



## Hepatoprotective effects of *Cassia tora* on CCl<sub>4</sub> induced liver damage in albino rats

A. Vetrivel Rajan<sup>1</sup>, N. Shanmugavalli<sup>2</sup>, C. Greety Sunitha<sup>3</sup> and V. Umashankar<sup>2</sup>

<sup>1</sup>Biocon India Pvt. Ltd., Bangalore; <sup>2</sup>Dept. of Bioinformatics, SRM University, Chennai, India

<sup>3</sup>PG Dept. of Biochemistry, VHNSN College, Virudhunagar, India

vallibiochem2000@yahoo.com

**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 glutamate 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 tora* 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<sub>4</sub>, 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<sub>2</sub><sup>•-</sup>) and hydroxyl radicals (OH). However, non-free radical species such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and singlet oxygen (O<sub>2</sub>) 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<sub>4</sub> -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

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 intraperitoneally injected with 30% carbon tetra chloride prepared in liquid paraffin. The six different drug treated groups were intraperitoneally 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<sub>4</sub> 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.

**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<sub>4</sub> are Serum Glutamic 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 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 <i>et al.</i>	1981
Reduced glutathione estimation	Moren <i>et al.</i>	1979
Glutathione peroxidase	Rotruck	1973
Glutathione -s - transferase	Habig <i>et al.</i>	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

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<sub>4</sub> -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 6<sup>th</sup> 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<sub>4</sub> - 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 malanalaldehyde

production. The vitamin E and glutathione were restored in drug treated groups. The present investigation also

Analysis of enzymes, proteins and vitamins in experimental animals

Fig.1. Marker enzyme level

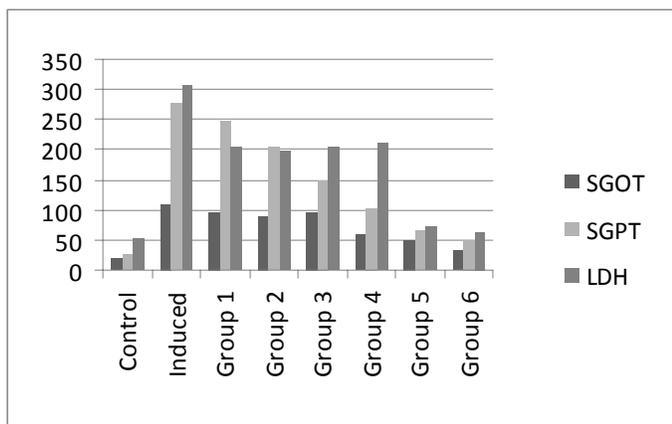


Fig.2. Protein and reduced Glutathione level

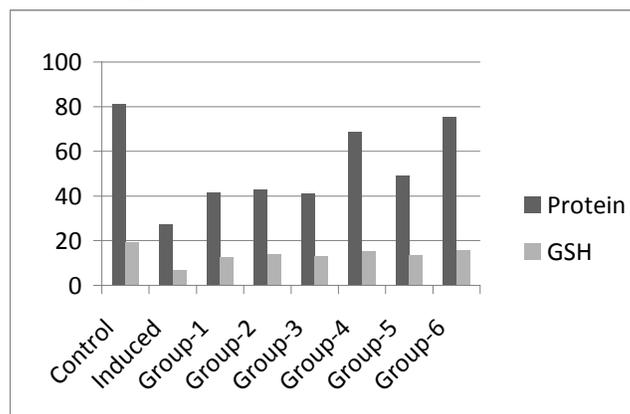


Fig.3 .Superoxide Dismutase and Glutathione- s- transferase level

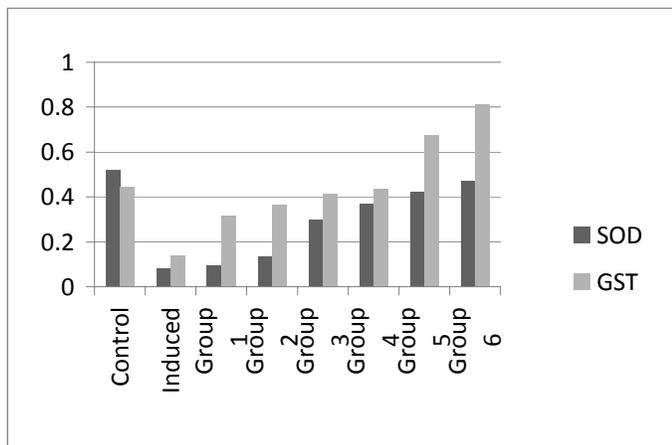


Fig.4. Vitamin-C and Glutathione peroxidase enzyme level

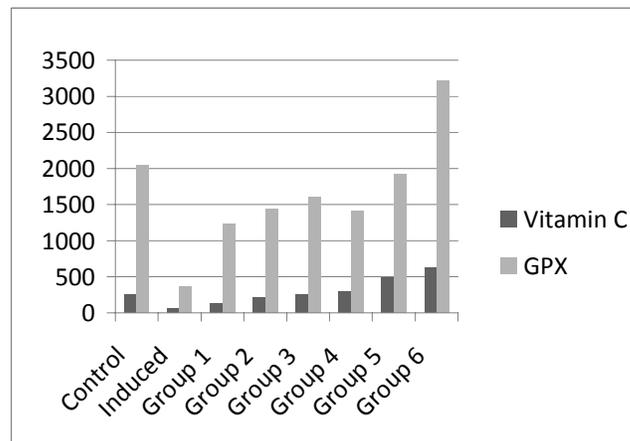


Fig.5. Catalase and Vitamin-E level

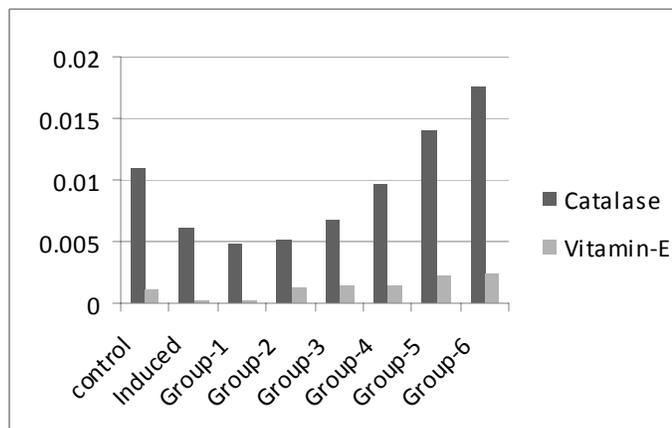
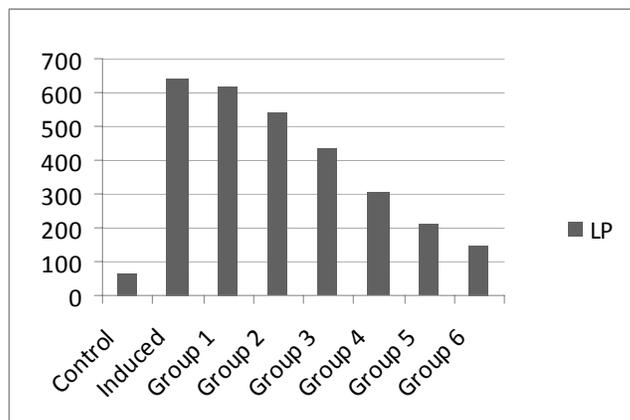


Fig.6 Lipid peroxidation level



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

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

### References

- Baker H, Frank C (1968) Determination of serum tocopherol. In: Clinical Biochemistry, eds. Varley H, Gowenlock AH and Bell M, NY. pp: 222-223.
- Buyukokuroglu ME, Gulcin I, Oktay M and Kufrevioglu OI (2001) *In vitro* antioxidant activity of Dantrolene sodium. *Pharmacol. Res.* 46, 491-494.
- Chang ST, Wang SH, Kang PL, Yang NS and Shyur LF (2001) Antioxidant activity of extracts from *Acacia confuses* bark and heartwood. *J.Agric. Food Chem.* 49, 3420-3424.
- Dahiru D, William ET and Nadro MS (2005) Protective effect of *Zizyphus mauritima* leaf extract on carbon tetrachloride - induced liver injury. *J. Biotechnol.* 4, 1177-1179.
- Dugan LR (1980) Natural antioxidants In: autoxidation in food and biological systems, eds Simic G & Karel M, Cambridge, USA. pp: 263-282.
- Gulcin I, Buyukokuroglu ME, Oktay M and Kufrevioglu OI (2002) On the *in vitro* antioxidant properties of melatonin. *L. Pineal Res.* 33, 167-171.
- Gulcin I, Oktay M, Kufrevioglu OI and Asian A (2002) Determination of antioxidant activity of lichen *Cetraia islandica* (l) Ach. *J. Ethanopharmacol.* 79, 325-329.
- Gulcin I, Buyukokuroglu ME, Oktay M and Kufrevioglu OI (2003) Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *palsiana* (Lamb) Holmboe. *J. Ethanopharmacol.* 86, 51-58.
- Hong - Bo Shao, Li-ye Chu, Zhao-Hua and Cong-Min Kang (2008) Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *Intl.J. Biol. Sci.* 4, 8-14.
- King J (1965) Practical Clinical Enzymology, *D Van Nostrand, London.*
- Kintzios and Spiridon E (2006) Terrestrial plant derived anticancer agents and plant species used in anticancer research. *Critical Rev. Plant Sci.* 25, 79-135.
- Langseth L (1995) Oxidant, antioxidant and disease prevention. JLSI Europe, Brussels, Belgium. pp:4-13.
- Lowry OH, Rosebrough NJ, Farral AL and Randall RJ (1951) Protein measurement with Folin phenol measurement. *J. Biol. Chem.* 193, 265-275.
- Moron MS, JN de Pierre and V. Mannervik (1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. *Biochem. Biophys. Acta.* 582, 67-68.
- Nishikimi M, Roe NA, Yagi K (1972) The occurrence of superoxide anion in the action of reduced phenazine methosuplphate and molecular oxygen. *Biochem. Biophys. Res. Commun.* 46, 849-853.
- Peery LM (1980) Medicinal plants of east and south east Asia. Cambridge MIT Press. pp: 205.
- Pellegrini N, Yang R and Rice-Evans C (1991) Screening of dietary carotinoids and carotenoid - rich fruit extracts for antioxidant activities applying 2,2'-azino-bis (3-ethylenbenzothiazoline-6-sulphonic acid) radical cation decolorization assay method. *Enzyme Molecules.* 299, 379-389.
- Perumal Sammy R, Ignacimuthu S and Patric Raja D(1999) Preliminary screening of ethnomedicinal plants from India. *J. Ethno Pharmacol.* 66, 235-240.
- Ratruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science.* 179, 588-590.
- Rehan Ahmad, Swayam Prakash Srivastava, Rakesh Maurya, Rajendran SM, Arya KR and Arvind K. Srivastava (2008) Mild antihyperglycaemic activity in *Eclipta alba*, *Berberis aristata*, *Betula utilis*, *Cedrus deodara*, *Myristica fragrans* and *Terminalia chebula*. *Indian J. Sci.Technol.* 1 (5), 1-6. Domain site: <http://www.indjst.org>
- Roe JH and CA Keuther (1953) The determination of ascorbic acid in whole blood and urine through 2,4-dinitrophenyl hydrazine derivative dehydro ascorbic acid. *J. Biol. Chem.* 147, 399-407.
- Shanmugavalli N, Umashankar V and Raheem (2009) Antimicrobial activity of *Vanilla planifolia*. *Indian J. Sci. Technol.* 2 (3), 37-40. Domain site: <http://www.indjst.org>.
- Sinha AK (1972) Calorimetric assay of catalase. *Anal. Biochem.* 47, 389-394.
- Stephen Bent (2008) Herbal medicine in the United States: Review of efficacy, safety and regulation. *J. General Intl. Med.* 23, 854-859.
- Tripathi YB, Singh AK and Dubey GP (2000) Antioxidant property of the bulb of *Scilla indica*. *Curr. Sci.* 80, 1267 -1269.
- Valanteo MR and Latah MS (2002) Antioxidant activity of Curologo echinoids in carbon tetra chloride induced hepatopathy in rats. *Ind. J. Clin. Biochem.* 17 (2), 80-87.
- Zhenbae JA, Tae Fei, Guo Ling, Tao Guanjum and Ding Xiaolin (2007) Antioxidant properties of extracts from *Cassia tora* L evaluated *in vitro*. *Food Sci. Technol.* 40, 1072-1077.

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