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Research Article

HEPATOPROTECTIVE EFFECTS OF *CASSIA TORA* WHOLE PLANT

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Abstract: Oral administration of a hydroalcholic extract of the *cassia tora* whole plant alleviated liver injury induced by silymarin. In the present study the effect of hydroalcholic extract of the *cassia tora* whole plant on blood and liver glutathione, serum marker enzymes, serum , against paracetamol induced damage in rats have been studied to find out the possible mechanism of hepatoprotection. Since results of biochemical studies of blood samples of paracetamol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by paracetamol and blood samples from the animals treated with the hydroalcholic extracts of *cassia tora* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extracts of *cassia tora* of whole plants could afford significant dose-dependent protection against paracetamol induced hepatocellular injury.

Key words: *cassia tora*, glutathione, hepatoprotection, paracetamol, serum marker enzymes.

Introduction:

Cassia tora Linn. (Cassia obtusifolia L.), is a small annual herbs or undershrub growing as common weed in Asian countries. It is found as a weed throught India, in wild state in Himachal Pradesh, Bihar, Orissa. Constitutes an Ayurvedic preparation “Dadhughnavati” which is one of the successful antifungal formulations *(acharya et al1975 and hatano et al 1999*). It is a well known Ayurvedic medicinal plant as a laxative, antiperiodic and is useful for

leprosy,ringworm, bronchitis and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic, haemorroids. It was reported that seeds of CT has antioxidant activity and contain many active substances including chrysophenol, emodin, rhein etc. *(huang et al 1993).* Many medicinal properties such as antimicrobial, antihepatotoxic and antimutagenic activities have been attributed to this plant *(wong et al 1989 and yen et al 1999).* Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses *(boyd et al 1966).* Paracetamol toxicity is due to the formation of toxic metabolites when a part of it's metabolized by cytochrome P-450. Introduction of cytochrome *(dahlin et al 1984)* or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity *(moron et al 1979, gupta et al 2006).* Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50, 000 new cases each year. In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity *(handa et al 1986).* Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders *(Sharma et al 2002).* The present attempt is two review and compiles updated information on various aspects of C.tora plant used in Indian system of medicine for variety of perpuses. It highlights the several pharmacological and experimental between the active constituents and uses in different fields.

Materials and Method

Plant collection: Healthy disease free, mature fresh whole plant sample were collected locally from Bilaspur, Chhattisgarh, India. Fresh whole plant was washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried without any contamination. The dried whole plant was then powdered using an grinder.

Preparation of Extracts

The whole plant *C.tora* was collected locally from Bilaspur, Chhattisgarh, India. The fresh whole plant were detached from the stems and dried at room temperature (27°C) for a week. They were then weighed several times until the weight was constant. The dried whole plant then ground into a fine powder with the help of grinder and the powder kept in an airtight amber container, for extraction procedure. The dried powder (25 g) of *C.tora* whole plant was extracted with a mixture of water and methanol in the ratio of 50:50 respectively. Extraction was continued at the temperature of 27°C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40°C. Concentrated extract was dried at 40°C in hot air oven. Dried extract was packed in an air tight container.

Animals:

Adult Albino rats (120–200 g) were used for hepatoprotective studies using water and methanol of the whole plant of *C.tora* administered orally. A set consisted of eight rats and each rodent was placed in a metabolic cage capable of collecting uncontaminated urine void of fecal matter. They were housed in polypropylene cages under standard conditions (23 ± 2 .C, humidity 60–70%, 12 h light/dark cycles). They were given standard pellet diet. For experimental, animals were kept fasting overnight but were allowed free access to water.

Paracetamol toxicity:

The hydroalcholic extract of *C.tora*, paracetamol, saline were given with the help of oral fidder. Three groups (Group I, Group II and III) of rats, six rats in each group were taken. The *C.tora* extract at a fixed dose (600 mg kg_ 1, P.O.) that was daily fed for seven days to one group (Group III) of rats and paracetamol (300 mg kg_ 1 P.O.) was administered on 5th day after 5th administration of the extract. The paracetamol treated group (Group II) received normal saline in place of *C.tora* extract. After 48h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood glutathione, reduced liver glutathione, serum marker enzymes and serum bilirubin using standard methods.

Assay of liver glutathione and blood glutathione: Blood was collected, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies. Freshly collected livers were washed with 0.9% NaCl, weighed

and homogenates were made in a ratio of 1g of wet tissue to 9ml of 1.25% KCl by using motor driven Teflon-pestle. Reduced glutathione (GSH) was estimated using DTNB *(sedlak et al 1968)*. The blood glutathione was estimated by the method of Beutler *(beutler et al 1963)*. The absorbance was read at 412 nm.

Serum marker enzymes:

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) *(retiman et al 1957, kind et al 1954)*, total serum protein (TSP) *(Bradford et al 1976)*, bilirubin *(jendrassik et al 1938).* were measured. Glycogen (GLY) *(hassid et al 1957)* and protein *(Bradford et al 1976)* were estimated in liver homogenate.

Statistical analysis:

The mean±S.E.M. statistical significance of difference of control and test data was determined by ANOVA. Simple oneway analyses of variance were used for different doses within a group. A probability value of 0.05 or less was taken to indicate statistical significance.

Results and Discussion:

Table 1: Effect of *C.tora* on blood and liver glutathione (GSH), serum marker enzymes (ALT, AST, ALP) in Paracetamol intoxicated albino rats.

Parameters	Group1	Group2	Group3
Blood GSH (mg%)	2.2±0.14	0.65±0.07	2.4±0.01
Liver GSH (_moles g_1 liver)	13.3±1.24	8.6±0.77	11.4±0.24
ALT (U mg_1 protein)	63.1±1.47	124±0.56	49.5±0.01
AST (U mg_1 protein)	71.6±1.47	187.05±0.58	54.7±0.15

ALP (KA unit	61.1±0.87	153.4±0.36	57.3±0.07
Bilirubin (mg%) (Total)	59.21±0.16	107.7±0.48	63.5±0.03
Body weight			
Before treatment (g)	133.3±0.12	134.3±0.25	131.2±0.07
After treatment (g)	140.7±0.61	141.4±0.12	143.4±0.23
Liver weight (g)	8.3±0.21	8.2±0.22	7.4±0.25

Results are mean of six observations ± S.E.M.

The glutathione levels in liver homogenate and in blood and serum marker enzymes are given in Table 1. The concentration of hydroalcholic extract of *C.tora* in animals treated with paracetamol was significantly ($p<0.001$) reduced in homogenate of liver and so was the level of glutathione in blood level ($p<0.001$) as compared with saline control animals. While glutathione in blood ($p<0.001$) when compared to its paracetamol treated control group. The abnormal high level ($p<0.001$) of serum ALT, AST, ALP and bilirubin observed (Table 1) in paracetamol induced liver toxicity. Treatment with hydroalcholic extract of *C.tora* reduced the enhanced level of serum ALT, AST, ALP and bilirubin. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses *(Mitchell et al 1973, Eriksson et al 1992).* Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations *(ahmed et al 2001, visen et al 1993).* Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. From our results, it can be speculated that (i) decreasing effect GSH, blood glutathione level in rat treated with paracetamol were due to hepatocellular damage and (ii) hydroalcholic extract of *C.tora* afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced the following hydroalcholic extract of *C.tora* by if self-act as a

free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1, 1- di phenyl-2-picrylhydrazyl radical (DPPH). Thus, by trapping oxygen related free radicals hydroalcholic extract of *C.tora* could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipids peroxidative processes leading to MDA formation *(gupta et al 2006a, gupta et al 2006b)* hydroalcholic extract of *C.tora* pretreatment exhibited a normal effect on the glutathione of the blood and liner cells. The extract significantly increased the hepatic and blood glutathione. Then results suggest that a significantly higher content glutathione in blood and liver would offer the tissue a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol infused hepatotoxicity. (c) The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with hydroalcholic extract of *C.tora* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells.

From our results, it can be concluded that decreased the levels of GSH, blood glutathione, serum marker enzymes level in albino rates treated- paracetamol was due to hepatocellular damage. The scientific research on Cassia tora suggests a huge biological potential of this plant. It is strongly believed that detailed information as presented in this review on the phytochemical and various biological properties of the extracts might provide detailed evidence for the use of this plant in different medicines. The phytochemical variations and efficacy of the medicinal values of C. tora is dependent on geographical locations and seasons. There is a demand to standardize the toxic properties of Cassia tora and their detailed clinical trials. After proper processing, identification and removal of the harmful properties of leaves, they may be utilized to prepare a good, Ayurvedic Formulations and Preparations. At the same time, the organic and aqueous extract of Cassia tora could be further exploited in the future as a source of useful phytochemical compounds for the pharmaceutical industry.

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