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Hepatoprotective effect of *Cassia tora* seeds on experimental animal model

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver toxicity. Our aim was to demonstrate the hepatoprotective effect of petroleum ether, methanol and aqueous extracts of *Cassia tora* seed with a view to explore its use for the treatment of hepatotoxicity in human. These extracts were used to study the hepatoprotective effect in paracetamol induced hepatotoxic model. In aqueous and methanol extracts treated groups there was statistical significant decrease in the levels of serum bilirubin, serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruvate transminase (SGPT) and serum alkaline phosphatase (SALP) as compared to the hepatotoxic group. In the histopathological study the hepatotoxic group showed hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis. Aqueous and methanol extracts treated groups showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. It can be concluded that the aqueous and methanolic extracts of *Cassia tora* seed were not able to revert completely hepatic injury induced by paracetamol, but it could limit the effect of these drug in liver. The effects of extracts were comparable with standard drug silymarin.

Keywords: Hepatoprotective, *cassia tora* seed, paracetamol.

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INTRODUCTION:

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders[1].

Cassia tora Linn. (Caesalpiniaceae) is a wild crop and grows in tropical region of India as a weed. According to Ayurveda the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, livertonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders[2,3]. Chemical component of *cassia tora* are anthraquinones[4], chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides. Naphthopyrones[5], rubrofusarin, norrubrofusarin, rubrofusaringentiobioside. Toralactone and torachrysone. Roots contains 1, 3, 5-trihydroxy-6-7-dimethoxy-2-methylanthroquinone and beta-sitosterol. Seeds contain Naptho-alpha-pyrone-toralactune, chrysophanol, physcion, emodin, rubrofusarin, chrysophonic acid-9-anthrone. Emodin, tricontan-1-0l, stigmasterol, sitosteral, D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin isolated from seeds. Antibacterial[6], anti-platelet aggregation[4], hepatoprotective[7], cAMP-phosphodiesterase inhibitory activities[8], antifungal, antiyeast, antiinflammatory[9], estrogenic and antiestrogenic[10], hypolimpidemic[11], antimutagenic[12] antioxidant[13] activities has been evaluated. Traditionally seeds are used as liver tonic and jaundice[2,4]. Therefore, in the present study an effort has been made to evaluate the hepatoprotective effect of *Cassia tora* Linn. In paracetamol induced model.

MATERIALS AND METHODS

Material and chemicals

Seeds of *Cassia tora* were collected during the month of October from the local areas of Surat, Gujarat, India. The samples were identified and authenticated by Dr. Minoo Parabia (Professor of Botany) Veer Narmad South Gujarat University, Surat, Gujarat, India. The voucher specimen (UTU/MPC/2011/62) preserve at Department of Pharmacognosy, Maliba Pharmacy College.

Uka Tarsadia University, Bardoli, Gujarat. The plant material was shade-dried, milled to powdered form and stored in airtight containers.

Paracetamol was gifted from Gufic Biosciences Ltd. (India) Navsari. Silymarin serve as standard was obtained from the Himalaya Drug Company, Bengluru. Standard kit of serum glutamate pyruvate transminase (SGPT), serum glutamate oxaloacetate transminase (SGOT), alkaline phosphatase (ALP) and bilirubin was obtained from Crest Biosystem Ltd, Santacruz. All other reagents used for the experiments were of analytical grade.

Preparation of extract

A coarsely powdered, air-dried seeds of *Cassia tora* were subjected to continuous extraction with petroleum ether in a soxhlet extractor[14]. After complete extraction, the solvent was distilled off and extract was concentrated on a water bath to a dry residue. The marc was dried completely at 50°C and again loaded in the extractor and further extracted successively with methanol. Finally, the marc was macerated with distilled water to obtain the aqueous extract. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. The different extracts were subjected to qualitative chemical investigation and were used for pharmacological studies.

Preliminary phytochemical screening

The extract was screened for preliminary phytochemical tests for the presence of carbohydrates, glycosides, flavonoids, saponins, fates and gums[15].

Animals

Wistar rats, 5-6 weeks old (100-190 g) were obtained from Maliba Pharmacy College, Uka Tarsadia University, Bardoli, Gujarat, India. Animals were kept under controlled environmental conditions (22 ± 0.5 °C relative humidity 65-67 %, 6 am to 6 pm alternate light–dark cycles, food and water *ad libitum*) in polypropylene rat cages covered with stainless steel grid and husk bedding. The animals were allowed to acclimatize for five days prior to commencement of experiment. The animal protocol was approved by the Institutional Animal ethical committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) New Delhi, India[16].

Toxicity Studies

Acute toxicity study was carried out according to OECD guidelines (Organization for economic co-operation and development). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration.

Animals were checked for morbidity, mortality and clinical signs like grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions. Based on results, doses will be selected for pharmacological activity[17].

Assessment of hepatoprotective activity

Paracetamol induced hepatotoxic model[18, 19] was used to assess the hepatoprotective effect of extracts. Rats were divided into 6 groups of 6 animals each. Group-1 served as control and received gum acacia (1ml/kg, *p.o.*) for 14 days. Group-2 was administered with (2gm/kg, *p.o.*) of paracetamol once a day for 14 days. Group-3 received standard drug silymarin as a single daily dose of (100mg/kg, *p.o.*) for 14 days with (2gm/kg, *p.o.*) of paracetamol once a day for 14 days. Group 4, 5, 6 received single daily dose of (200 mg/kg, *p.o.*) of petroleum ether, methanol, aqueous extract for 14 days respectively with (2gm/kg, *p.o.*) of paracetamol once a day for 14 days. On the 15th day of the respective treatment the rats were anaesthetized by light ether anesthesia and the blood was withdrawn by making intra-cardiac puncture to the rats. It was allowed to coagulate for 30 min at 37°C and serum was separated by centrifugation at 2500rpm for 10 minutes. The serum was used to estimate serum glutamate pyruvate transminase (SGPT), serum glutamate oxaloacetate transminase (SGOT), serum alkaline phosphatase (SALP) and serum bilirubin.

Histopathological studies:

Liver slices were fixed for 12 hrs in Bouin's solution, processed for paraffin embedding following standard micro techniques. 5µm sections of liver stained with alum haematoxylin and eosin were observed microscopically for histopathological changes.

Statistical analysis

Results of biochemical estimation are reported as mean ± SEM of six animals in each group. Statistical analysis was performed using Dunnet's't' test and ANOVA. $P<0.001$ was considered statistically significant.

RESULTS AND DISCUSSION

In the present investigation preliminary phytochemical analysis of *Cassia tora* seeds extracts revealed the presence of carbohydrates, glycosides, flavanoids and saponins in methanolic and aqueous extract and fats and gums in petroleum ether extract. In the toxicity study of petroleum ether, methanol and aqueous extracts there was no mortality or clinical signs up to dose level of 2000mg/kg b.w. as safe dose and one tenth of these doses (200mg/kg b.w) were selected for the evaluation of hepatoprotective effect. There was significant elevation of SGOT, SGPT, SALP

and bilirubin levels in the paracetamol treated groups (group II). In groups orally treated with petroleum ether, methanol and aqueous extracts of *Cassia tora* and silymarin above activities of enzymes were found to be significantly (P<0.001) decreased as compare to the paracetamol treated group. (Table-1)

Table. 1 - Effect of petroleum ether, methanol and aqueous extracts of *Cassia tora* seeds on paracetamol induced hepatotoxicity in rats

Group	**Serum biochemical parameters**			
	SGPT (IU/L)	**SGOT (IU/L)**	**SALP (IU/L)**	**Serum Bilirubin (IU/L)**
Control	46.83 ± 1.078	54.5 ± 0.619	35.48 ± 0.47	2.173 ± 0.048
Paracetamol (2ml/kg/day)	151.5 ± 2.527	163.7 ± 2.06	99.82 ± 3.00	12.52 ± 0.686
Silymarin (5ml/kg + paracetamol)	53.5 ± 2.446*	70.33 ± 2.616*	53.43 ± 0.9*	5.252 ± 0.54
Pet ether extract (200mg/kg)+ paracetamol	78.5 ± 2.262	97.50 ± 2.814	91.0 ± 2.352	11.02 ± 0.533
Methanol extract (200mg/kg)+ paracetamol	66.67 ±1.202	80.17 ± 2.301*	67.15 ± 3.992*	5.98 ± 0.574*
Aqueous extract (200mg/kg)+ paracetamol	57.67*± 1.892	70.83 ± 2.301*	53.82 ± 0.833*	5.51 ± 0.577*

Values are mean ±SEM from 6 animals in each group.

P values *<0.001 when compared with normal control group

Morphological observations showed an increased size and enlargement of the liver in paracetamol treated groups. These changes were reversed by treatment with silymarin and also *Cassia tora* extract at the doses tested. Histopathological studies (figure.1) showed paracetamol to produced extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with methanolic and aqueous extract of *Cassia tora* produced mild degenerative changes and absence of centrilobular necrosis when compared with control. All these results indicate a hepatoprotective potential of the extract.

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs,

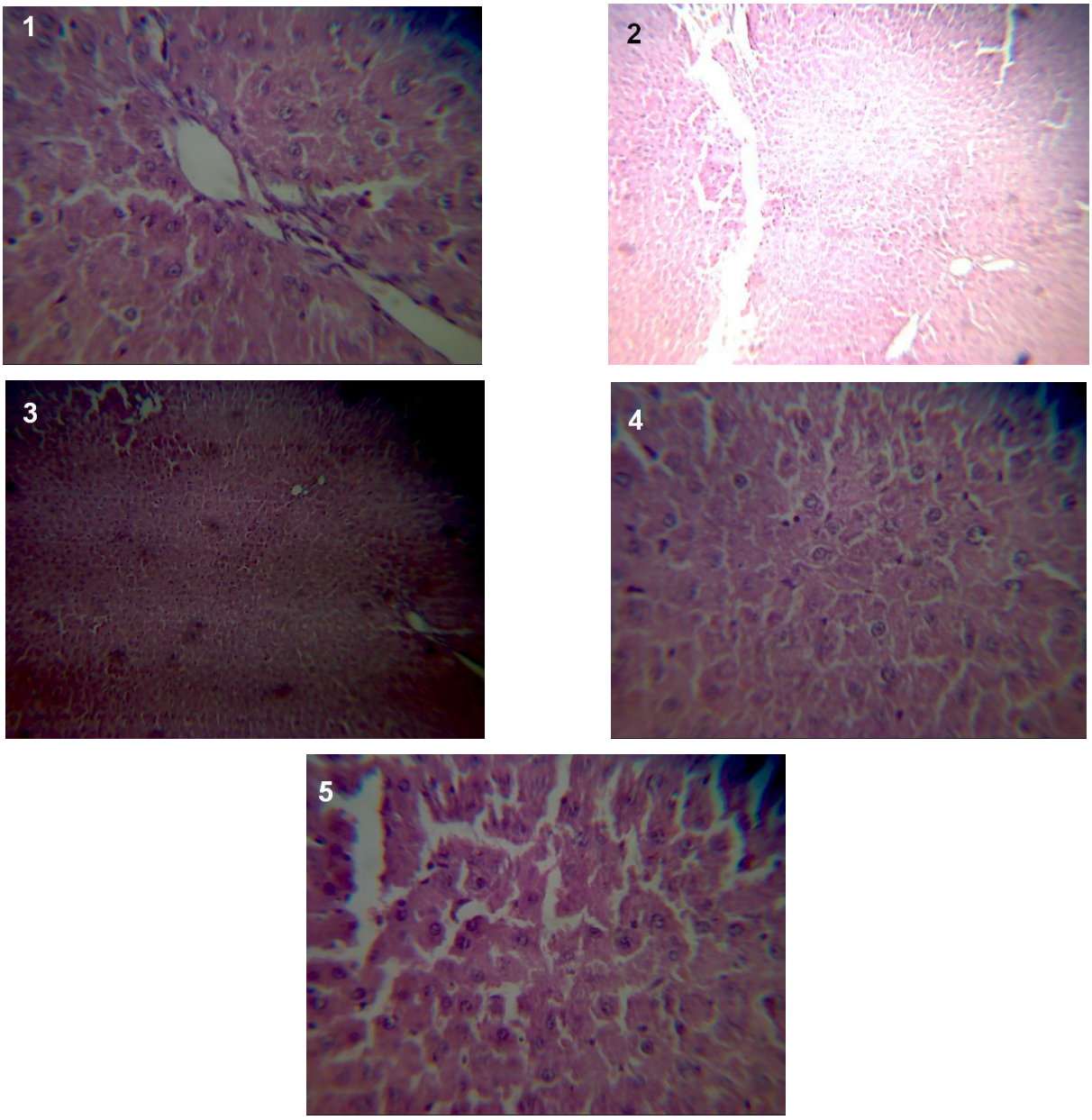

Figure 1. Effect of *cassia tora* seeds on histopathological changes in rat liver after 14 days of paracetamol treatment.

[1] Liver from rat treated with saline shows normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein
[2] Liver from rat treated with paracetamol exhibited severe hepatocyte degeneration and necrosis
[3] Liver treated with silymarin (100 mg/kg, *p.o.*) plus paracetamol shows normal architecture with mild hepatocyte degeneration
[4 & 5] Liver treated with methanolic and aqueous extract of cassia tora seed (200 mg/kg, p.o.) plus paracetamol shows mild hepatocyte degeneration.

prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments[20].

Paracetamol is a widely used antipyretic and analgesic, produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450, to a highly reactive metabolite N-acetyl-P+benzoquinoneimine (NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or –SH group of protein and alters the homeostasis of calcium after depleting GSH[21]. The compound, Sodium nitroprusside is known to decompose in aqueous solution pH (7.2) producing NO·. Under aerobic conditions, NO· reacts with oxygen to produce stable products (nitrate and nitrite) ions. This leads to reduction of nitrite concentration in the assay media[22]. Hepatocellular necrosis leads to very high level of aspartate transaminase and alanine transaminase released from liver to blood. Between the two, alanine transaminase is a better index of liver injury as its activity represents 90% of total enzyme present in the body. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damage caused by paracetamol. ALP activity on the other hand is related to the functioning of hepatocytes and increase in its activity is due to its increased synthesis in presence of increased biliary pressure[23,24]. The data in Table-1 reveal the decreased level of serum transaminase in animals treated with *Cassia tora* extracts indicating the stabilization of plasma membrane and hepatoprotection against the effect of paracetamol and decreased ALP concentration evidences the normal functioning of hepatic cells.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by paracetamol. Treatment with methanolic and aqueous extract of seeds of *cassia tora* produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency.

CONCLUSION:

In the present investigation preliminary phytochemical analysis of *Cassia tora* seeds extracts revealed the presence of carbohydrates, glycosides, flavonoids, saponins, fats and gums. Flavonoids, saponins and glycosides are well known for their anti-oxidant and hepatoprotective activities. In this study aqueous extract showed protective effect against toxicity induced by paracetamol, which may be attributed to the individual or combined effect of phytoconstituents present in it. Based on the above results of the pharmacological screening, it can be concluded

that the aqueous seed extract of *Cassia tora* possesses more significant hepatoprotective effect. The result confirms the folklore claim for the seed extract of *Cassia tora* in treating jaundice.

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