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Research Article

HEPATOPROTECTIVE ACTIVITY OF METHANOL EXTRACT OF STEM BARK OF PROSOPIS CINERARIA LINN AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY

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ABSTRACT

Objectives: To evaluate the hepatoprotective activity of methanolic extract of stem bark of *Prosopis cineraria* linn were studied against Wister rats with liver damage induced by carbon tetrachloride (CCl_4).

Methods: The hepatoprotective effect of the methanolic extract is measured against Wister rats with liver damage induced by carbon tetrachloride (CCl_4) through measuring the serum levels of Alanine Aminotransferase (ALT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and total bilirubin to a significant extent. The methanolic extract was screened for toxicity by oral toxicity studies according to OECD guidelines 423. LD_{50} was calculated for selection of dose. The liver samples were dissected out, blotted off blood, washed with saline and also stored it in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Results: Results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities. A significant increase ($p<0.05$) in serum SGPT, SGOT, ALP and total protein levels was observed in animals treated with CCl_4 (2 ml/kg s.c.) as compared to normal pretreatment with MEPC (200 mg/kg and 400 mg/kg p.o) for 5 days decreases the above parameters significantly ($p<0.05$) as compared to CCl_4 treated group.

Conclusion: The methanolic extracts of stem bark of *Prosopis cineraria* linn showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extract of stem bark of the plant could afford significant dose-dependent protection against CCl_4 induced hepatocellular injury.

Keywords: Prosopis cineraria linn, Carbon tetrachloride, Alanine Aminotransferase, Serum Glutamate Pyruvate Transaminase and Serum Glutamate Oxaloacetate Transaminase.

INTRODUCTION

The liver is a vital organ of human body detoxification of the exogenous xenobiotic, drugs, viral infection and chronic alcoholism. The liver is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis cirrhosis and alcoholic liver disease [2-3]. Excess consumption of certain drugs like antibiotics, chemotherapeutic agents, acetaminophen, and exposure to some chemicals such as peroxidised oils, aflatoxin, CCl4, alcohol etc make liver vulnerable to variety of disorders viz., jaundice, hepatitis etc which are the two major hepatic disorders that account for high death rate [4]. Treatment for these disorders is done by using drugs from different sources including traditional herbal medicines. These traditional herbal medicines are believed to be better than other in treating liver disorders without any scientific proof. Therefore it is necessary to explore and develop such herbal medicines scientifically [5]. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders [6-7]. Therefore, much hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [8-9]. Silymarin is a powerful antioxidant said to protect liver cells (and other cells in the body and brain) from toxins. Silymarin apparently promotes liver cell protein synthesis and decreases the oxidation of glutathione. Silymarin may potentially be beneficial in a number of diseases involving liver disease [10].

MATERIALS AND METHODS

The stem bark of *Prosopis cineraria* was collected from Cuddalore district of Tamilnadu. The botanical identities were determined and authenticated by Dr. G.V.S. Murthy, Scientist 'F', Head of office, Botanical Survey of India, Southern Regional Center, Coimbatore. In the present work, the authenticated shade dried Stem bark of *Prosopis Cineraria linn,* approximately (500 g), were powdered to coarse particle size no. 40 and subjected to extraction with methanol in a Soxhlet extractor for 48 hrs. The total methanol extract was filtered and concentrated to dryness at 40^0 C. Finally the end product was coded as MEPC. The acute toxicity for MEPC was determined on swiss albino male mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OECD guideline No.423 given by CPCSEA was adopted for toxicity studies. Healthy albino rats (150-250gm, 12-14 weeks age) were housed in cages with free access to standard rat chow diet and water adds libitum. Finally this was acclimatized to the surroundings for one week prior to the experiment. Animals were harbored on a light/dark cycle (12/12 hr) at a constant temperature (25^0C) and relative humidity (50%). The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). As per the guidance of committee the control and supervision of experiments on Animals (CPCSEA) were followed.

In the dose response experiment, Wister rats (150-250) were randomly assigned into 5 groups of 6 individuals each.

- ✓ Group: I-Animals (-ve control) were administered 1 ml of distilled water p.o., for 5 days

✓ Group:II-Animals (+ve control) were administered 1 ml distill water p.o., for 5 days

✓ Group:II- Animals were administered with Silymarin 50 mg/kg p.o., for 5 days

✓ Group:IV-Animals were administered with Methanolic extract 200 mg/kg p.o., for 5 days

✓ Group:V- Animals were administered with Methanolic extract 400 mg/kg p.o., for 5 days

Group: 1 received olive oil (1ml/kg) s.c., on 2nd and 3rd day

Group: II, III, IV and V received CCl_4: olive oil (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day, after 30 min of vehicle, 50mg/kg silymarin, 200mg/kg of MEPC and 400mg/kg MEPC administration. Animals were sacrificed on the 6th day under mild ether anesthesia. Blood samples were collected by retro orbital plexus route for evaluating the serum biochemical parameters like SGOT, SGPT, ALP and Total bilirubin. The liver samples were dissected out, blotted off blood, washed with saline and also stored it in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically [10].

Histopathology

Small pieces of liver tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope for analyzing the altered architecture of the liver tissue due to CCl_4 challenge and improved liver architecture due to pretreatment with test extracts and standard drug [11].

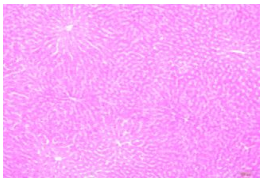
Statistical analysis

Results were expressed as Mean±Sem, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test by using graph pad instat software. P value less than 0.05 was considered to be statistically significant. *p<0.01, **p<0.05, when compared with CCl_4 treated group.

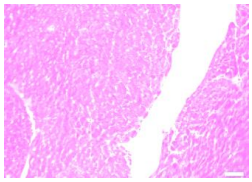
Table 1: Hepatoprotective activity of Stem Bark of *Prosopis Cineraria* linn

S. No.	Groups	Treatment	SGOT Level (U/L)	SGPT Level (U/L)	ALP Level (U/L)	Total bilirubin (mg/dl)
1.	Group-I	Normal	96.34±1.22	32.04±0.12	19.20±0.14	0.42±0.10
2.	Group-II	CCl_4	176.4±0.24*	129.28±1.10*	92.76±0.98*	0.94±0.48*
3.	Group-III	Silymarin	118.20±0.24**	34.26±0.24**	30.24±0.72**	0.50±0.74**
4.	Group-IV	MEPC 200 mg/kg	138.84±0.22**	78.24±0.28**	62.45±0.74**	0.64±0.91**
5.	Group-V	MEPC 400 mg/kg	126.72±0.86**	46.28±0.78**	42.78±0.44**	0.52±0.88**

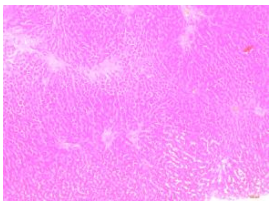
Each value represents Mean±Sem, (n=6). Values in the parentheses indicate 'P'value.*P<0.01; **P<0.05; compared to CCl_4 group. One way ANOVA followed by Dunnett.'s multiple comparison tests.

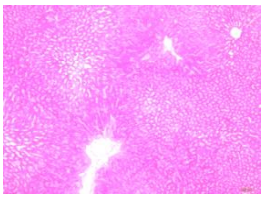


(1.a) Microscopical view of Normal liver cells



(1.b) Microscopical view of CCl_4 induced liver damage





(1.c) Microscopical view of MEPC treated liver cells

RESULTS AND DISCUSSION

SGOT- Serum Glutamate Oxaloacetate Transaminase

SGPT- Serum Glutamate Pyruvate Transaminase

ALP- Alanine Aminotransferase

MEPC- Methanolic extract of *prosopis cineraria* linn

The acute toxicity study was carried out in male swiss albino mice for MEPC according to fixed dose method of OECD guideline No: 423. Hence, no mortality was observed upto 2000 mg/kg of dose in mice. Therefore 1/10th and 1/5th (200 mg/kg, 400 mg/kg) dose was taken as effective dose for all further *In-vivo* studies. A significant increase (p<0.05) in serum SGPT, SGOT, ALP and total protein levels was observed in animals treated with CCl_4 (2 ml/kg s.c.) as compared to normal pretreatment with MEPC (200 mg/kg and 400 mg/kg p.o) for 5 days decreases the above parameters significantly (p<0.05) as compared to CCl_4 treated group. Silymarin pretreatment produced significant decreases (p<0.05) in the above parameter when compared to CCl_4. (Tab-1). Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus where as CCl_4 treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of MEPC at higher doses (400 mg/kg p.o.) significantly normalized these defects in the histological architecture of the liver (Figure-1)

CONCLUSION

Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs [12]. The rise in serum levels of

AST, ALT and cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [13]. When rats were treated with carbon tetrachloride it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidease in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid per-oxidation [14-16]. The study shows that MEPC at higher dose (400 mg/kg p.o.) have markable hepatoprotective activity compared with standard drug Silymarin. The histopathological findings reveal that the phytoconstituents like flavanoids and tannins which are present in the plant extract showed excellent protection to liver architecture almost to the level of the Silymarin treated groups, showing its potent hepatoprotective effects in animal model. Thus, the extract also reveals the significant hepatoprotective activity in a dose dependent manner by the reducing the elevated level of biochemical enzyme when they are treated with CCl_4. The plant contain flavanoids have an antioxidant properties and found to be useful for the treatment of liver damage [17]. MEPC has proven that hepatoprotective activity may be due to presence of flavanoids and tannins. Further research is sought to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

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