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

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ABSTRACT
In the present study, *Calotropis procera* (Asclepiadaceae) was evaluated for its possible hepatoprotective and antioxidant potential. Hepatoprotective activity of the methanol extract (MCP) of the root was determined using paracetamol-induced liver injury in rat. The animals were weighed each and divided in groups of five. Liver damage was achieved by injecting Paracetamol (2 g/kg). The treatment groups pretreated with MCP Group-IV (200mg/kg) and Group-V (400mg/kg). 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. Alteration in the levels of biochemical markers of hepatic damage like serum transaminases (AST, ALT), alkaline phosphatase (ALP), bilirubin, cholesterol, high density lipoprotein (HDL) and tissue GSH were tested in all the groups. The MCP extract exhibited a significant hepatoprotective effect by lowering the elevated serum levels of AST, ALT, ALP, total and direct serum bilirubin, cholesterol and significantly (p<0.05) increased HDL and moderately increased total protein and albumin in a dose dependent manner. These biochemical observations were supplemented by histopathological examination of liver sections. Further, the effects of the active fractions 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 dose dependent manner by modifying the levels of reduced glutathione, superoxide dismutase, catalase activity and malondialdehyde equivalent, an index of lipid peroxidation of the liver. These findings suggest the use of this plant for the treatment of liver toxicity in oriental traditional medicine.

KEY WORDS: *Calotropis procera*, Paracetomol, Hepatoprotective activity, Albino rats

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 limited therapeutic options and disappointing therapeutic success of the modern medicine, uses of herbal drugs has increased worldwide. Numerous medicinal plants and their formulations used for liver disorders in ethnomedical 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 (Asclepiadaceae) 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 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, antipyretic and analgesic activity. Dried leaves used as an expectorant, as antiinflammatory, 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 capsule root bark powder is effective in diarrhoea and asthma. The previous pharmacological studies include reports of anticancer, antifungal and Insecticidal activity of *C. procera*. The flowers of the plant possessed hepatoprotective activity, anti-inflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal 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, calotropursenyl acetate and calopfriedelenyl; a nor-diterpenyl ester, calotroternyl ester oleane and triterpenes like calotropopaleanyl ester, procerelan A and B and cardiac glycosides calotropigenin, calotropin, uscharin, calotoxin and calactin. The plant also has been investigated for cardenolides and anthocyanins. The root bark also found to possess α-amyrin, β-amyrin, lupeol, β-sitosterol and flavanols like quercetin-3-rutinoside. The rich source of phytoconstituents and there are no scientific bases or reports in modern literature regarding usefulness of root as hepatoprotective agent prompts us to evaluate root of plant for its possible hepatoprotective activity. In the course of searching for hepatoprotective agents from medicinal plants, the Methanol extract of root of *C. procera* was evaluated against paracetamol induced hepatic damage.

**MATERIALS AND METHODS**

**Plant material**

Fresh, well-developed plants of *C. procera* were collected from *C. procera* root was collected from the rural area of north Karnataka. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, Karnataka, India. A voucher specimen (021/2008) has been deposited at the museum of our college. The root was collected in the month of May 2008 and shade dried at room temperature.

**Preparation of extract**

Dried root bark powder (200 g) was extracted with methanol by soxhlet apparatus for 5 h. The methanolic extract of *C. procera* (MCP) was tested for qualitative phytoconstituents and indicated the presence of tri-terpenoids and their glycosides, flavanoids, alkaloids and steroids. Hepatoprotective activity of the methanolic extract was studied in two doses (200mg/kg, 400mg/kg).

**Animals**

Albino rats (150-200 g) were procured from National Institute of Mental Health and Neuro Sciences, Bangalore India. After procuring the animals were acclimatized for 10 day’s 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 study by paracetamol induced hepatotoxicity**

The method of Chattopadhyaay was used in the study. Animals were divided into five groups of 6 animals each. The first group received saline 1 ml/kg for one week (control). The group II received saline 1 ml/kg for one week (positive control). The groups III, IV and V received silymarin (100 mg/kg p.o.) and 200 mg/kg and 400 mg/kg of *C. procera* methanolic extract respectively once a day for seven days. On the
fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV and V were administered with paracetamol 2 g/kg orally. On the seventh day after 2 h of respective treatments the blood samples were collected for the estimation of biochemical marker enzymes.

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, aminotransferases (AST, ALT), alkaline phosphatase (ALP) and Bilirubin (Total and Direct) total protein (TP), cholesterol and HDL using Span diagnostic kits. The liver was immediately dissected out and the liver-tissue was used for estimation of malondialdehyde equivalent, an index of lipid peroxides (LPO), reduced glutathione (GSH), Super Oxide Dismutase (SOD) and Catalase Activity (CAT). A section of liver was processed for histological studies.

**Assessments of oxidative stress**

**Preparation of tissue antioxidant**

The livers were rinsed with ice cold distilled water followed by sucrose solution (0.25 M), 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 lipid per oxidation (LPO) by measuring the release of malondialdehyde (MDA) by the method of Slater and Sawyer (1971), Super Oxide Dismutase (SOD) and Catalase Activity (CAT) 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, tukey’s method; using Graph pad prism statistical software, version 5.0) and were considered statistically significant at p<0.05.

### RESULT AND DISCUSSION

Paracetamol has enhanced the levels of aminotransferases (AST, ALT), bilirubin (both total and direct bilirubin levels), Alkaline phosphatase level (ALP), total cholesterol, whereas HDL and tissue GSH levels are decreased significantly. Treatment with silymarin and 200 mg/kg and 400 mg/kg of *C. procera* root (methanolic extract) has significantly brought down the elevated levels of AST, ALT, ALP, bilirubin, cholesterol and also significantly (p<0.05) enhanced the decreased levels of tissue GSH and HDL in a dose dependent manner. Paracetamol is normally eliminated mainly as sulfate and glucoronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquinime. However, upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinime (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leading to increased lipid peroxidation and liver damage. In our experiments it is observed that tissue GSH levels in the paracetamol group is decreased to the extent of around 70%. This clearly indicates that there is a significant hepatic damage due to paracetamol. This is further evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like AST, ALT, bilirubin, and cholesterol. Treatment with silymarin and *C. procera* root (methanolic extract) has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels which was significant (p<0.05) in group V compared to Group IV. It may be concluded...
that the hepatoprotective effect of *C. procera* root (methanolic extract) is due to the prevention of the depletion in the tissue GSH levels. Upon literature review it is found that the root of *C. procera* contains quercetin-3-rutinoside and other flavonoids which are still present in the methanolic extract. Therefore there is a possibility that the root extract may possess antioxidant property, which may be involved in the hepatoprotective property. In addition it is necessary to carry-out further studies to rule out if treatment with methanolic extract is able to inhibit oxidation of paracetamol to highly reactive NAPQI. The results were further substantiated with the histopathological study, the reduced hepatic damage or improvement in the hepatic architecture in a dose dependent manner.

REFERENCES
8. Al-YahyaMA, Al-MeshallA, MossaJS, Tariq M. Phytochemical and pharmacological studies on *Calotropis procera*. Proceeding of the 3rd International Conference of Traditional and Folk Medicine, July 8-12, Lecatecas, Mexico. 1985; july:8-12
Table 1: Effect of the *Calotropis procera* flowers Methanolic extract (MCP) on biochemical parameters in paracetamol-induced hepatic injury in rats

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<tr>
<td>AST (U/ml)</td>
<td>68.572±0.001a</td>
<td>281.212±0.014b</td>
<td>74.572±0.002c</td>
<td>161.252±0.011d</td>
<td>86.866±0.002e</td>
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<td>ALT (U/ml)</td>
<td>131.72±0.001a</td>
<td>403.14±0.013b</td>
<td>135.253±0.015a</td>
<td>280.66±4.496c</td>
<td>135.663±16.63a</td>
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<tr>
<td>ALP (U/ml)</td>
<td>135.532±0.009a</td>
<td>436.317±0.027a</td>
<td>157.992±8.338c</td>
<td>286.41±0.018d</td>
<td>181.66±0.016e</td>
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<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.92±0.00a</td>
<td>3.417±0.001b</td>
<td>1.034±0.001a</td>
<td>1.444±0.001c</td>
<td>1.07±0.002a</td>
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<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.25±0.00a</td>
<td>0.685±0.00b</td>
<td>0.255±0.00a</td>
<td>0.379±0.00c</td>
<td>0.308±0.00d</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>103.457±0.009a</td>
<td>157.838±0.012b</td>
<td>116.195±0.013c</td>
<td>136.34±0.012d</td>
<td>118.683±0.015c</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>47.135±0.001a</td>
<td>28.458±0.011b</td>
<td>45.12±0.001c</td>
<td>33.405±0.001d</td>
<td>41.255±0.001e</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 *Calotropis procera* flowers Methanolic extract (MCP) on antioxidant parameters in paracetamol-induced hepatic injury in rats

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<tr>
<td>MDA(nmol/mg protein)</td>
<td>0.22±0.02a</td>
<td>0.32±0.04a</td>
<td>0.26±0.02a</td>
<td>0.32±0.04a</td>
<td>0.27±0.02a</td>
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<tr>
<td>SOD(activity/mg protein)</td>
<td>0.49±0.01b</td>
<td>0.57±0.01b</td>
<td>0.54±0.01b</td>
<td>0.57±0.01b</td>
<td>0.51±0.01b</td>
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<tr>
<td>CAT(activity/mg protein)</td>
<td>0.44±0.02c</td>
<td>0.37±0.01c</td>
<td>0.45±0.01c</td>
<td>0.37±0.01c</td>
<td>0.38±0.01c</td>
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<tr>
<td>GSH(μ mol/mg protein)</td>
<td>0.934±0.001d</td>
<td>0.316±0.001d</td>
<td>0.565±0.001d</td>
<td>0.454±0.001d</td>
<td>0.545±0.001d</td>
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Values are mean±SEM; *P<0.05* , Values with different superscripts differ significantly different from group-I
**Fig. A:** Effect of the *C. procera* root methanolic extract (MCP) on biochemical parameters in paracetamol induced hepatotoxicity in rats

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

- MDA = nmol/mg protein; SOD = activity/mg protein; CAT = activity/mg protein; GSH = µmol/mg protein.
- AST = U/ml; ALT = U/ml; ALP = U/ml; LDH = U/ml; C = mg/dl
- HDL C = mg/dl
Fig. 1: Microphotograph of normal control rat liver section (x 200)

Fig. 2: Microphotograph of rat liver section treated with CCl₄ (x 200)

Fig. 3: Microphotograph of liver section of MCP (200 mg kg⁻¹; p.o.) and paracetamol treated rat (x 200)

Fig. 4: Microphotograph of liver section of MCP (400 mg kg⁻¹; p.o.) and paracetamol treated rat (x 200)

Fig. 5: Microphotograph of liver section of Silymarin and paracetamol treated rat (x 200)