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Hepatoprotective activity of *cassia tora* on carbon tetrachloride induced hepatotoxicity

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ABSTRACT

In the present study to investigate the Hepatoprotective activity of *Cassia tora* on carbontetrachloride induced hepatotoxicity in rats. Carbon tetrachloride (CCl_4) treated rats showed a significant elevation in the serum activities of ALT, AST and acid phosphatasewhile significantly decreasing the levels of total protein and albumin as compared to the normal control rats, thereby indicating liver damage. Administration of *Cassia tora* leaf at doses of 500mg/kg, significantly prevented the rise in the levels of the marker enzymes as well as it significantly prevented the decrease in the serum levels of total protein and albumin. The diminished rise of serum enzymes, together with the diminished fall in the levels of total protein and albumin in the extract treated groups, is a clear manifestation of the hepatoprotective effect of the extract.

Keywords: Liver, Carbon tetrachloride, *Cassis tora*, enzymes, oxidative stress

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INTRODUCTION

Liver, the largest gland is a vital organ It is the metabolic "engine-room of the body". Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver, and as such it is often exposed to maladies resulting in a number of clinical syndromes. Many chemicals, foods, drugs and infections (parasitic, bacterial, viral or fungal) can cause variety of liver diseases such as hepatitis, jaundice, cirrhosis, liver cancer, etc. Because of variations in liver dysfunctions and difficulties encountered in reaching to a proper diagnosis, a physician is rarely able to provide specific treatment. Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Further, detoxification of a variety of drugs and xenobiotics occurs in the liver itself. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious disorders (Pandey Govind, 1980, 1990)..

Drug induced liver injury is a major health problem that challence not only health care professional but also the paramachetical industry and drug regulatory agencie according to the united states acute liver failure study group (Ostapowicz *et al.*, 2002) account for more than 50% acute liver failure including hepato toxicity casused by over dose of ocitaminophon (apap 39%) and idio syncratic liver injury triggered by other drugs (13%).

Carbon etrachloride (CCl_4), a hepatotoxin, has been used extensively for decades to induce liver injury in various experimental models to elucidate the mechanisms behind hepatotoxicity (Hardin, 1954). Experimentally induced cirrhotic response in the rat by CCl_4 is shown to be superficially similar to human cirrhosis of the liver (Tamayo, 1983). CCl_4 called also perchloromethane or tetrachloromethane, is a colourless, non-inflammable volatile liquid with a distinct odour and immiscible with water, is produced by chlorination of methane, ethane, propane or propene. The molecular weight of this compound is 153.82 Da. Although this compound has been prohibited in the US since 1970 to use as a dry-cleaning agent owing to its profound toxicity, it is still produced in large quantities for various purposes globally because an interdict has not yet been imposed for overall use. However, a significant reduction in the manufacture of this compound has been observed in the last several decades. At its infancy this solvent is used in pharmaceutical preparations, such as anaesthetics. It has been known for a long time that inhalation of the vapour of this compound can depress the central nervous system activity and cause degeneration of liver and kidneys through exerting a destructive and poisonous effect to the cells and organs as many other well known toxins do.

Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary healthcare needs. Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honored tradition that is respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha. The earliest mention of the use of plants in medicine is found in the Rigveda which was written between 4500 and 1600 BC. During British period due to Western culture our Traditional art of natural healing is disappeared slowly. Now it is reappearing due to realization of its importance in curing diseases without any side effect (Dahanukar *et al*., 2000). In the present study to investigate the Hepatoprotective activity of *Cassia tora* on carbontetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals:

Carbon tetrachloride Sodium hydroxide and Trichloro Acetic acid (TCAs) and Diethyl ether were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Plant material and preparation of extract

The leaves of *Cassia tora* were collected from Tamil University, February 2015 at Thanjavur. The collected whole plant of *Cassia tora* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Cassia tora* was macerated with 50% methanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

Experimental design

Body weights of the animals were recorded and they were divided into **3** groups of 6 animals each as follows. **Group 1:** Normal control rats fed with standard diet and served as a control.**Group 2:** Rats were induced with hepatocellular damage by receiving suspension of Carbon tetrachloride (CCl_4) in olive oil (1:2,v/v, 1ml of CCl_4 i.p./kg body weight) was given every 72 hrs for 7 consecutive days. **Group 3:** Rats were treated with *Cassia tora* orally (through intragastric tube) at the dose of 500 mg/kg body weight for every day in addition to CCl_4 was given every 72 hrs for 7 consecutive days.

Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

Phytochemical analysis

Phytochemical analysis for major phyto constituents of the plant extract was undertaken using standards methods as described. The plant extract were screened for the presence of biologically active compounds like . Saponin, Flavonoids, Steroids,Alkaloids and Polyphenol etc.

Biochemical estimation

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al.* (1979). The serum GOT was estimated by the method of

Reitman and Frankel (1957). The serum GPT was estimated by the method of Reitman and Frankel *(*1957). Acid phosphatase activity was measured by the method of Annon (1963). Protein was estimated by the method of Lowry *et al*. (1951). Albumin was estimated by the method of Rodkey (1965).

Statistical Analysis:

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by student "t" test and p< 0.001 were considered to be significant.

RESULTS

The present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Cassia tora* investigated a summarized in Table 1. The present study was carried out to evaluate the Hepatoprotective activity of *Cassia tora* in rats. The observations made on different groups of experimental animals were compared as follows.

Table 1 Phytochemical screening of *Casssia tora*

Test	Result
Saponin	+
Flavonoids	+
Steroids	++
Alkaloids	+
Polyphenol	++

(+)Presence (-) Absence

Hepatoprotective activity

The present study was carried out to evaluate the Hepatoprotective activity of *Cassia tora* leaf. The observations made on different groups of experimental and control animals were compared as follows.

Table I represents the levels of MDA and GSH in serum of normal and experimental rats. Group II CCl_4 intoxicated rats showed a significant increased in the level of MDA when compared to Group I rats. Group III CCl_4 intoxicated rats treated with *Cassia tora* leaf significantly decreased in the level of MDA when compared to group II.

Group II CCl_4 intoxicated rats showed a significant decreased in the level of GSH when compared to Group I rats. Group III CCl_4 intoxicated rats treated with *Cassia tora* leaf significantly increased in the level of GSH as compared to group II.

Table I Effect of *Cassia tora* leaf on MDA and GSH in experimental rats

Parameters	Group I	Group II	Group III
MDA (mg/dl)	7.045 ± 0.95	12.27 ± 1.901*	6.83 ± 1.08**
GSH (mg/dl)	1.77 ± 0.49	0.72 ± 0.38*	1.829 ±0.44**

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

Table II represents the levels of protein in serum of normal and experimental rats. Group II CCl_4 intoxicated rats showed a significant decreased in the level of protein when compared to Group I rats. Group III CCl_4 intoxicated rats treated with *Cassia tora* leaf significantly increased in the level of protein when compared to group II.

Table II Effect of *Cassia tora* leaf on protein in experimental rats

Parameters	Group I	Group II	Group III
Protein (gm/dl)	7.43 ± 1.33	5.53 ± 0.96*	6.98 ± 0.90**
Albumin (gm/dl)	3.96 ± 0.64	2.39 ± 0.58*	3.42 ± 0.51**

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

Table III represents the activity of SGOT and SGPT in serum of normal and experimental rats. Group II CCl_4 intoxicated rats showed a significant increased in the activity of SGOT when compared to Group I rats. Group III CCl_4 intoxicated rats treated *Cassia tora* leaf significantly decreased in the activity of SGOT when compared to group II.

Group II CCl_4 intoxicated rats showed a significant increased in the activity of SGPT when compared to Group I rats. Group III CCl_4 intoxicated rats treated with *Cassia tora* leaf significantly decreased in the activity of SGPT as compared to group II.

Group II CCl_4 intoxicated rats showed a significant increased in the activity of acid phosphatase when compared to Group I rats. Group III CCl_4 intoxicated rats treated with *Cassia tora* leaf significantly decreased in the activity of acid phosphatase as compared to group II.

Table III Effect of *Cassia tora* leaf on SGOT, SGPT and acid phosphataseactivities in experimental rats

Parameters	Group I	Group II	Group III
SGOT (IU/dl)	36.01 ± 4.20	53.40 ±6.66*	38.05 ±5.56**
SGPT (IU/dl)	31.88 ± 5.36	61.29 ± 2.43*	32.40 ± 3.87**
Acid phosphatase (IU/dl)	4.07 ± 0.73	6.91 ± 0.68*	5.58 ±0.99**

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I (p<0.001)
** Significantly different from Group II (p<0.001)

DISCUSSION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complimentary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Wolf, 1999). To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *Cassia tora* leaf. Hence, to justify the herbal claims we have evaluated the hepatoprotective effects of *Cassia tora* leaf CCl_4 induced hepatotoxicity in rats. The hepatoprotective activity of the plant reported in this study would provide scientific evidence of its claimed medicinal properties.

It has been known for a long time that a part of the liver injury caused by this solvent may have originated through the free radical reactions to the metabolism of CCl_4 in the liver and subsequent initiation of lipid peroxidation (Comporti, 1985). This ultimately causes the body to experience oxidative stress and seems to play a major role in the pathogenesis of both acute and chronic liver damage. During the last decades, application of this compound has been further shown to be an excellent tool for the study of experimental oxidative injury due to its rapid metabolism in the liver to a free radical and following deleterious effects in the liver. Recently it has been shown that administration of CCl_4 to rats leads to distinctive formation of both non-enzymatically and enzymatically derived eicosa noids (Morrow *et al*., 1990, 1992b).Since several excellent reviews have dealt with CCl4 induced liver toxicity and lipid peroxidation (Recknagel, 1967; Poli *et al*., 1987).

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloro methylperoxyl free radical leads to initiate the process of lipid peroxidation., the destruction of Ca^{2+} homeostasis, and finally, results in cell death (Clawson, 1989; Recknagel et al., 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel and Glende, 1973; Reckengel et al., 1991; Wolf et al., 1980). MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Ray and Husain, 2002). Hepatotoxic compounds like CCl_4 are known to cause marked elevation in serum enzyme activities. In the present study, treatment with *Cassia tora* leaf attenuated the increased content of MDA in serum and liver (Table- 1).

Glutathione is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydroperoxides. Glutathione status is a highly sensitive indicator of cell functionality and viability. Glutathione is responsible for the regulation of lipidperoxidation (Pastore *et al*., 2003). The toxic activation of CCl_4 via the CYP2E1 (Cytochrome *P450* 2E1) pathway, the detoxification pathway involves GSH conjugation of the trichloromethyl radical, a CYP2E1-mediated CCl_4 metabolite. Previous studies on the mechanism of CCl_4-induced hepatotoxicity have shown that GSH plays a key role in detoxifying the reactive toxic metabolites of $CCl_4$and that liver necrosis begins when the GSH stores are markedly depleted (Recknagel et al., 1991; Williams and Burk, 1990). GSH is largely mediated through the activity of GST, and forms adducts with the toxic metabolites of CCl_4. Moreover, GSH contribute to the detoxification of CCl_4, and it has been suggested that one of the principal causes of CCl_4-induced liver injury lipid peroxidation caused by its free radical derivatives (Recknagel et al., 1991). Our results show that a treatment with *Cassia tora* leaf significantly inhibited lipid peroxidation (Table 1) and significantly reduces CCl_4-induced hepatic and serum GSH depletion. This was attributed to the decreased bioactivation of CCl_4caused by the *Cassia tora* leaf treatment.

In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) is largely used. Serum activities of AST, ALT and Acid phosphatase (ACP) are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in serum. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and

loss of functional integrity of the cell membrane in the liver (Wolf, 1999). The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in the bone, intestine and the liver (Thapa and Walia, 2007). Serum total protein and albumin levels, on other hand, are related to the function of hepatic cells i. e they reveal the functional status of the hepatic cells. Decreased levels of total protein and albumin are indicative of the failure of the biosynthetic function of the hepatocyte. (Crawford, 2004).

In the present study, the CCl_4 treated rats showed a significant elevation (Table- 3) in the serum activities of ALT, AST and acid phosphatasewhile significantly decreasing the levels of total protein and albumin as compared to the normal control rats, thereby indicating liver damage. The CCl_4-induced liver damage was confirmed by the histopathological examination of CCl_4-treated rat liver, which revealed steatosis, centrilobular necrosis, vacuolisation and fibrosis. Administration of *Cassia tora* leaf at doses of 500mg/kg, significantly prevented the rise in the levels of the marker enzymes as well as it significantly prevented the decrease in the serum levels of total protein and albumin. The diminished rise of serum enzymes, together with the diminished fall in the levels of total protein and albumin in the extract treated groups, is a clear manifestation of the hepatoprotective effect of the extract.

All the variables tested as LPO, GSH, vitamin C & E, Albumin, Protein, Bilirubin, ALP, SGOT and SGPT recorded a significant alteration observed in CCl_4 treated rats. However treatment with herbal extract restored the level to near normal was observed. The potential hepatoprotective activity of *Cassia tora* leaf is due to the presence of phytochemical constitution present in plant. Some of these phytochemicals have possessed hepatoprotective activity.

REFERENCES

Annon K. (1963) The colorimetric determination of phosphatase- Sigma Technical Bulletin, Sigma chemicals co., St Louis, USA. pp104-108.

Beuge J.A and Aust S.D (1978). The thiobarbituric acid assay. Methods in Enzymology 52: pp 306-307.

Clawson, G.A., 1989. Mechanism of carbon tetrachloride hepatotoxicity. Pathology and immunology research 8, 104–112.

Comporti Nethercott S. (1985) Job-loss and family morbidity: a study of a factory closure. *J R Coll Gen Pract* 35:510-514.

Crawford JM. Liver and biliary tract. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease. 7th ed. Pennysylvania: Saunders, an imprint of Elsevier; c2004. p. 877–937.

Dahanukar SA., Kulkarni AR and Rege NN. (2000) Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology: 32: S81-S118.

Hardin, B.L., 1954. Carbon tetrachloride poisoning a review. Ind. Med. Surg. 23, 93_ 105.

Lowry OH., Rosenbrough NJ., Farr AL and Randall RJ. (1951) Protein measurement with the Folin's reagent. Journal of Biological Chemistry 193: 265-276.

Moron MS., DsePierre JW and Manerwik KB. (1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochimica et Biophysica Acta 582: pp67-68.

Morrow, J.D., Jollow, D.J. (1990).Lipids versus proteins as major targets of prooxidant, directacting hemolytic agents. *Toxicol. Sci.* **88**:274–283.

Ostapowicz (2002) Ethnomedical knowledge of plants used by Kunabi tribe of Karnataka in India. *Fitoterapia.* **73(4):** 281-287.

Pandey Govind P. Hepatogenic effect of some indigenous drugs on experimental liver damage. PhD thesis. Jabalpur, MP, India: JNKVV; 1990.

Pandey Govind P. Pharmacological studies of Livol with special reference to liver function. MVSc & AH thesis. Jabalpur, MP, India: JNKVV; 1980.

Pastore A., Federici G., Bertini E and Piemonte F. (2003) Analysis of glutathione: Implication in redox and detoxification. *Clinica chimica Acta*. 333: 19-39.

Poli G., Albano E and Dianzai MU. (1987) The rol of lipid peroxidation in liver damage. Chemistry and Physics of Lipids. 45: 117-142.

Ray G and Husain SK. (2002) Oxidants, antioxidants and carcinogenesis. Indian Journal of Experimental Biology. 40: 1213-1232.

Reckengel R.O, Glende Jr, E.A, Britton R.S.(1991) Free radical damage and lipid peroxidation. In: meeks, R.G., Harrison, S.D., Bull, R.J. (Eds.), Hepatotoxicology. CRC Press, Florida. **401–436**.

Recknagel R.O, Glende Jr, E.A.(1973) Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. CRC Critical Review Toxicology 2, **263–297**.

Recknagel, R.O, Glende E.A, Dolak J.A, Waller R.L, (1989). Mechanism of carbon tetrachloride toxicity. Pharmacological Therapy 43, **139–154**.

Recknagel, R.O., Glende Jr., E.A., 1967. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. CRC Critical Review Toxicology 2, 263–297.

Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvate transaminases. Am J Clin Pathol. 1957; 28: 56-63.

Rodkey FL. (1965) Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry. 11: 478-479.

Tamayo, R.P., 1983. Is cirrhosis of the liver experimentally produced by CCl4 an adequate model of human cirrhosis. Hepatology 3, 112_ 120.

Thapa BR, Walia A. Liver Function Tests and their Interpretation. Indian Journal of Pediatrics 2007; Jul; 74:663-71.

Williams A.T, Burk R.F. (1990). Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Semin. Liver Dis. 10, 279–284.

Wolf C.R, Harrelson Jr, W.G, Nastainezyk W.M, Philpot R.M, Kalayanaraman B and Mason R.P.(1980). Metabolism of carbon tetrachloride in hepatic micro some and reconstitution monoxygenase .

systems and its relationship to lipid peroxidation. Molecular pharmacology 18, 558–558.

Wolf, P.L., 1999. Biochemical diagnosis of liver diseases. Indian Journal of Clinical Biochemistry 14, 59–90.

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