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Hepatoprotective effects of *Cassia tora* on CCl_4 induced liver damage in albino rats

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Abstract: The objective of the present study is to determine the hepatoprotective effects of *Cassia tora* against carbon tetra chloride induced liver damage in albino rats. The efficacy of the treatment was estimated by the serum level marker enzymes: serum glutamatic oxaloacetic transaminase, serum glumate pyruvate transaminase and lactate dehydrogenase. The treatment also includes the estimation of enzymatic antioxidants: superoxide dismutase, glutathione peroxidase, glutathione-S transferase and catalase; non-enzymatic antioxidants: vitamin C and vitamin E. The results of this study reveal the remarkable increase of marker enzymes in induced rats and decreased level in *cassia torra* treated ones. Furthermore, the level of enzymatic and non-enzymatic antioxidant level were elevated in treated rats compared to induced ones.

Keywords: Antioxidants, *Cassia tora*, CCl_4, liver damage, albino rats.

Introduction

Many of the developing countries practice traditional medicine as its main source of healthcare, which is usually of plant origin (Rehan Ahmad *et al*., 2008; Stephen Bent, 2008). Today, nearly 88% of the global populations switch to plant derived medicines as their first line of defense for maintaining health and combating diseases (Kintzios *et al*., 2006). At present there are about 60 types of medicinal plants that have already been promoted to use in primary healthcare and classified according to their pharmacological actions such as peptic ulcers, anti-flatulence, laxative, anti-diarrhoea and anti-herpetic (Viomolos *et al*., 2003). In future, the discovery of novel therapeutic agents will be only dependent on plant origin (Perumalsamy *et al.,* 1999).

Free radicals are highly reactive compounds with uneven number of electron in their outermost orbit. These can react with cellular compounds like unsaturated fatty acids and can generate new free radicals which results in irreversible biochemical injury like membrane damage, apoptosis and cell necrosis. Antioxidants scavenge free radicals and quench the subsequent reactions, hence protecting the macromolecules and cellular environment from toxicity and degeneration (Hong-Bo Shao *et al.,* 2008). The oxygen consumption inherent in cell growth leads to the generation of series of reactive oxygen species (ROS). These ROS are molecules such as superoxide anion radicals (O_2^+) and hydroxyl radicals (OH). However, non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) are formed *in vivo* also. Both the oxygen species play a positive role in energy production, phagocytes, regulation of cell growth, intercellular signaling and synthesis of biologically important compounds. However, ROS may also be very damaging; they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases (Valentao *et al.,* 2002; Gulcin *et al*., 2003) ROS are continuously produced during normal physiologic events and are removed by antioxidant defense mechanisms (Buyukokuroglu *et al*., 2001; Chang *et al.,* 2001; Gulcin *et al*., 2002a, 2002b).

A number of plant products include polyphenolic substances such as, flavanoid and tannins are antioxidative substances usually have a phenolic moiety in their molecular structure (Shanmugavalli *et al*., 2009). They have been found among flavanoid, tocopherol and catechin. Organic acids, carotenoids, protein hydrolysates and tannins can also act as antioxidants or have synergistic effects (Dugan, 1980; Langseth, 1995). Certain plant species belonging to the genus *Cassia* (Leguminosae) have been used for medicinal purposes in Asian countries (Perry, 1980). *Cassia* is a native plant in southeast Asia, Africa, Northern Australia and Latin America (Parsons & Cuthbertson, 1992). *Cassia alata, C. fistula* and *C. tora* are recommended for primary health care in Thailand to treat ringworm and skin diseases (Farnsworth & Bunyaprapatsara, 1992). Many reports have shown that some of the *Cassia* species have acquired antimicrobial substances and antioxidant activity (Zhenbae *et al.,* 2007). Hence, the current study was directed towards determining the hepatoprotective effect of *Cassia tora* against CCl_4 -induced liver damage in albino rats.

Materials and methods

Extraction

Five hundred grams of dried leaf powder of *Cassia tora* collected in an around Chennai with reference to the voucher specimen in our lab were taken in Hopkins flask and extracted using 1500ml of sterile distilled hot water and 90% methanol. The immersed leaf powder was kept in shaker (60 rpm) for a week and filtered through Whatmann No. 1 filter paper. The extract was then concentrated using simple distillation and lyophilisation method and stored in sterile vials at 4°C (Bhakta *et al.,* 1999).

Induction of liver damage

The albino rats of both sexes, approximately weighing 50gms, were purchased from Kings Institute, Chennai and were divided into eight groups viz., Group-I acts as Control, Group- II is the set of rats in which the liver damage is induced by carbon tetra chloride. Group

Indian Journal of Science and Technology Vol.2 No 3 (Mar. 2009) ISSN: 0974- 6846

III, IV, V, VI, VII and VIII is a mixture of carbon tetra chloride induced and treated ones with the leaf extract;

each groups consists of 6 rats. The control group was treated with saline; induced group was intra-peritonially injected with 30% carbon tetra chloride prepared in liquid paraffin. The six different drug treated groups were intraperitonially injected with 30% carbon tetrachloride and as well as treated with methanolic leaf extract of *cassia tora* orally in the concentration of 100mg to 600mg/Kg body weight respectively. After every 72hrs, the rats were injected with CCl_4 for 21 days and after last injection the animals were kept for fasting and subsequently after 12hrs, the animals were anesthetized and sacrificed. The liver was dissected and the protein content of various subcellular fractions was estimated by the method of Lowry *et al.* (1951). Followed by which the marker enzyme and antioxidant estimations were carried out in blood according to Table1.

Table 1. Methods for marker enzyme estimation

Marker enzyme	Method	year
Serum Lactate dehydrogenase, SGPT, SGOT	King	1965
Ascorbic acid estimation	Roe & Keuther	1953
Vitamin E estimation	Varley *et al.*	1981
Reduced glutathione estimation	Moren *et al.*	1979
Glutathione peroxidase	Rotruck	1973
Glutathione -s - transferase	Habig *et al.*	1974
Superoxide dismutase	Misra & Fridovech	1972
Catalase	Beers & Seizer	1952

Table 2. marker enzyme level in control, induced and drug treated groups

Animal groups	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)
Control	19.10	27.80	52.31
Induced	108.14	275.76	307.90
GI	95.50	249.11	205.27
GII	88.19	205.08	198.44
GIII	95.50	147.15	204.13
GIV	60.67	101.96	212.14
GV	48.31	64.88	73.35
GVI	31.45	48.66	63.11

Results

Hepatoprotective effects of Cassia tora

The hepatoprotective effect of *C. tora* (methanolic extract of dried leave powder) was studied and the data presented as follows: The marker enzymes for liver damage induced by CCl_4 are Serum Glutamatic Oxaloacetic Transaminase, Serum Glumate Pyruvate Transaminase and Lactate Dehydrogenase. These enzymes were observed in control, induced and treated group of rats (Table 2, Fig.1). The level of all three were lower in control group, the induced group showed elevated level in comparison with control and treated groups. The treated group showed gradual decrease of marker enzymes proportionate to drug concentration.

Table 3. Protein and non enzymatic antioxidant level in control, induced and drug treated groups

Animal groups	Protein mg/g	GSH µg/mg	Vitamin C µg/mg	Vitamin E µg/mg
Control	81.397	19.65	251.59	0.0012
Induced	27.64	7.06	61.5	0.0001
GI	41.54	12.93	125.79	0.0001
GII	43.16	13.98	212.45	0.0013
GIII	41.17	13.31	248.79	0.0014
GIV	68.75	15.39	296.31	0.0014
GV	49.26	13.72	497.59	0.0022
GVI	72.57	15.90	626.18	0.0024

Table 4. Antioxidant enzymes level in control, induced and drug treated groups

Animal groups	SOD u/min/mg	GPx µg/min/mg	GST moles/ min/mg	CAT µg/min/mg
Control	0.5215	2053.12	0.445	0.0061
Induced	0.0836	369.29	0.142	0.11
GI	0.0944	1233.34	0.317	0.0049
GII	0.1353	1433.49	0.364	0.0052
GIII	0.2991	1601.21	0.415	0.0050
GIV	0.3704	1417.27	0.437	0.0053
GV	0.4236	1927.72	0.674	0.0057
GVI	0.4703	3213.64	0.812	0.0057

Generally, in CCl_4 -induced conditions the protein level will be decreased compared to control as well as drug treated group. The present reveals the lower level of protein in induced group. The treated groups showed a gradual increase which was close to the control (Table 3, Fig. 2)

The non-enzymatic antioxidant glutathione, Vitamin C and Vitamin E was observed in elevated level in drug treated group when compared with the induced group. The level of the above in treated group 6 was close to the control in comparison with other drug treated groups (Table 3, Fig. 2, 4, 5).

The antioxidant enzymes Super Oxide Dismutase, Glutathione peroxidase, Glutathione-S Transferase and Catalase were studied in the present investigation. The observed data showed elevated level in the drug treated group than the induced group. The SOD, GPx, GST and Catalase level was of higher concentration in drug treated 6th group and was close to the control (Table 4, Fig. 3, 4, 5).

The malondialdehyde production in drug treated groups was decreased in comparison to the induced and controlled group (Table 5, Fig. 6).

Discussion

The hepatoprotective effects of *Cassia tora* leaf extract on carbon tetrachloride- induced liver injury in the present study showed increase in liver marker enzymes SGPT, SGOT and ALP in CCl_4 - induced rats and the production of malanoaldehyde was also found to be elevated. Whereas, the *Cassia tora* leaf extract treated group showed decreased level of the same marker enzymes and malanolaldehyde production. The vitamin E and glutathione were restored in drug treated groups. The present investigation also

Indian Journal of Science and Technology Vol.2 No 3 (Mar. 2009) ISSN: 0974- 6846

Analysis of enzymes, proteins and vitamins in experimental animals

Fig.1. Marker enzyme level

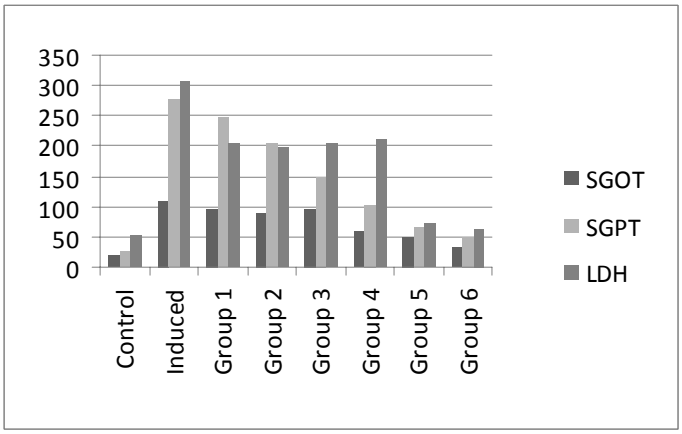


Fig.2. Protein and reduced Glutathione level

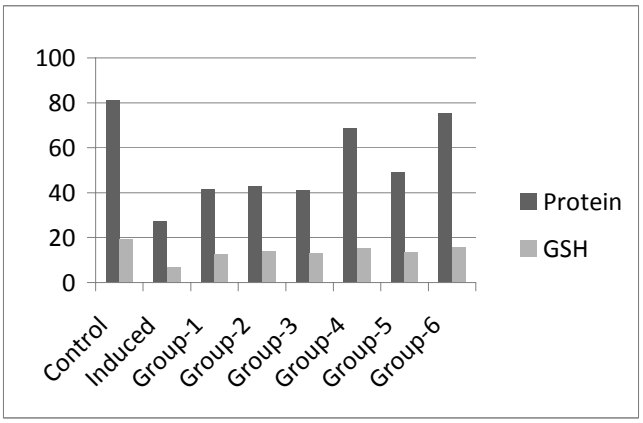


Fig.3 .Superoxide Dismutase and Glutathione- s- transferase level

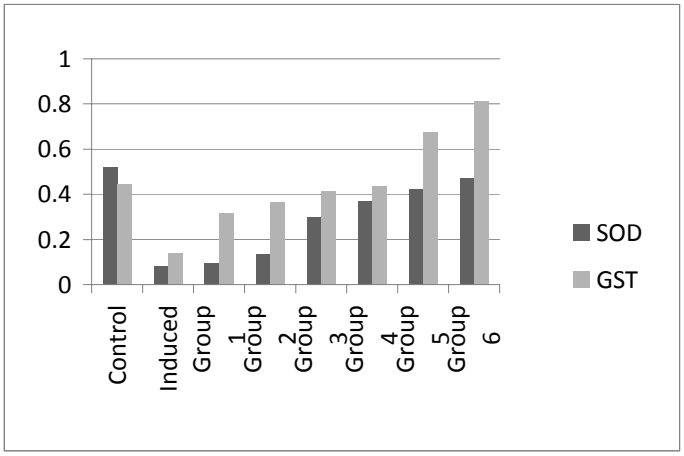


Fig.4. Vitamin-C and Glutathione peroxidase enzyme level

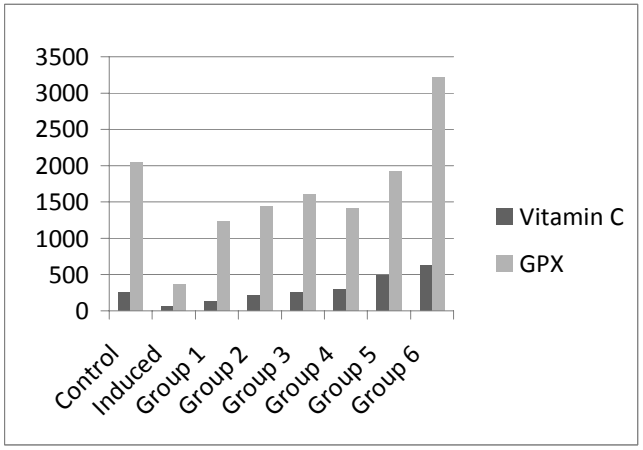


Fig.5. Catalase and Vitamin-E level

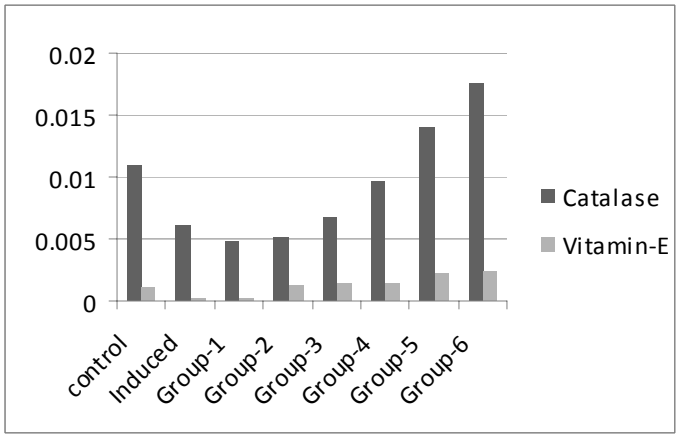
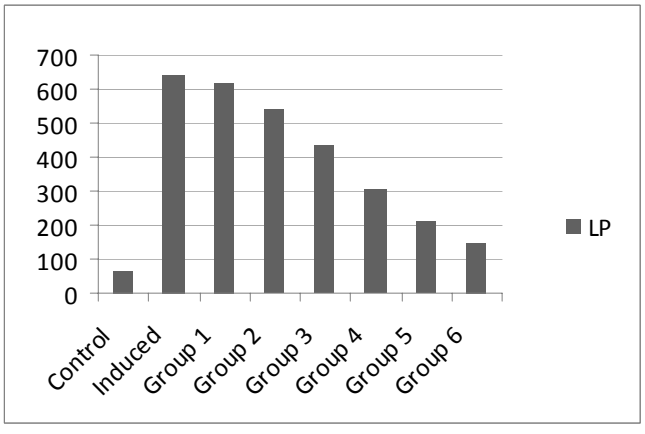


Fig.6 Lipid peroxidation level



falls in line with the previous study (Dahiru *et al*., 2005) showing the level of glutathione, vitamin E and enzymatic oxidants elevated in treated groups when compared with control and induced groups. The serum level of liver marker enzyme SGPT, SGOT and LDH was also found to be decreased in *C. tora* extraction treated groups.

The present study showed elevated level of antioxidants in drug treated group, whereas decreased in induced and controlled group. The protein level was also increased in drug treated group in comparison with induced group of experimental analysis. The lipid peroxidation was highly elevated in induced group than the control and drug treated group. A similar study was carried out by Tripathi *et al.,* (2000) wherein, they used ferrous sulphate as inducers and stress producing agents. Hence the present study demonstrates the protective effects of C*. tora* against hepatotoxicity because of the effective free radical scavenging that

Indian Journal of Science and Technology Vol.2 No 3 (Mar. 2009) ISSN: 0974- 6846

accounts for antioxidant property and shall be validated for safe use in humans by clinical trials.

Table 5. Lipid peroxidation level in control, induced and drug treated groups

Animal groups	Lipid peroxidation moles/mg
Control	63.86
Induced	639
GI	618.91
GII	543.59
GIII	435.53
GIV	307.0
GV	209.57
GVI	145.72

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