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Research Article

ANTIHEPATOTOXIC EFFECT OF *HOLMSKIOLDIA SANGUINEA*

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ABSTRACT

The present study seeks to evaluate the alcoholic extract of aerial part of *H.Sanguinea* for its anti-hepatotoxic effect against carbon tetrachloride (CCl_4) induced hepatic damage in rats and phytochemical analysis.The phytochemical results depicted the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrate, glycosides and phenols.The activity was evaluated by using some biochemical parameters such as serum glutamate pyruvate transaminase (SGPT), alkaline phosphates (ALP), serum glutamate oxaloacetate transaminase (SGOT) total bilirubin and gama glutamate transpeptidase(GGTP).The histopathological changes of liver sample were compared with respective control. The alcoholic extract showed significant hepatoprotective activity.

Keywords: *Holmskioldia sanguinea*; Hepatoprotective; Carbon tetrachloride; Histopathological studies; Biochemical parameters.

INTRODUCTION

Holmskioldia sanguinea Retz. (Verbenaceae), known as Kapni in Hindi, is a large scrambling shrub distributed in the subtropical and Himalayan regions from Kumaon to Bhutan and the only species of the genus found in India[1-2]. It is reportedly used for pain relief, an anticancer agent, diuretic, CNS depressive and anti-inflammatory properties.[3-4]Phytoconstituents like diterpenoids, andrographolides[5] and neoandrographolide, some known lipids, wogonin, oroxindin, friedelin, friedelinol. β-sitosterol glucoside, β-amyrin and new lipids, 27-methylnonaeicosanol were isolated[6]. However there is no report on the hepatoprotective activity of *H. Sanguinea.* So the present study was undertaken to evaluate hepatoprotective effect of alcoholic extract of *H. Sanguinea* and separate a bio-active fraction from inactive fractions against carbon tetrachloride (CCl_4) induced hepatic damage in rats.

MATERIALS AND METHODS

Plant material

The leaves of *H. Sanguinea* were collected from the district Shillong, India in the month of December 2006. A voucher specimen number HS-63419 has been deposited in the National Botanical Research Institute herbarium. The fresh leaves were kept for shade drying. Dried leaf material was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder. Powdered material was preserved in an air tight container.

Preparation of Extracts

Shade dried *H. Sanguinea* leaf powder was subjected to successive extraction in a soxhlet extractor using ethanol[7]. The extracts were filtered and the filtrates were concentrated under reduced pressure to obtain the extracts as solid residues (yield w/w=7.5%)[8].Finaly ethanol free clear residue was used for the study.

Phytochemical studies

Preliminary phytochemical test of *H. Sanguinea* ethanolic extract was performed for phytochemical analysis of alkaloids, tannins, resions, terpenoids, anthraquinone, carbohydrates, flavonoids, saponins, glycosides and phenols.[9]

Animals

Male Wistar rats weighing between 150-200g were employed for assessing the anti-hepatotoxic activity. The animals were maintained in the Institute animal house under standard laboratory condition of light and temperature. Food pellets and tap water were provided ad *libitum*. The study protocols were prepared according to the guidelines specified by Institutional animal ethics committee.

Hepatoprotective studies

Rats were divided into four groups; each group consisting of six animals. Hepatoprotective activity of *H. Sanguinea* was evaluated using CCl_4-induced model. Group one was kept on normal diet and served as control; the second group receive CCl_4 (1.25ml/kg) orally; the third and fourth groups received silymarin (100 mg/kg) and alcoholic extract of *H. Sanguinea* (250 mg/kg) once a day, for seven days[10]. On the seventh day, CCl_4 was given by oral route 30 min after the administration of silymarin and test drug.After 36h of CCl_4 administration, blood was collected and the separated serum was analyzed for various biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT),serum glutamic pyruvate transaminase (SGPT)[11] alkaline phosphate[12], total bilirubin[13]and gamma glutamate transpeptidase(GGTP)[14]were analysed.The liver was examined grossly, weighed and stored in 10% formalin and were processed for paraffin embedding using the standard micro technique[15] Sections of 5µm thickness of the liver stained with alum hemotoxylin and eosin were observed microscopically for histopathological studies.

Statistical analysis

All values were expressed as mean ± SEM and data were analyzed by student's t-test.

Phytochemical methods

Preliminary phytochemicals tests for alkaloids, tannins, resions, terpenoids, anthraquinone, carbohydrates, flavonoids, saponins, glycosides and phenols were carried out on the extracts. The methods were based on those reported by[9]. The results are presented in (Table-1).

Table 1: Phytochemicals screening of the alcoholic extract of *H.Sanguinea*

Phytochemicals extract	Alcoholic
Carbohydrate	+
Alkaloids	+
Tannin	+
Terpenoids	+
Glycosides	+
Saponins	-
Flavonoids	+
Resins	-
Anthraquinons	-
Phenols	+

+: Present, - : Not present

RESULTS AND DISCUSSION

The phytochemical studies carried out on the extracts revealed that the extract contained a wide array of phytochemicals which include carbohydrate, tannins, flavonoids, alkaloids, sterols, glycosides and phenols (Table-1). The absence of resion, saponins and anthraquinones was also observed. It is a fact that the phytochemical constituent can help one to speculate on the medicinal value of the plant.Flavonoids, tannins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system. The presence of these phytochemicals in the alcoholic extracts suggest that the plant is pharmacologically active, supporting the claim by traditional.Phytochemical results of alcoholic extract suggested that carbohydrate, alkaloids, terpenoids, tannins, flavonoids, glycocides and phenols can be isolated from the plants.

The result of biochemical parameters revealed the elevation enzyme level in CCl_4-treated group indicating that CCl_4-induces damage to the liver (Table-2)

Table 2: Effect of alcoholic extract of *H. Sanguinea* on CCl_4 treated rats

Design of Treatment	Dose (mg/kg)	SGPT U/L	SGOT U/L	ALP U/L	Total Bil mg%	GGTP U/L
Control	-	131.5±1.9	45±0.8	165.6±3.79	0 .70±0.03	123.0±4.11
CC_4	1.25ml/kg	217.3±4.5	341±3.8	386±18.25	2.10±0.01	251.1 ±5.30
Silymarin+ CCl_4	100	138±2.17**	59±3.1*	178±4.27*	0.8±0.07*	109.5 ±5.19**
Alcoholic Extract+CCl_4	250	145±1.16*	64±2.8*	196.5±4.32*	0.93±0.02*	140.5±3.71**

N=6 animals in each group; *P<0.001; **P<0.001 when compared with control.

Values are expressed as mean ± SEM.

Liver tissue rich in both transaminase increases in patients with acute hepatic diseases. SGPT, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver diseases[15-16].A significant reduction was observed (P<0.001)in SGPT, SGOT, ALP total bilirubin and GGTP levels in the group treated with selymarin and alcoholic extract of *H. Sanguinea*. The enzyme levels were almost restored to the normal. It was observed that the size of the liver was enlarged in CCl_4 intoxicated rats but it was normal in drug treated groups.

A significant reduction [P<0.001] in liver weight support this finding (Table-3). Histopathological examination of the liver section of the rats treated with toxicants showed intense centrilobular necrosis and vacuolization. The rats treated with silymarin and extract along with toxicant showed significance of protection against these toxicants to considerable extent as evident from formation normal hepatic cords and absence of necrosis and vacuoles.

Table 3: Effect of alcoholic extract of *H. Sanguinea* on Liver weight variation

Design of treatment	Dose (mg/Kg)	Liver wt/100g Body weight(g)
Control	-	3.3 ±0.10
CCl_4	1.25ml/kg	6.7 ±0.26
Silymarin+CCl_4	100	3.7 ±0.25*
Alcoholic extract+ CCl_4	250	4.8 ±0.03*

Extract+ CCl_4 250 4.8 ±0.03*

N=6 animals in each group; *P<0.05 when compared with control.

Values are expressed as mean ± SEM.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical[17].These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membranes lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipids peroxidative degradation of bio membranes is one of the principal causes of hepatotoxicity of CCl_4[18] .This is evidenced by an elevation in the serum marker enzymes, namely SGPT, SGOT, ALP, total bilirubin and GGTP.

The efficacy of any hepatoprotective drug is dependent on its capacity either reducing the harmful effect or restoring the normal hepatic physiology, which has been disturbed by a hepatotoxin. The silymarin and the plant extract decreased the CCl_4-induced elevated levels of the enzymes in the third and fourth groups, indicating the enhancement of structural integrity if hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in serum bilirubin after treatment with the extract in CCl_4 intoxicated rats indicated the effectiveness of the extract in normal functional status of the liver. Histopathological analysis is in good agreement with biochemical changes. The chemical constituents of *H. Sanguinea*, responsible for their hepatoprotective activities are not known. However, the preliminary phytochemical studies reveal the presence of flavonoids and terpenoids in alcoholic extract of *H. Sanguinea.* various flavonoides have been reported for their hepatoprotective activity[19-20]. Therefore, the possible mechanism of hepatoprotective effect of *H. Sanguinea* may be due to its flavonoides and terpenoids content. On the basis of the study, results conclude that *H. Sanguinea* exhibited significant hepatoprotective activity. Further studies are needed to isolate the active principle of the *H. Sanguinea* and established chemical nature for their hepatoprotective properties.

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