

Research Article



Hepatoprotective Activity of Leaves Extract of *Bauhinia acuminata* (Linn) against CCl₄ induced Hepatotoxicity in Albino rats.

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ABSTRACT

The purpose of this study was to see if ethanolic and aqueous extracts of *Bauhinia acuminata* (Linn.) had any hepatoprotective activity against CCl₄-induced albino rats. The levels of biochemical parameters (SGOT, SGPT and ALP) were reduced and the levels of hepatic antioxidant enzymes such as GSH and CAT were decreased whereas the level of hepatic lipid peroxidation (MDA) was elevated by CCl₄ induction in Albino rats when compared with the normal group. The ethanolic and aqueous extract of *Bauhinia acuminata* (Linn.) and Silymarin treated animal groups showed significant decrease in activities of different biochemical parameters like SGOT, SGPT, ALP and lipid peroxidase i.e., MDA level which were elevated by carbon tetrachloride (CCl₄) intoxication, at doses of 200 and 400 mg/kg and increased the level of antioxidant enzymes such as GSH and CAT. The high dose of ethanolic extracts (400 mg/kg) was more effective as compared to low dose (200 mg/kg). So, it was concluded from the result that the ethanolic extract of *Bauhinia acuminata* (Linn.) possesses significant hepatoprotective activity compared to aqueous extracts against CCl₄ induced hepatotoxicity in rats.

Keywords: *Bauhinia acuminata* (Linn.), CCl₄, hepatoprotective, hepatotoxicity, SGPT, SGOT, ALP.

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INTRODUCTION

The most serious ailment is liver disease, which is primarily caused by toxic substances (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc.). Despite tremendous advances in allopathic medicine, there is no effective hepatoprotective medicine available. Plant drugs are well-known for their importance in the treatment of liver diseases. Many plants and polyherbal formulations are appealed to have hepatoprotective properties. More than 87 medicinal plants are used in various mixtures in the preparation of thirty-three patented herbal formulations in India.¹⁻⁴ Cellular necrosis, arise in tissue lipid peroxidation, and a decrease in tissue GSH levels are all associated with liver damage. Serum levels of many biochemical markers such as SGPT/ALT, SGOT/AST, triglycerides, and alkaline phosphates are also elevated. Because of their high medicinal value, these species have piqued the interest of phytochemical and pharmacological researchers.

They are well known in folk medicine for their laxative and purgative uses.⁵ *Bauhinia acuminata* leaves have

antidiabetic action.⁶ It is used traditionally as treatment of headache and high blood pressure by its flower and to relieve coughs by its root as well as various skin diseases, worms, tumors.⁷ The presence of kaempferol, ursolic acid, and apigenin in *B. acuminata* was revealed by paper chromatography of flavonoids.⁸ The major constituents of *B. acuminata* (L.) leaf oil was identified as Phytol, Sesquiterpenoids, -carophyllene, and carophyllene oxide.⁹

MATERIALS AND METHODS

Plant material

Fresh *Bauhinia acuminata* (Linn.) plants were collected in Kolhapur, Maharashtra, and authenticated by the Botanical Survey of India in Pune. A mechanical grinder was used to grind the plants into coarse powder after they had been cleaned and shade dried.

Experimental animal

Male Albino rats weighing between 150-220 gm were procured from the Animal House, Appasaheb Birnale College of Pharmacy, Sangli for the present study. The animals were randomly assigned to treatment groups and housed in cages with paddy husk as bedding.

The animals were kept at a temperature of 24±2°C and a humidity of 30-70%. A light: day cycle of 12:12 was used. All animals had unrestricted access to water and were fed standard commercial pelleted rat chaw. The (IAEC) reviewed all of the investigational procedures and protocols used in this study, and they were all as per the



IAEC's guidelines. Animal handling was carried out in accordance with Good Laboratory Practice (GLP). The Institutional Animal Ethics Committee granted ethical approval, and the experiment was performed in accordance with the Indian National Science Academy's guidelines for the use and care of experimental animals. R. No: (IAEC/ABCP/19/19-20)

Preparation of plant extract

Using the Soxhlet apparatus, the coarse powder plant material was extracted with ethanol and water to obtain semisolid mass, the solvent was removed under reduced pressure. To determine the phytoconstituents in the extract, standard methods were used for preliminary phytochemical screening.¹⁰ Formalized paraphrase. The extract was discovered to contain alkaloid, flavonoids, glycosides, steroids, and tannins.

Acute toxicity Study

Toxicity was demonstrated by the absence of diarrhoea, drowsiness, convulsions, writhing, respiratory distress, and mortality after oral administration of extracts up to 2000 mg/kg body weight.

Experimental design

Evaluation of *in-vivo* Hepatoprotective activity of aqueous & ethanolic extract of *Bauhinia acuminata* (Linn.)

A total of 42 animals were equally divided into 7 groups which contains six animals each.

In the experiment, Albino Wistar rats were randomly assigned into seven groups of six animals each.

Group I - Served as Normal, Received distilled water 1 ml/kg p.o. for Seven days.

Group II - Served as Disease control, Received (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval

Group III - Served as Standard, Received Silymarin 100 mg/kg orally daily for Seven days and (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval.

Group IV - Served as Aqueous extracts of BA200mg/kg p.o., for Seven days and (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval.

Group V- Served as Aqueous extracts of BA400mg/kg p.o., for Seven days and (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval.

Group VI - Served as Ethanolic extracts of BA 200mg/kg p.o., for Seven days and (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval.

Group VII- Served as Ethanolic extracts of BA 400mg/kg p.o., for Seven days and (CCl₄ in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. for 72 hours interval.

On day seven, all animals except Group 1, received orally of 50% CCl₄ in olive oil (1 ml/kg p.o.).

24 hours after CCl₄ treatment (8th day) of the experiment, Blood (2-3ml) samples was collected in blood collecting tube from the retro orbital plexus of all the rats, under light anesthesia mild ether anesthesia blood was allowed to coagulate for 30 minutes at 37°C. Serum was isolated by centrifugation at 2500 rpm at 37°C for 15 minutes and analyzed for SGOT, SGPT and ALP.

Measurement of serum biochemical parameters

Blood samples were collected and centrifuged for 15 minutes at 2500 rpm. The serum was tested for SGPT/ALT, SGOT/AST and ALP. The assays were performed using a colorimetric method and commercially available kits.^{11, 12}

Histopathology

The rats were sacrificed after their blood was collected, and their livers were removed and fixed in a 10% buffered formaldehyde solution for 1 week. The paraffin sections were then processed (Automatic tissue processor, Auto technique) and cut into 3–4mm slices with rotary microtomes. After that, the slices were stained with Hematoxylin and Eosin dye and examined for histopathological changes.¹³

Estimation of antioxidant enzymes

The liver was detached, weighed (1 g), and homogenised in 10 ml of ice-cold phosphate buffer (50 mM, pH 7.4). Catalase (CAT) and reduced glutathione (GSH) activities were measured using a standard colorimetric method.^{14, 15, 16}

Estimation of Lipid peroxidation level

The concentration of MDA, a measure of the intensity of lipid peroxidation, was measured using the Ohkawa et al. method in the form of thiobarbituric acid reacting substances (TBARS).¹⁷

Statistical analysis

The results were expressed as the mean value±SEM. Group comparisons were performed by using one-way analysis of variance (ANOVA) test.



RESULTS AND DISCUSSION

Table 1: Effect of AQ and ETH extracts of leaves of BA on selected serum biochemical parameters in CCl₄ induced hepatotoxicity in rats.

Groups	SGOT(U/L)	SGPT(U/L)	ALP(U/L)
Normal Control(d.w 1ml/kg p.o.,)	98.16 ± 0.142	58.33 ± 0.211	161.5 ± 0.513
CCl ₄ + liquid paraffin (1 ml/kg i.p.)	273.66 ± 0.33	239±0.516	235.5 ± 0.43
Standard 100 mg/kg	103.5 ± 0.43***	72.166 ± 0.477***	170.67 ± 0.33***
AQ BA 200 mg/kg	256.67 ± 0.33*	138.17 ± 0.40*	231.83 ± 0.48*
AQ BA 400 mg/kg	242.33 ± 0.49*	210.83 ± 0.48*	227.5 ± 2.44*
ETH BA 200 mg/kg	238.33±0.49**	200.33 ± 1.08**	219.16±0.48**
ETH BA 400 mg/kg	217.5 ± 0.76**	159.16 ± 0.70**	213.67±0.80**

Values are expressed as Mean ± SEM (n=6), Data was analyzed using one-way ANOVA by using Graph Pad Prism 8.4.3 for Windows. (* p<0.05, ** p<0.01, ***p<0.001).

Abbreviation: AQ: Aqueous, ETH: Ethanol, CCl₄: Carbon tetrachloride, BA: *Bauhinia acuminata*(Linn.), SGOT/AST: Serum Glutamic Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, ALP: Alkaline Phosphatase.

Biochemical estimation

As presented in Table 1, intoxication with CCl₄ caused a significant increase in the serum levels of SGPT, SGOT, and ALP compared to the Normal group (p<0.001). Pre-treated with the ethanolic and aqueous extract of *Bauhinia acuminata* (Linn), at the doses of 200 and 400 mg/kg/d,

significantly decreased the CCl₄-elevated serum levels of SGPT/ALT, SGOT/AST, and ALP (p<0.001). *Bauhinia acuminata* (Linn). Analysis of the serum levels of SGPT/ALT, SGOT/AST, and ALP confirmed no significant differences in the biochemical parameters between the control group and the animals treated with the extract at a dose of 400 mg/kg/d (p>0.05).

Table 2: Effect of AQ and ETH extracts of BA on Antioxidant enzymes in CCl₄ induced hepatotoxicity in rats.

Groups	MDA (nmol/mg)	CAT (U/mg)	GSH
Normal Control (d.w 1ml/kg p.o.,)	12.66 ± 0.95	142.5 ± 0.76	18.56 ± 0.55
CCl ₄ + liquid paraffin (1 ml/kg i.p.)	26.33 ± 0.95	86.5 ± 0.76	11.08 ± 0.46
Silymarin 100 mg/kg	13.6 ± 0.70***	134.33 ± 0.99***	17.52 ± 0.47***
AQ(200 mg/kg)	19.5 ± 0.96*	110.33 ± 0.76*	12.60 ± 0.42*
AQ(400 mg/kg)	18.83 ± 0.60*	128.83 ± 0.83*	13.41 ± 0.46*
ETH(200 mg/kg)1	16.16 ± 1.30*	120.66 ± 1.65*	15.37± 0.79*
ETH(400 mg/kg)	14.16 ± 0.70**	132.33 ± 1.23**	16.48± 0.74**

Values are expressed as Mean ± SEM (n=6), Data was analyzed using one-way ANOVA by using Graph Pad Prism 8.4.3 for Windows. (* p<0.05, ** p<0.01, ***p<0.001).

LP- Lipid peroxidation (mmoles of malondialdehyde (MDA) formed /mg, GSH- Glutathione reductase (µg of glutathione consumed/ µg/min/mg), CAT- Catalase (unit/mm/mg protein)

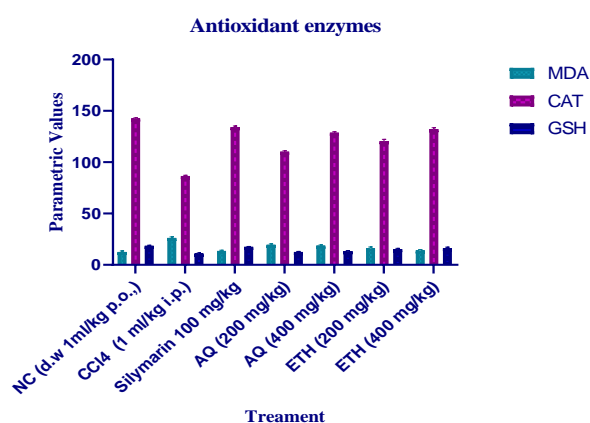


Figure 1: Effect of AQ and ETH extracts of BA on Antioxidant enzymes.

Determination of antioxidant enzymes in liver tissue homogenate.

Effect on Catalase and SOD levels

As shown in Table 2, there was significant decrease (p<0.001) in Catalase and GSH values in CCl₄ induced group as compared to Normal Control group whereas groups treated with Standard drug, AQ and ET showed significant (p<0.01, p<0.001) increase in the Catalase and GSH values when compared with the Disease Control.

Effect on MDA levels

As shown in Table: 2the serum concentration of MDA were significantly enhanced following injection of CCl₄ compared to the Normal group (p<0.001). According to the results, the extract at doses of 200 and 400 mg/kg/d, considerably reduced the MDA.



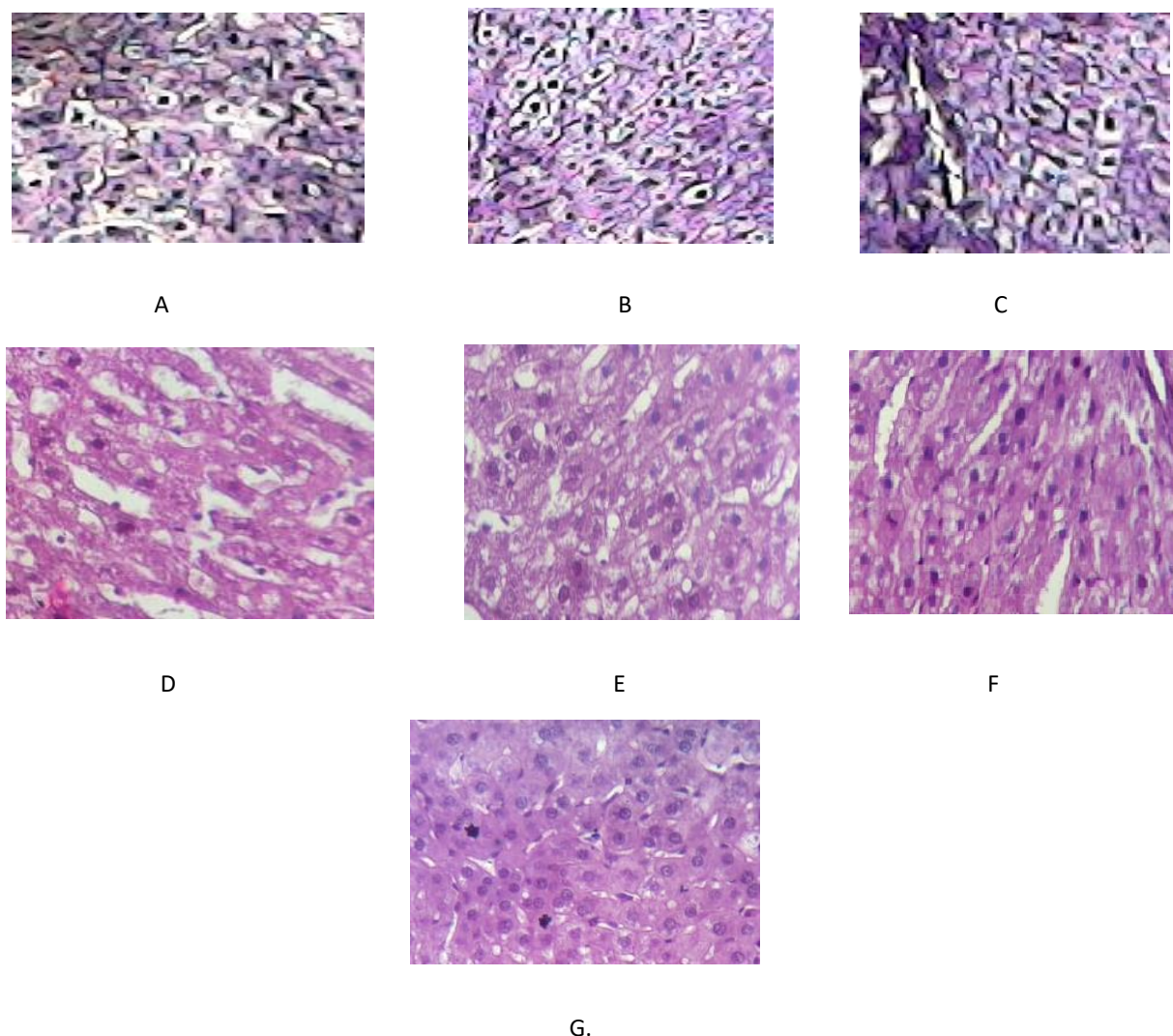


Figure 2: Photograph of liver section of rat treated with ETH and AQ Extract of BA.

Note:(A) Normal: showing normal histology of rat liver. (B) CCl_4 Induced: Control showing extensive necrosis, fraying of cell margins, and portal triditis with mononuclear lymphoplasmocytic inflammatory infiltration. (C) Standard: Silymarin-treated mouse liver showing normal appearance of hepatocytes. (D) AQ extract of *Bauhinia acuminata* 200mg/kg few hepatocytes show vacuolar degeneration. (E) AQ extract of *Bauhinia acuminata* 400mg/kg showing higher grade of vacuolar degeneration. (F) ETH extract of *Bauhinia acuminata* 200mg/kg shows very mild central vein dilation. (G) ETH extract of *Bauhinia acuminata* 400mg/kg shows showed significant recovery from necrosis, fatty changes, sinusoid congestion and lymphocytic infiltrations which is comparable to normal.

The results of body weight and hepatoprotective activity of ETH and AQ extract of *Bauhinia acuminata* (Linn.) on CCl_4 treated rats are shown in Table 1 and Table 2. The hepatic enzymes SGPT/ALT, SGOT/AST, ALP were significantly ($P < 0.001$) increased in CCl_4 treated animals when compared to control. The ethanolic extract of *Bauhinia acuminata* (Linn.) treatments significantly ($P < 0.01$) reversed the

levels of SGPT/ALT, SGOT/AST and ALP. Silymarin (100 mg/kg) treated animals also showed significant decrease in levels of SGPT/ALT, SGOT/AST and ALP and also Antioxidant enzymes like MDA, Catalase and Reduced Glutathione at 400 mg/ml and 200mg/kg when compared to CCl_4 alone treated rat.(shown in Fig:1)

Histopathological examination

A section of liver was collected and immediately fixed in 10% formalin before being dehydrated in ascending grades of alcohol (ethanol) of 70%, 80%, and 95% and absolute alcohol for two changes each. The tissues were cleaned with xylene before being embedded in paraffin wax. Using a rotary microtome, serial sections of 5-6 microns thickness were obtained and stained with hematoxylin and eosin. The stained sections were analyzed under a microscope.¹⁸ Formalized paraphrase When CCl_4 -treated mice were compared to negative controls, the morphological examination of rat liver tissue revealed visible darkened nodules, a gross and irregular surface, indicating severe hepatocellular damage. Pre- and post-

treatment with ETH and AQ extract at 200 and 400 mg/kg, respectively, as well as Silymarin at 100 mg/kg protected the liver from CCl₄-induced damage. The ethanol extract showed a protective effect at the dose of 400 mg/kg. (Figure no: 1)

The current study's findings confirmed BA hepatoprotective effect in CCl₄-induced liver toxicity. In animal models of liver disease, CCl₄ has been widely used to induce hepatic injuries.¹⁹⁻²¹ Formalized paraphrase CCl₄ causes experimental damage that histologically resembles viral hepatitis. CCl₄ is one of the most important and widely used hepatotoxic agents in research study of liver related disorders. The active metabolite of CCl₄, trichloromethyl radical, is largely responsible for its hepatotoxic effects.²² These radicals bind covalently to macromolecules and cause Peroxidative degradation of endoplasmic reticulum membrane lipids rich in polyunsaturated fatty acids.²³ Formalized paraphrase this process causes an excess of lipid formation and accumulation in tissues such as the liver. Lipids from peripheral adipose tissue are translocated to liver for accumulation.²⁴ Recent hepatoprotective drugs have the ability to inhibit the aromatase activity of cytochrome P₄₅₀ thereby favoring liver regeneration.²⁵ Due to hepatocyte necrosis or abnormal membrane permeability, SGOT/AST and SGPT/ALT are normally found in high concentrations in the liver; these enzymes are released from the cells, and their levels in the blood rise. SGPT is a sensitive marker of acute liver damage, and it is unusual for it to be elevated in non-hepatic diseases. SGPT is a more specific parenchymal enzyme of the liver than SGOT.²⁶ Evaluation of liver function can be made by estimating the activities of serum SGPT, SGOT and ALP .which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the bloodstream in proportion to the severity of the liver damage.

The elevated levels of these marker enzymes were observed in rats treated with group II CCl₄. The improved levels of these enzymes are caused by the extensive liver damage caused by the toxin. The reduced level of SGPT/ALT, SGOT/AST, and ALP observed as a result of leaves extract administration during the current study could be attributed to the presence of flavonoids.²⁷

The report of histopathological studies backs up the results of the biochemical studies, demonstrating that the hepatic damage caused by CCl₄ during intoxication is reduced in the liver samples of animals treated with an ETH extract of *Bahunia acuminata* (Linn.). Histological examination of control animal liver sections revealed the presence of normal hepatocytes with well-preserved cytoplasm, a prominent nucleus and distinct sinusoidal spaces. CCl₄ treated groups liver tissue cells show severe damage to liver tissue by cell necrosis. Interestingly, the administration of ETH extract of *Bahunia acuminata*(Linn.). The ability of an ETH extract of *Bahunia acuminata*(Linn.) to protect against liver necrosis is demonstrated in this study. When compared to the other dose, an ETH extract

of *Bahunia acuminata*(Linn.) at a dose of 400 mg/kg b.w. was more effective and resulted in the restoration of normal histological appearance. Which clearly indicates the protective and curative effect of ethanolic extract of *Bahunia acuminata* (Linn.) against CCl₄-induced hepatic damage. The standard, Silymarin-treated rats liver section showed almost normal liver with no sign of necrosis.²⁸

CONCLUSION

Serum biochemical markers and histopathological studies in the ETH and AQ extract pre- and post-treated groups support the hepatoprotective effect and provide support for the traditional use of *Bahunia acuminata* (Linn.) for the treatment of liver disorders. Larger doses of both extracts demonstrated remarkable hepatoprotective activity, comparable to Silymarin. Larger doses of both extracts demonstrated remarkable hepatoprotective activity, comparable to Silymarin. The ability of ETH extract to maintain the normal functional status of the liver has been demonstrated in both extracts. Based on the preliminary findings, we conclude that the ETH leaf extract of *Bahunia acuminata* (Linn.) is one of the herbal remedies for liver disease.

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