



Research Article

HEPATOPROTECTIVE ACTIVITY OF *CROTALARIA JUNCEA* AGAINST THIOACETAMIDE INTOXICATED RATS

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Abstract: The petroleum ether extract of *Crotalaria juncea* seed at low and high dose (100mg/kg and 500mg/kg) were tested for its efficacy against thioacetamide induced acute hepatic damage in rats. The different groups of rats were administered with thioacetamide (100mg/kg, s.c.). Drug Silymarin (100 mg/kg,) was used as reference standard. The rats were monitored for biochemical changes of serum Glutamate Oxaloacetate Transaminase (SGOT), serum Glutamate Pyruvate Transaminase (SGPT), serum Alkaline Phosphatase (ALP), and bilirubin (total and direct). Activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase in liver tissue homogenate (LTH) and Histopathological changes were observed. From the experimental results it was proved that the *crotalaria juncea* seed extract (CJSE) possesses hepatoprotective potency in a dose dependent manner by reducing the elevated levels of marker enzymes and by increasing the decreased antioxidant enzyme activity.

Keywords: *Crotalaria juncea*, Hepatoprotective, Silymarin, Thioacetamide.

INTRODUCTION

The liver is one of the major organ in the body responsible for maintaining the homeostasis of body. It is having significant role in growth, fight against disease, nutrient supply, energy provision, reproduction and it gives protection against the hazards of harmful drugs and chemicals. Because of the complex nature, it is susceptible to many adverse effects from wide variety of things like alcohol, infections from hepatitis viruses, cancer and other metabolic disorders¹. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are still on rise. Due to less potency and the chances of severe side effects reliable liver protective drugs are explicitly inadequate in allopathic medicine which exhorted the scientists to explore herbal remedies. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate². Drugs from natural sources are showing remarkable benefit with the negligible side effects against the different pathological conditions. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders.

The *Crotalaria juncea* is a herb which is traditionally used for many aliments, it is popularly known as sunn hemp belongs to the family Leguminosae, subfamily Papilionaceae which is one of the 550 species of the genus widely distributed in the tropical and subtropical regions. Sunn hemp is an erect, stiff branched, half-woody herb, usually about 1 meter high, with all the parts finely hairy³. *Crotalaria* species documented for the presence of linoleic acid (62.36%), steroids, flavanoids, phenols, glycosides and triterpinoides apart from that it contains some of the interesting compounds which include monocrotaline, riddelline, seneciphylline, senecionine, trichodesmine, chodesmine, galactose specific lectin and cardiogenin 3-O-[OH]-d-xylopyranosid⁴. *Crotalaria juncea* seed traditionally known for the nutritional and medicinal potential such as a blood purifier, abortifacient, astringent, demulcent, emetic,

purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis⁵. Modern scientific studies already documented the potential of seed for antispermatogenic, anti-ovulatory and contraceptive activities, seed oil demonstrated antioxidant, anti-inflammatory and antibacterial activities⁶. *Crotalaria* leaves for anti-inflammatory, anti-ulcerogenic activities and the aerial part for moderate antifungal activity⁷.

In the absence of reliable liver protective drugs in modern medicine, there are numbers of medicinal preparations in the ayurvedic system of Indian medicine recommended for the treatment of liver disorders. Their usage is in vogue since centuries and are quite often claimed to offer significant relief. However, no scientific information is available regarding the hepatoprotective effect of *C.juncea* seed. Since, antioxidants are known to reduce the development of chemically induced liver damage, the effect of petroleum ether extract of seeds of *C.juncea* (CJSE) has been evaluated for hepatoprotective activity against paracetamol induced liver damage using rat as experimental animals.

MATERIALS AND METHODS

Chemicals

Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and Bilirubin (total and direct) kits were purchased from Robonik India Pvt Ltd, Mumbai. Other chemicals used were obtained from SD Fine chemicals Ltd (Mumbai, India). All chemicals used in the present study were of analytical grade.

Experimental Animals

Rats of either sex weighing 170-250 g were housed at 25° ± 5°C, relative humidity 50 ± 5% in a well-ventilated animal house under 12:12h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard and Supervision on

Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (SDCP/IAEC-12/2011-12).

Plant material

Crotalaria juncea seeds were collected during May 2012 from the surrounding of Mangalore. The plant material was taxonomically identified and authenticated by Dr suja G. Nair, Department of Dravyaguna, Parassinikkadavu Ayurveda Medical College, Kerala. Seeds were dried and powdered coarsely. Pulverized crude powder was extracted by soxhlet apparatus by using the solvent petroleum ether for 36 h. *C. juncea* seeds petroleum ether extract (CJSE) was then concentrated under vacuum at low temperature to obtain as pale yellow colour oil.

Phytochemical estimations of the extract⁸

Dried seed extract of seeds *Crotalaria juncea* was subjected to qualitative analysis to investigate the presence of various phytochemical constituents such as fatty acid, terpenes and sterols.

Acute toxicity studies

The extract was dissolved in distilled water by using Sodium carboxy methyl cellulose and used for the animal experiments. The dose selection of CJSE was based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines following the limit test procedure⁹. The animals were fasted over night prior to the studies. Mice were divided into two groups of six each. Test dose of 2 g/kg body weight and 5 g/kg body weight were given orally to either group of mice. Mice were observed for 72 hours for mortality. 1/10th and 1/50th of the maximum safe dose were selected as high and low doses respectively.

Experimental protocol

The animals were divided into 5 groups of six animals each. Group I and Group II received distilled water for 7 days and termed as normal control and toxic control respectively. Group III served as standard, were administered Silymarin (100 mg/kg/day, *p.o.*). Group IV and Group V termed as low and high dose treated group were treated with *Crotalaria juncea* seed extract (CJSE 100 mg/kg/day, *p.o.* and 500 mg/kg/day, *p.o.*). All the groups received assigned treatment for 7 days.

Thioacetamide (TAA) induced liver necrosis in rats¹⁰

After treatment of animals from group II to IV according to the protocol, a single dose of TAA was administered subcutaneously (100mg/kg) after dilution with 40% sucrose on fifth day. 48 hrs after the administration TAA, blood samples were collected by retro-orbital puncture method and serum was used for assay of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin (Total and direct). Then the animals were sacrificed and livers from each group were isolated and homogenized with sucrose solution (0.25M) for estimation of super oxide dismutase (SOD) and catalase.

Histological studies

Liver from each group were isolated and fixed immediately in 10% neutral formalin solution. The liver sections were stained with hematoxylin and eosin and histological changes were observed microscopically.

Statistical analysis

Results were expressed as mean \pm SE. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P < 0.05$ was considered significant.

Table 1: Effect of Silymarin and CJSE on serum ALT, AST, ALP, bilirubin (total and direct) in TAA induced liver necrosis in rats.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	97.11 \pm 3.53	223.96 \pm 13.75	156.92 \pm 2.29	0.26 \pm 0.07	0.19 \pm 0.04
TAA control	235.73 \pm 7.07 ***	924.17 \pm 12.65 ***	570.89 \pm 12.67 ***	1.09 \pm 0.07 ***	0.72 \pm 0.02 ***
Silymarin (100 mg/kg)	127.32 \pm 5.32* ###	269.38 \pm 10.15 ###	318.30 \pm 14.06***###	0.20 \pm 0.01 ###	0.24 \pm 0.02###
CJSE-100	138.73 \pm 4.09**###	323.20 \pm 7.38*** ###	325.30 \pm 9.12*** ###	0.64 \pm 0.01 ** ###	0.47 \pm 0.01 ***###
CJSE-500	120.27 \pm 4.20 ###	227.57 \pm 3.05 ###	232.88 \pm 5.40** ###	0.35 \pm 0.02 ###	0.26 \pm 0.01 ###

All values are mean \pm SEM, n=6, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared to normal control. ### $P < 0.001$ compared to TAA control.

Table2; Effects on SOD and Catalase in liver tissue homogenate

Treatments	SOD (unit/mg protein)	Catalase (unit/mg protein)
Normal control	7.56 ± 0.19	0.56 ± 0.03
Toxic control	0.43 ± 0.05***	0.07 ± 0.00***
Standard	7.14 ± 0.13###	0.43 ± 0.01###
CJSEE-100	6.56 ± 0.34*###	0.27 ± 0.03***##
CJSEE-500	7.66 ± 0.23###	0.34 ± 0.04***##

All values are mean ± SEM, n=6, *p< 0.05, **p< 0.01, ***p< 0.001 when compared to normal control; ##P<0.01, ###P<0.001 when compared to toxic control group.

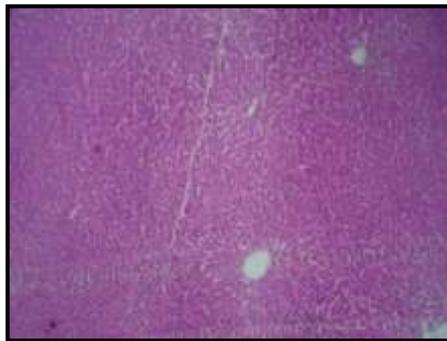


Figure-1a: Normal control: Normal Texture of liver tissue

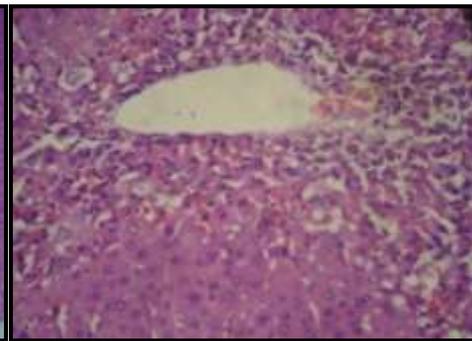


Figure-1b: Toxic control: Moderate to severe tissue degranulation

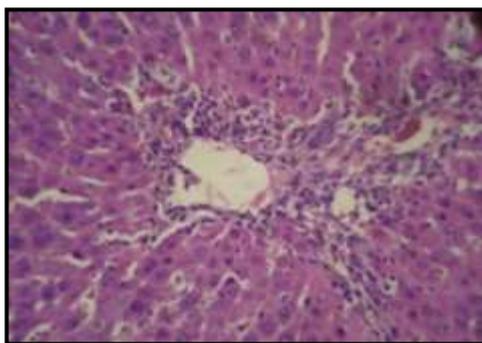


Figure-1c: Standard drug : Mild to Moderate liver tissue degranulation

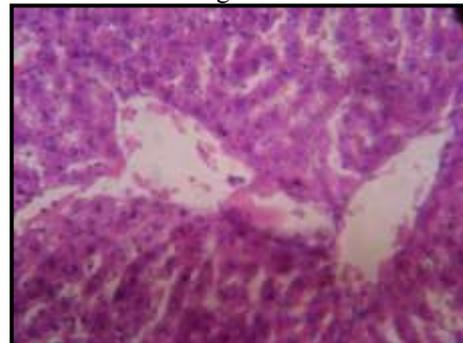


Figure-1d: CJSE-100: Moderate degranulation of liver tissue

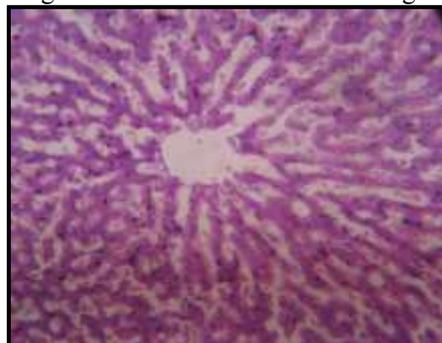


Figure -1e: CJSE-500: Mild liver tissue degranulation

Figure -1: Haematoxylin and eosin (H&E) stained section of liver in TAA induced liver toxicity. Photographed at magnification400X.

RESULT

Effect on serum enzymes level

By performing the experimental protocol it was recorded that toxic control group demonstrated extremely significant (P <0.001) increase in marker enzymes such as AST, ALT,

ALP and bilirubin (total and direct) when compare to vehicle control.

The pre-treatment of Standard (Silymarin), both low dose and high dose of CJSE exhibited an extremely significant (P<0.001) reduction of elevated values such as AST, ALT,

ALP and bilirubin (total and direct) when compare to toxic group.

Effect on SOD

In this experimental model, comparison of normal control with toxic control revealed an extremely significant ($P < 0.001$) decrease of SOD activity in LTH. The experimental group toxic control when compared with the prophylactic treated groups like standard, low dose and high dose of CJSE demonstrated an extremely significant ($P < 0.001$) increase of SOD activity in LTH.

Effect on Catalase

Animals treated with TAA demonstrated extremely significant ($P < 0.001$) reduction in Catalase activity in LTH compared to normal control group.

Toxic control group on comparison with standard and high dose treated group demonstrated extremely significant ($p < 0.001$) and low dose treated group demonstrated moderately significant ($P < 0.01$) elevation in reduced activity of Catalase activity in LTH.

DISCUSSION

The mechanism behind in thioacetamide induced hepatic toxicity is thought to be associated with its toxic metabolites which interfere with the movement of RNA from the nucleus to cytoplasm which may cause membrane injuries. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. Hepatic damage associated with toxic metabolite of thioacetamide is evident from the increased level of biomarkers and reduced level of antioxidant enzyme system such as SOD and catalase in the liver tissue homogenate. It also decreases the volume of bile and its content.

In this model *Crotalaria juncea* seed extract had proven significant protection by decreasing the increased level of serum biomarkers and increasing the reduced level of antioxidant enzymes. The possible reason may be antioxidant activity which may neutralize reactive metabolite of TAA. The hepatoprotective effect was once again supported by histological changes produced by different groups.

In the present study, both high and low dose (500 and 100 mg/kg *p.o.*) reported significant level of protection. The predicted reason is the antioxidant property due to the presence of fatty acids such as linolenic acid, linoleic acid

and oleic acid which has been witnessed as the chief chemical constituent justifying the therapeutic potential. Some of the reported activities of *Crotalaria juncea* namely antioxidant property and anti-inflammatory may be attributed to the hepatoprotective property of the plant. However, further studies are required to understand the exact mechanism behind the hepatoprotective effect of *Crotalaria juncea* seed extract.

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