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HEPATOPROTECTIVE ACTIVITY OF *CROTALARIA JUNCEA* AGAINST PARACETAMOL INTOXICATED RATS

Chakraborty M*[1],
Rahila KC[1], Bhatt L[1], Kamath JV[1]

[1]Department of Pharmacology, Shree Devi College of Pharmacy, Mangalore-574142, India

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 paracetamol induced acute hepatic damage in rats. The different groups of rats were administered with paracetamol (2 gm/kg, p.o.). 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, Paracetamol*

Correspondence to Author

MANODEEP CHAKRABORTY

Department of Pharmacology, Shree Devi College of Pharmacy, Mangalore-574142, India

Email: manodeep.chakraborty@gmail.com

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 disorders[1]. 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 rate. 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 disorder[2].

The *Crotalaria juncea* is a herb which is traditionaly 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 hairy[3]. Crotalaria species documented for the presence of linoleic acid (62.36%), steroids, flavanoids, 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-xylopyranosid[4]. *Crotalaria juncea* seed traditionally known for the nutritional and medicinal potential such as a blood purifier, abortificient, astringent, demulcent , emetic, purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis. Modern scientific studies already documented the potential of seed for antispermatogenic, anti-ovulatory and contraceptive activities, seed oil demonstrated antioxidant, anti-inflammatory and antibacterial activities. Crotalaria leaves for anti-inflammatory, anti-ulcerogenic activities and the aerial part for moderate antifungal activity[5].

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 [6]. 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 petrolium 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 METHOD

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-11/2011-12).

Plant material

Crotalaria juncea seeds were collected during May 2012 from the surrounding of Manglore. 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 extract [7]

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 procedure[8]. 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. $1/10^{th}$ and $1/50^{th}$ 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. GroupI and GroupII received distilled water for 5 days and termed as normal control and toxic control respectively. GroupIII served as standard, were administered Silymarin (100 mg/kg/day, *p.o.*). GroupIV and GroupV 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 5 days.

Paracetamol induced (PCM) acute hepatitis in rats[9]

After treatment of animals from group II to IV according to the protocol, PCM was administered in three divided dose by oral route (2g/kg) after dilution with 40% sucrose on fifth day. 48 hrs after the administration PCM, 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 ± 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.

Effect on serum enzymes level

By executing the experimental protocol it was documented that toxic control (PCM) exhibited extremely significant ($P<0.001$) increases in marker enzymes such as AST, ALT, ALP and bilirubin (Total bilirubin and Direct bilirubin) when compared to normal control.

The pre-treatment group of standard and low dose of CJSE showed extremely significant ($P<0.001$) reduction in elevated levels of AST, ALT, ALP, and bilirubin (total and direct bilirubin). High dose of CJSE showed extremely significant ($P<0.001$) decreased in AST, ALT, ALP and Total bilirubin while direct bilirubin revealed moderately significant ($P<0.01$) reduction when compared with toxic group.

Effect on SOD

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

Effect on Catalase

Animals treated with paracetamol illustrated extremely significant ($P<0.001$) decrease of Catalase activity in LTH when compared to normal control group. On comparison with toxic control group, the prophylactic treated groups like standard, low and high dose of CJSE demonstrated extremely significant ($P<0.001$) increase of Catalase activity in LTH.

Table 7: Effect of silymarin and CJSE on serum ALT, AST, ALP, bilirubin (total and direct) in PCM induced liver toxicity in rats.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	68.07 ± 6.04	223.9613.57	156.83 ±2.27	0.18 ±0.01	0.16 ± 0.01
PCM control	370.67 ± 16.40 ***	441.44 ± 15.66 ***	348.40 ±10.08 ***	0.72 ± 0.04***	0.66 ± 0.13 ***
Silymarin (100mg/kg)	99.91 ± 8.11 ###	263.57 ±4.64 ###	166.57 ± 5.97 ###	0.12 ± 0.00 ###	0.15 ± 0.00 ###
CJSE-100	153.95 ±5.57 *** ###	318.41 ± 8.20*** ###	265.II ±8.83*** ###	0.35 ± 0.06* ###	0.26 ± 0.00 **###
CJSE-500	116.13 ± 3.57*###	290.92 ±4.71** ###	211.89 ± 9.39** ###	0.16± 0.00 ###	0.20 ± 0.00 ###

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 compared to PCM control.

Table 8: Effects on SOD and Catalase in liver tissue homogenate

Treatments	SOD (unit/mg protein)	Catalase (unit/mg protein)
Normal control	7.45 ± 0.17	0.59± 0.03
Toxic control	0.43 ± 0.04***	0.07 ± 0.01***
Standard	7.42± 0.22###	0.46 ± 0.01*###
CJSEE-100	7.08 ± 0.09###	0.45 ± 0.01**###
CJSE-500	7.63 ± 0.09###	0.51 ± 0.01###

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

DISCUSSION

In paracetamol induced hepatotoxicity, the liver damage is due to its toxic metabolite. Paracetamol is normally eliminated mainly as sulfate and glucoronide. Upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules is oxidized to highly reactive N-acetyl-p-benzoquineimine (NAPQI) by cytochrome-450 enzymes. Semi Quinone a radical obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage. Significant hepatic damage due to paracetamol is evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, and bilirubin (total and direct). Decreased level of enzymatic antioxidants is a clear manifestation of excessive formation of

free radical during the metabolism of the PCM and activation of lipid peroxidation system.

The documented results proved that *Crotalaria juncea* seed extract is able to provide hepatoprotection by reducing the elevated level of biomarkers and by increasing the reduced level of antioxidant enzyme. The probable mechanism of this action may be due to free radical scavenging activity which provides protection to liver by scavenging reactive molecule produced by PCM metabolism. The hepatoprotective effect was supported by histological changes produced by the experimental animal of 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 antiinflammatory 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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