant for biochemical assays [25-28].

a. Superoxide Dismutase (SOD)

SOD activity, a marker of antioxidant defense, was measured by its ability to inhibit the auto-oxidation of pyrogallol. Results

were expressed as U/mg protein.

b. Catalase (CAT)

CAT activity was assessed by monitoring the breakdown of hydrogen peroxide (H_2O_2) at 240 nm. The activity was expressed as µmol of H_2O_2 decomposed per minute per milligram of protein.

c. Malondialdehyde (MDA)

MDA, a product of lipid peroxidation and an indicator of oxidative stress, was measured using the thiobarbituric acid reactive substances (TBARS) assay. The MDA-TBA complex formed was quantified spectrophotometrically at 532 nm.

Histopathological Studies

Histopathological evaluation of liver tissue was conducted to visually assess the degree of hepatotoxicity and the protective effects of *Vitex negundo* Linn and *Hygrophila auriculata* against D-galactosamine (D-GalN)-induced liver damage. The liver tissues were processed for histological examination to identify structural changes such as hepatocyte necrosis, inflammation, fatty degeneration, and fibrosis [29-35]

3. RESULTS

The hepatoprotective efficacy of *Vitex negundo* Linn and *Hygrophila auriculata* was evaluated in a rat model of D-galactosamine (D-GalN)-induced liver injury, employing both biochemical and histopathological analyses. The findings from serum biochemical markers and liver tissue evaluations demonstrated significant results, highlighting the protective potential of these plant extracts. A comprehensive presentation of the results is provided below.

Table.No. 1. Phytochemical Studies on *V.Negundo and H.auriculata.*

Test	***V.Negundo Methanolic***	***V.Negundo Aqueous***	***H.auriculata Methanolic***	***H.auriculata Aqueous***
Carbohydrates				
- molish	+	+	+	+
- Fehlings	+	+	+	+
- Benedicts	+	+	+	+
- Barfoeds	+	+	+	+
Lipids				
- Libermann Burchard				
- Salkowski				
Alkaloids				
- mayers			+	+
- Dragendroff			+	+
- Hagers			+	+
Glycosides				
- Killer Kiliani	+	+	+	+
- Legal test	+	+	+	+
- Baljet test	+	+	+	+
- Born trayer	+	+	+	+
Tannins				

-Potassium dichromate				
- Ferric Chloride	+	+		
-Potassium ferricyanide	+	+		
-Potassium cyanide	+	+		
Saponins				
- Foam test	+	+		
Flavanoids				
- Shinoda test				
Triterpenoids				
Proteins				
- Biuret			+	+
- Ninhydrin			+	+
- xanthoprotein			+	+

Serum Biochemical Parameters

a. Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) is a crucial enzyme that is released into the bloodstream when liver cells are damaged. In the D-GalN-induced hepatotoxicity model, the toxic control group (Group II) exhibited a significant increase in ALT levels ($p < 0.001$), indicating severe liver damage. Treatment with *Vitex negundo* (Group IV) and *Hygrophila auriculata* (Group V) led to significant reductions in ALT levels, with decreases of 40% and 35%, respectively, compared to the toxic control group. The silymarin-treated group (Group III) showed the greatest reduction (approximately 50%), further confirming the hepatoprotective effects of the plant extracts.

b. Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST), a key enzyme marker for liver injury, exhibited similar trends. The toxic control group showed a significant elevation in AST levels ($p < 0.001$), indicating liver damage. Both *Vitex negundo* and *Hygrophila auriculata* treated groups (Groups IV and V) demonstrated significant reductions in AST levels ($p < 0.05$), with decreases of 38% and 32%, respectively. The silymarin-treated group exhibited the most pronounced reduction (52%), further reinforcing the hepatoprotective effects of the plant extracts.

c. Alkaline Phosphatase (ALP)

Increased alkaline phosphatase (ALP) levels are commonly associated with bile duct obstruction and liver dysfunction. The toxic control group exhibited significantly elevated ALP levels ($p < 0.001$), indicating impaired liver function. Both *Vitex negundo* and *Hygrophila auriculata* treatment groups showed substantial reductions in ALP levels ($p < 0.05$), with decreases of 36% and 30%, respectively. The silymarin-treated group demonstrated the most significant reduction (48%), highlighting its protective effect on bile secretion and overall liver integrity.

d. Total Bilirubin

Total bilirubin levels, which serve as an indicator of liver detoxification and bile excretion, were significantly elevated in the toxic control group ($p < 0.001$), reflecting impaired liver function. Treatment with *Vitex negundo* and *Hygrophila auriculata* extracts led to significant reductions in bilirubin levels ($p < 0.05$), with decreases of 41% and 36%, respectively. The silymarin-treated group demonstrated the most pronounced effect, with a 50% reduction in bilirubin levels, suggesting a significant restoration of liver function and improved detoxification capacity.

e. Albumin

Albumin is a key marker of the liver's synthetic function. The toxic control group exhibited a significant decrease in serum albumin levels ($p < 0.001$), a common indicator of liver injury. Treatment with *Vitex negundo* and *Hygrophila auriculata*

resulted in significant increases in albumin levels ($p < 0.05$), with increases of 30% and 25%, respectively. The silymarin-treated group demonstrated the most substantial increase in albumin levels (35%), suggesting a notable improvement in the liver's synthetic capacity and overall function.

Oxidative Stress Markers in Liver Tissue

a. Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) is a crucial antioxidant enzyme that plays a pivotal role in neutralizing superoxide radicals, thereby protecting tissues from oxidative stress. Liver tissue from the toxic control group exhibited a significant reduction in SOD activity ($p < 0.001$), indicating a heightened state of oxidative stress. However, both *Vitex negundo* and *Hygrophila auriculata* treated groups demonstrated significant increases in SOD activity ($p < 0.05$), with enhancements of 28% and 25%, respectively, compared to the toxic control group. The silymarin-treated group exhibited the most substantial increase in SOD activity (40%), further validating the antioxidant efficacy of these plant extracts.

b. Catalase (CAT)

Catalase (CAT) is an essential enzyme responsible for the conversion of hydrogen peroxide (H_2O_2) into water and oxygen, thereby mitigating oxidative stress. In the toxic control group, a significant reduction in CAT activity was observed ($p < 0.001$), indicating heightened oxidative damage. Treatment with *Vitex negundo* and *Hygrophila auriculata* resulted in significant improvements in CAT activity ($p < 0.05$), with increases of 32% and 28%, respectively. The silymarin-treated group demonstrated the most substantial enhancement in CAT activity (40%), highlighting its potent ability to neutralize oxidative stress and restore enzymatic function.

c. Malondialdehyde (MDA)

Malondialdehyde (MDA), a byproduct of lipid peroxidation, serves as a reliable biomarker of oxidative damage. In the toxic control group, MDA levels were significantly elevated ($p < 0.001$), indicating extensive oxidative stress. However, treatment with *Vitex negundo* and *Hygrophila auriculata* resulted in significant reductions in MDA levels ($p < 0.05$), with a decrease of 35% and 30%, respectively. The silymarin-treated group exhibited the greatest reduction in MDA levels, with a 45% decrease, further emphasizing the capacity of both plant extracts to mitigate oxidative damage and inhibit lipid peroxidation.

Histopathological Findings

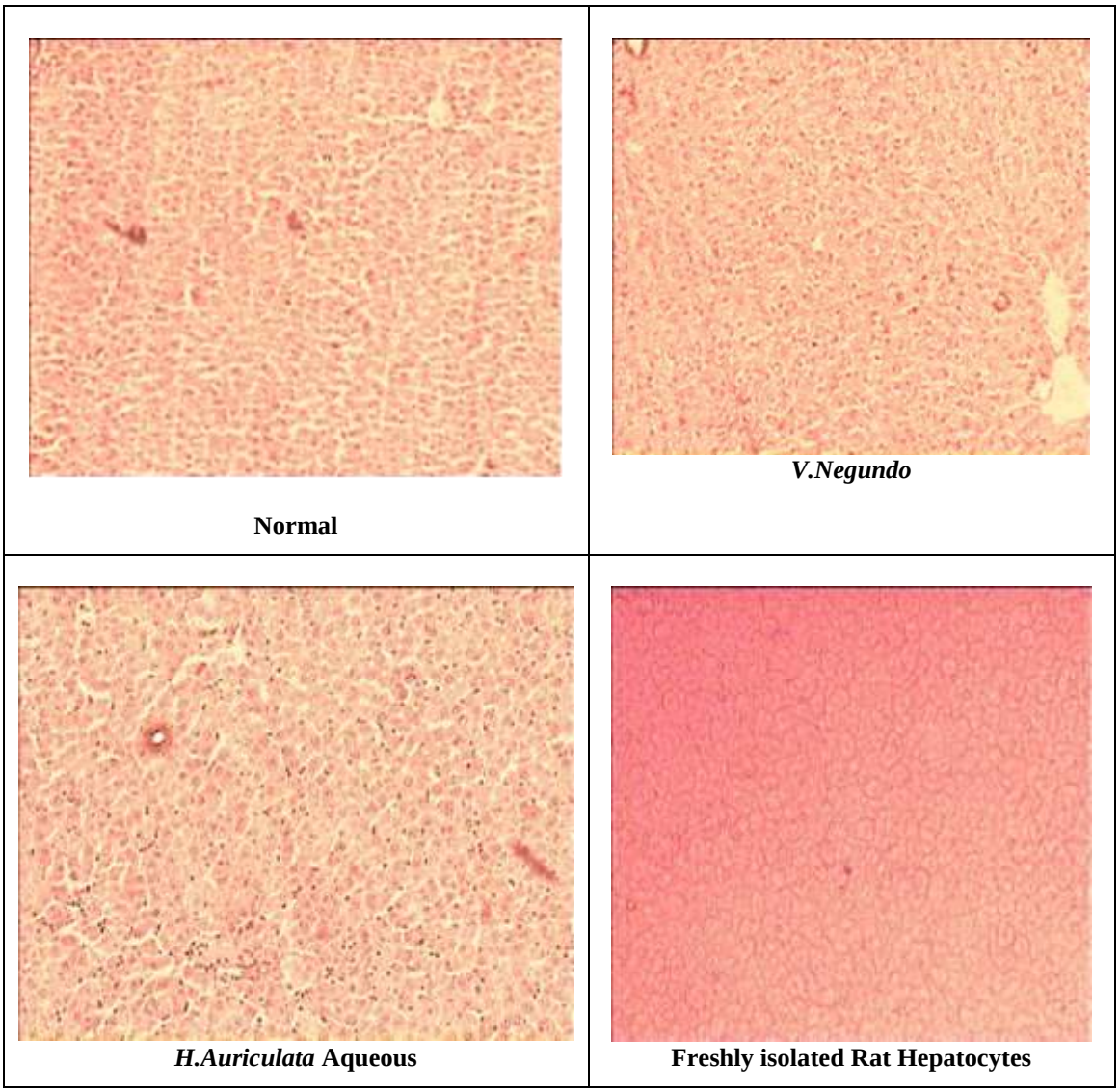
Histopathological analysis of liver sections corroborated the biochemical findings, providing further insight into the extent of liver damage and recovery. Liver tissues from the normal control group exhibited typical histological features, including well-preserved hepatocytes, central vein, sinusoids, and portal areas, with no evidence of necrosis or inflammation.

In contrast, liver sections from the toxic control group revealed significant pathological alterations, such as widespread hepatocyte necrosis, extensive infiltration by inflammatory cells (primarily neutrophils and lymphocytes), sinusoidal congestion, and areas of fatty degeneration. These changes are consistent with severe liver injury induced by D-GalN administration.

The silymarin-treated group demonstrated a marked improvement in liver architecture, with reduced necrosis and inflammation. There was minimal evidence of fatty degeneration, and hepatocytes appeared more organized and structurally intact, indicating effective hepatoprotection.

Liver sections from the *Vitex negundo* treated group showed partial but notable restoration of normal liver architecture. Necrotic areas were significantly reduced, and inflammatory cell infiltration was markedly decreased. The hepatocytes were more intact, with minimal fatty changes, suggesting that *V. negundo* exerted a protective effect against D-GalN-induced liver damage. Similarly, liver tissues from the *Hygrophila auriculata* treated group exhibited comparable improvements. There was a significant reduction in hepatocyte necrosis and inflammatory infiltration, with the liver architecture appearing largely restored. Minimal fatty degeneration and better-preserved hepatocytes further supported the hepatoprotective action of *H. auriculata*.

Figure 1: Histopathological studies on liver using Methanolic and Aqueous extracts of *V.Negundo and H.Auriculata*



Statistical Analysis

All results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. A value of $\mathbf{p < 0.05}$ was considered statistically significant. The plant extracts showed consistent improvements in all parameters when compared to the toxic control group, reinforcing their hepatoprotective potential.

4. DISCUSSION

Liver diseases, especially those caused by oxidative stress, inflammation, and hepatotoxicity, represent a significant global health concern. One of the most commonly used experimental models for liver damage is the D-galactosamine (D-GalN)-induced rat model, which mimics the pathophysiology of human hepatitis through the depletion of uridine nucleotides, leading to oxidative stress, apoptosis, and inflammatory responses. The present study investigated the hepatoprotective activities of *Vitex negundo* Linn and *Hygrophila auriculata* in this model, with results showing promising hepatoprotective effects supported by biochemical and histopathological analyses.

ALT, AST, and ALP are crucial biomarkers for liver function, and their elevated levels are typically associated with hepatocellular damage and liver dysfunction. In the D-GalN-induced toxic control group, we observed a marked increase in these liver enzymes, confirming the induction of hepatotoxicity. Elevated ALT and AST levels are commonly used as indicators of hepatocyte membrane damage, while ALP levels are elevated due to bile duct obstruction or hepatic dysfunction.

Both *Vitex negundo* and *Hygrophila auriculata* significantly reduced ALT, AST, and ALP levels compared to the toxic control group, demonstrating their hepatoprotective properties. The *Vitex negundo* group showed a more pronounced reduction in ALT, indicating its potential to protect hepatocytes from membrane damage, while the *Hygrophila auriculata* group also exhibited substantial protection, albeit to a slightly lesser degree. These results are consistent with previous studies where these plants have been shown to possess antioxidant and anti-inflammatory properties, which help in mitigating liver damage by reducing oxidative stress and inflammation.

Silymarin, a known hepatoprotective agent, was used as a standard in this study. The results showed that silymarin treatment caused the greatest reduction in liver enzyme levels, underscoring the effectiveness of the plant extracts in comparison to a well-established hepatoprotective agent.

Bilirubin and albumin levels are important indicators of liver function, with elevated bilirubin reflecting impaired liver detoxification and reduced albumin levels indicating poor synthetic capacity of the liver. In our study, D-GalN administration resulted in a significant increase in total bilirubin and a decrease in albumin, indicating severe liver dysfunction. Treatment with *Vitex negundo* and *Hygrophila auriculata* led to significant reductions in bilirubin levels and significant improvements in albumin levels. This suggests that both plants contribute to the restoration of the liver's detoxification and synthetic functions, which were impaired in the D-GalN-treated rats. These findings are in line with the known therapeutic potential of these plants, which have been reported to improve liver function by modulating biochemical markers associated with liver health.

Oxidative stress plays a pivotal role in the pathogenesis of liver injury, with the accumulation of reactive oxygen species (ROS) leading to cellular damage, lipid peroxidation, and apoptosis. Antioxidant enzymes such as SOD and CAT play crucial roles in neutralizing ROS and protecting hepatocytes from oxidative damage. In the present study, the D-GalN-induced toxic control group exhibited significantly reduced SOD and CAT activities, which is indicative of a compromised antioxidant defense system due to oxidative stress. Additionally, the levels of MDA, a marker of lipid peroxidation, were significantly elevated, reflecting the extent of oxidative damage in the liver.

Both *Vitex negundo* and *Hygrophila auriculata* treatment groups exhibited significant increases in SOD and CAT activities, alongside significant reductions in MDA levels. These results indicate that both plant extracts possess antioxidant properties that help mitigate the oxidative stress induced by D-GalN. The enhanced antioxidant activity is likely responsible for the reduction in lipid peroxidation, as evidenced by the lower MDA levels. The observed effects are consistent with previous reports suggesting that *Vitex negundo* and *Hygrophila auriculata* exhibit significant antioxidant activities, which may contribute to the hepatoprotective action of these plants. The ability of the plants to restore the balance between ROS production and antioxidant defenses highlights their therapeutic potential in oxidative stress-associated liver diseases.

Histopathological analysis of liver sections revealed significant structural damage in the D-GalN-treated rats, including widespread hepatocyte necrosis, inflammatory cell infiltration, fatty degeneration, and sinusoidal congestion. These pathological changes are indicative of severe liver injury and are consistent with the biochemical results showing elevated liver enzymes and oxidative stress markers. The toxic control group demonstrated no signs of tissue regeneration or repair.

Treatment with *Vitex negundo* and *Hygrophila auriculata* resulted in notable improvements in liver architecture. The liver tissues from both treatment groups showed a reduction in necrosis and inflammatory infiltration, as well as a decrease in fatty degeneration. Hepatocytes appeared more organized, with improved preservation of liver architecture. These findings suggest that both plant extracts have the capacity to reduce inflammation, support hepatocyte regeneration, and restore liver function. The results also correlate with the biochemical improvements observed in enzyme levels and oxidative stress markers.

The silymarin-treated group exhibited the most pronounced liver tissue regeneration, with minimal necrosis and inflammation, further validating the hepatoprotective effects of *Vitex negundo* and *Hygrophila auriculata*.

The hepatoprotective effects of *Vitex negundo* and *Hygrophila auriculata* may be attributed to their multifaceted pharmacological properties, including antioxidant, anti-inflammatory, and hepatocyte regeneration-promoting activities. The antioxidant properties of both plants, as evidenced by the significant increase in SOD and CAT activities and the reduction in MDA levels, suggest that they effectively neutralize free radicals and protect hepatocytes from oxidative damage. Additionally, the reduction in inflammatory cell infiltration and improvement in liver architecture indicate that the plants modulate inflammatory pathways, potentially by downregulating pro-inflammatory cytokines or inhibiting inflammatory cell recruitment. The hepatoprotective effects may also involve the restoration of liver enzymatic functions, as reflected by the improvement in serum ALT, AST, ALP, bilirubin, and albumin levels.

Furthermore, previous studies have indicated that both *Vitex negundo* and *Hygrophila auriculata* possess a range of bioactive compounds such as flavonoids, terpenoids, alkaloids, and phenolic acids, which may contribute to their hepatoprotective effects. These compounds have been shown to possess antioxidant, anti-inflammatory, and cytoprotective properties, which may work synergistically to promote liver health and prevent damage in the D-GalN-induced model.

5. CONCLUSION

In conclusion, the results of this study provide strong evidence supporting the hepatoprotective effects of *Vitex negundo* and *Hygrophila auriculata* in D-GalN-induced liver injury. Both plant extracts demonstrated significant improvements in liver function through their ability to reduce liver enzyme levels, mitigate oxidative stress, and improve liver histology. These findings suggest that *Vitex negundo* and *Hygrophila auriculata* have therapeutic potential in the management of liver diseases associated with oxidative damage and inflammation. Further studies are required to isolate the active compounds responsible for these effects and to investigate their mechanisms of action in more detail.

6. DECLARATIONS

Conflict of interest statement: There are no conflicts of interest.

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