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

1Jeeva Jothi Dhanasekaran and 2Mathangi Ganapathy
1Centre for Advanced Studies in Botany, University of Madras, Chennai, India
2Centre for Biotechnology, Anna University, Guindy Campus, Chennai-600 025, India

Corresponding Author: Mathangi Ganapathy, Centre for Biotechnology, Anna University, Guindy Campus, Chennai-600 025, India. Tel: 044-22356772 Fax: 044-22350299

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, antihelmintic, 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 HepG₂ 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 reversible changes in CAME treated experimental animal liver tissue section.

Key words: CAME, *Cassia auriculata*, ABTS⁺-2, 2’-azo-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 alloxan-induced 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 cm² 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 CO₂ 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 CCl₄, 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 intraperitoneal route and simultaneously treated with CAME crude extract orally (600 mg kg⁻¹ b.wt.). The rats were injected with 30% CCl₄ 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 (Cmave 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 flavonoids.

**Cytotoxicity activity and DNA fragmentation:** Cytotoxic effect of *Cassia auriculata* methanolic leaf extract was done in Human Hepatocellular carcinoma cell lines (HepG₂) and the IC₅₀ 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 HepG₂ 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: 1 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

<table>
<thead>
<tr>
<th>Cassia auriculata (μg mL⁻¹)</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>56.34±5.98</td>
<td>61.58±6.21</td>
<td>65.67±6.58</td>
<td>70.56±7.58</td>
</tr>
<tr>
<td>400</td>
<td>54.13±5.87</td>
<td>59.63±6.01</td>
<td>63.12±6.17</td>
<td>65.78±7.12</td>
</tr>
<tr>
<td>200</td>
<td>53.12±5.23</td>
<td>57.94±5.45</td>
<td>60.19±5.67</td>
<td>63.84±6.84</td>
</tr>
<tr>
<td>100</td>
<td>50.17±5.01</td>
<td>54.23±5.12</td>
<td>58.65±5.45</td>
<td>60.78±6.09</td>
</tr>
<tr>
<td>50</td>
<td>39.58±3.27</td>
<td>53.97±4.58</td>
<td>55.32±4.87</td>
<td>58.98±6.33</td>
</tr>
<tr>
<td>25</td>
<td>34.56±3.43</td>
<td>48.01±4.25</td>
<td>51.33±4.64</td>
<td>55.07±5.78</td>
</tr>
<tr>
<td>12.5</td>
<td>29.07±3.12</td>
<td>32.11±4.18</td>
<td>47.89±4.23</td>
<td>50.17±5.73</td>
</tr>
</tbody>
</table>

Results are presented as the Mean±SD

**Serum biochemical parameters:** Marker enzymes Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Lactate dehydrogenase and γ-glutamyl transpeptidase
Table 2: Effect of CAME on liver marker enzymes, total liver protein, non enzymatic antioxidants and enzymatic antioxidants in CCl4-induced toxicity experimental rats

<table>
<thead>
<tr>
<th>Liver marker enzyme</th>
<th>G1-control</th>
<th>G2-CCl4 induced</th>
<th>G3-drug alone</th>
<th>G4-post treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>83.51±0.06</td>
<td>275.84±0.20*</td>
<td>72.41±0.05**</td>
<td>102.31±0.07***</td>
</tr>
<tr>
<td>SGPT</td>
<td>67.38±0.05</td>
<td>375.66±0.28*</td>
<td>58.40±0.04**</td>
<td>87.38±0.00***</td>
</tr>
<tr>
<td>GGT</td>
<td>67.35±0.00054</td>
<td>375.66±0.00062*</td>
<td>58.40±0.00048**</td>
<td>98.38±0.00011***</td>
</tr>
<tr>
<td>LDH</td>
<td>97.76±0.07</td>
<td>253.34±0.19*</td>
<td>123.82±0.09**</td>
<td>116.12±0.08***</td>
</tr>
<tr>
<td>Pro</td>
<td>8.40±0.06</td>
<td>27.64±0.02*</td>
<td>8.71±0.06**</td>
<td>11.54±0.03***</td>
</tr>
<tr>
<td>LPO</td>
<td>63.87±0.04</td>
<td>639.10±0.48*</td>
<td>54.37±0.04**</td>
<td>145.74±0.10***</td>
</tr>
<tr>
<td>GSH</td>
<td>19.65±0.01</td>
<td>7.06±0.005*</td>
<td>24.60±0.018**</td>
<td>15.96±0.011***</td>
</tr>
<tr>
<td>Vit C</td>
<td>2.51±0.18</td>
<td>61.51±0.046*</td>
<td>257.14±0.19**</td>
<td>626.28±0.47***</td>
</tr>
<tr>
<td>Vit E</td>
<td>0.00171±0.000220</td>
<td>0.00014±2.96*</td>
<td>0.00396±0.000342**</td>
<td>0.00228±0.000393***</td>
</tr>
<tr>
<td>CAT</td>
<td>98.71±0.07</td>
<td>27.10±0.02*</td>
<td>102.31±0.07**</td>
<td>89.71±0.06***</td>
</tr>
<tr>
<td>SOD</td>
<td>0.5215±0.000393</td>
<td>0.9834±0.00035*</td>
<td>0.6521±0.000491**</td>
<td>0.4703±0.0000254***</td>
</tr>
<tr>
<td>GPx</td>
<td>127.32±0.09</td>
<td>46.29±0.03*</td>
<td>157.36±0.11**</td>
<td>112.65±0.08***</td>
</tr>
<tr>
<td>GST</td>
<td>0.9781±0.000736</td>
<td>0.1700±0.000128*</td>
<td>1.7805±0.000134**</td>
<td>0.8121±0.0000611***</td>
</tr>
</tbody>
</table>

Values are Means±SD (n = 6). *p<0.001 as compared with normal control group; **p<0.001 as compared with normal control group; ***p<0.01 as compared with CCl4 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); GGT: γ-Glutamyl transpeptidase (Unit L⁻¹); Pro: Protein (mg g⁻¹ of wet tissue); LPO: μ moles 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 dismutase (units/min/mg protein); GPx: Glutathione peroxidase (μoles of GSH oxidized/min/mg protein under incubation condition); GST: Glutathione-s-transferase (1 μoles of CDNB conjugated/min/mg); CAT: Catalase (μoles of H2O2 consumed/min/mg).

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 CCl₄ 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 tetrachloride 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). Enumerated 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 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; Sheweta 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 CCl₄ 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 CCl₄ damage on cell membranes. But in the case of untreated CCl₄ induced group, liver histopathology shows more vaculated 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 of 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 CCl₄ induced liver damaged rats.

ACKNOWLEDGMENTS

M.G. developed the concept and designed the experiments. The experiments were done by J.D, analysis and interpretation of data by M.G. The authors wish to express their gratitude to Centre for Advanced Studies In Botany, University of Madras, Chennai-600 025, India for providing Laboratory facilities and to Dr. Vijayalakshmi, Vaishnavi Histopathology and Cytology Centre, Chennai, India for interpreting the histopathological variation. The authors also wish to express their gratitude to Prof R.B. Narayanan (Centre for Biotechnology, Anna University, Chennai-25) on his critical comments on the manuscript.

REFERENCES


