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Hepatoprotective Effect of *Cassia auriculata* L. Leaf Extract on Carbon Tetrachloride Intoxicated Liver Damage in Wister Albino Rats

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ABSTRACT

Cassia auriculata L. belongs to Fabaceae and is widely distributed in India. It is used in traditional medicine, typically for skin disease, as a purgative, laxative, antihelminthic, antidiabetic and antioxidant from the ancient period. There have been reports of antidiabetic and medicinal properties of *C. auriculata* dried leaf and flowers. The present investigation was done to find whether the methanolic extract of *Cassia auriculata* leaf had hepatoprotective effect against carbon tetrachloride induced liver damage in Wister albino rats and to estimate the total antioxidant content, total phenolic content and total flavanoids of *C. auriculata* methanolic leaf extract. The methanol extract was used to treat the carbon tetra chloride induced liver damage on Wister albino rats for 60 days. *In vitro* antioxidant activity was studied using $ABTS^+$ free radical scavenging method. The total content of phenolic compounds and flavanoids was also estimated by spectrophotometric method. The *in vitro* cytotoxicity activity was conducted on $HepG_2$ cell line at increasing concentration and the apoptotic activity was determined. The animals were treated at 600 mg kg^{-1} b.wt. The blood serum was used for liver function test. Serum Lactate Dehydrogenase (LDH), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and γ-Glutamyl Transpeptidase (GGPT), Lipid Peroxidation (LPO) and liver tissue total protein were ($p<0.001$) significantly decreased in post treated animals. The non enzymatic antioxidant Reduced glutathione (GSH), Vitamin C (Vit C), Vitamin E (Vit E) and enzymatic antioxidant Glutathione Peroxidase (GPx), Glutathione-s-Transferase (GST), Superoxide Dismutase (SOD) and Catalase (CAT) were increased significantly in plant extract post treated group. The histopathological studies showed fine revertible changes in CAME treated experimental animal liver tissue section.

Key words: CAME, *Cassia auriculata,* $ABTS^+$-2, 2'-azio-bis (3 ethylbenzenethiazoline 6 sulphonic acids) free radical, cytotoxicity, antioxidant activity, carbon tetra chloride

INTRODUCTION

The use of plants and plant products for medicinal purposes has been well documented over the years. Plant derived medicines have been a part of the evolution of human healthcare for thousands of years. At present there are a large number of medicinal plants that have already been promoted for use in primary health care and classified according to their pharmacological actions

such as peptic ulcers, anti-flatulence, laxative, anti-diarrhoeal and anti-hepetic. The abundance of secondary metabolites and chemicals in plants state that still more therapeutic agents can be discovered from plants (Perumal Samy *et al*., 1999).

The existence of various organic compounds like hormones and antimicrobial principles in many plants as their essences and volatile oils have been well documented and these have been extensively used medicinally in ayurveda and aroma therapy. Plants are known to contain innumerable biologically active compounds (Alade and Irobi, 1993) which possess antibacterial properties (Branter *et al*., 1996).

Medicinal components from plants play an important role in conventional as well as western medicine. One hundred and nineteen secondary metabolites derived from plants are globally used as drugs; 15% of all angiosperms have been investigated chemically and of that, 74% pharmacologically active components have been discovered. These increasing medicinal interests highlight the importance of proper conservation of the biodiversity and cultural diversity of the ecosystem in order to safe guard and perpetuate our interdependence of plants as a source of medicine (Perumal Samy *et al*., 1999).

Cassia auriculata L. belongs to the Fabaceae family and is used in traditional medicine, typically for skin disease, as a purgative, laxative, antihelminthic, antidiabetic and antioxidant from the ancient period. The pharmacological actions of *Cassia auriculata* L. (Ayyanar and Ignacimuthu, 2008) as well as the antidiabetic and hypolipidemic efficacy of various parts (root, stem, leaves and flowers) of Cassia auriculata on alloxaninduced diabetic rats (Uma Devi *et al*., 2006) have been documented.

The present investigation was done to find whether the methanolic extract of *Cassia auriculata* leaf had hepatoprotective effect against carbon tetrachloride induced liver damage in Wister albino rats and to estimate the total antioxidant content, total phenolic content and total flavanoids of *C. auriculata* methanolic leaf extract.

MATERIALS AND METHODS

The leaves of the experimental plant *Cassia auriculata* was collected from Padappai, Chennai, in Jan 2007-Mar-2007, dried at room temperature for 10 days and powdered for further study. The chlorophyll and pigment content of the leaves was removed by extracting with petroleum ether, chloroform and acetone (1:1:1). The crude extract was obtaining by Sohxlet apparatus using 90% methanol. The concentrated extract was stored in sterile vials at 4°C for further studies.

Estimation of phytochemical contents: Estimation of total antioxidant (Re *et al*., 1999), total phenolic content (Price and Butler, 1977) and total flavanoids (Lamaison and Carnat, 1990) were conducted.

Cell lines and culture medium: Human hepatocellular carcinoma cell lines (HepG2) were purchased from National Center for Cell Science (NCCS, Pune). The cells were cultured in 75 cm^2 flask containing HepG2. All experiments were performed using cells from passage 25 or less. The methanolic extract of *C. auriculata* in the concentrations of 200-1.5 μg mL^{-1} were used for the experiment and the stock was maintained at -20°C.

Cytotoxicity study (MTT assay): The survivals of cells were determined by MTT assay as described by Mosmann (1983). The plant extract amended cell cultured plates were incubated in

a CO_2 incubator for 4 h. After 4 h incubation period, the inhibition of cell growth induced by the tested fractions was detected by eluting the dye with DMSO and Optical Density (OD) was measured using a 96 well micro plate reader (BIO-RAD, model 680, USA) at 570 nm.

Analysis of DNA fragmentation: Two million cells were treated with indicated amount of compound for 12-24 h. The cells were then scraped with the medium and centrifuged at 1500 rpm for 8 minute and the DNA was extracted from individual concentration by Yokozawa and Dong (2001) method and studied by gel electrophoresis in 1.5% agarose gel.

Experimental design: Male Wister albino rats (180 to 225 g) were purchased the rats were divided into four different group's consisting of 6 rats *viz.,* I. Control group receiving only sterile physiological saline, II. Group induced with CCl_4, III-treated with CAME alone and IV-induced + drug treated group. Group IV were intoxicated with 30% carbon tetra chloride prepared in liquid paraffin through intraperitonial route and simultaneously treated with CAME crude extract orally (600 mg kg^{-1} b.wt.). The rats were injected with 30% CCl_4 for 60 days at every 72 h interval and after the last injection, the animals were kept fasting for a night. The animals were anesthetized and sacrificed by decapitation method. The blood collected from each experimental animal group was used for obtaining serum to study the marker enzyme. The livers excised from the sacrificed animals were washed with physiological saline, fixed, embedded in wax and sections were made using microtome.

Blood Serum was used for testing the liver functions like Serum Lactate Dehydrogenase, SGPT, SGOT (King, 1965), γ-glutamyl transpeptidase (Indirani and Hill, 1977) as per the standard procedure. Liver tissues were homogenized and used for estimation of total protein (Lowry *et al.*, 1951) Vitamin C (Omaye *et al.*, 1979), Vitamin E (Quaife and Dju, 1949), Reduced glutathione (Moron *et al.*, 1979), Glutathione peroxidase (Rotruck *et al.*, 1973), Glutathione-s-transferase (Habig *et al.*, 1974), Superoxide dismutase (Misra and Fridovich, 1972), Catalase (Sinha, 1972) and lipid peroxidation (Ohkawa *et al.*, 1979) as per the normal protocol. Histopathological studies were done using the liver collected from experimental animal groups. The tissues were fixed in 10% formal saline, embedded in wax and anatomical sections were taken using microtome. The sectioned liver tissues were then stained in haematoxylin-eosin and comparison studies were made by observation of the slides. The results were expressed by mean values±SEM calculated for each parameter using SPSS 10.0 for Windows. For determining the significant inter-group difference each parameter was analyzed separately and one-way Analysis of Variance (ANOVA).

RESULTS

Phytochemical contents: The experimental plant *Cassia auriculata* leaves methanol extract was used for estimating the total antioxidant content, total flavanoid content and total phenolic content (Fig. 1) and the result showed the experimental plant consisting of significant quantity of total antioxidants, total phenol and total flavanoids.

Cytotoxicity activity and DNA fragmentation: Cytotoxic effect of *Cassia auriculata* methanolic leaf extract was done in Human Hepatocellular carcinoma cell lines ($HepG_2$) and the IC_{50} concentration was observed at 24 h in 25 μg mL^{-1} of CAME extract and the DNA fragmentation study was also observed using DNA gel electrophoresis (Fig. 2). The 25 μg mL^{-1} of CAME extract showed good apoptotic activity than the other concentrations in $HepG_2$ cell line (Table 1).

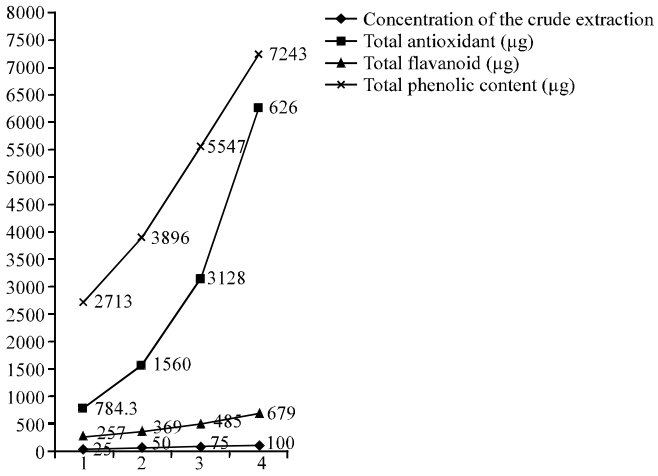

Fig. 1: Total antioxidant, phenolic and flavanoid content of *Cassia auriculata* dried leaves methanol extract (µg g^{-1} of dried leaf powder)

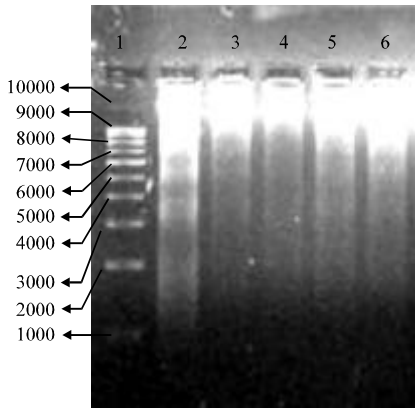

Fig. 2: Effect of CAME on HepG2 cell line (DNA fragmentation). Lane 1: I Kb base pair DNA ladder; Lane 2: DNA from treated cells with 25 µg mL^{-1} at 48 h; Lane 3: DNA from treated cells with 50 µg mL^{-1} at 48 h; Lane 4: DNA from treated cells with 100 µg mL^{-1} at 48 h; Lane 5: DNA from treated cells with 50 µg mL^{-1} at 24 h; Lane 6: DNA from untreated cells

Table 1: Cytotoxic effect of *Cassia auriculata* methanol leaf extract

Cassia auriculata (µg mL^{-1})	12 h	24 h	48 h	72 h
800	56.34±5.98	61.58±6.21	65.67±6.58	70.56±7.58
400	54.13±5.67	59.60±6.01	63.12±6.17	65.78±7.12
200	53.12±5.23	57.94±5.45	60.19±5.67	63.68±6.84
100	50.17±5.01	54.23±5.12	58.65±5.45	60.78±6.59
50	39.58±3.27	53.97±4.58	55.32±4.87	58.98±6.23
25	34.56±3.43	48.01±4.25	51.23±4.64	55.67±5.78
12.5	29.07±3.12	32.11±4.18	47.89±4.23	50.17±5.73

Results are presented as the Mean±SD

Serum biochemical parameters: Marker enzymes Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Lactate dehydragenase and γ-glutamyl transpeptidase

Table 2: Effect of CAME on liver marker enzymes, total liver protein, non enzymatic antioxidants and enzymatic antioxidants in CCl_4-induced toxicity experimental rats

Liver marker enzyme	G1-control	G2-CCl_4 induced	G3-drug alone	G4-post treated
SGOT	83.51±0.06	275.84±0.20*	72.41±0.05**	102.31±0.07***
SGPT	67.35±0.05	375.66±0.28*	58.40±0.04**	87.38±0.06***
GGPT	67.35±0.00054	375.66±0.00062*	58.40±0.00048**	98.89±.00011***
LDH	97.76±0.07	253.34±0.19*	123.82±0.09**	116.12±0.087***
Pro	8.40±0.06	27.64±0.02*	8.71±0.06**	11.54±0.03***
LPO	63.87±0.04	639.10±0.48*	54.37±0.04**	145.74±0.10***
GSH	19.65±0.01	7.06±0.005*	24.60±0.018**	15.90±0.011***
Vit C	251.63±0.18	61.51±0.046*	257.14±0.19**	626.28±0.47***
Vit E	0.00171±0.000220	0.00014±2.96*	0.00266±0.000342**	0.00228±0.000293***
CAT	98.71±0.07	27.10±0.02*	102.31±0.07**	89.71±0.06***
SOD	0.5215±0.000393	0.0834±0.00035*	0.6521±0.000491**	0.4703±0.000354***
GPx	127.32±0.09	46.29±0.03*	157.26±0.11**	112.65±0.08***
GST	0.9781±0.000736	0.1700±0.000128*	1.7803±0.00134**	0.8121±0.000611***

Values are Mean±SD (n = 6). *p<0.001 as compared with normal control group; **p<0.001 as compared with normal control group; ***p<0.001 as compared with CCl_4 induced group. SGOT: Serum glutamate oxaloacetic transaminase (IU L^{-1}); SGPT: Serum glutamate pyruvate transaminase (IU L^{-1}); LDH: Lactate dehydrogenase (units mL^{-1} of lavage fluid); GGPT: γ-Glutamyl transpeptidase (Unit I^{-1}); Pro: Protein (mg g^{-1} of wet tissue); LPO: (μ moles of TBA mg^{-1} protein); GSH: Reduced glutathione (μg mg^{-1} protein); Vit C: Vitamin C (μg mg^{-1} of protein); Vit E: Vitamin E (μg mg^{-1} of protein); SOD: Superoxide dimutase (unites/min/mg protein); GPx: Glutathione peroxidase (moles of GSH oxidized/min/mg protein under incubation condition); GST: Glutathione-s-transferase (η moles of CDNB conjugated/min/mg); CAT: Catalase (μ moles of H_2O_2 consumed/min/protein)

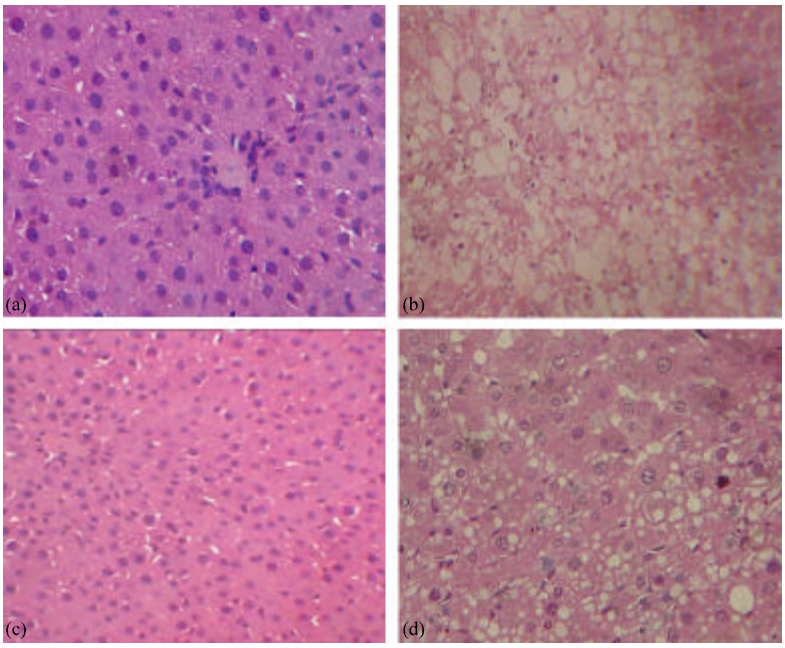

Fig. 3: Histopathological evidence showing the effect of CAME on the treated tissue. (a) control, (b) induced, (c) drug alone and (d) post treated

showed (p<0.001) decreasing level in drug treated group than in the induced rat group and (p<0.001) elevated level in induced group (Table 2). The total protein level and lipid peroxidation

of MDA production were significantly decreased ($p<0.001$) in drug treated group (Table 2) than the induced rat group. This biochemical changes indicate in liver shows recovery of damaged liver by *C. auriculata* dried leaf extract. The non antioxidant vitamin C, vitamin E and reduced glutathione shows in elevated level ($p<0.001$) in post treated rat group while comparing with CCl_4 induced group and nearest to control group (Table 2). The enzymatic antioxidant enzymes (Table 2) superoxide dismutase, glutathione-s-transferase, glutathione peroxidase and catalase level was ($p<0.001$) significantly increased level in post treated rat group than the carbon tetra chloride induced group.

The group I animal is normal control and the liver sections showed normal tissue cell architecture with clear central vein. There are no any symptoms for tissue damage (Fig. 3a). The group II animals liver section showed loss of vascularization with well damaged veins, loss of tissue architecture, ballooning and fatty changes (Fig. 3b). The liver sections of Group III animals treated with plant extract alone showed no abnormal tissue or damage and were comparable to normal control group (Fig. 3c). Group IV animals' liver section shows fewer fatty changed cells and very few number of ballooning cells, restore cells and less number of fragmented tissues (Fig. 3d).

DISCUSSION

Cassia auriculata dried flower and leaf of the plants are being used for medicinal treatment (Sawhney *et al*., 1978; Joshi, 1986) and the flower and seed has been shown to have antidiabetic activity (Jain and Sharma, 1967). Enumerable scientific data are available on *Cassia auriculata* for antiviral activity and anti spasmodic activity (Dhar *et al*., 1968), anti pyretic activity (Vedavathy and Rao, 1991), emollient effect (Nanba *et al*., 1994) and phytochemicals reported on *C. auriculata* include an alkane-Nonacosane-6-one (Lohar *et al*., 1981); Saponins (Gedeon and Kinel, 1956) and tannins (Balasooriya *et al*., 1982). Also, the leaf extract of *Cassia auriculata* was found to lower the serum glucose level in normal rats (Sabu and Subburaju, 2002). The pathological effect of administering carbon tetrachloride and its effect on liver tissues, as also the role of halogenated hydrocarbons on liver damage had already been described (Brattin *et al*., 1985; Recknagel, 1983; Reynolds and Moslen, 1979; Sheweita *et al*., 2001). There are very few reports on *Cassia auriculata* for liver damage or liver injury and this present investigation was carried out to evaluate the potential activity against CCl_4 induced liver damage on Wister albino rats and also the extract was evaluated for its total phytochemical content and cytotoxicity activity. The results clearly revealed the extract has antioxidant activity and also, good yield of phytochemical contents by spectrophotometric method. The *C. auriculata* flowers showed antioxidant activity based on scavenging of ABTS radical cations and DPPH radical (Kumaran and Karunakaran, 2007). The cytotoxic activity of *C. auriculata* leaf extract was proved at 24 h in 25 μg mL^{-1} and showed few DNA fragments on gel electrophoretic studies which indicate apoptotic activity on hepatocarcinoma cell line HepG2 while compared with other concentration of leaf extracts and this is a new report for the present experimental plant.

In the present investigation, the MDA production was significantly lower on CAME extract treated groups, apparently indicating the protective role against CCl_4 damage on cell membranes. But in the case of untreated CCl_4 induced group, liver histopathology shows more vacuolated fatty changes, loss of cell membrane architecture and condensed nuclear material than the control group. The liver marker enzymes increasing activity is indicative of hepatic injury or hepatic damage (Weber *et al*., 2003; Lin *et al*., 1997; Rees and Spector, 1961). Conversely, the reduction in liver marker enzyme activity and subsequent recovery from damage has also been evaluated, especially

the action o f S-allylcysteine and its reaction with GST (Hatono *et al.*, 1996). In the present findings, the marker enzymes significantly ($p<0.001$) reduced in plant extract treated group than the induced group and very clearly showed that this is a preventive action on damaged liver by *C. auriculata* leaf extract. This supports the other observations made on the medicinal properties of the plant. Further, the cytotoxic activity of *C. auriculata* leaf extract was proved at 24 h in 25 μg mL^{-1} and showed few DNA fragments on gel electrophoretic studies which indicate apoptotic activity on hepatocarcinoma cell line HepG2 while compared with other concentration of leaf extracts and this is a new report for the present experimental plant.

In present study, the data's clearly showed the protective role against cell membrane damage and oxidative stress by obtained data lipid peroxidation. The histopathological observation showed considerable reverting changes in liver sections of the CAME extract treated group than in the untreated induced group.

From the present investigation, *C. auriculata* leaf extract showed strong hepatoprotective effect on CCl_4 induced liver damaged rats.

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