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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *BAUHINIA PURPUREA* LINN

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ABSTRACT:

Present study was carried out to investigate different extracts of *Bauhinia purpurea* (B.P) for its hepatoprotective activity against CCl_4 induced hepatotoxicity. The different parts of *Bauhinia purpurea* were collected authenticated and were subjected to extraction using solvent alcohol. Healthy wistar albino rats (150-200g) of male sex were used for the *in-vivo* investigations. Liver damage was induced by administration of 30% CCl_4 suspended in olive oil (1ml/kg body weight). Activities of liver marker enzymes, glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), acid phosphatase (AP), alkaline phosphatase (ALP), total albumin (TA), total bilirubin (TB), Total protein (TP), direct bilirubin (DB) at a dose (100mg/kg, 200mg/kg and 400 mg/kg) ethanol extract of stem, root, leaves, and flower of B.P. The results obtained shown a significant hepatoprotective effect in comparison with the standard (sylimarin). It is also confirmed by liver histopathology of treated animals. The present study demonstrated the extracts of B.P have hepatoprotective effect against CCl_4 induced hepatotoxicity.

KEYWORDS: Bauhinia purpurea, CCl_4 and Hepatoprotective.

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INTRODUCTION

Liver diseases are considered as fatal & life threatening. It creates a serious challenge to public health. Liver diseases are due to infection and/or exposure of liver to various toxic substances such as drugs or alcohol. Some times over dosage of drugs can also lead to liver damage. Nowadays due to inadequacy of liver protective agents, researchers and traditional medicine practitioners concentrate in herbal based remedies for various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders. There was no safe hepatoprotective drug available for the treatment of liverdisorders[1,2] . Therefore, many folk remedies from plant source are used for the protection of hepatic damages starting from ancient period. Hence the present work was carried out to screen the hepatoprotective activity of *Bauhinia purpurea* Linn.

Bauhinia purpurea may possess antibacterial, antidiabetic, analgesic, anti-inflammatory, anti-diarrheal, anticancerous, nephroprotective and thyroid hormone-regulating activity[3]. Water extracts of the leaves of *Bauhinia purpurea* have been shown to have anti-ulcer activity in animals in the 'ethanol-induced gastric ulcer model'. Water extracts did not show any signs of toxicity when given to rats orally at doses up to 5000 mg/kg[4].

The use of natural products as medicinal agents presumably predates the earliest recorded history. *Bauhinia purpurea* is a species of flowering plant is used in several traditional medicine systems to cure

various diseases. A wide range of chemical compounds including 5,6-Dihydroxy-7-methoxyflavone 6-*O*- β –D xylopyrano-Side, bis [3',4'-dihydroxy-6-methoxy-7,8-furano-5',6'-mono-methylalloxy]-5-C-5-biflavonyl and (4'-hydroxy-7-methyl3-C-α-L-rhamnopyranosyl)-5-C-5-(4'-hydroxy-7-methyl-3-C-α-D-glucopyranosyl) bioflavonoid, bibenzyls, dibenzooxepins, mixture of phytol fatty esters, lutein, β-sitosterol, isoquercitin and astragalin etc. The present review discusses phyto-chemistry, pharmacology, medicinal properties and biological activity of *B. purpurea* and its usage in different ailments[3].

MATERIALS AND METHODS

Drugs & Chemicals: Silymarin is obtained from Sigma chemicals, Mumbai. CCl_4 was procured from S.D Fine chem. Ltd, Mumbai. Suspensions of *Bauhinia purpurea* extracts were prepared in 1% tween 80. All chemicals used in the study were obtained chemically and were of analytical grade.

Experimental Animals: Adult male Wister albino rats weighing 150-200g, procured from central animal house KSHEMA Deralakatte, Mangalore and were housed in a clean polypropylene cage with not more than four animals per cage and maintained under Standard laboratory conditions (temperature 25± 2°C with dark/light cycle 12/12 h). They were fed with standard diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days prior to experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Evaluation of hepatoprotective activity: CCl4 is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury, Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphatase (ALP), Serum total bilirubin (TB), Total protein (TP)[5].

Experimental design: In this study, total 90 Albino rat of Wistar strain weighing 150-200 g were selected and divided of into fifteen groups of six rats.

Group I: Control group (Normal untreated rats)

Group 2: (Hepatotoxin Control) received a single dose of 0.2ml/kg i.p of CCl_4 diluted with 0.2ml of olive oil in 1:1 ratio for 14 days alternatively.

Group 3: Hepatoprotective agent control) animals were administered with CCl4 for 14 days & followed by the treatment with 100mg/kg of known hepatoprotective agent (silymarin) for 21 days.

Group 4,5,6,7,8,9,10,11,12,13,14,15 (Test): were administered with single dose of 0.2ml/kg of CCl_4 along with vehicle alternative days for 14 days and it was followed by the treatment with (100 mg/kg, 200 mg/kg & 400 mg/kg of ethanolic extract of *B. purpurea Linn.* stem bark, root, leaves and flowers extracts orally for 21 days. On 22nd day, blood was collected by carotid artery under mild ether anesthesia; serum was collected by allowing the blood samples to coagulate for 30 min at 37^0C followed by centrifugation (3000 rpm for 15 min) and subjected for determination of biochemical parameters like total bilirubin[6], SGPT SGOT[7] and ALP[8] by semi auto analyzer kits. **[Table 1]**

Table 1: Hepatoprotective activity of *B. purpurea* by CCl4 induced hepatotoxicity

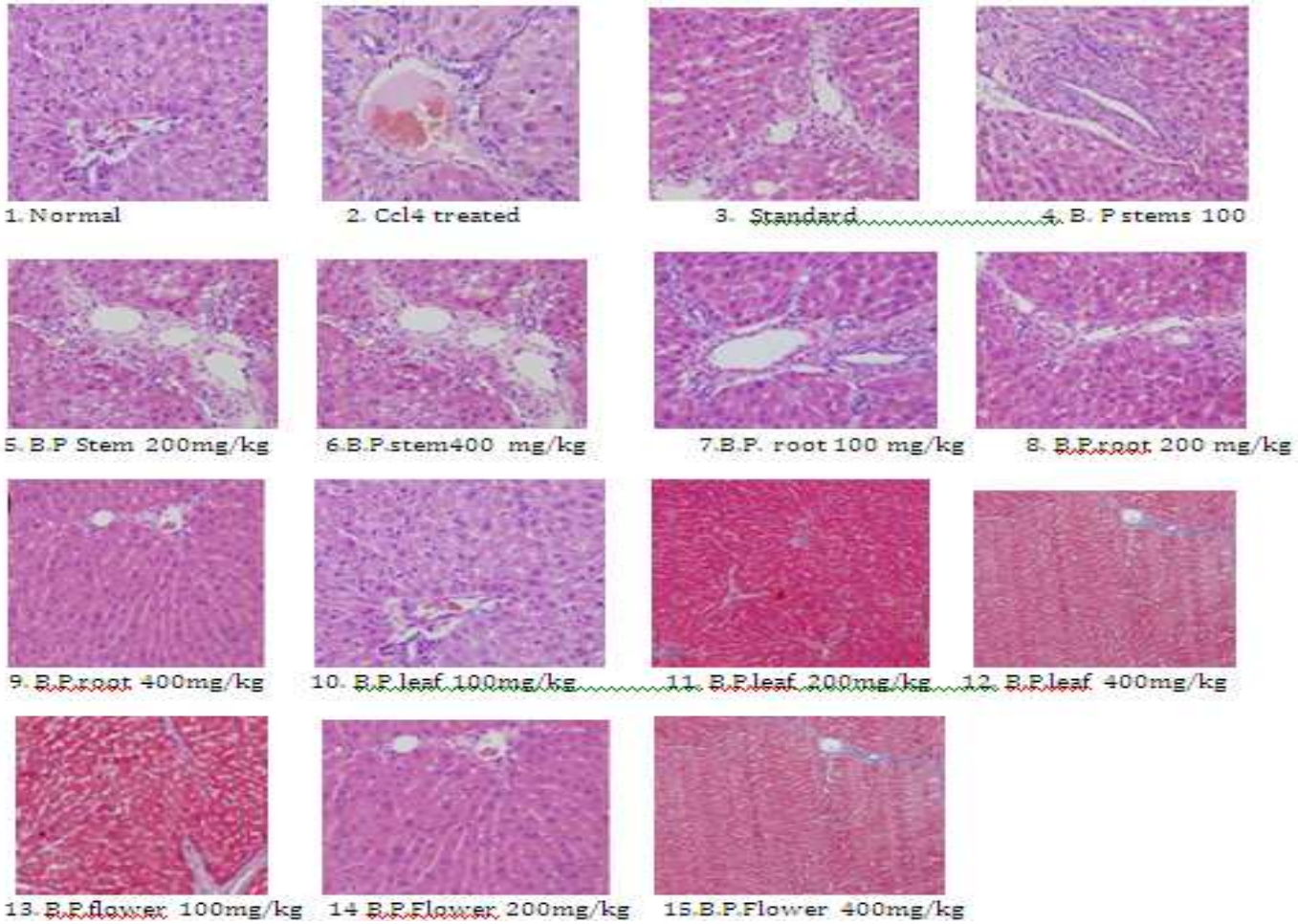
Groups	SGOT	SGPT	ALP	Total bilirubin	Total protein
Normal	48.5±2.1	23.17 ±1.2	420.8±2.6	0.4717±0.007	10.5± 0.34
Control	169.8±3.2	99.33 ±3.5	1549±25.26	2.383±0.13	5.667± 0.21
Std Silymarin 100 mg/kg	57.83±1.3**	33.5 ±1.4**	534.5±15.4**	0.7167±0.04**	8.5± 0.34**
BP St 100mg/kg	109.3±3.3**	61 ±2.9**	1268±48.42**	1.45±0.05**	7.333± 0.21**
BP St 200mg/kg	85.33±1.9**	48.5 ±2.9**	1139±22.56**	1.067±0.02**	7.5± 0.22**
BP St 400mg/kg	63.33±1.6**	40 ±1.4**	1016±18.68**	0.9833±0.05**	8.333± 0.21**
BP Rt 100mg/kg	129.8±3.04*	91.83 ±1.2	1279±35.65**	2.383±0.06	6.75± 0.30
BP Rt 200mg/kg	107.5±3.4**	66.33 ±4.4**	1097±28.87**	2.133±0.06	7.333± 0.24*
BP Rt 400mg/kg	89.5±2.9**	61.33 ±1.4**	877.8±29.05**	1.533±0.09**	7.75± 0.42**
BP Lf 100mg/kg	155.5±3.3	99 ±1.4	1550±29.94	2.417±0.03	5.75± 0.25
BP Lf 200mg/kg	142.2±3.6*	87.67 ±1.08	1354±19.81*	2.133±0.06	6.083± 0.23
BP Lf 400mg/kg	111.3±2.7**	79 ±0.93*	1089±42.68**	1.95±0.05**	6.667± 0.35
BP Fl 100mg/kg	164.2±5.9	98.17 ±1.07	1542±24.51	2.5±0.04	5.75± 0.21
BP Fl 200mg/kg	161.2±3.20	94.33 ±1.202	1521±35.87	2.4±0.04	5.75± 0.17
BP Fl 400mg/kg	137.3±2.7*	86.33 ±2.7	1439±27.85	1.9±0.11**	5.417± 0.15

** Represents statistical significance vs. control ($P < 0.01$).
*** Represents statistical significance vs. control ($P < 0.001$).
B.P: Bauhinia purpurea Linn., St: stem, Rt: root, Lf: Leaf., Fl: Flower.

Histopathology of liver

A portion of the liver was cut into two to three pieces approximately of 6mm3 size and fixed in phosphate buffered10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5µm thickness were cut and stained with haematoxylin–eosin. The stained sections were made into permanent slides and examined[9] under high resolution Microscope (motic) model: AP40) with photographic facility and photomicrographs were taken. **Fig 1-15.**

Statistical analysis: The results were analysed by calculating, mean, median mode ±SEM. The values were compared with control group p value is calculated, one way ANOVA followed by Dunnet's test.



RESULTS

Biochemical parameters: The extract showed a decrease in the levels of ALP, ASP, TB and DB ($p < 0.001$) and SGOT ($p < 0.001$), SGPT, TP and AP ($p < 0.001$) Table No: 1 when compared to standard sylimarin. The alcoholic extracts showed a dose dependent decrease in the enzyme levels of ALP, ASP, SGOT, SGPT ($p < 0.001$).TP, ($p < 0.001$), TB ($p < 0.001$) and TB ($p < 0.001$). The extracts showed a dose dependent decrease in the enzyme levels of ALP, SGOT, SGPT ($p<0.001$).TP, TB, ($p < 0.001$), and TB ($p < 0.001$). The histopathology of the liver samples revealed a significant dose dependent protection by the extracts against CCl4 induced hepatotoxicity. The fatty degeneration, lymphocytic infiltration, steatosis and necrosis from ballooning degeneration to necrosis changes were analyzed. The maximum protection was seen with alcoholic 200, 400mg/kg stem, root and standard sylimarin (100mg/kg I.P.).

DISCUSSION

The present study revealed a significant decrease in the serum enzyme levels which can be attributed to hepatoprotection. BP extract was found to decrease the levels of ALP, ASP significantly and there is a dose dependent decrease in the elevated SGOT and SGPT levels of the extracts when compared to CCl_4 group. CCl_4 treated Liver showed perivenular necrosis, steatosis with degree of steatosis being variable from ballooning degeneration to necrosis. Central lobular vacuoles, frequently dilated and congested central veins were seen with dilatation of surrounding sinusoids, which contradicted to the observations of standard sylimarin, alcoholic extracts showed a clear portal tract and central vein with normal lobular architecture and decreased cell degeneration indicating the hepatoprotective action of extracts of *B.purpurea*. The histopathological studies further confirmed the above results presented in fig 1-15.Therefore, from the above study the extracts of *Bauhinia purpurea* exhibited potent hepatoprotective activity against CCl_4 induced liver toxicity which can be ascribed to its ability to decrease the oxidative damage.

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