Research Article

# Hepatoprotective Activity of Hydroalcoholic and Ethyl Acetate Extract of Abutilon Indicum Leaf on Rats

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**ABSTRACT**

The present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic and ethyl acetate extract of leaf of *Abutilon indicum* (AI) in CCl4 induced toxicity in Sprague dawley rats. Leaves of AI were collected, and subjected to continuous hot extraction in a Soxhlet apparatus at temperature not exceeding 60°C with solvents like hydroalcoholic ethanol:Water (9:1) and ethyl acetate separately. Liver damage was induced in rats by administering $CCl_4$ intraperitoneal (i. p.) mixed with olive oil in the ratio 1: 1 at the dose of 1 ml $CCl_4$/kg b. wt. of each animal. The hydroalcoholic extract at the dose of 200 mg/kg and 400 mg/kg b. wt and ethyl acetate extract 200 mg/kg b. wt was evaluated by inducing hepatotoxicity with $CCl_4$ and using silymarin (100 mg/kg) as the reference standard. Biochemical parameters like, AST, ALT, ALP, total protein, triglyceride and serum bilirubin level were analysed. A section of liver was subjected to histopathological studies. Based on the above comperative studies, it is reported that the ethyl acetate extract of *Abutilon indicum* possess greater hepatoprotection as compared to hydroalcohlic extract against $CCl_4$ induced hepatotoxicity in albino rats.

**Keywords:** *Abutilon indicum,* Soxhlet apparatus, Biochemical parameters.

## INTRODUCTION

In India different parts of medicinal plants have been used for curing various diseases from ancient times. In this regard, one such plant is *Abutilon indicum* (AI).[1] The *Abutilon* L. genus of the Malvaceae family is one such medicinal plant used in the treatment of liver disorders in folk medicine. It also has hypoglycemic, antimicrobial, antimalarial, antidiabetic activities, etc.[2-4] This has triggered the authors and the present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic and ethyl acetate extract of *Abutilon indicum* against liver disorders induced by $CCl_4$ in Sprague dawley rats. Biochemical parameters like AST, ALT, ALP, total protein, triglyceride and serum bilirubin were determined to assess the hepatoprotective effect of hydroalcoholic and ethyl acetate extract against $CCl_4$ induced liver disorders. The study revealed that these extracts significantly reduced AST, ALT, ALP, triglyceride and serum bilirubin levels. The preliminary findings suggest that the plant *Abutilon indicum* possess potential hepatoprotective activity. The present study scientifically validated the traditional use of *Abutilon indicum* for liver disorders.

## MATERIALS AND METHODS

### Plant material

*Abutilon indicum* leaves were collected in the month of Aug 2013 and authenticated at Blatter Herbarium, St. Xavier's College, Mumbai, by Dr. Rajendra D. Shinde, and its identity was confirmed to be *Abutilon indicum* (L.) Sweet by comparing with herbarium specimen no. 16461.

### Plant extract preparation

Leaves of the plant was air-dried, protected from direct sunlight, and then powdered. The powdered plant material was extracted with ethanol : water (9:1) and ethyl acetate separately, in a Soxhlet apparatus. The each extracts were then concentrated under reduced pressure on a rotary evaporator to dryness to give the crude residue. The crude residues were employed for further investigation. The extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, flavonoids, alkaloids, glycosides, saponins, Tannins, phenols, proteins etc.[5]

### Animals

Sprague dawley female rats (150-200g weight) were used for toxicological studies and pharmacological studies respectively. These animals were procured from the registered breeder Glenmark pvt ltd, Mahape, Navi Mumbai, India. The animals were maintained in hygienic conditions in animal house in groups of 6 in clean plastic cages containing husk bedding. The animals were fed standard pellet food (Amrut brand pelleted standard feed manufactured by Nav Maharashtra Chakan Oils Ltd., Pune, Maharashtra) and had free access to water *ad libitum.* The animal house conditions maintained were: Temperature (25±1)°C, Relative humidity (65±10) % and 12 hrs (light) and 12 hrs (dark) cycles. The animals were allowed to acclimatize to animal house conditions for 6-7 days period prior to the experiments. The institution's animal house is registered with Govt. Of India, having registration No.25/1999/CPCSEA and conforms to the Indian National Science Academy guidelines for the use and care of experimental animal research. All experimental protocols involving animal studies were

placed before the Institutional Animal Ethics Committee. The committee granted approval after carefully reviewing the research project through protocol no. 131404.

**Hepatoprotective activity**

Sprague dawley female rats (150-200g weight) were used for the study. They were housed in plastic cages with not more than six animals per cage and maintained under standard conditions. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals. Animals was randomly divided into six groups with six animals in each group

Group I was served as Control group receiving only distilled water and food orally

Group II was served as Toxicant receiving only $CCl_4$ intraperitoneal (i.p) to assist assessing severity of toxicity

Group III, Group IV, Group V & Group VI were received *A.indicum* hydroalcoholic extract 200mg/kg, 400 mg/kg, Ethyl acetate extract 200mg/kg and Standard- Silymarin 100mg/kg respectively thrice at 12 hours interval.

After which $CCl_4$ mixed with olive oil in the ratio (1:1) was administered in a dose of 1ml/kg body weight for two days to all animal groups except group I.

Blood was collected after 36 hours of $CCl_4$ treatment from all groups by puncturing the retro-orbital plexus. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and was analyzed for various biological & biochemical parameters.[6]

Animals were then sacrificed under deep ether anesthesia and their livers were removed for histopathological studies**.**

**Statistical analysis**

Experimental results were grouped according to the treatment, and the arithmetic average was calculated for each group from the values for each individual with that group. This average was expressed as the mean ± the standard error of the mean (SEM) for six determinations. Experimental data were analyzed statistically by one-way analysis of variance (ANOVA). Bonferroni's multiple comparison test was used to determine significant differences between means. The p value corresponding to the test statistic value was reported to denote the degree of significance. PRISM software was used for statistical analysis.

## RESULTS AND DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against $CCl_4$ as hepatotoxin to prove its claims in folklore practice against liver disorders. $CCl_4$ induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. The extent of hepatic damage is assessed by the level of various biochemical parameters in circulation and histological evaluation. It is well known that carbon tetrachloride is converted by cytochrome P450 mixed function oxygenases in smooth endoplasmic reticulum of liver into toxic metabolite, mainly trichloromethyl radical ($CCl_3$). This free radical in the presence of oxygen may cause peroxidation of lipids on target cell resulting in extensive damage to liver.[7]

The results of biochemical parameters revealed to the alteration of enzyme levels in $CCl_4$ treated group indicating that $CCl_4$ induces damage to the liver. Table 1 show that $CCl_4$ causes a significant increase in AST level from control 120 ± 15.28IU/L to 304 ± 10.58 IU/L after CCl4 intoxication. Administration of two doses of hydroalcoholic and ethyl acetate extract at the dose level of 200 mg/kg, 400 mg/kg and 200 mg/kg in $CCl_4$ intoxicated rats caused reduction in AST level to 212 ± 12.22 IU/L, 158 ± 6.028 IU/L and 190± 10.58 IU/L respectively (P<0.001).

Further Table 1 reveals that $CCl_4$ causes a significant increase in ALT level from control 73 ± 5.132 IU/L to 126 ± 3.464 IU/L after intoxication. Administration of two doses of hydroalcoholic and ethyl acetate extract at the dose level of 200 mg/kg, 400 mg/kg and 200 mg/kg in $CCl_4$ intoxicated rats caused reduction in ALT level to 102.7 ± 1.764 IU/L, 94.33 ± 2.333 IU/L and 100 ± 2.887 IU/L (P<0.01, P<0.001 and P<0.01 respectively). ALP level in the control group increased from 82.5 ± 2.598 KA to 150.9 ± 1.069 KA in $CCl_4$ intoxicated rat as shown in Table 1. Administration of two doses of hydroalcoholic and ethyl acetate extract at the dose level of 200 mg/kg, 400 mg/kg and 200 mg/kg in $CCl_4$ intoxicated rats led to lowering of the ALP level to 124.3 ± 1.202 KA, 98.9 ± 2.654 KA and 120 ± 2.654 KA (P<0.01, P<0.001 and P<0.001 respectively). Destruction of hemoglobin yields bilirubin which is conjugated in the liver to diglucoroxide and excreted in the bile. Bilirubin accumulates in plasma when liver insufficiency exists or biliary obstruction is present or rate of hemolysis increases. The serum bilirubin level increased from 0.88 ± 0.023 mg/dL in the control group to 1.66 ± 0.0305 mg/dL after $CCl_4$ intoxication as shown in Table 1. Administration of two doses of hydroalcoholic and ethyl acetate extract at the dose level of 200 mg/kg, 400 mg/kg and 200 mg/kg in $CCl_4$ intoxicated rats caused reduction in serum bilirubin to 1.2 ± 0.1155 mg/dL, 1 ± 0.0503 mg/dL and 1.2 ± 0.1155 mg/dL (P<0.01, P<0.001 and P<0.01 respectively). Hepatotoxicity leads to increase in serum triglycerides level. The serum triglycerides level increased from 90 ± 2.887 in the control group to 133 ± 3.606 after $CCl_4$ intoxication as shown in Table 1. Administration of two doses of hydroalcoholic and ethyl acetate extract at the dose level of 200 mg/kg, 400 mg/kg and 200 mg/kg in $CCl_4$ intoxicated rats caused reduction in serum triglyceride to 100 ± 6.429, 98 ± 1.155 and 104 ± 3.215 (P<0.01, P<0.001 and P<0.01 respectively). In this study, $CCl_4$ administration to rats leads to a marked elevation in the levels of serum enzymes like AST, ALT, ALP, serum bilirubin and serum triglycerides level.

**Table 1:** Effect of *Abutilon indicum* extract on serum enzymes (AST, ALT and ALP), triglycerides and bilirubin in CCl4 intoxicated rats.

| Biochemical parameters | Group I Normal Control | Group II Toxicant $CCl_4$ 1ml/kg | Group III Hydro Alc. extract 200mg/kg | Group IV Hydro Alc. extract 400mg/kg | Group V Ethyl acetate extract 200mg/kg | Group VI Silymarin 100mg/kg |
|---|---|---|---|---|---|---|
| **AST (IU/L)** | 120±15.28 | 304±10.58[a] | 212±12.22[***] | 158±6.028[***] | 190±10.58[***] | 148±5.196[***] |
| **ALT (IU/L)** | 73±5.132 | 126±3.464[a] | 102.7±1.764[**] | 94.33±2.333[***] | 100±2.887[**] | 83.33±1.667[***] |
| **ALP (KA)** | 82.5±2.598 | 150.9±1.069[a] | 124.3±1.202[**] | 98.9±2.654[***] | 120±7.211[***] | 94.1±1.159[***] |
| **Bilirubin (mg/dL)** | 0.88±0.023 | 1.66±0.0305[a] | 1.2±0.1155[**] | 1±0.0503[***] | 1.2±0.1155[**] | 0.96±0.023[***] |
| **Triglycerides** | 90±2.887 | 133±3.606[a] | 100±6.429[**] | 98±1.155[***] | 104±3.215[**] | 81±3.215[***] |

Values are mean ± SEM; *N* = 6 in each group, One-way ANOVA followed by Bonferroni's multiple comparison test is applied for statistical analysis.
*P* values: a < 0.001 when Toxicant Control compared with Normal Control; *** < 0.001 when Experimental groups compared with Toxicant Control; ** < 0.01 when Experimental groups compared with Toxicant Control

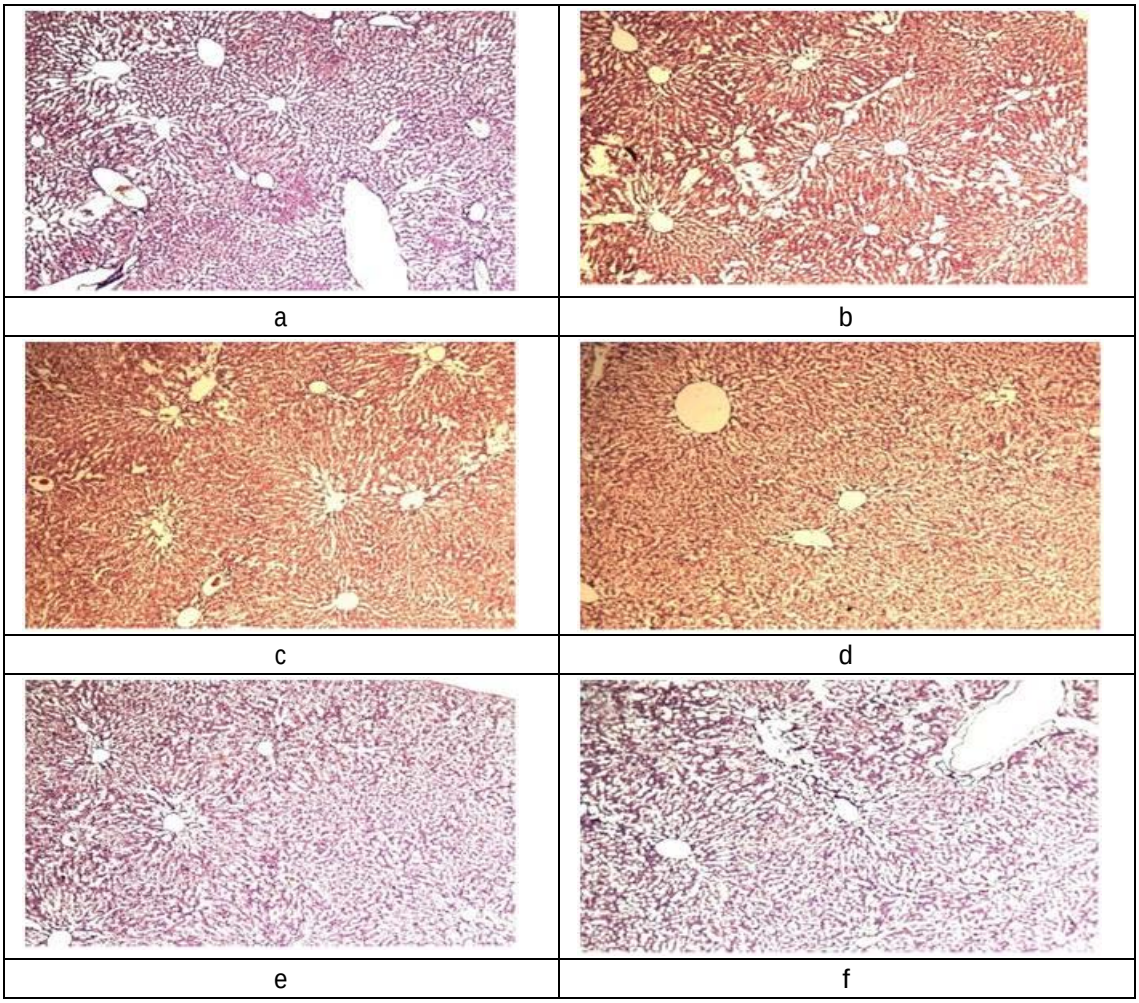


**Figure 1:** Histopathological observations of rat liver sections of representative animal from each group in $CCl_4$ induced hepatotoxicity and restore by Silymarin and *Abutilon indicum* extracts.

(a) **Normal group.** showed normal cellular architecture with distinct hepatic cells and compact arrangement with prominent nuclei and sinusoidal spaces.

(b) **Rats intoxicated with $CCl_4$ 1 ml/kg b. wt.** showed disarrangement of normal hepatic cells with marked multifocal centrilobular necrosis, large number of fatty vacuoles, moderate diffuse granular degeneration with high lymphocytic infiltration in necrotic zone.

(c) **Rats treated with $CCl_4$ + hydroalcoholic extract 200 mg/kg b. wt.** showed a mild to moderate degree of centrilobularmicrovesicular necrosis and moderate diffuse granular degeneration with mild lymphocytic infiltration. Moderate regenerative cells and fatty vacuoles were noted.

(d) **Rats treated with $CCl_4$ + hydroalcoholic extract 400 mg/kg b. wt.** showed mildly multifocal centrilobular necrosis and minimal diffuse granular degeneration with fewer numbers of fatty vacuoles, minimal degree of lymphocytic infiltration.

(e) **Rats treated with $CCl_4$ + ethyl acetate extract 200 mg/kg b. wt.** showed mildly multifocal centrilobular necrosis and minimal diffuse granular degeneration with fewer numbers of fatty vacuoles, minimal degree of lymphocytic infiltration Regeneration of a mild to moderate degree was noted.

(f) **Rats treated with $CCl_4$ + Standard silymarin 100 mg/kg b. wt.** showed a mild to moderate multifocal centrilobular necrosis, mild degree of centrilobular lymphocytic infiltration with minimal diffuse granular degeneration. Also seen was a regeneration of hepatocytes with few numbers of fatty vacuoles. Regenerative foci of mild to moderate degree were noted in these areas.

This might be due to release of these enzymes from the cytoplasm, into the blood stream rapidly after rupture of the plasma membrane and cellular damage.[8] Treatments with ethyl acetate extract at the dose of 200 mg/kg and hydroalcoholic extract at the dose of 400 mg/kg significantly reduced the levels of these marker enzymes in $CCl_4$ treated rats. This implies that the extract tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. Effective control of ALP and bilirubin, points towards an early improvement in the secretary mechanism of the hepatic cells. Decrease in serum bilirubin and triglycerides level after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver.

## CONCLUSION

Herbal therapies have greater safety index at modest dose levels and negligible side effects, which is altogether a benefit for treating such hepatic disorders. Herbs can help alleviate intensity of side effects caused by conventional drugs. Based on the results obtained, it may be concluded that the ethyl acetate extract of *Abutilon indicum* leaves possess greater hepatoprotection as compared to hydroalcohlic extract. As the results indicated, the extract possesses significant hepatoprotective activity. A study of effect of extract on immunological parameters, like TNF-alpha, interleukin, etc is required to be conducted. Also, a thorough study of clinical trials is required to be performed. After carrying out these studies, the plant may be considered as a low cost, potent, herbal medicine for liver disorders.

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## ABBREVIATIONS

| | |
|---|---|
| $CCL_4$ | : Carbon tetrachloride. |
| Hydro Alc | : Hydroalcoholic. |
| AST | : Aspartate transaminase. |
| ALT | : Alanine aminotransferase. |
| ALP | : Alkaline phosphatase. |

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