Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

EVALUATION OF HEPATOPROTECTIVE EFFECT OF *CALOTROPIS PROCERA* (AIT) R.BR ROOT EXTRACT AGAINST CCL_4 INDUCED HEPATO-OXIDATIVE STRESS IN ALBINO RATS

Patil Prakash[1]*, K Prasad[2], M Nitin[3], M Vijay Kumar[4], K Sreenivasa Rao[5]

[1]Department of Pharmacology, RRKS College of Pharmacy, Bidar, Karnataka, India
[2]Department of Pharmacology, Vishnu College of Pharmacy, Bhemavaram, Karnataka, India
[3]Department of Pharmacology, HKE College of Pharmacy, Gulbarga, Karnataka, India
[4]Department of Veterinary Pharmacology & Toxicology, Veterinary College, KVAFSU, Bidar, Karnataka, India
[5]Department of Pharmaceutics, RRKS College of Pharmacy, Bidar, Karnataka, India

Received on: 02/01/2011 Revised on: 22/01/2011 Accepted on: 03/02/2011

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 (CCl_4) 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 CCl_4 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 ($p<0.05$) hepato-protective 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.

***Corresponding author**

Prakash Patil, Department of Pharmacology, RRK College of Pharmacy, Bidar, Karnataka, India-585401
Email: patil_pnp@yahoo.co.in

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[1]. Due to limited therapeutic options and disappointing therapeutic success of the modern medicine, use of herbal drugs has increased worldwide[2]. Numerous medicinal plants and their formulations used for liver disorders in ethano-medical practices and in traditional system of medicine in India[3]. 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[4]. 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 large bushy shrub, more common in southwestern and central India and western himalayas[5]. In India the *C. procera* holds pride of place largely because of its other uses and economical values[6]. The plant is also known for its use in folk medicines. Traditionally, the plant has been used as Antifungal[7], antipyretic[8] and analgesic activity[9]. Dried leaves used as an expectorant, as anti-inflammatory[10], for treatment of paralysis and rheumatic pains[11]. Dried latex and dried root used as an antidote for snake poisoning. It is also used as an abortificient[12], for treatment of piles[13] and intestinal worms[14]. The tender leaves of plant are also used to cure migraine[15]. The capsulated root bark powder is effective in diarrhoea[14] and asthma[14]. The previous pharmacological studies include reports of anticancer[15], antifungal[16] and Insecticidal[15] activity of *C. procera*. The flowers of the plant possessed hepatoprotective activity[17], anti-inflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity[18,19]. The latex of the plant was reported to possess analgesic and wound healing activity[20,21], anti-inflammatory activity[22] and antimicrobial[23]. The roots are reported to have anti-fertility[24] and anti-ulcer activities[25].

Earlier chemical examination of this plant has shown the presence of triterpenoids, calotropursenyl acetate and calopfriedelenyl; a norditerpenyl ester, calotropternyl ester oleanene triterpenes like calotropoleanyl ester, procerleanol A and B[26] and cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin and calactin[6] The plant also has been investigated for cardenolides[27] and anthocyanins[6]. The root bark also found to possess α-amyrin[28], β-amyrin[29], lupeol, β-sitosterol[28] and flavanols like quercetin-3-rutinoside[30]. The rich source of phytoconstituents and there are no scientific bases or reports in modern literature regarding usefulness of root bark as hepatoprotective agent prompts us to evaluate root bark of plant for its possible hepatoprotective activity.

In the course of searching for hepatoprotective agents from medicinal plants, the MeOH extract of root bark of *C. procera* was evaluated against carbon tetrachloride 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. 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 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 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 CCl_4 at the dose of 0.8 mL kg^{-1} (1:1 of CCl_4 in olive oil) orally. Twenty four hours after CCl_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 transminases (AST and ALT), Alkaline phosphatase (ALP) and Billirubin (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 Supernant 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 supernant (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 CCl_4 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 CCl_4 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, billirubin (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 CCl_4 (1:1 in olive oil, 0.8 mL kg^{-1}) intraperitonially. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles. CCl_4 also plays a significant role in depletion 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. CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles

REFERENCES

1. Reddy BP, Kokate CK, Rambhau D, Venkateshwarlu V, Murthy VN. Antihepatotoxic activity of some ayurvedic prepartions. Indian J. Pharma. Sci. 1993;55:137-40.
2. Stickel F, Schuppan D. Herbal medicine in the treatment for liver diseases. Dig. Liver Dis. 2007;39:293-04.
3. Sethuraman MG, Lalitha KG, Kapoor BR. Hepatoprotective activity of *Sarcostemma brevistigma* against carbon tetrachloride-induced hepatic damage in rats. Curr. Sci. 2003;84:1186-87.
4. Girish B, Koner B, Jayathi S, Rao KR, Rajesh B, Pradhan S. Hepatoprotective activity of six herbal formulations in the CCl_4 induced liver toxicity in mice. Indian J. Med. Res. 2009.
5. Phondke GP. The Wealth of India, Raw Materials. Vol. 2, Council of Scientific and Industrial Research, New Delhi.ISBN: 81-85038-00-7. 1992;556.
6. Ahmed KKM, Rana AC, Dixit VK. Calotropis species (Ascelpediaceae): A comprehensive review. Pharmacog. Maga. 2005; (I): 48-52.
7. Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. Fitotrapia. 1997;68:371-73.
8. Al-Yahya MA, Al-MeshalI A, Mossa JS, 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
9. Mohsin A, Shah AH, Alaha MA, Tariqi MO, Ageel AM. Analytic anti-pyretic activity and phytochemical screening of some plants used in traditional Arab systems of medicine. Fitoterapai.1989;60:174-77.
10. Kapur SK, Sarin YK.. Medico-botanical survey of medicinal and aromatic plants of Katra valley (J.K. State), India. Indian Drugs.1984;22:4-10.
11. Sebastian MK, Bhandari MM. Medico-ethno botany of mount Abu, Rajasthan, India. J. Ethnopharmacol. 1984;12:223-30.
12. Basu A, Sen T, Ray RN, Chaudhuri A. Hepatoprotective effects of calotropis procera root extract on experimental liver damage in animals. Fitoterapia.1992;63:507-14.
13. Gupta DK, Ali M, Bhutani KK. Triterpenoids from calotropis procera root bark. Indian J. Chem. 1996;35:1079-84.
14. Singh VP, Sharma SK, Khare VS. Medicinal plants from ujjain district, madhya pradesh Part II. Indian Drugs Pharm. Ind. 1980;5:7-12.
15. Ahmed UAM, Zuhua S, Bashier NHH, Muafi H, Zhongping H, Yuling G. Evaluation of insecticidal potentialities of aqueous extracts from *Calotropis procera* ait. Against *Henosepilachna elaterii* rossi. J. Applied Sci. 2006;(6):2466-70.
16. Hassan SW, Bilbis FL, Ladan MJ, Umar RA, Dangoggo SM. Evaluation of antifungal acivity and phytochemical analysis of leaves, roots and stem barks extracts of *Calotropis procera* (asclepiadaceae). Pak. J. Bio. Sci.2006;9:2624-29.
17. Setty SR, Quereshi AA, Swamy AH, Patil T, Prakash T. Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. Fitoterapia. 2007;78:451-54.
18. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J. Ethnopharmacol. 1989;27:129-40.
19. Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. J. Ethnopharmacol. 2000;73:293-97.
20. Dewan S, Sangraula H, Kumar VL. Preliminary studies on the analgesic activity of latex of *Calotropis procera*. J. Ethnopharmacol.2000;73:307-11.
21. Rasik AM, Raghubir R, Gupta A, Shukla A, Dubey MP. Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. J.
22. Arya S, Kumar VL. Antiinflammatory efficacy of extracts of latex of *Calotropis procera* against different mediators of inflammation. Mediators Inflammation. 2005; 4:228-32.
23. Sehgal R, Arya S, Kumar VL. Inhibitory effect of extracts of latex of *Calotropis procera* against *Candida albicans*, a preliminary study. Indian J. Pharmacol. 2005;37;334-35.
24. Kamath JV, Rana AC. Preliminary study of antifertility activity of *Calotropis procera* root in female rats. Fitoterapia. 2002;73:111-15.
25. Basu A, Sen T, Pal S, Muscalo N, Capasso F, Choudhuri AKN. Studies on antiulcer activity of the chloroform fraction of *Calotropis procera* root extract. Phytother. Res. 1997;11:163-63.
26. Ansari SH, Ali M. Norditerpenic ester and pentacyclic triterpenoids from root bark of *Calotropis procera* (Ait) R. Br. Pharmazie.2001; 56:175-77.
27. Seiber JN, Nelson CJ, Lee CJ. Cardenolides in the latex and leaves of seven *Asclepias* species and *Calotropis procera*. Phytochem. 1982;21:2343-48.
28. Saber AH, Maharan GH, Rizkallah MM, Ste Saber AH, GH Maharan and MM.Rizkallah. Sterols and pentacyclic triterpenes of *Calotropis procera*. Bull. Fac. Pharm. Cairo Univ. 1969;7:91-104.
29. Saxena VK, Saxena YP. Isolation and study of triterpenoids from *Calotropis procera*. J. Res. Indian Med. Yoga Homeopathy. 1979;14:152-54.
30. Lal SD, Kumar P, Pannu DS. Quercetin-3-rutinoside in *Calotropis procera*. J. Sci. Res.1985;7:141-42.

Table 1: Effect of the *C. procera* root methanolic extract (MCP) on biochemical parameters in CCl_4 induced hepatotoxicity in rats

Parameter	Group-I	Group-II	Group- III	Group-IV	Group-V
AST (U/ml)	48.553±0.0a	296.473±0.02b	57.775±0.0c	117.198±0.01d	89.091±0.0e
ALT (U/ml)	95.293±0.0a	481.167±1.08b	131.715±0.02c	221.877±0.05d	152.637±0.01e
ALP (U/ml)	126.14±0.08a	250.502±0.01b	91.792±0.00c	122.935±0.01d	81.846±0.00e
	0.992±0.00a	4.242±0.01b	1.237±0.00c	2.083±0.00d	1.543±0.00e
Total bilirubin (mg/dl)	0.185±0.00a	1.496±0.00b	0.376±0.00c	0.72±0.00d	0.546±0.00e
Direct bilirubin (mg/dl)					
Cholesterol (mg/dl)	104.257±0.71a	167.653±0.01b	118.098±0.01c	137.14±0.01d	120.938±0.01e
HDL(mg/dl)	47.436±0.00a	27.605±0.00b	44.792±0.00c	33.853±0.00d	40.623±0.00e

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 CCl_4 induced hepatotoxicity injury in rats

Parameter	Group-I	Group-II	Group- III	Group-IV	Group-V
MDA(nmol/mg protein)	0.22±0.02a	0.32±0.04b	0.27±0.02c	0.29±0.04d	0.25±0.02e
SOD(activity/mg protein)	0.49±0.01a	0.57±0.01b	0.51±0.01c	0.52±0.01d	0.51±0.01e
CAT(activity/mg protein)	0.44±0.02a	0.37±0.01b	0.38±0.01c	0.39±0.01d	0.42±0.01e
GSH(µ mol/mg protein)	0.934±0.001a	0.437±0.001b	0.844±0.001c	0.677±0.001d	0.816±0.001e

Values are mean±SEM;*P<0.05 ,Values with different superscripts differ significantly different from group-I

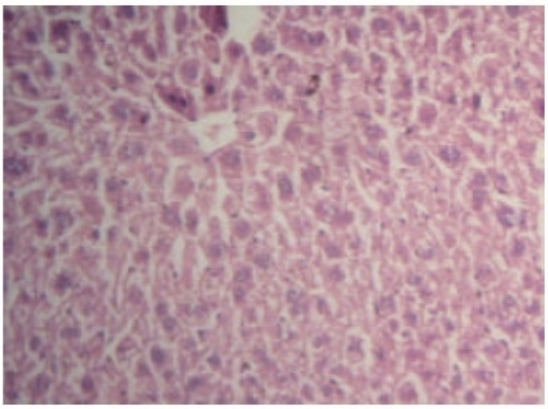

Fig. 1: Microphotograph of normal control rat liver section (x 200)

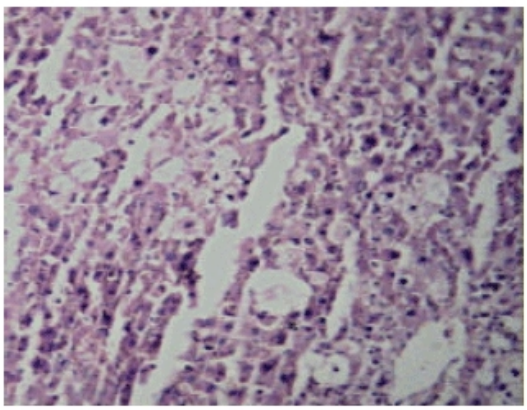

Fig. 2: Microphotograph of rat liver section treated with CCl_4 (x 200)

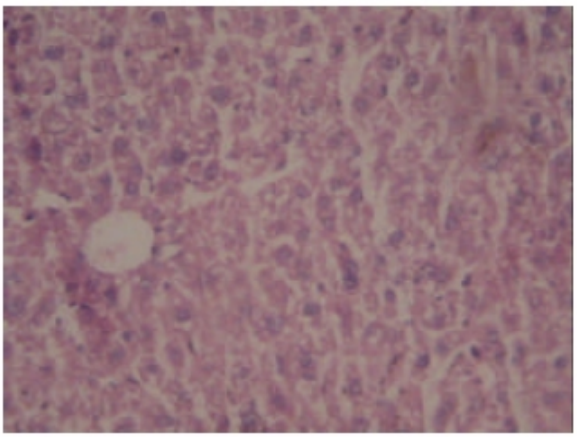

Fig. 3: Microphotograph of liver section of MCP (200 mg kg^{-1}; p.o.) and CCl_4 treated rat (x 200)

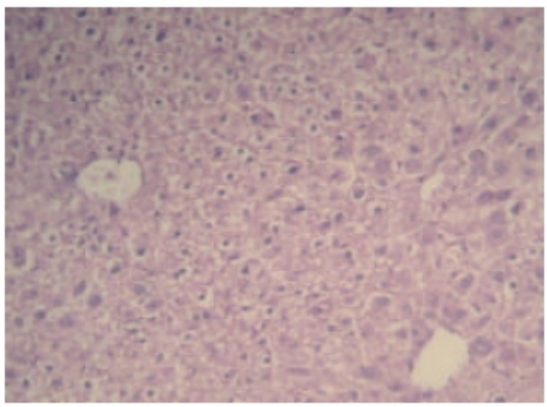

Fig. 4: Microphotograph of liver section of MCP (400 mg kg^{-1}; p.o.) and CCl_4 treated rat (x 200)

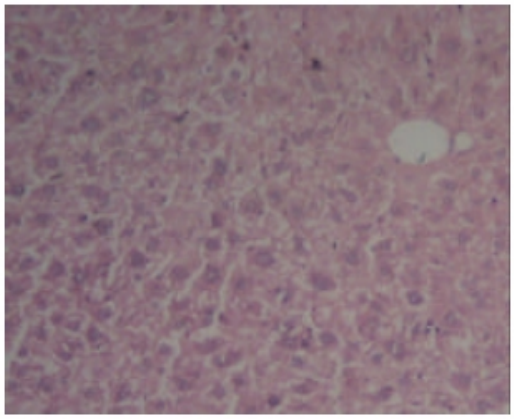

Fig. 5: Microphotograph of liver section of Silymarin and CCl_4 treated rat (x 200)

Fig.A

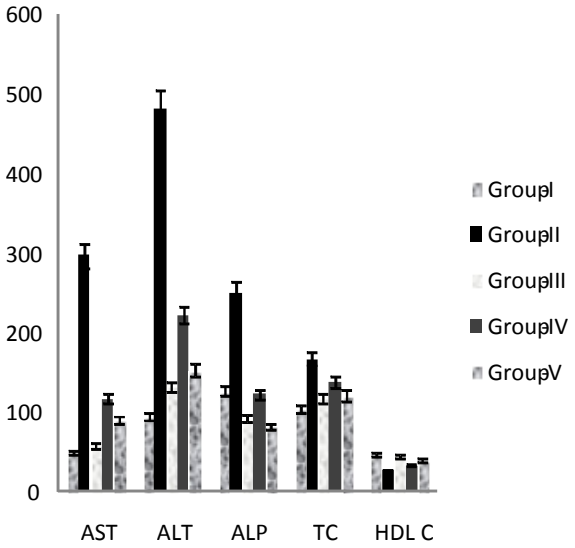

Fig.B

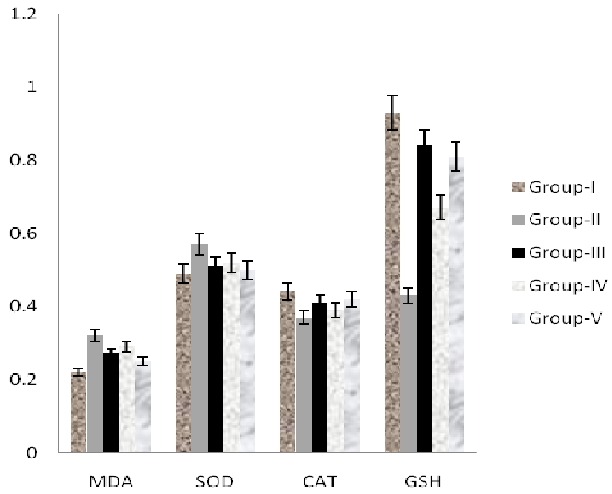

Fig.A: Effect of the C. procera root methanolic extract (MCP) on biochemical parameters in CCl_4 induced hepatotoxicity in rats

Fig.B: Effect of the C. procera root methanolic extract (MCP) on antioxidant parameters in CCl_4 induced hepatotoxicity in rats

Source of support: Nil, Conflict of interest: None Declared