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Hepatoprotective Effects of *Acacia arabica* Bark Extract Against Paracetamol and Alcohol-Induced Liver Injury in Wistar Rats: Biochemical and Histopathological Evaluation

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ABSTRACT
Paracetamol overdose and chronic alcohol consumption are major causes of hepatic injury worldwide. Natural hepatoprotective agents rich in phenolic and flavonoid compounds are increasingly investigated for their protective roles. Previous phytochemical and antioxidant evaluations have confirmed that *Acacia arabica* bark extract contains high phenolic and flavonoid content with potent antioxidant activity. This study investigated the hepatoprotective effects of *A. arabica* bark extract in paracetamol- and alcohol-induced hepatotoxicity models in Wistar rats.
Hepatotoxicity was induced in rats using paracetamol (640 mg/kg) or 40% ethanol. Animals were pre-treated with *A. arabica* extract (400 mg/kg) or standard drugs (silymarin or Liv.52). Liver biomarkers such as AST, ALT, ALP, bilirubin, total protein, albumin, and globulin were measured. Histopathological examination was conducted to evaluate tissue injury.
Paracetamol significantly elevated AST and ALT levels, increased bilirubin, and caused severe hepatic degeneration. Pre-treatment with *A. arabica* restored ALT, normalized bilirubin, and improved ALP levels. In the alcohol model, ethanol markedly elevated AST and ALT and produced fatty changes, which were significantly reduced by *A. arabica*. Histopathology showed that the treated groups had well-maintained liver cell structure
The above results indicate that *A. arabica* has liver-protective effects, which are mediated by its antioxidant activity, cell membrane stabilisation, and inflammation., and probable modulation of Nrf2/HO-1 and NF-κB pathways. The extract demonstrates potential for development as a natural hepatoprotective agent.

INTRODUCTION:

Liver injury caused by pharmaceutical agents, xenobiotics, and chronic alcohol consumption is a major global health concern. Paracetamol toxicity results in excessive formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI), which rapidly consumes hepatic glutathione reserves and triggers redox imbalance within the cell, mitochondrial dysfunction, and hepatic necrosis. Similarly, chronic alcohol intake leads to CYP2E1 activation, formation of acetaldehyde, and excess production of reactive oxygen species (ROS), resulting in inflammatory responses, membrane lipid damage, and steatotic alterations.

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Herbal hepatoprotective agents are gaining interest because many plant-derived phenolics and flavonoids exhibit antioxidant, anti-inflammatory, and cytoprotective properties. *Acacia arabica* is rich in tannins, flavonoids, gallic acid derivatives, and catechins. The present investigation is a continuation of our previously published work in which the phytochemical profile and antioxidant potential of this plant were established [1].

On the basis of these observations, this research was able to study the liver-protective properties of *A. arabica* bark extract on rats administered with paracetamol and ethanol. It was demonstrated by the fact that the investigation evaluated the main biochemical markers and studied tissue-level modifications to demonstrate its protective potential.

MATERIALS AND METHODS:

Plant Material and Extraction :

The *Acacia arabica* bark was obtained, dried under shade, crushed and remixed using Soxhlet apparatus. A crude extract upon which the result was obtained was concentrated and kept until further use. [1]

Experimental Animals

Wistar albino rats (180220 g) were got in the Animal Facility of Teerthanker Mahaveer Medical College and Research Centre, Teerthanker Mahaveer University in Moradabad. Animals were housed under standardized laboratory conditions (25 ± 2°C; 12-h light/dark cycle) throughout the study period. All experimental procedures adhered to the Institutional Animal Ethics Committee (IAEC) guidelines and were approved under Protocol no. CCSEA/1205/2025/15, in accordance with CCSEA regulations for the care and use of laboratory animals.

Toxicity assessment and dose selection

Previous toxicity evaluations indicated that *Acacia arabica* bark extract is well tolerated within its therapeutic window. Accordingly, and in alignment with dose ranges documented for comparable polyphenol-rich botanical extracts, a dose of 400 mg/kg was selected for the present *in-vivo* hepatoprotective assessment [2]

Paracetamol-Induced Hepatotoxicity

A total of 24 healthy adult Wistar rats of both sexes were randomly allocated into four groups, with six animals in each group. Animals received the assigned treatments once daily by oral gavage for 14 days. The experimental design was as follows:
Group 1 (Normal control): Animals received normal saline (5 mL/kg, p.o.).
Group 2 (Negative control): Animals received paracetamol at 640 mg/kg (p.o.), prepared in 1% carboxymethyl cellulose.
Group 3 (Paracetamol + *A. arabica* extract): Animals were administered A. arabica bark extract (400 mg/kg, p.o.) along with paracetamol (640 mg/kg, p.o.).
Group 4 (Paracetamol + silymarin): Animals received silymarin (50 mg/kg, p.o.) together with paracetamol (640 mg/kg, p.o.).
At 24 hours after the final paracetamol administration, anaesthesia was induced using thiopental sodium (200 mg/kg, i.p.). Blood was collected via cardiac puncture for biochemical evaluation. The liver was excised immediately after euthanasia for gross examination and histopathological analysis [3]

Alcohol-Induced Hepatotoxicity

Eighteen adults male Wistar albino rats were included in the study. After acclimatization, The animals were maintained under standard laboratory conditions at 22 ± 2 °C with a 12-hour light–dark cycle. They were given free access to a standard pellet diet and drinking water.
The rats were randomly assigned to three groups, with six animals in each group. All treatments were given once daily by oral gavage for 30 days as follows:
Group 1 – Ethanol control: Received 40% ethanol at 3 g/kg.
Group 2 – Ethanol + *Acacia arabica* extract: Received *A. arabica* bark extract (200 mg/kg) two hours before the ethanol dose (3 g/kg of 40% ethanol).
Group 3 – Ethanol + Liv-52: Received Liv-52 (0.216 mL/kg) along with ethanol (3 g/kg of 40% ethanol).

The dose of *A. arabica* extract was chosen based on previous reports showing it to be safe for use in rats. The dose of ethanol used was selected after initial tests to ensure that liver damage is achieved without inducing death. [4]

Biochemical Analysis

Serum biochemical markers were evaluated to determine liver function in both experimental models. Standard diagnostic kits were used to estimate the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), total and direct bilirubin, total protein, and albumin. Globulin values were calculated from total protein and albumin concentrations. These biochemical indicators were used collectively to assess the degree of hepatic injury and to determine the protective effects of the respective treatments [5]

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Histopathology

The liver was removed and preserved for at least 24 hours in formalin (10% formaldehyde in water) for the histological investigation. A microtome (RM2235 Rotary Microtome) was used to prepare and cut the paraffin sections into sections with a thickness of 5µm. Haematoxylin-eosin dye was then used to stain the microtome slices. Photos of the slides were taken and examined [6]

Statistical Analysis

All the statistical analysis were done using Graph Pad Prism 6.0. All the data were analysed to get mean ± SD (standard deviation). One way ANOVA test was carried out followed by Tukey's multiple range list. The 'p' value was set as $p < 0.05$ as significant.

RESULTS:

Paracetamol-Induced Hepatotoxicity

Paracetamol treatment caused a marked rise in AST and ALT levels in comparison to the normal group, and total bilirubin also increased to 0.5 mg/dL. ALP levels showed a noticeable decline. Administration of *A. arabica* extract helped bring ALT closer to normal, lowered bilirubin, and improved ALP levels, as presented in Table 1.

In the normal control group (Group A), liver sections showed a normal appearance with clearly defined hepatocytes, regular sinusoids, and an intact central vein. In contrast, the paracetamol-treated group (Group B) displayed severe liver injury, including ballooning of cells, heavy inflammatory cell infiltration, and disruption of normal cell structure. Rats pretreated with silymarin (200 mg/kg, Group C) showed noticeable improvement, with liver tissue appearing close to normal and only mild inflammatory changes. Group D having pretreatment of *Acacia arabica* extract (400mg/kg) preserved hepatic lobular pattern showed almost a normal pattern with slight lymphocytic infiltration. (**Error! Reference source not found.**).

Table 1: Biochemical parameters of PCM induced hepatotoxic model

TEST	Normal	Negative	Treated (	Positive		Units	Bio. Ref. Interval
AST (SGOT)	56.45 ± 8.65	160.43 ± 12.31**	178.4 ± 20.32**	110.6 ± 9.65*		U/L	64 - 185
ALT (SGPT)	44.2 ± 6.32	70.5 ± 8.33*	40.12 ± 6.39ns	37.54 ± 7.23ns		U/L	40 - 87
GGTP	2.5 ± 0.54	1.4 ± 0.48ns	1.2 ± 0.01ns	1.35 ± 0.11ns		U/L	5 - 40
Alkaline Phosphatase	317 ± 12.32	140.47 ± 15.36**	110.9 ± 10.39**	97.4 ± 9.77**		U/L	237 - 518
Bilirubin Total	0.17 ± 0.01	0.5 ± 0.01*	0.1 ± 0.01ns	0.3 ± 0.01*		mg/dL	0.1 - 0.7
Bilirubin Direct	0.2 ± 0.01	0.08 ± 0.01ns	0.08 ± 0.02ns	0.05 ± 0.01ns		mg/dL	> 0.3
Bilirubin Indirect	0.2 ± 0.01	0.04 ± 0.01ns	0.02 ± 0ns	0.02 ± 0.01ns		mg/dL	0.2 – 0.8
Total Protein	6.3 ± 0.25	4.25 ± 0.22ns	6.87 ± 0.01ns	5.62 ± 0.3ns		g/dL	5.6 - 7.6
Albumin	3.9 ± 0.66	2.87 ± 0.35ns	3.33 ± 0.04ns	2.54 ± 0.02ns		g/dL	2.8 - 4.5
Globulin	1.99 ± 0.87	3.2 ± 0.65ns	3.54 ± 0.55ns	2.1 ± 0.08ns		g/dL	1.5 – 3.5
A : G Ratio	1.95 ± 0.01	0.89 ± 0.01ns	0.94 ± 0.02ns	1.2 ± 0.01ns			

Values are displayed as Mean ± SD; n = 6. Statistical analysis was done using one-way ANOVA followed by Tukey's test versus control. *p < 0.05; p < 0.01; ns = not significant

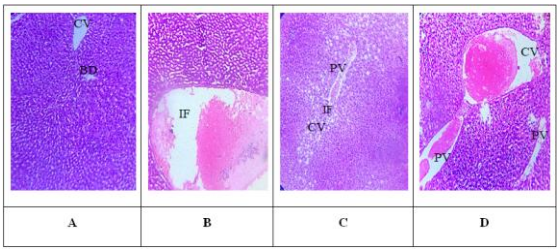

Figure 1: (A) Normal control showing intact hepatic architecture; (B) Paracetamol-treated control showing prominent inflammatory infiltration and cellular damage; (C) Silymarin-treated group showing improved liver structure; (D) *A. arabica* extract–treated group showing a near-normal lobular pattern. CV: central vein; PV: portal vein; IF: inflammatory infiltrate; BD: bile duct. H&E stain, ×100

Alcohol-Induced Hepatotoxicity

The use of alcohol led to a significant increase in AST and ALT in the negative control group relative to normal ones, which showed liver damage. Total bilirubin and total protein, albumin and globulin also exhibited an increase and decrease respectively. The *A. arabica* extract treatment made these values closer to normal and reduced AST, ALT, and bilirubin levels and enhanced protein parameters. The standard group showed levels of enzyme and protein as well which were more comparable to those of the normal group. (Table 2).

Liver sections from the normal control group showed a normal arrangement of hepatocytes with clear sinusoids and a well-defined central vein. The ethanol-treated group displayed noticeable fatty changes along with infiltration of inflammatory cells, although cell death was not prominent. In the standard group treated with Liv-52, most of the

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ethanol-induced changes were reduced, with fewer fat deposits and less inflammatory infiltration. The *Acacia arabica* extract–treated group showed a pattern similar to the ethanol group but with only mild degeneration of hepatocytes (Figure 1).

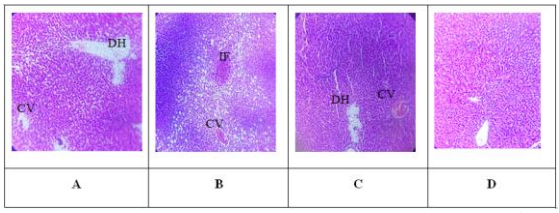

Figure 1: (A) Negative (ethanol-treated) group showing fatty changes and inflammatory infiltration; (B) Standard group (Liv-52 treated) showing reduced inflammatory changes and improved hepatic architecture; (C) Acacia arabica extract–treated group showing mild hepatocyte degeneration with comparatively preserved structure. (D) Control group. CV: central vein; DH: degenerated hepatocytes; IF: inflammatory infiltrate. Hematoxylin and eosin stain×100.

Table 2: Biochemical parameters of alcohol induced hepatotoxic model

TEST	Normal	Negative	Treated	Positive	Units	Bio. Ref. Interval
AST (SGOT)	81 ± 9.96	204.8 ± 13.36**	191.8 ± 13.36**	197.47 ± 8.95**	U/L	64 - 185
ALT (SGPT)	78 ± 7.63	156.4 ± 8.97**	40.12 ± 8.99^{ns}	46.22 ± 9.32^{ns}	U/L	40 - 87
GGTP	1.5 ± 0.45	1.4 ± 0.55^{ns}	1.5 ± 0^{ns}	1.4 ± 0.01^{ns}	U/L	5 – 40
Alkaline Phosphatase	252 ± 10.64	87.33 ± 14.68**	56.6 ± 6.38**	70.5 ± 5.67**	U/L	237 - 518
Bilirubin Total	0.69 ± 0.01	0.8 ± 0.01^{ns}	0.5 ± 0.01^{ns}	0.07 ± 0^{ns}	mg/dL	0.1 - 0.7
Bilirubin Direct	0.2 ± 0.01	0.05 ± 0^{ns}	0.02 ± 0^{ns}	0.06 ± 0^{ns}	mg/dL	> 3
Bilirubin Indirect	0.2 ± 0.01	0.6 ± 0.01^{ns}	0.48 ± 0^{ns}	0.56 ± 0^{ns}	mg/dL	0.2 – 0.8
Total Protein	6.2 ± 0.68	9.02 ± 0.11^{ns}	8.06 ± 0.01^{ns}	7.5 ± 0.25^{ns}	g/dL	5.6 - 7.6
Albumin	4.6 ± 0.52	4.6 ± 0.21^{ns}	3.92 ± 0.04^{ns}	3.5 ± 0.02^{ns}	g/dL	2.8 - 4.5
Globulin	2.8 ± 0.85	4.43 ± 0.54^{ns}	4.14 ± 0.55^{ns}	4.2 ± 0.7^{ns}	g/dL	1.5 – 3.5
A : G Ratio	1.64 ± 0.01	1.03 ± 0.01^{ns}	0.95 ± 0.02^{ns}	0.83 ± 0.01^{ns}		

All values are expressed as Mean ± SD (Standard deviation); n=6; One-Way ANOVA followed by Tukey's test v/s Control. *p<0.05; **p<0.01; ns=Not significant.

DISCUSSION:

The liver injury observed in both the paracetamol and alcohol models can be understood in terms of oxidative stress, inflammation, and drug/chemical metabolism. In the case of Paracetamol (acetaminophen) overdose, the formation of the toxic metabolite N-acetyl p-benzoquinone imine (NAPQI) leads to glutathione depletion, mitochondrial dysfunction, and a surge in reactive oxygen species (ROS) that cause hepatocyte damage. This process has been attributed to the significant increases in serum markers AST, ALT, ALP, bilirubin, in the paracetamol-treated group and histological evidence of ballooning and inflammatory infiltration. [7]

Chronic ethanol exposure in alcohol model raises hepatic enzyme activity (CYP2E1), upsets the mitochondrial functioning, enhances the production of ROS, lipid peroxidation, fat deposition in hepatocytes (steatosis), and infiltration. Such biochemical alteration coincides with our results of increased liver enzymes and bilirubin, decreased protein content, and histopathological conditions of fatty changes, and inflammatory infiltration of the ethanol-treated group. [8]

Acacia arabica bark extract improved the biochemical profiles and histology in both the models. This suggests that the extract may exert its protective effects via several mechanisms: (i) scavenging of ROS and reduction in oxidative stress, (ii) stabilization of hepatocyte membranes thereby preventing leakage of enzymes, (iii) reduction of inflammatory cell infiltration and release of pro-inflammatory cytokines, and (iv) enhancement of endogenous antioxidant defence systems such as glutathione, SOD, and catalase. Intervention with such botanical extracts has also been shown in other studies to ameliorate oxidative damage and inflammation in hepatic injury models. For example, natural antioxidants were shown to reduce ALT/AST, ameliorate lipid peroxidation and restore antioxidant enzyme levels [9]

The comparison with reference standards (silymarin in the paracetamol model, and Liv-52 in the alcohol model) provides context showing that while standard therapies are effective, Acacia arabica offers promising protection, potentially as an affordable herbal alternative or adjuvant. Almost all liver enzyme levels have normalised and liver tissue demonstrates better structure, which is a good sign of the extract as a potential treatment [10]

Mechanistically, the improvements could be because of reduced liver cell deaths and apoptosis, replenished glutathione, less CYP2E1 activation in

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the alcohol model and less mitochondrial impairment. Less inflammation and the improved lobular structure are observed in the histology, which is correlated with the reduced oxidative and inflammatory stress. Restored liver cell functioning is also indicated by lowering bilirubin and ameliorating protein synthesis [11]

Therefore, it is possible to claim that the data indicate a two-pronged hepatoprotection: antioxidative (suppressing of the reactive oxygen species and lipid peroxidation) and anti-inflammatory (suppressing of the immune cell infiltration and cytotoxic release). This synergistic effect is especially applicable, in light of the literature-described pathways of pathogenesis of liver injury in both paracetamol and ethanol-induced liver injury.

CONCLUSION:

The investigation showed that the *Acacia arabica* bark extract prevents the damage of liver by paracetamol and alcohol. After treatment of the rats, there was an increase in the liver enzymes, reduced bilirubin, and positive protein values. Their liver tissues were found to be healthier having fewer inflammation and less damaged cells. These improvements suggest that *A. arabica* may protect the liver by reducing oxidative stress and inflammation. Although the standard drugs also worked well, the plant extract produced similar positive effects.

Overall, *Acacia arabica* appears to be a safe and promising natural option for supporting liver health, and further studies can help explore its benefits in more detail.

Author Contributions:

Conceptualization, methodology, investigation: Ashwani Gupta; Supervision: Mayur Porwal; Writing—original draft: Ashwani Gupta; Writing—review and editing: Mayur Porwal.

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Conflicts of Interest

The authors declare no conflict of interest.

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