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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF TRADITIONAL HERBAL FORMULATION

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ABSTRACT

An indigenous herbal formulation containing *Cichorium intybus* (Wild chicory seed*), Sphaeranthus indicus (*East Indian globe thistle*), Rosa gallica (*Red rose petals*), Swertia chirata (*Chirata*), Coriandrum sativum (*Coriander*), Smilax chinensis (*China root*), Rheum emodi (*Indian rhubarb*) and* water was investigated for hepatoprotective activity on experimentally induced liver injury with carbon tetrachloride (CCl_4) (0.7 mL/kg body weight, intra peritoneal for 7 days). The hepatoprotective activity of the test formulation was evaluated by the assay of liver functioning biochemical parameters (total bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (SALP) and liver weight). The toxic effect of carbon tetrachloride was controlled significantly by restoration of the levels of total bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (SALP) and liver weight as compared to the normal (control) and the standard drug (Liv52) treated groups. Histology of the liver sections of the animals treated with the test formulation showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity.

Key words: Hepatoprotective, Traditional herbal formulation, Carbon tetrachloride.

INTRODUCTION

Liver is a vital organ with diverse functions. It plays an important role not only in the metabolism, synthesis and storage but also in the detoxification of many endogenous and exogenous compounds and converting them to less toxic substances for excretion. However, continuous exposure to a variety of xenobiotics, therapeutic agents and environmental pollution leads to various disorders of organs, especially liver[1].

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The major clinical manifestation of liver disorder is Jaundice. Despite the extra ordinary capacity of regeneration of this organ, a slight injury may lead to fatal complication. Hepatocellular Jaundice may occur due to the liver cell damage caused by hepatic viruses, bacterial toxins or hepatotoxic chemicals[2].

From ancient times plants from different families have been used in herbal formulations for the treatment of various ailments especially that of liver. There are more than 600 commercial preparations available from the crude plant extracts, available as formulations for the treatment of liver ailments[3].

Exploration of chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically[4,5].

In the present study, an indigenous herbal formulation containing *Cichorium intybus* (Wild chicory seed*), Sphaeranthus indicus (*East Indian globe thistle *), Rosa gallica (*Red rose petals*), Swertia chirata (*Chirata*), Coriandrum sativum (*Coriander*), Smilax chinensis (*China root*), Rheum emodi (*Indian rhubarb*) and* water[6-9], which is claimed to have the potential to treat various liver diseases, fistula, inflammation etc, was selected to evaluate the hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity in albino rats.

EXPERIMENTAL

Materials and method

The formulation was obtained as a gift sample from a traditional practioner Mr. Abdullah K. Hyderabad.

Animals

Male wistar rats weighing 150-200 g were used for assessing the hepatoprotective activity. They were maintained at standard housing conditions and fed with commercial diet and provided with water *ad libitum* during the experiment. The institutional animal ethical committee permitted this study.

Acute toxicity study

Albino mice of either sex, weighting 20-25 g and at 90 days age were used to determine the dose. Animals were fasted for 18 hours with water *ad libitum* and the test formulation was administered by oral route to pairs of mice in ascending and widely spaced doses. 2 mL/kg body weight, 4mL/kg body weight, 8 mL/kg body weight. 16 mL/kg body weight and 20 mL/kg body weight.

Animals were observed continuously for two hours and occasionally further four hours and finally over night mortality was recorded by "Up and down method"[10].

No mortality was observed even after administration of a dose of 20 mL/kg body weight. Thus based upon the studies, doses of 2.5 mL/kg body weight, 5 mL/kg body weight and 10 mL/kg body weight were selected for the assessment of hepatoprotective activity.

Hepatoprotective activity

The experimental animals were divided in six groups of six rats each. The animals in group–I served as control and received normal saline. Group II served as toxic control and received carbon tetrachloride diluted with liquid paraffin in a ratio of 1 : 1 (0.7 mL/kg body weight, intra peritoneal) on the first day to produce toxicity in the liver [11, 12]. Group III was given a single dose of carbon tetrachloride on the first day (0.7 mL/kg body weight, intra peritoneal) and then Liv52 standard (1 mL/kg body weight, orally) for 7 days, Group IV received a single dose of carbon tetrachloride on the first day (0.7 mL/kg body weight, intra peritoneal) and then the test formulation at the dose of 2.5 ml/ Kg body weight, orally for 7 days. Group V received a single dose of carbon tetrachloride on the first day (0.7 mL/kg body weight, intra peritoneal) and then the test formulation at the dose of 5 mL/kg body weight, orally for 7 days. Group VI received a single dose of carbon tetrachloride on the first day (0.7 mL/kg body weight, intra peritoneal) and then the test formulation at the dose of 10 mL/kg body weight orally for 7 days.

On the 8^{th} day, animals were sacrificed by cervical dislocation and blood was collected directly by cutting the carotid artery. Serum was separated by centrifugation and kept at lower temperature until the measurement of biochemical parameters [13,14]. Liver was excised and fixed in formalin, the weight of each liver was recorded and then subjected to histopathological studies[15,16].

Table 1 : Effects of traditional herbal formulation on different biochemical parameters in the serum of rats and weight of liver in different groups

Group	SGPT IU/L	SGOT IU/L	SALP IU /L	Total bilirubin mg/dL body weight	Liver weight in g/100 g
(I) Control	63.63 ± 4.56	169.1 ± 9.74	185 ± 11.20	0.760 ± 0.06	4.285 ± 0.16
(II) CCl_4	160.1 ± 4.26$^{\#\#}$	307.6 ± 12.37$^{\#\#}$	308.5 ± 12.36$^{\#\#}$	1.24 ± 0.18$^{\#}$	5.256 ± 0.08$^{\#\#}$
(III) CCl_4+Liv 52	85.06 ± 2.61**	198.8 ± 5.46**	190.6 ± 5.83**	0.76 ± 0.02*	4.214 ± 0.005**
(IV) CCl_4 +Test Formulation (2.5 mL/kg body weight)	84.1 ± 1.16**	196.6 ± 4.64**	188.8 ± 3.16**	0.74 ± 0.16*	4.282 ± 0.18**
(V) CCl_4 + Test Formulation (5 mL/kg body weight)	80.18 ± 2.45**	190.4 ± 1.16**	184.6 ± 4.46**	0.77 ± 0.07*	4.265 ± 0.10**
(VI) CCl_4 + Test Formulation (10 mL/kg body weight)	78.16 ± 2.14**	187.8 ± 4.40**	182.8 ± 4.16**	0.76 ± 0.02*	4.260 ± 0.6**

SGPT, Serum glutamate pyruvate transaminase; SGOT, Serum glutamate oxaloacetate transaminase: SALP, alkaline phosphatase

Results are reports as mean ± SEM, for ANOVA

#$p < 0.01$, $^{\#\#}$ $p < 0.001$ when compared with Group I

* $p < 0.01$, ** $p < 0.001$ when compared with Group II

Histopathological studies

The livers were quickly removed after autopsy and fixed in 10% formalin[17]. The rats were sacrificed and the livers removed were washed with normal saline. Small pieces of tissues were embedded in paraffin wax. The sections of about 5-6 mcm were cut, stained and then observed under microscope for histopathologicial changes in liver and their pictography were taken.

Statistical analysis

Results of liver functioning biochemical parameters were reported as mean ± S.E.M. of six animals in each group. Significant inter group difference were determined statistically by subjecting the data to ANOVA followed by Dunnet's multiple comparison test and $p < 0.01$ were considered significant.

Table 2: Histopathological changes in liver of wistar rats

Groups	Treatment	Microscopic observations
I	Control	Liver samples show normal architecture without any degeneration necrosis or inflammation.
II	CCl_4 (Toxic control)	Prominent centrilobular necrosis with prominent and enlarged central vein, periportal inflammation with fatty deposition, reflecting liver damage.
III	CCl_4 + Liv 52	A significant reduction in portal inflammation, clearly visible central vein and absence of necrosis and fatty deposition.
IV	CCl_4 + Test formulations (2.5 mL/kg body weight	Liver histology was normal. Central vein appeared clearl with the disappearance of fatty infiltration and necrosis, indicating a potent hepatoprotective activity.
V	5 mL/kg body weight	
VI	and 10 mL/ kg body weight)	

RESULTS AND DISCUSSION

The administration of CCl_4 to the animals resulted in marked increase in the levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, total bilirubin and liver weight as compared to normal (control) group. The results are shown in Table 1. Histological sections of CCl_4 treated animals showed severe hapatotoxicity evidenced by prominent centrilobular necrosis with prominent and enlarged central vein and periportal inflammation with fatty deposition as compared to normal hepatic architecture of the control group. The results are shown in Table 2.

The CCl_4 has been used as a tool to induce hepatotoxicity in experimental animals[18,19]. The toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum bilirubin reflected the depth of Jaundice and the increase in the transaminase and alkaline phosphates was the clear indication of cellular leakage and the loss of functional integrity of the cell membranes[20].

Administration of the test formulation showed protection against the toxic effects of CCl_4 as shown in the Table 1. Histopathological sections of test formulation treated animals suggest the recovery against the CCl_4 induced necrosis by returning to the normal hepatic architecture as shown in the Table 2.

The test formulation decreased the CCl_4 induced elevated enzyme levels, suggesting the protection of structural integrity of hepatocytes cell membrane or regeneration of the damaged liver cells by the test formulation. The results suggest that the traditional herbal formulation possess a potent hepatoprotective activity against CCl_4 induced liver damage in rats. Hence, justifying its use in traditional practice.

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