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RESEARCH ARTICLE

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HEPATOPROTECTIVE EFFECT OF *CASSIA SOPHERA* LEAVES EXTRACT AGAINST PARACETAMOL INDUCE HEPATIC INJURY IN RATS.

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ABSTRACT

Ethanol extract of *Cassia sophera* (Caesalpiniaceae) was evaluated for hepatoprotective activity in rats. The plant extract (500 mg/kg, p.o.) showed a remarkable hepatoprotective activity against paracetamol induced hepatotoxicity as judged from the serum markers for liver damage. Paracetamol induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin and decrease in total protein. Treatment of rats with ethanol extract (500 mg/kg) significantly altered serum marker enzymes levels to near normal against paracetamol treated rats. The activity of the extract was comparable to the standard drug Liv-52 (5 ml/kg, p.o.) Histopathological changes of liver sample were compared with respective control. Results indicate that *Cassia sophera* possesses hepatoprotective effect on paracetamol induced hepatotoxicity in rats.

KEYWORDS

Cassia sophera, Paracetamol, biochemical parameters and hepatotoxicity.

INTRODUCTION

Liver is a vital organ regulating important metabolic functions. A number of chemical agents and drugs which are used on a routine basis cause cellular as well as metabolic liver damage [1]. Paracetamol gets converted into reactive toxic metabolite by hepatic microsomal cytochrome P450 and causes hepatotoxicity. The traditional system of medicine like ayurveda, siddha and unani plays pivotal role in the treatment of liver disorder [2]. Polyherbal formulation such as Liv-52 has been proved to be evident as hepatoprotective agents in various liver disorders [3-4].

Cassia *sophera* L. (Caesalpiniaceae) is a medicinal plant of Bangladesh and Indian sub continent, which is widely used as folk medicine for the treatment of many diseases. 'Kasondi' is described in Unani literature to be repulsive of morbid humors (specially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic[5-7]. In ethno botanical literature, it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children [8-10]. The chemical analysis of the seed of *C. sophera* revealed the presence of ascorbic acid, dihydroascorbic acid and B-sistosterol [11]. Antioxidant principles like Flavone-8-C-glycoside and anthraquinone have been identified in leaves of *C. Sophera* (CS) [12-14].

Taking into consideration the traditional claim of CS being used in liver diseases and the chemical constituents which may prove beneficial acting as antioxidant and preventing liver disorder; the present study aims to investigate hepatoprotective effect of CS leaves against paracetamol-induce hepatic injury in rats.

MATERIALS AND METHODS

i. *Plant material*

As a plant material the leaves of CS were collected from Junner (Pune) district of Maharashtra state, India and identified at the Botanical serve of India, Pune. The sample of plant has been deposited in the department of Pharmacognocy at Dr. D.Y. Patil I.P.S.R., Pune. The voucher specimen no of plant is SSDH-1. Leaves were shade dried and coarsely ground to make powder. The powdered material was extracted with 95% ethanol, dried and then dried extract was adsorbed on silica gel (60-120) and fractionated successively with chloroform (CSCHR), ethyl acetate and finally with ethanol (CSETH) by using soxhlet apparatus.

ii. *Animals*

Male Wister albino rats of weighing about 200-250 g were obtained from Manoharbhai Patel Institute of pharmacy (B.Pharm) Gondia, Maharastra were housed individually in polypropylene cages and fed on standard pellet diet (Trimurti feed, Nagpur) and provided with water *ad libitum* during the experiment. The animals were maintained under standard laboratory conditions (temperature 24-28^{o}C, relative humidity 60-70% and 1:1 dark and light cycle). Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Commit.

iii. *Evaluation of hepatoprotective activity*

The acute toxicity studies were carried out as per stair case method suggested by OECD [15, 16] .The dose of 5000 mg/ kg, p.o. was found to be safe with no signs of toxicity.

The animals were divided into 4 groups, each group comprising 6 animals.

Group I served as control and received 2 ml/kg of saline daily for 7 days orally. Group II rats were similarly treated as group I. Group III rats were received liv-52 (5 ml/kg p. o.) for 7 days and Group IV were treated with alcohol extract of CS at a dose of 500 mg/kg respectively for 7 days. On the seventh day paracetamol (2 g/kg, i.p.) was administered, 30 min after the last dose to all rats except rats in group I [17].

After 36 h, all the rats were sacrificed under light ether anaesthesia; blood was collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and used for the biochemical assays like SGOT, SGPT and Serum bilurubin etc. Histopathological studies of liver were performed after scarifying animals.

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dennett test compared with induced control group. P values <0.05 were considered as significant.

RESULTS

Table 1

Effect of ethanolic extract of CS on paracetamol induced hepatotoxicity in rats

Group (n=6)	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (mg/dl)	Total bilurubin (mg/dl)
I	Control	111.15±1.28	101.01±1.38	452.66±3.249	7.45±0.09	0.47±0.01
II	IC	206.73±4.66##	234.06±1.75##	852.06±2.39##	4.10±0.08##	0.83±0.04##
III	CS 250	134.76±3.82**	140.01±1.43**	493.01±1.480**	5.433±0.04**	0.75±0.01**
IV	CS 500	126.12± 3.15**	135.65± 3.45**	477.45 ± 5.10**	6.10±1.12**	0.61±0.11**
V	Liv-52	114.01±2.12**	133.36±1.82**	479.01±2.21**	6.90±0.09**	0.59±0.07**

*Values are expressed as mean± SEM. N=6 rats each group. Data was analysed by one way ANOVA followed by Dunneets test where **p<0.001 when compared with induced group. Control and induced group was analysed using students 't' test where ##p<0.001.*

Histopathological Slides

Figure 1

Section of the liver tissue of control rats showing normal histology, portal triad consisting of portal vein (V), portal artery (arrow), hepatic duct (arrowhead) (H & E, 100 x)

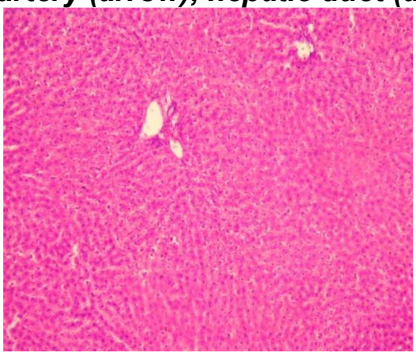


Figure 2
Section of the liver tissue of rats treated with Paracetamol showing necrosis (N) and fatty vacuole (F) (H & E, 100 x)

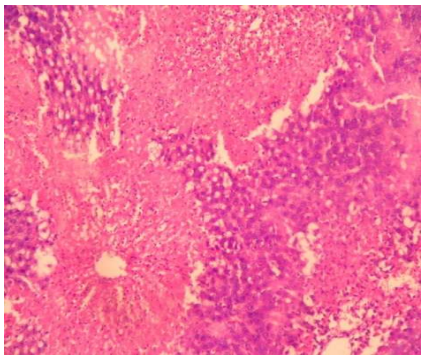


Figure 3
Section of the liver tissue of Liv-52-treated rat showing normal hepatocytes, necrosis (N), portal triad showing portal vein (V), portal artery (arrow), hepatic duct (arrowhead) (H & E, 100 x)

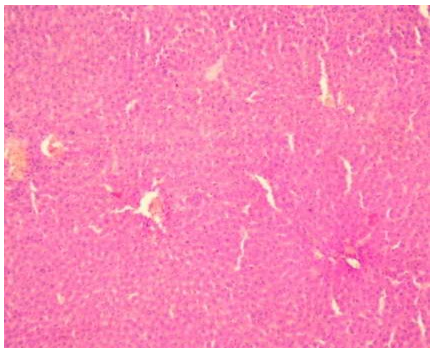
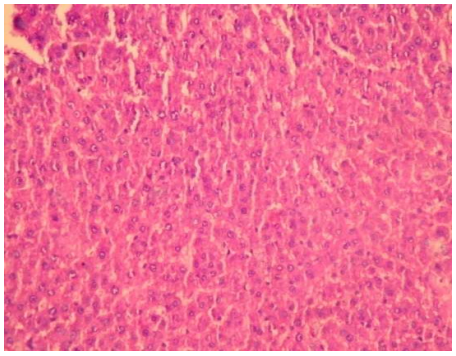


Figure 4
Section of the liver tissue of ethanol extract-treated rat showing normal arrangements of hepatocytes around the central vein (V), absence of necrosis, fatty vacuoles (H & E, 100 x)



The administration of paracetamol to the animals resulted in marked increase in serum aminotransferase (AST and ALT), total bilurubin and serum alkaline phosphatase

activity. However serum total protein level was decreased indicating liver damage.

The toxic effect of paracetamol was controlled in animals treated with CS by way of restoration of levels of the liver function biochemistry similar to that of the standard drug Liv-52. The ethanolic extract of CS showed the significant hepatoprotective activity.

Histopathological profile of control animals showed normal architecture of hepatocytes (Fig. 1). Group II animals exhibited intense centrilobular necrosis (N), vacuolization (F) and macro vesicular fatty change (Fig 2). The section of liver taken from animals treated with standard drug showed the hepatic architecture, which was similar to that of control (Fig. 3). The animal treated with ethanol extract exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Fig. 4).

DISCUSSION

The paracetamol has been used as a tool to induce hepatotoxicity in experimental animals [18]. This toxic chemical caused peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. Serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane [19].

Administration of ethanol extract of CS showed significant hepatoprotective activity, which was comparable with the standard drug Liv-52. Many phytochemical reports revealed that the ethanolic extract of the plant was found to contain higher concentrations of flavones and anthraquinone glycoside. Antioxidant principles like Flavone glycoside and anthraquinone were identified in leaves of *C. Sophera* which was found to be useful in the treatment of liver damage [5].

The earlier investigators reported the hepatoprotective activity of the flavonoid compound, which claimed to have free radical scavenging and anti lipid peroxidant activities against CCl^4-induced hepatic toxicity [19, 20]. In the present investigation, the ethanolic extract of CS has demonstrated significant hepatoprotective activity probably due to the higher content of flavonoids [20].

In accordance to the findings, it is comprehensible that CS has potential to be active as hepatoprotective in paracetamol induced hepatic injury. CS should further screened for detailed study regarding hepatoprotective activity.

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