EVALUATION OF HEPATOPROTECTIVE EFFECT OF CALOTRIPS PROCERA (AIT) R.BR ROOT EXTRACT AGAINST CCL₄ INDUCED HEPATO-OXIDATIVE STRESS IN ALBINO RATS

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ABSTRACT
In the present study, Calotropis procera (Asclepediaceae) was evaluated for its possible hepatoprotective and antioxidant potential. Hepatoprotective activity of the methanol extract (MCP) of the root bark was determined using carbon tetrachloride (CCL₄) induced liver injury in rats. MCP extract evaluated, at an oral dose of 200 and 400 mg kg⁻¹. The animals were weighed each and divided in groups of six. Liver damage was achieved by injecting CCL₄ in olive oil (1:1) 0.8 mL kg⁻¹. The treatment groups pretreated with above extracts. Silymarin was used as reference standard drug. At the end of 7 days, blood was collected, liver extracted, weighed, processed for histopathological assessments and for antioxidant activity. The MCP exhibited a significant (p<0.05) hepatoprotective effect by lowering the elevated serum levels of serum transaminases (AST and ALT), Alkaline phosphatase (ALP), total and direct serum bilirubin, cholesterol and significantly increasing high density lipoprotein (HDL) and moderately increasing total protein and albumin. These biochemical observations were supplemented by histopathological examination of liver sections. Further, the effects of the MCP extract on antioxidant enzymes also have been investigated to elucidate the possible mechanism of its hepatoprotective activity. The MCP extract exhibited a significant effect (p<0.05) in a dose dependent manner by modifying the levels of reduced glutathione, super oxide dimutase, catalase activity and malondialdehyde equivalent, an index of lipid peroxidation of the liver. These findings suggest that the MCP extract exhibited a dose dependent significant effect on hepatoprotective and antioxidant potential which revitalidates the use of this plant for the treatment of liver toxicity in oriental traditional medicine.

KEY WORDS: Calotropis procera, CCL₄, Hepatoprotective activity, Albino rats.

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INTRODUCTION
Liver is a vital organ of the body. It plays a pivotal role in the metabolism, secretion and storage. Any type of the injury or impairment of its functions may leads to many type of complication in one’s health. Unfortunately, Hepatic dysfunction due to ingestion or inhalation of hepatotoxins is increasing worldwide. Management of liver disease is still a challenge to the modern medicine. Due to limited therapeutic options and disappointing therapeutic success of the modern medicine, use of herbal drugs has increased worldwide. Numerous medicinal plants and their formulations used for liver disorders in ethano-medical practices and in traditional system of medicine in India. In this modern age it is very important to provide scientific proof to justify the medicinal uses of herbs. Efficacy of the drugs should be tested by standard experimental methods and there should be adequate data from studies to validate the therapeutic potential. In the present study, in order to search for a new natural remedy for hepatic disorder, the Calotropis procera root bark was evaluated for its possible hepatoprotective activity. The genus Calotropis R. Br (Asclepediaceae) is distributed in tropical and subtropical region of Asia and...
Africa, while in India it is represented by two species viz., *Calotropis procera* and *Calotropis gigantea*. *C. procera* is a large bushy shrub, more common in southwestern and central India and western Himalayas. In India the *C. procera* holds pride of place largely because of its other uses and economical values. The plant is also known for its use in folk medicines. Traditionally, the plant has been used as Antifungal, anti-inflammatory, analgesic and anesthetic activity. Dried leaves used as an expectorant, as anti-inflammatory, for treatment of paralysis and rheumatic pains. Dried latex and dried root used as an antidote for snake poisoning. It is also used as an abortifacient, for treatment of piles and intestinal worms. The tender leaves of plant are also used to cure migraine. The capsulated root bark powder is effective in diarrhoea and asthma. The previous pharmacological studies include reports of anticcancer, antifungal and Insecticidal activity of *C. procera*. The flowers of the plant possess hepatoprotective activity, anti-inflammatory, anti-inflammatory, analgesic, antimicrobial properties and larvicial activity. The latex of the plant was reported to possess analgesic and wound healing activity, anti-inflammatory activity and antimicrobial activity. The roots are reported to have anti-fertility and anti-ulcer activities.

Earlier chemical examination of this plant has shown the presence of triterpenoids, calotropenseryl acetate and calopfiedeleinyl; a norditerpenyl ester, calotropternyl acetate and their glycosides, flavanoids, alkaloids and steroids. Hepetoprotective activity of the methanolic extract of *C. procera* (MCP) was tested for qualitative phytoconstituents and indicated the presence of triterpenoids and their glycosides, flavanoids, alkaloids and steroids. Hepetoprotective activity of the methanolic extract was studied.

**Animals**
Albino rats (150-200 g) were procured from National Institute of Mental Health and Neurosciences, Bangalore India. After procuring the animals were acclimatized for 10 days under standard husbandry conditions, room temperature (27 ± 3°C), relative humidity (65 ± 10 %) and 12 hours light / dark cycle. They were allowed free access to standard dry pellet diet (Gold mohr, Lipton India Ltd., Bangalore, India) and water *ad libitum* under strict hygienic conditions. All the described procedure were reviewed and approved by the Institutional Animal Ethical Committee.

**Hepatoprotective activity**
Animals were divided into five groups each of six animals. Group I and II served as normal and intoxicated control, respectively and received only the vehicle (0.5% Tween-80; 1 mL kg\(^{-1}\) p.o.). Group III animals were treated with standard silymarin at an oral dose of 100 mg kg\(^{-1}\), group IV and V received the MCP at an oral dose of 200 mg kg\(^{-1}\) and 400 mg kg\(^{-1}\). The treatment was continued for 7 days, once daily. On the day of 2nd, 4th and 6th for groups II, III, IV and V 30 min post-dose of extract administration animals received CC\(_4\) at the dose of 0.8 mL kg\(^{-1}\) (1:1 of CC\(_4\) in olive oil) orally. Twenty four hours after CC\(_4\) administration, blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and analyzed for various biochemical parameters, serum transaminases (AST and ALT), Alkaline phosphatase (ALP) and Bilirubin (Total and Direct) using Span diagnostic kits.

**Assessments of oxidative stress**

**Preparation of tissue antioxidant**
The livers were rinsed with ice cold distilled water followed by sucrose solution (0.25 M). And again rinsed with distilled water and immediately stored at -20°C till further biochemical analysis. One gram of liver tissue homogenized in 10 mL of ice cold Tris-hydrochloride...
buffer. The prepared homogenates were centrifuged and used for the assay of determination of lipid peroxidation (LPO) by measuring the release of malondialdehyde (MDA) by the method of Slater and Sawyer (1971) and the estimation of reduced glutathione enzyme (GSH).

**Post Mitochondrial Supernatant preparation (PMS)**

The homogenates were centrifuged at 800 rpm for 5 min at 4°C to separate debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 min at 4°C to get the post mitochondrial supernatant (PMS) which was used to assay catalase (CAT) and superoxide dismutase enzyme (SOD) activity.

**Histopathological study**

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin. Histological observations were made under light microscope.

**Statistical analysis**

The results are expressed as Means±SD. The differences between experimental groups were compared by one-way ANOVA (toxic control versus treatment, tukeys method; using Graph pad prism statistical software, version 5.0) and were considered statistically significant at p<0.05.

**RESULTS**

In the present study, it was seen that administration of 
CCl₄ elevates the levels of serum marker enzymes AST, ALT and ALP (Table 1). It can also be seen from the Table 1 that the animals pretreated with methanolic extract of *C. procera* (200 and 400 mg kg⁻¹; p.o.) showed significant (p<0.05) decrease in the serum enzyme values compared to those of toxic control values. The animals treated with toxic doses of CCl₄ showed markedly elevated values of the serum AST, ALT, ALP, total and direct bilirubin, cholesterol and decreased total protein, albumin and HDL compared to normal control, indicating acute hepato-cellular damage. Pretreatment with MCP (200 and 400mg kg⁻¹; p.o.) fraction significantly (p<0.05) decreased the value of AST, ALT, ALP, bilirubin (total and direct) and cholesterol and prevented diminution of HDL value in a dose dependent manner. It suggested clear indication of the improvement of the functional status of the liver cells. Both the fraction showed marginal improvement in the values of total protein and albumin. The MCP extract also exhibited a significant effect(p<0.05) in a dose dependent manner by modifying the levels of reduced glutathione, super oxide dimutase, catalase activity and malondialdehyde equivalent, an index of lipid peroxidation of the liver. (Table 2)

**DISCUSSION**

Although *Calotropis procera* is reported to possess varied medicinal uses as discussed earlier, there is no previous report about the hepatoprotective activity of the root extract of the plant. The present investigation reports the hepatoprotective activity of the MCP in two selective doses (200 and 400 mg kg⁻¹; p.o.)

In the present study, hepatotoxicity model in albino rats was successfully produced by administering CCl₄ (1:1 in olive oil, 0.8 mL kg⁻¹) intraperitonially. It is well established that hepatotoxicity by CCl₄ is due to enzymatic activation to release CCl₃ radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles. CCl₄ also plays a significant role in depletition of Intracellular antioxidant reduced glutathione (GSH), increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzymes activity. As the damage marker enzymes AST and ALT are cytoplasmic in location they get released in serum. So increase in the level of AST, ALT, and ALP, total and direct bilirubin, cholesterol and HDL is conventional indicator of liver injury. CCl₄ significantly (P<0.05) decrease the levels of SOD, GSH and catalase in liver. The level of MDA which is produced as a result of lipid peroxidation is significantly (P<0.05) increased.

As discussed in results, MCP showed significant (p<0.05) hepatoprotective activity on CCl₄ induced hepatotoxic animals in a dose dependent manner. MCP extract significantly(p<0.05) reduced the elevated levels of the different enzyme values and also showed appreciable increase in the levels of GSH, SOD and catalase whereas decreased the lipid peroxidation in a dose dependent manner. Plant demonstrated superoxide scavenging activity therefore it may be inferred that the antioxidant property of the plant may prevent formation of free radical and so inhibit the lipid peroxidation and offers the hepatoprotection against CCl₄ toxicity. Further, the improvement of GSH level also indicate that probably the natural tissue protection mechanism is kept intact and oxidative degeneration of the liver tissues is prevented by the MCP extract supplementation. The results for antioxidant study suggest that the reason for hepatoprotective effect of the extracts may be that *C. procera* contains terpenoids and flavanoids which might have scavenged the free radicals offering hepatoprotection. Further, the results also supported by histopathological examination of liver sections of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig. 1). Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and...
fatty degeneration were observed in CCl4 intoxicated animals (Fig. 2). The liver sections of the rats treated with MCP (200 mg kg\(^{-1}\); p.o.) (Fig. 3), MCP (400 mg kg\(^{-1}\); p.o.) (Fig. 4) and Silymarin (Fig. 5) followed by CCl4 intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles.

REFERENCES


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Table 1: Effect of the *C. procera* root methanolic extract (MCP) on biochemical parameters in CCl₄ induced hepatotoxicity in rats

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<td>AST (U/ml)</td>
<td>48.553±0.0a</td>
<td>296.473±0.02b</td>
<td>57.775±0.0c</td>
<td>117.198±0.01d</td>
<td>89.091±0.0e</td>
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<td>ALT (U/ml)</td>
<td>95.293±0.0a</td>
<td>481.167±1.08b</td>
<td>131.715±0.02c</td>
<td>221.877±0.05d</td>
<td>152.637±0.01e</td>
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<tr>
<td>ALP (U/ml)</td>
<td>126.14±0.08a</td>
<td>250.502±0.01b</td>
<td>91.792±0.00c</td>
<td>122.935±0.01d</td>
<td>81.846±0.00e</td>
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<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.992±0.00a</td>
<td>1.496±0.00b</td>
<td>0.376±0.00c</td>
<td>0.72±0.00d</td>
<td>0.546±0.00e</td>
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<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.185±0.00a</td>
<td>1.496±0.00b</td>
<td>0.376±0.00c</td>
<td>0.72±0.00d</td>
<td>0.546±0.00e</td>
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<td>Cholesterol (mg/dl)</td>
<td>104.257±0.71a</td>
<td>167.653±0.01b</td>
<td>118.098±0.01c</td>
<td>137.14±0.01d</td>
<td>120.938±0.01e</td>
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<tr>
<td>HDL(mg/dl)</td>
<td>47.436±0.00a</td>
<td>27.605±0.00b</td>
<td>44.792±0.00c</td>
<td>33.853±0.00d</td>
<td>40.623±0.00e</td>
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Values are mean±SEM; *P<0.05 ,Values with different superscripts differ significantly different from group-I

Table 2: Effect of the *C. procera* root methanolic extract (MCP) on antioxidant parameters in CCl₄ induced hepatotoxicity in rats

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<td>MDA(nmol/mg protein)</td>
<td>0.22±0.02a</td>
<td>0.32±0.04b</td>
<td>0.27±0.02c</td>
<td>0.29±0.04d</td>
<td>0.25±0.02e</td>
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<td>SOD(activity/mg protein)</td>
<td>0.49±0.01a</td>
<td>0.57±0.01b</td>
<td>0.51±0.01c</td>
<td>0.52±0.01d</td>
<td>0.51±0.01e</td>
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<tr>
<td>CAT(activity/mg protein)</td>
<td>0.44±0.02a</td>
<td>0.37±0.01b</td>
<td>0.38±0.01c</td>
<td>0.39±0.01d</td>
<td>0.42±0.01e</td>
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<tr>
<td>GSH(µmol/mg protein)</td>
<td>0.934±0.001a</td>
<td>0.437±0.001b</td>
<td>0.844±0.001c</td>
<td>0.677±0.001d</td>
<td>0.816±0.001e</td>
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Values are mean±SEM; *P<0.05 ,Values with different superscripts differ significantly different from group-I
Fig. 5: Microphotograph of liver section of Silymarin and CCl₄ treated rat (x 200)

Fig. A: Effect of the C. procera root methanolic extract (MCP) on biochemical parameters in CCl₄ induced hepatotoxicity in rats

Fig. B: Effect of the C. procera root methanolic extract (MCP) on antioxidant parameters in CCl₄ induced hepatotoxicity in rats

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