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R E S E A R C H A R T I C L E

Hepatoprotective Activity of Ethanolic *Bauhinia Acuminata.L* Extract against Cci_4-Induced Liver Damage in Rats

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A B S T R A C T

The present study was aimed at evaluating the hepatoprotective activity of Bauhinia acuminata.L against Carbon tetra chloride induced hepatotoxicity in rats. Hepatic damage was induced by administration of CCL4 (1.5ml/kg b.w, i.p) in combination with olive oil (1:1) as a single dose. The extent of liver damage was studied by measuring liver tissue damage markers like SGOT, SGPT, ALP & BILIRUBIN (TOTAL & DIRECT) levels. Bauhinia acuminata.L showed the hepatoprotective activity by lowering CCL4 induced elevation of SGOT, SGPT, ALP & BILIRUBIN levels ($p<0.01$) and was confirmed by the histopathological examination of liver tissues treated with test compound. This result strongly supports the protective effect of EEBA against liver injury.

Keywords: Bauhinia acuminata.L, Hepatoprotective, CCl4, Silymarin, Hepatotoxicity.

A R T I C L E I N F O

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1. Introduction

Liver is main organ responsible for the biosynthesis, uptake and degradation of proteins & enzymes. It plays a central role in transforming, clearing chemicals and is susceptible to the toxicity from these agents. Certain medical agents taken in over doses& sometimes even when introduced with in therapeutic ranges may injure the organ [1]. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures [2].The use of herbal products for the treatment of liver diseases is completely an old approach of various traditional system of medicine [3]. Plant drugs play a important role in the management of liver diseases all over the world [4]. There are numerous traditional medicinal plants reported to have hepatoprotective properties such as *Silybum marianum*

(Milk thistle)[5], *Zingiber officinale* (Ginger)[6], *Cucurbita pepo* L. (Pumpkin)[7], *Citrus reticulata* (Mandarin)[8], *Andrographis paniculata* (King of bitters)[9], and so on. Many of these are showed significant hepatoprotection. *Bauhinia acuminata. L* with the common name of 'white orchid tree' is a plant belonging to the family of fabaceae[10].Traditionally, the leaves and bark of *Bauhinia acuminata.L* have been widely used for various medicinal purposes such as cold, cough, biliousness and skin diseases etc…[11]. Therefore, this study was conducting with the aim of assaying the effects of *Bauhinia acuminata*.L on liver enzymes in the rats damaged by ccl_4.

Fig 1: Bauhinia acuminata. L

2. Materials and Methods

Chemicals:

SGOT, SGPT, ALP, BILIRUBIN were purchased from Excel diagnostics Pvt Ltd., India. Chemical substances including ccl_4 bought from SD fine chemical Ltd, India. Sodium citrate and Normal saline purchased from Finar chemicals Ltd. Ahmadabad, Formaldehyde purchased from E-Merck , Mumbai, India. Silymarin bought from Microlabs Ltd. Olive oil purchased from Oleoforfait S.A. Alcolea, Spain. Ethanol from Avantor performance material Pvt. Ltd.

Plant collection and Extraction: The Leaves of *Bauhinia acuminata.L* was collected from local areas of karimnagar, Telangana, India and authenticated by BSI, Deccan Regional Centre , Attapur, Hyderabad [Authentication no: BSI/DRC/2017-18/Tech./700]. The samples were prepared for extraction. The powdered leaves of *Bauhinia acuminata.L* extract was prepared by using solvent ethanol and extracted by soxhlet apparatus[13]. The extracts were used for further experimentation.

The design of the study:

Hepatotoxicity Induction: In order to induce liver damage in rats, CCL_4 was solved in Olive oil with the ratio of 1:1 & from the obtained mixture 1.5mg/kg was injected to them intraperitoneally.

Grouping of rats:

20 rats were randomly assigned to 5 groups each consisting of 4 rats.Group –I: rats were orally administered with distilled water for 14 days and on 14th day single dose of olive oil (1.5 mg/kg i.p) as a normal control.Group- II: rats were orally administered with distilled water for 14 days and on 14th day single dose of olive oil and ccl_4 mixture (1.5 mg/kg i.p) Group – III: rats were pre-treated with plant extract 200mg/kg orally for 14 days and on 14th day single dose of olive oil and ccl_4 mixture (1.5mg/kg i.p) Group – IV: rats were pre-treated with plant extract 400mg/kg orally for 14 days & on 14th day single dose of olive oil and ccl_4 mixture (1.5mg/kg i.p) Group – V: rats were pre-treated with standard drug (100mg/kg) orally for 14 days and on 14th day single dose of olive oil and ccl4 mixture (1.5 mg/kg i.p) as a positive control.

Serum analysis: Treatment with Bauhinia acuminata plant extract was continued for 14 days. And on 15th day blood samples were collected and centrifuged at 10,000RPM for 10mins and serum was separated. Serum was analyzed for liver damage biomarkers. The animals were then dissected, the liver were carefully removed, washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies. [14]

Biochemical estimations

Different biochemical parameters like Aspartate transaminase [AST], Alanine transaminase [ALT], Alkaline phosphatase[ALP], Bilirubin[Total & Direct] levels were determined by using commercially available kits [Excel diagnostics Pvt. Ltd,India] .

Histopathological Examination

At the end of study, the abdomen was cut; open to isolate liver from each animal. Isolated liver tissue were washed with normal saline and fixed in 10% formalin solution, dehydrated in graded ethanol and embedded in paraffin wax. Sections were prepared and stained with hematoxylin and eosin. The slides thus prepared were observed for histopathological features under microscope [15].

Statistical analysis

Statistical significance was determined by one way ANOVA test followed by Dunnet's T test and P values less than 0.05 were considered as significant [16].

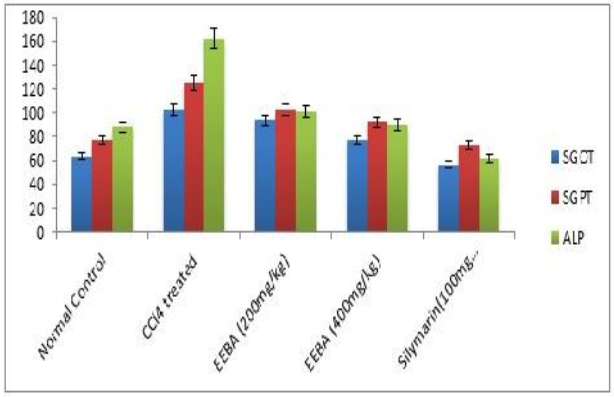
3. Results and Discussion

Histopathological examination of liver:

The results of the study indicated the hepatoprotective activity of *Bauhinia Acuminata.L* which are supported by the histopathological examination of rat livers treated with CCl_4. Histopathological observations performed in this study supported the results obtained from the serum enzyme assays. The results of the histopathological examination of rat livers treated with CCl_4 alone and along with silymarin and test drugs were shown in (Figure 4). Histopathological profile of the control animals showed no pathological significance (Figure 4A). Carbon tetrachloride treated animals showed large hepatic lobules, multiple focal areas of necrosis, loss of cell boundaries, blood sinusoids were dilated and congested (Figure 4B). The animals treated with *Bauhinia Acuminata.L* (200mg/kg and 400mg/kg) exhibited significant dose dependent liver protection against the toxicant as evident by the presence of mild to moderate hepatic cords, complete regeneration of cells and absence of necrosis (Figure 4C, D).The sections of liver taken from the animals treated with standard drug Silymarin showed the small hepatic lobule, normal hepatic architecture, which was similar to that of control (Figure 4E). CCl_4 induced hepatic injury is a commonly used model for the hepatoprotective drug screening. Hepatic enzymes such as Aspartate transaminase, Alanine transaminase, alkaline phosphatase and Bilirubin (Total & Direct) levels were

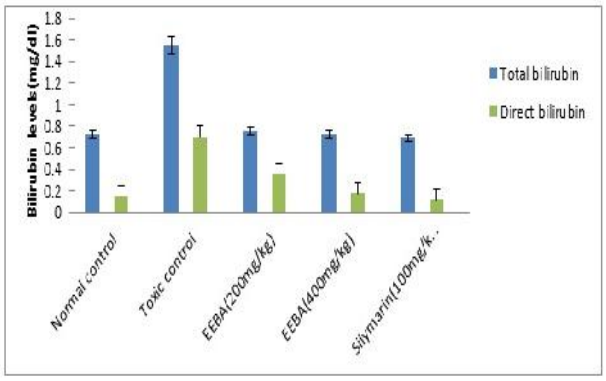
investigated and showed an increase in CCl_4 induced rats when compared to control group. Animal groups treated with 200&400mg/kg of *Bauhinia acuminata.L* showed a significant dose dependent inhibition (P<0.01) of the elevated SGPT, SGOT, ALP & BILIRUBIN (Total & Direct) levels induced by the CCl_4 induction. This may suggest that treatment with *Bauhinia acuminata.L* accorded a protection against CCl_4 induced increase in serum liver damage marker levels in a dose dependent manner. Pre-treatment with silymarin and Ethanolic exract of *Bauhinia acuminata.L* (EEBA) significantly prevented the biochemical changes induced by CCl_4. The hepatoprotective effect provided by EEBA 400mg/kg was found to be greater than that of 200mg/kg treatment. (Table No.1). Medicinal plants used for centuries as remedies for human diseases because they contain natural compound which play a dominant role in the development of novel drug lead for treatment and prevention of diseases [11]. CCL_4 induced hepatic injury is a commonly used as an experimental method for the study of hepatoprotective effects of various drugs. CCL_4 induced liver injury depends on a toxic agent that has to be metabolized by the liver NADPH-cytochrome p-450 enzyme system to a highly reactive intermediate. It has been suggested that this toxic intermediate is the trichloromethyl radical ($CCL_3^{\cdot}$) that produces damage to the liver. This free radical attacks lipids on the membrane of endoplasmic reticulum initiating lipid peroxidation and eventually leads to liver damage [17].

The hepatoprotective activity of *Bauhinia acuminata* was also supported by the histopathological examinations of rat livers treated with CCL_4 and *Bauhinia acuminata*. Pretreatment with *Bauhinia acuminata* protected the liver by the toxicant at doses of 200 and 400mg/kg body weight which indicate the hepatoprotective activity. In addition, the possible hepatoprotective mechanism of *Bauhinia acuminata* has not been reported yet. In conclusion, from the overall result of the biochemical and histopathological examinations it could be inferred that *Bauhinia acuminata* showed the hepatoprotective activity. Hepatocellular necrosis caused by CCL_4 leads to the elevation of serum marker enzymes mainly AST, ALT, ALP and bilirubin (Total and Direct) and are the conventional indicators of liver injury. The present study showed a significant increase in AST, ALP, ALT and bilirubin after exposure of CCL_4 indicating the likely occurrence of liver damage. Treatment with *Bauhinia acuminata* extract at doses of 200 and 400mg/kg reduced these elevated serum enzyme markers indicating the hepatoprotective activity of plant extract against CCL_4 induced liver damage in a dose dependent manner (p< 0.01) and its hepatoprotective activity was comparable with know standard drug, Silymarin.



**P<0.01, **P<0.001 Vs CCl4 control.

Fig 2: Effect of Bauhinia Acuminata. L leaf extract on SGOT, SGPT & ALP in carbon tetrachloride-induced Hepatotoxicity in rats



**P<0.01, **P<0.001 Vs CCl4 control.

Fig 3: Effect of Bauhinia Acuminata.L leaf extract on Bilirubin levels in carbon tetrachloride-induced Hepatotoxicity in rats

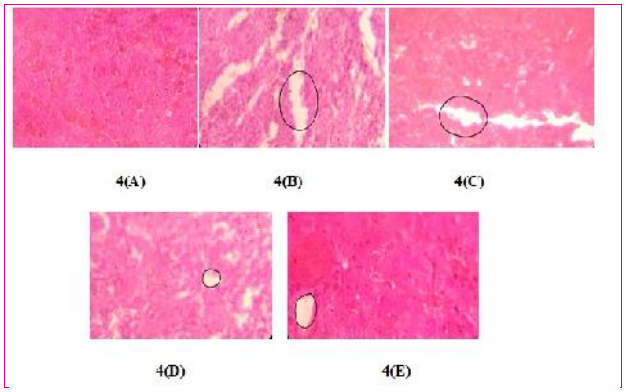


Fig 4: Hepatoprotective activity of Bauhinia Acuminata .L against CCl4-induced hepatotoxicity in rats

Table 1: Effect of Bauhinia Acuminata.L on various biochemical parameters in Carbon tetrachloride-induced Hepatotoxicity in rats

S.No	Treatment	Bio chemical parameters				
		AST(IU/L)	ALT(IU/L)	ALP(U/L)	Bilirubin(mg/dl)	
					Total	Direct
1	Normal control	63.3±3.4	77.2±1.39	87.7±3.06	0.72±0.01	0.15±0.01
2	Toxic control	102.9±3.6	125.5±2.7	162.4±4.8	1.55±0.04	0.70±0.02

3	EEBA (200mg/kg)	93.5±1.4**	102.5±4.3**	101.6±1.6**	0.75±0.01**	0.36±0.01**
4	EEBA (400mg/kg)	77.2±1.3**	92.2±1.3**	90±3.3**	0.73±0.02**	0.18±0.01**
5	Silymarin (100mg/kg)	56.5±2.57***	72.3±1.3***	61.6±2.8***	0.69±0.01***	0.12±0.01***

4. Conclusion

In conclusion, EEBA showed dose dependent protection against CCl_4 induced acute liver injury, maximum effect was observed in the dose of 200&400mg/kg.Further studies should be carried out to isolate the potential chemical constituents of ethanolic extract of *Bauhinia acuminata.L* & to find its mechanism of action in the treatment.

Conflict of Interest: We declare no conflict of interest.

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