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HEPATOPROTECTIVE AND ANTIOXIDANT POTENTIAL OF *AQUILARIA MALACCENSIS* LEAF EXTRACT AGAINST ETHANOL-INDUCED LIVER INJURY IN RATS

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ABSTRACT

The present study was undertaken to standardize and evaluate the hepatoprotective potential of the ethanolic extract of *Aquilaria malaccensis* (Agarwood) leaves against ethanol-induced hepatotoxicity in rats. The dried leaves were extracted with ethanol and subjected to preliminary phytochemical screening, physicochemical evaluation and chromatographic standardization. The extract showed the presence of carbohydrates, gums and mucilages, saponins, starch, proteins, amino acids, phenolic compounds and reducing sugars, and exhibited a characteristic TLC Rf value of 0.56. In vitro antioxidant activity was assessed using the DPPH free-radical scavenging assay, in which the extract demonstrated concentration-dependent activity with an IC_{50} value of 305 µg/mL. Acute oral toxicity testing according to OECD Guideline 423 indicated that the extract was tolerated up to 4000 mg/kg body weight. Hepatoprotective activity was evaluated in ethanol-induced hepatotoxic rats using ethanolic Agarwood leaf extract at 200 and 400 mg/kg, while silymarin 200 mg/kg served as the standard drug. Ethanol administration produced marked elevations in serum SGOT, SGPT, alkaline phosphatase and total bilirubin, indicating significant hepatic injury. Treatment with the extract significantly reduced these altered biochemical parameters toward normal values. Histopathological examination supported the biochemical findings and demonstrated improved hepatic architecture, reduced tissue damage and evidence of hepatocyte regeneration in extract-treated animals. Overall, the findings indicate that the ethanolic extract of *Aquilaria malaccensis* leaves possesses significant hepatoprotective

activity, which may be associated with its antioxidant and phenolic constituents. The study supports further investigation of Agarwood leaves as a potential natural source of hepatoprotective agents.

KEYWORDS: *Aquilaria malaccensis*; Agarwood; hepatoprotective activity; ethanol-induced hepatotoxicity; antioxidant activity; DPPH; silymarin; phytochemical screening; liver enzymes; histopathology.

1. INTRODUCTION

The liver is highly susceptible to toxic injury because of its central role in the metabolism and detoxification of drugs, chemicals, alcohol and other xenobiotics. Among these, excessive or prolonged alcohol consumption is an important cause of hepatic injury. Ethanol is primarily metabolized in the liver, and its metabolism can promote oxidative stress, lipid accumulation, inflammatory responses and hepatocellular damage. Persistent injury may eventually contribute to the development of alcoholic liver disease and progressive impairment of hepatic function. Ethanol-induced hepatic damage is commonly associated with increased production of reactive oxygen species (ROS), depletion of endogenous antioxidant defences and oxidative damage to cellular lipids and proteins. Damage to hepatocyte membranes also results in leakage of intracellular enzymes into the circulation. Consequently, serum markers such as aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase (ALP) and bilirubin are widely used to assess hepatic injury. Histopathological examination provides additional evidence of structural alterations and is useful for confirming the extent of hepatoprotection.

The involvement of oxidative stress in liver injury has encouraged the investigation of medicinal plants containing natural antioxidants as potential hepatoprotective agents. Plant-derived phenolic compounds and other secondary metabolites may exert protective effects through free-radical scavenging, reduction of oxidative damage and stabilization of hepatocellular membranes. Therefore, identification and scientific evaluation of medicinal plants with antioxidant and hepatoprotective properties remain an important area of pharmacological research. *Aquilaria malaccensis* Lam., commonly known as Agarwood, is a medicinally important plant best known for its highly valued aromatic resinous wood. However, its leaves also represent a renewable source of potentially bioactive phytoconstituents. Different parts of the plant have been used traditionally for medicinal purposes, suggesting its potential for further pharmacological investigation. Compared with

the extensive utilization of Agarwood resin and wood, the therapeutic potential of its leaves, particularly their hepatoprotective activity, requires further experimental evaluation.

A systematic investigation of a medicinal plant extract should combine biological evaluation with appropriate phytochemical characterization, standardization and safety assessment. Preliminary phytochemical screening and chromatographic profiling can provide information regarding the chemical characteristics of the extract, while antioxidant studies may help identify a possible basis for its biological activity. Therefore, the present study was undertaken to evaluate the hepatoprotective potential of the ethanolic extract of *Aquilaria malaccensis* leaves (EEBR) against ethanol-induced hepatotoxicity in rats. The extract was subjected to phytochemical and chromatographic evaluation, DPPH free-radical scavenging assay and acute oral toxicity assessment. Its hepatoprotective activity at 200 and 400 mg/kg was evaluated using silymarin as the standard drug, based on changes in serum SGOT, SGPT, ALP and total bilirubin together with histopathological examination of liver tissue. The study aimed to provide experimental evidence for the hepatoprotective potential of Agarwood leaves and a scientific basis for their further investigation as a source of natural hepatoprotective agents.

2. MATERIALS AND METHODS

2.1 Plant Material, Extraction and Standardization

Leaves of *Aquilaria malaccensis* (Agarwood) were collected from Madhya Pradesh, India, cleaned, shade-dried and coarsely powdered. Approximately 500 g of powdered material was subjected to successive Soxhlet extraction using petroleum ether, chloroform, acetone and finally 98% ethanol. The ethanolic extract (EEBR) was filtered, concentrated under reduced pressure using a rotary evaporator and stored until further use. Preliminary phytochemical screening was performed using standard qualitative tests for major phytoconstituents, including carbohydrates, alkaloids, glycosides, proteins, amino acids, saponins, phenolics, tannins, terpenoids and steroids. Physicochemical parameters including total ash, water-soluble ash, acid-insoluble ash, water- and alcohol-soluble extractive values and loss on drying were determined by standard pharmacognostic procedures. Chromatographic evaluation of EEBR showed a characteristic TLC spot with an Rf value of 0.56.

2.2 Antioxidant Activity and Acute Toxicity

The in vitro antioxidant activity of EEBR was evaluated by the DPPH free-radical scavenging assay. Different concentrations of the extract were mixed with methanolic DPPH

solution (3.6×10^{-5} M), incubated at 37°C for 30 min and absorbance was measured at 517 nm. Silymarin was used as the reference antioxidant, and percentage radical-scavenging activity and IC_{50} values were determined. Acute oral toxicity was evaluated in Wistar albino rats according to OECD Guideline 423 (Acute Toxic Class Method). EEBR, suspended in 3% carboxymethyl cellulose, was administered orally and the animals were monitored for behavioural changes, signs of toxicity and mortality for 14 days. No mortality was observed up to 4000 mg/kg, and doses of 200 and 400 mg/kg were selected for the hepatoprotective study.

2.3 Experimental Design and Hepatoprotective Study

Wistar albino rats (150–200 g) were acclimatized for one week under standard laboratory conditions with free access to food and water. The animals were divided into five groups. Group I served as the normal control and received water (5 mL/kg, p.o.); Group II served as the toxic control and received 40% v/v ethanol (2.0 mL/100 g body weight, p.o.); Group III received ethanol plus silymarin (200 mg/kg, p.o.); and Groups IV and V received ethanol along with EEBR at 400 and 200 mg/kg, p.o., respectively. All treatments were administered once daily for 21 days. The study was conducted under institutional ethical supervision in accordance with CPCSEA requirements.

2.4 Biochemical and Histopathological Evaluation

Twenty-four hours after completion of treatment, blood was collected from the retro-orbital plexus under anaesthesia and serum was separated by centrifugation at 2500 rpm for 15 min. Hepatic injury was assessed by estimating serum SGOT/AST, SGPT/ALT, ALP and total bilirubin using standard biochemical methods and commercial diagnostic kits. AST and ALT were estimated by the Reitman–Frankel method, ALP using the p-nitrophenyl phosphate method and bilirubin by the Jendrassik–Grof method. After blood collection, the animals were sacrificed under anaesthesia and liver tissues were collected for histopathological examination. Liver sections were evaluated for hepatocellular degeneration, necrosis, inflammatory infiltration, sinusoidal congestion, preservation of hepatic architecture and hepatocyte regeneration. Body and liver weights were also recorded as supportive parameters of hepatoprotection.

3. RESULTS AND DISCUSSION

3.1 Phytochemical and Chromatographic Evaluation

Preliminary phytochemical screening of the ethanolic extract of *Aquilaria malaccensis* leaves (EEBR) revealed the presence of carbohydrates, gums and mucilages, saponins, starch, proteins and amino acids, phenolic compounds, and reducing sugars, whereas alkaloids, fixed oils and fats, tannins, phytosterols and glycosides were not detected. The presence of phenolic constituents and saponins is of particular interest because these classes of phytochemicals may contribute to antioxidant and membrane-protective effects. Chromatographic evaluation of EEBR showed a characteristic spot with an Rf value of 0.56, providing a preliminary chromatographic fingerprint of the extract.

Table 1: Preliminary phytochemical profile of EEBR.

Phytoconstituent	Result	Phytoconstituent	Result
Carbohydrates	+	Alkaloids	−
Gums and mucilages	+	Saponins	+
Fixed oils and fats	−	Tannins	−
Starch	+	Proteins and amino acids	+
Phytosterols	−	Phenolic compounds	+
Glycosides	−	Reducing sugars	+

+ = present; − = absent

3.2 DPPH Free-Radical Scavenging Activity

EEBR exhibited concentration-dependent DPPH radical-scavenging activity. At concentrations ranging from 100 to 1000 µg/mL, inhibition increased from 9.14% to 80.14%. Silymarin showed inhibition ranging from 20.30% to 78.26% over the same concentration range. The IC_{50} values were 305 µg/mL for EEBR and 585 µg/mL for silymarin, indicating appreciable free-radical scavenging capacity of the extract under the experimental conditions.

Table 2: DPPH radical-scavenging activity of EEBR and silymarin.

Concentration (µg/mL)	Silymarin (% inhibition)	EEBR (% inhibition)
100	20.297 ± 0.01	9.14 ± 0.00
200	32.657 ± 0.31	38.72 ± 0.21
400	47.403 ± 0.25	63.41 ± 0.25
600	57.052 ± 0.66	72.26 ± 0.02
800	72.084 ± 0.79	79.34 ± 0.45
1000	78.261 ± 0.52	80.14 ± 0.16
IC_{50} (µg/mL)	585	305

The progressive increase in DPPH inhibition suggests the presence of constituents capable of donating hydrogen atoms or electrons to free radicals. The phenolic constituents identified

during phytochemical screening may contribute to this activity. Since oxidative stress is an important component of ethanol-induced hepatocellular damage, the observed antioxidant effect provides a plausible basis for at least part of the hepatoprotective activity of EEBR. However, the DPPH assay alone cannot establish the mechanism of hepatoprotection.

3.3 Effect of EEBR on Ethanol-Induced Hepatotoxicity

Administration of ethanol produced marked alterations in serum biochemical markers of hepatic function. Compared with the normal-control group, the ethanol-treated group showed increased SGOT (1760 ± 1.02 to 2097.90 ± 2.468), SGPT (1875 ± 2.11 to 2263.36 ± 1.46), ALP (35.06 ± 0.12 to 53.47 ± 0.01) and total bilirubin (1.48 ± 0.01 to 2.61 ± 0.16 mg/dL), confirming the induction of hepatic injury. Treatment with silymarin (200 mg/kg) markedly restored these parameters toward normal values. Similarly, EEBR at both 200 and 400 mg/kg reduced the ethanol-induced elevations in hepatic biochemical markers. The 200 mg/kg dose showed particularly pronounced normalization of SGOT, SGPT and ALP, whereas the 400 mg/kg dose produced greater improvement in total bilirubin. Thus, the hepatoprotective response was evident at both doses, although a uniform dose-dependent relationship was not observed.

Table 3: Effect of EEBR on biochemical parameters in ethanol-induced hepatotoxicity.

Treatment group	SGOT	SGPT	ALP	Total bilirubin (mg/dL)
Normal control	1760 ± 1.02	1875 ± 2.11	35.06 ± 0.12	1.48 ± 0.01
Ethanol control	2097.90 ± 2.468	2263.36 ± 1.46	53.47 ± 0.01	2.61 ± 0.16
Silymarin 200 mg/kg	1759.53 ± 2.33	1870.98 ± 2.65	37.80 ± 0.03	1.47 ± 0.01
EEBR 200 mg/kg	1735.64 ± 1.73	1883.34 ± 2.22	35.00 ± 0.72	1.82 ± 0.03
EEBR 400 mg/kg	1750.26 ± 1.99	2090.66 ± 2.32	39.98 ± 0.11	1.53 ± 0.01

Values are expressed as mean ± SEM.

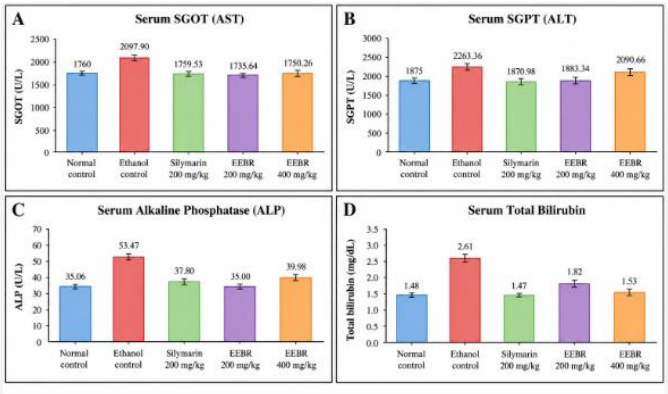

Figure: Effect of EEBR (200 and 400 mg/kg) and silymarin (200 mg/kg) on serum biochemical markers in ethanol-induced hepatotoxicity.

The elevation of SGOT and SGPT following ethanol administration indicates loss of hepatocellular membrane integrity and leakage of intracellular enzymes into the circulation. Their reduction following EEBR treatment suggests stabilization of hepatocyte membranes and attenuation of ethanol-induced cellular injury. Similarly, increased ALP and bilirubin indicate disturbances in hepatic and biliary function. The marked reduction of these parameters after EEBR administration suggests improvement in hepatic functional integrity. The protective effect may be related, at least partly, to the antioxidant constituents of the extract. Ethanol metabolism promotes generation of reactive oxygen species and oxidative damage to hepatocellular membranes. The free-radical scavenging activity observed in the DPPH assay, together with the presence of phenolic compounds, therefore supports a possible antioxidant contribution to the hepatoprotective effect. Nevertheless, because EEBR is a complex plant extract, its activity may involve several mechanisms and cannot be attributed exclusively to antioxidant action. The original results likewise show substantial reversal of ethanol-induced elevations in all four biochemical parameters following EEBR administration.

3.4 Histopathological Findings and Overall Hepatoprotective Effect

Histopathological examination supported the biochemical findings. Liver sections from normal-control animals showed preserved hepatic architecture and normal hepatocytes, whereas ethanol-treated animals exhibited prominent hepatocellular damage and congestion. The silymarin-treated group showed substantial preservation of hepatic architecture. EEBR-treated groups also demonstrated improved tissue organization and evidence of regenerative hepatocytes, although some sinusoidal congestion and scattered mononuclear inflammatory cells remained.

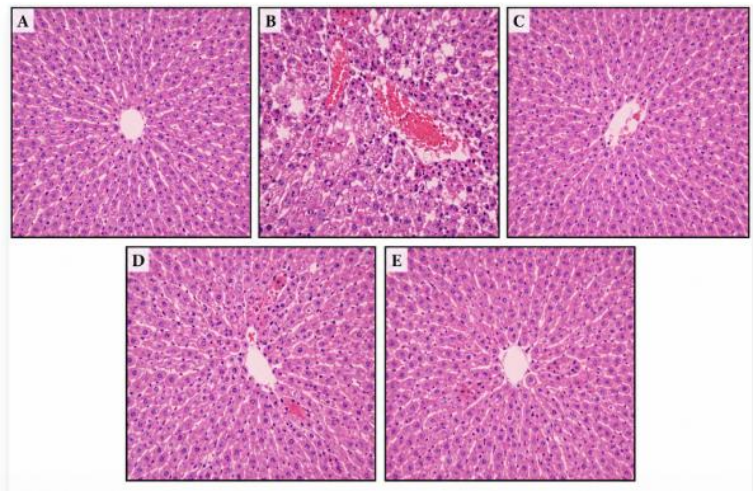

Figure: Representative histopathological sections of rat liver: (A) **normal control showing preserved hepatic architecture;** (B) **ethanol control showing hepatocellular damage and congestion;** (C) **silymarin 200 mg/kg showing restoration of hepatic architecture;** (D) **EEBR 200 mg/kg and** (E) **EEBR 400 mg/kg showing regenerative hepatocytes and attenuation of ethanol-induced hepatic injury.**

The agreement between biochemical and histopathological findings strengthens the evidence for the hepatoprotective effect of EEBR. Ethanol-induced elevations in serum liver markers were accompanied by visible hepatic tissue damage, while treatment with EEBR improved both biochemical parameters and liver morphology. Overall, the results demonstrate that ethanolic extract of *Aquilaria malaccensis* leaves attenuated ethanol-induced hepatic injury in rats. The extract showed significant free-radical scavenging activity, reduced elevated SGOT, SGPT, ALP and total bilirubin levels, and improved hepatic histoarchitecture. These effects may be associated with phenolic and other bioactive constituents present in the extract and support further investigation of Agarwood leaves as a potential source of natural hepatoprotective agents.

4. CONCLUSION

The present study demonstrates that the ethanolic extract of *Aquilaria malaccensis* leaves (EEBR) possesses significant protective activity against ethanol-induced liver injury in rats. The extract exhibited appreciable free-radical scavenging activity and significantly reduced ethanol-induced elevations in SGOT, SGPT, ALP and total bilirubin. These biochemical improvements were supported by histopathological findings showing better preservation of hepatic architecture and evidence of hepatocyte regeneration in EEBR-treated animals. The hepatoprotective effect may be partly associated with the antioxidant and other bioactive

phytoconstituents present in the extract, particularly phenolic compounds. Overall, the findings provide experimental evidence supporting *A. malaccensis* leaves as a promising natural source of hepatoprotective agents. Further studies involving isolation and characterization of active constituents, evaluation of specific molecular mechanisms, and detailed safety studies are required to establish their potential therapeutic application.

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