Pharmacognosy Magazine [Phcog Mag.]
Vol 5, Issue 20 (Suppl.), Oct-Dec, 2009 Page 394-399
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 09 Sep, 2009
Modified: 12 Nov, 2009
Accepted: 22 Nov, 2009

PHCOG MAG.: Research Article

Hepatoprotective potential of methanol extract of *Clerodendrum infortunatum* Linn. against CCl_4 induced hepatotoxicity in rats.

Sannigrahi Santanu[1]*, Mazumder Upal Kanti[2], Pal Dilipkumar[3], Mishra Silpi Lipsa[4]

[1] *St. Peter's Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, Andhra Pradesh- 506001, India.*
[2] *Department of Pharmaceutical Technology, Jadavpur Univesity, Kolkata-700032, West Bengal, India*
[3] *Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, Orissa-757086, India.*
[4] *Institute of Pharmacy and Technology, Salipur, Cuttack, Orissa - 754202, India*

*** Communicating author:** *Santanu Sannigrahi, Asst. Professor St. Peter's Institute of Pharmaceutical Sciences, Hanamkonda, Warangal Andhra Pradesh – 506001, India Tel: +91-9177257087; Fax: +91-870-2567304. E-mail: santanuin@rediffmail.com (S. Sannigrahi)*

ABSTRACT

Hepatoprotective potential of methanolic extract of *Clerodendrum infortunatum* Linn. (MECI), which was widely used in Indian indigenous system of medicine, was studied against carbon tetrachloride induced hepatotoxicity in rats. Methanol extract at the dose of 100 and 200 mg/kg was administered daily along with carbon tetrachloride once in 72 hours for 14 days. The study was evaluated by assaying the serum biochemical parameters glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin and total protein. Malondialdehyde (MDA) level, as well as reduced glutathione (GSH) content and catalase activity (CAT) was determined to explain the possible mechanism of the activity. The substantially elevated serum enzymatic levels of AST, ALT, ALP and total bilirubin were restored towards normalization significantly by the extract. Silymarin was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced haptotoxicity in rats. MDA concentration was decreased, while the liver antioxidative enzyme activity was elevated in all the MECI treated rats. All the results were compared with standard drug silymarin. In addition, histopathology of liver tissue was investigated to observe the morphological changes, showed the reduction of fatty degeneration and liver necrosis. The results of this study revealed that methanol extract of *C. infortunatum* has moderate hepatoprotective activity. This effect may be due to the ability of the extract to inhibit lipid peroxidation and increase in the anti-oxidant enzymatic activity.

Keywords: *Clerodendrum infortunatum,* hepatoprotective, anti oxidant, carbon tetrachloride.

INTRODUCTION

Clerodendrum infortunatum Linn. (Family: Verbanaceae), a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiolate, exstipulate, coriacious, hairy leaves with a disagreeable odour (1). Different species of *Clerodendrum* genus have been traditionally used over centuries and their antioxidant and hepatoprotective potential have already been proved (2–6). *Clerodendrum infortunatum* is very common throughout the plains of India, found widely in West Bengal. Various parts of the plant are used by tribes in colic, scorpion sting and snake bite, tumors and certain skin diseases (1). The leaves are slightly bitter, cure inflammation, skin diseases and good in small pox (7). The plant was found to contain triterpenes, steroids and flavonoids (8–11). The antioxidant (12), antimicrobial (13), anti-malarial (14), anthelmintic (15) and analgesic (16) activities of the plant has further created an upsurge in investigations on the plant.

The liver is the key organ of paramount importance and appears to be sensitive target site for substances modulating biotransformation. Carbon tetrachloride (CCl_4) has been widely used in animal models to investigate chemical toxin-induced liver damage. The magnitude of liver damage by disease or hepatotoxins is generally measured by the level of glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin and some pathological characteristics like fatty liver, cirrhosis and necrosis. Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India (17). Herbs play a vital role in the management of various liver disorders (18). Some plant hepatoprotectives are considered as important leads for development of several herbal formulations (19). In the absence of a reliable liver protective drug in the modern medicine, a number of medicinal preparations in Ayurveda are recommended for the treatment of liver disorders (20–21).

Therefore the objective of the present study was designed to evaluate the hepatoprotective potential of methanol extract of *Clerodendrum infortunatum* (MECI) against CCl_4 induced oxidative damage in rats.

EXPERIMENTAL

Reagents and chemicals

Aspartate aminotransferase (GOT), alanine aminotransferase (GPT), alkaline phosphatatse (ALP), total bilirubin, and total protein determination kits were purchased from Span Diagnostics Ltd., Surat, India. All the reagents and solvents used in the study were of analytical grade and commercially available includes 5'-dithio-*bis*-2-nitrobenzoic acid (DTNB) and carbon tetrachloride (Sisco Research Laboratory, Mumbai), trichloro acetic acid, thiobarbituric acid (Loba Chemie, Mumbai).

Plant material and extraction

Fresh leaves of the plant were collected in the month of December, 2005 and identified by Dr. H. J. Chowdhury, Joint Director, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (DKP 02/2005) has been deposited in the laboratory for further reference. After collection the leaves were washed properly and fungal leaves were picked out. Air-dried and powdered leaves (1.5 kg) were extracted successively with petroleum ether (60–80°C) and methanol using Soxhlet apparatus. The solvents were distilled off and evaporated to dryness *in vacuo* to leave the crude methanol extract (95 g). Weighed amount of MECI was suspended in Tween 80 prior to administration.

Phytochemical analysis

Preliminary phytochemical analysis of methanol extract was performed as per the standard protocol described by Khandelwal (22).

Animals

Adult male Wistar albino rats weighing 150-200 g were used for the present investigation. They were housed in clean polypropylene cages and were fed with standard pellet diet and water *ad libitum* with a 12-h light-dark cycle. All the studies were approved by Jadavpur University Animal Ethical Committee, Kolkata, India.

Acute toxicity studies

The LD_{50} value of the methanol extract was calculated according to the methods of Litchfield and Wilcoxon (23). Animals were divided into different groups (10 in each group) and treated with aliquot doses of the extracts orally (100, 200, 300, 400, 500, 550 and 600 mg/kg). The mortality and symptoms of toxicity referred to as CNS behavioural activities were observed and recorded.

Study protocol

Animals were divided into five groups (n=6) and treated as follows. Group I treated as normal group which received liquid paraffin. Group II to group V were treated with CCl_4 in liquid paraffin (1:2) at the dose of 1 ml/kg body weight intraperitoneally once in every 72 h for 14 days. Methanol extract of *C. infortunatum* (MECI) at the doses of 100 mg/kg and 200 mg/kg, b.w. were administered orally to the animals in group three and four daily for 14 days. Group V received silymarin as a standard drug at the dose of 25 mg/kg, b.w. orally for 14 days.

Preparation of serum

Animals were anaesthetized under light ether anesthesia 24 h after the last treatment. The thoracic region was opened to expose the heart. Blood was obtained via cardiac puncture by means of a 5 mL hypodermic syringe and needle and placed in ice-cold 2 mL microcentrifuge tubes. It was allowed to clot and then centrifuged at 5000 rpm for 5 min. The serum samples were collected and left standing on ice until required estimation. Liver tissues were collected for biochemical estimation of different antioxidant enzyme activity and histopathological study.

Biochemical estimations

Serum biochemical parameters like aspartate aminotransferase (GOT), alanine aminotransferase (GPT), alkaline phosphatatse (ALP), total bilirubin and

total protein were determined by using commercially available kits (Span Diagnostic Limited, Surat, India).

Lipid peroxidation

Liver tissues were homogenized in 1.15% KCl and the homogenate were centrifuged at 10000 g at 4 °C for 20 min. From this microsomal fraction, lipid peroxidation in liver was ascertained by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid reactive substance method according to Ohkawa et al (24). The levels of lipid peroxides were expressed as 'n' moles of thiobarbituric acid substances (MDA)/gm of tissue using extinction co-efficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Glutathione content (GSH)

Tissue fragments was thawed and homogenized on ice in 1 mL of homogenizing buffer (250 mM sucrose, 20 mM Tris-HCl, 1mM dithiothreitol, pH 7.4), using glass-teflon homogenizers. The homogenates were centrifuged at 15,000 rpm at 4 °C for 2 h. The reduced glutathione was determined by the method of Ellman (25).

Catalase activity (CAT)

Liver tissue was homogenized in M/150 phosphate buffers in ice and centrifuged at 2000 rpm for 10 min at 4 °C. From the supernatant, Catalase activity was assayed by the method of Aebi (26).

Histological study

Liver tissues were fixed in 10% buffered formaldehyde and processed for histological examination by conventional methods (27) and stained with hematoxylin and eosin dye and finally, observed under a photomicroscope and morphological changes such as cell necrosis, ballooning degeneration, fatty changes or inflammation of lymphocytes were observed.

Statistical analysis

The results were expressed as mean ± S.D. The statistical significance of differences between groups was determined by one way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among groups by using GraphPad Prism4, La Jolla, CA 92037, USA. Differences of $p<0.05$ were considered statistically significant.

RESULT AND DISCUSSION

Preliminary phytochemical analysis of methanol extract of *C. infortunatum* showed the presence of saponins, terpenoids and flavonoids. The acute toxicity of MECI was found to have the LD_{50} value of 450 mg/kg.

Biochemical estimations

The effect of methanol extract of *C. infortunatum* on serum biochemical parameters was shown on Table 1. Administration of CCl_4 alone resulted in significantly (*p<0.01*) increase of all the serum biochemical parameters like aspartate aminotransferase (GOT), alanine aminotransferase (GPT), alkaline phosphatatse (ALP) and total bilirubin. Treatment with MECI at a dose of 100 mg/kg significantly decreased ALT (63.41 IU/L, *p<0.01*), ALP (293.4 IU/L, *p<0.01*) and bilirubin (3.12 IU/L, *p<0.01*) where as AST (304.4 IU/L) was not significantly reduced. Treatment with MECI at 200 mg/kg significantly (*p<0.01*) reduced ALT, ALP, bilirubin and AST (*p<0.05*). Treatment with MECI restored the decreased level of protein caused by CCl_4. The increase of all the serum enzymes and bilirubin indicates the cellular leakages and loss of functional integrity of cell membrane in liver. Decreased level of GOT, GPT, ALP and bilirubin suggested that MECI preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by CCl_4.

MDA levels

Effect of MECI on lipid peroxidation has been shown on Table 2. MDA contents in liver homogenate of CCl_4 treated group (65.35 nM/mg tissue) was significantly (*p<0.01*) increased than the normal rats (25.61 nM/mg tissue). Lipid peroxidation was inhibited with the treatment of MECI

Table 1. Effects of methanol extract *C. infortunatum* (MECI) and silymarin on serum biochemical parameters#.

Biochemical parameters	Normal	CCl_4 1 ml/kg	MECI 100 mg/kg	MECI 200 mg/kg	Silymarin 25 mg/kg	F value
ALT	33.84 ± 4.1*a*	91.76 ± 18.37	63.41 ± 5.02*a*	61.17 ± 4.72*a*	41.34 ± 12.15*a*	27.88
AST	109.94 ± 18.05*a*	372.72 ± 72.95	304.4 ± 18.38^{ns}	297.9 ± 13.95*b*	187.0 ± 53.38*a*	30.32
ALP	120.6 ± 25.53*a*	400.74 ± 34.07	293.4 ± 5.84*a*	278.6 ± 10.16*a*	161.70 ± 21.79*a*	128.8
Total protein	8.39 ± 1.19*a*	4.72 ± 0.54	6.08 ± 0.48*b*	6.12 ± 0.24*b*	7.63 ± 0.47*a*	19.89
Total bilirubin	1.29 ± 0.19*a*	4.66 ± 0.24	3.12 ± 0.16*a*	2.9 ± 0.27*a*	2.12 ± 0.13*a*	180.3

#Values are mean ± s.d. (n = 6)

a $p<0.01$,

b*p<0.05, ns* = not significant

Table 2: Effects of methanol extract of *C. infortunatum* on lipid peroxidation, GSH content and CAT activity*.

Biochemical parameters	Normal	CCl_4 1 ml/kg	MECI 100 mg/kg	MECI 200 mg/kg	Silymarin 25 mg/kg	F value
MDA content (nM/gm tissue)	25.61 ±1.77*a*	65.35 ±1.95	57.02 ±10.69*b*	49.12 ±5.17*a*	44.15 ±2.09*a*	44.24
GSH level (µM/mg tissue)	11.01 ±2.08*a*	7.67 ±1.27	9.08 ±1.33^{ns}	10.04 ±0.55*b*	10.20 ±0.53*a*	4.913
CAT activity (U/mg tissue)	274.4 ±7.81*a*	152.1 ±9.71	201.6 ±11.25*a*	209.9 ±20.38*a*	251.4 ±8.32*a*	73.13

*values are mean ± s.d. (n=5)

[a]*p<0.01,*

[b]*p<0.05, ns* not significant

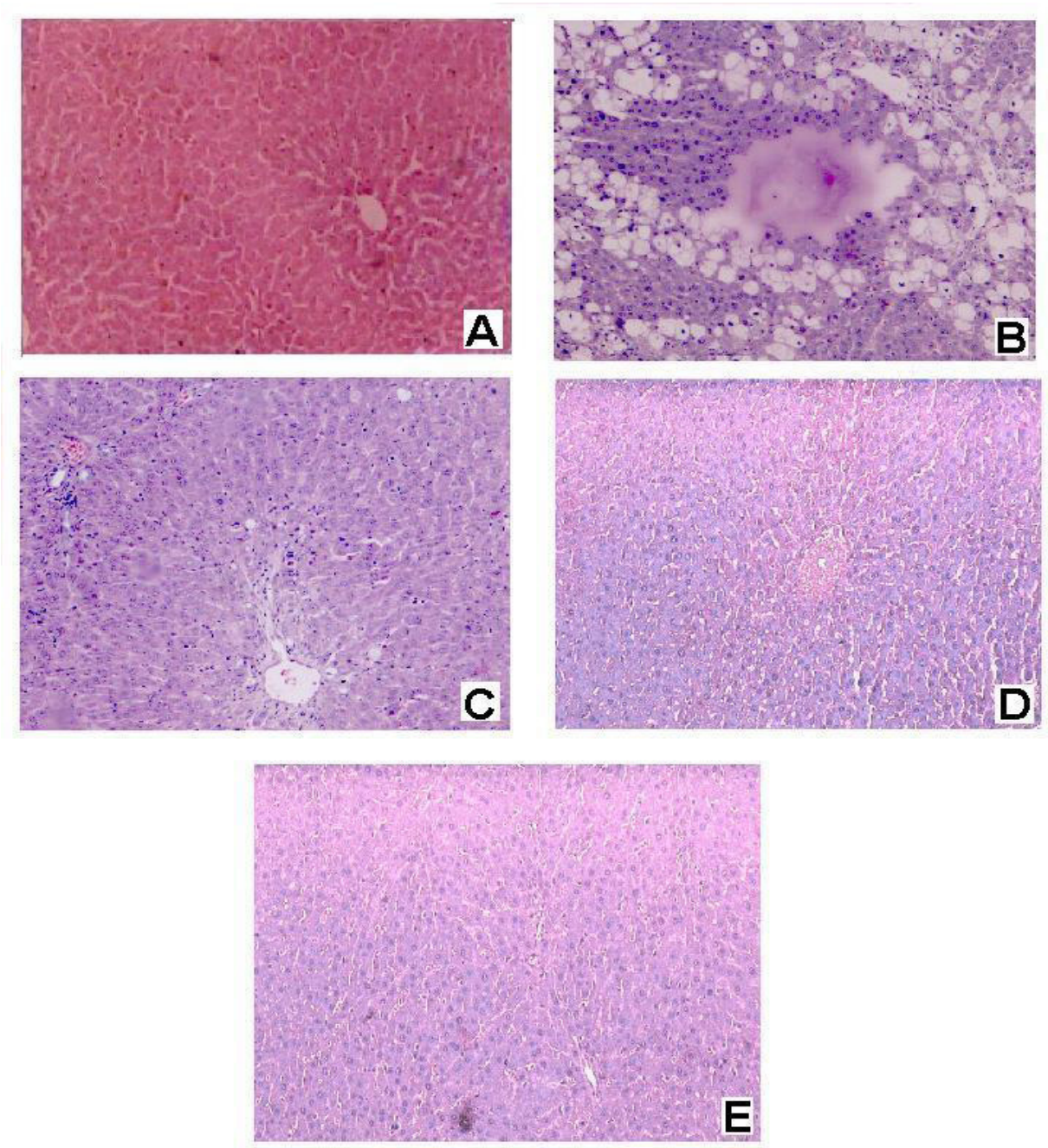

Figure 1: Histopathological examination of liver tissues of different experimental groups (A) normal rats (B) CCl_4 treated rats (C) rats treated with MECI (100 mg/kg) along with CCl_4 (D) rats treated with MECI (200 mg/kg) along with CCl_4 (E) rats treated with silymarin (25 mg/kg) along with CCl_4.

at 100 mg/kg (57.02 nM/mg tissue, $p<0.05$) and MECI at 200 mg/kg (49.12 nM/mg tissue, $p<0.01$). Metabolism of CCl_4 leads to formation of reactive intermediates which induce lipid peroxidation and increase MDA content. It has been shown that MDA is mutagenic to human cells (28) and play a significant role in DNA damage, sister-chromatid exchanges (SCEs) and carcinogenesis (29). The significant reduction in MDA content in animals treated with MECI suggests the protection of liver through its inhibitory action on lipid peroxidation.

GSH content

Table 2 showed the effect of MECI on the GSH content of liver homogenate. Treatment with CCl_4 alone was significantly ($p<0.01$) reduced the total GSH content of liver homogenate (7.67 µM/mg tissue) compared to normal rats (11.01 µM/mg tissue). GSH content was significantly ($p<0.05$) increased on treatment with MECI at 200 mg/kg (10.04 µM/mg tissue). Glutathione can be considered as an important reductant and protects cells against free radicals, peroxides and other toxic compounds. Liver injury by consuming alcohol (30) or by taking drugs like acetaminophen (31), lung injury by smoking (32) and muscle injury by intense physical activities (33) are all known to be correlated with low tissue levels of GSH. The GSH reduction in control group may be explained by increased utilization of GSH for removal of ROS. In our experiment MECI, prevents the depletion of GSH which caused detoxification of free radicals produced following carbon tetrachloride intoxification.

CAT activity

CAT activities of liver homogenate of rats for experimental groups are shown on table 2. The CAT activity in the liver tissue homogenates of CCl_4 treated rats (152.1 U/mg tissue) was significantly ($p<0.01$) lower than that of normal rats (274.4 U/mg tissue). In the MECI and silymarin treated rats the CAT activity was significantly ($p<0.01$) higher than the CCl_4 treated rats. CAT is the key component of antioxidant defense system, which decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Increase in CAT activity by MECI suggesting the improvement of enzymatic defense system against free radicals.

Histological studies

The hepatoprotective activity of MECI was supported by histological assessment, in which the normal rats did not showed any abnormal state in the liver architecture (Fig. 1A). Rats treated with CCl_4 showed excess fatty generation and nodules formation (Fig. 1B), whereas, the MECI and silymarin treated group showed reduction in all the abnormal architecture (Fig. 1C, 1D,1E). So, hepatoprotective activity also can be explained on the basis of histological examination.

CONCLUSION

On the basis of the experimental results, it can be concluded that *Clerodendrum infortunatum* have moderate hepatoprotective activity. The hepatoprotective effect may be due to inhibition of lipid peroxidation and increasing the activity the content of enzymatic defense system, which causes the recuperation of biological parameters and the integrity of the tissue. The hepatoprotective activity of methanol extract of *C. infortunatum* may be due to presence of flavonoids, terpenoids and saponins in the extract. The present results can be accounted for the traditional uses of the plant in treating some common diseases.

REFERENCES

1. Nadkarni K.M. and Nadkarni A.K. *Indian Materia Medica*, (Popular Publications, Mumbai: 2002) 353.
2. Gopal N. and Sengottuvelu S. Hepatoprotective activity of *Clerodendrum inerme* against CCl_4 induced hepatic injury in rats. *Fitoterapia*. **79**: 24–26 (2008).
3. Vidya S.M., Krishna V., Manjunatha B.K., Mankani K.L., Ahmed M. and Singh S.D. Evaluation of hepatoprotective activity of *Clerodendrum serratum* L. *Indian J Exp Biol*. **45**: 538–42 (2007).
4. Chae S., Kim J.S., Kang K.A., Bu H.D., Lee Y., Seo Y.R., Hyun J.W. and Kang S.S. Antioxidant activity of isoacteoside from *Clerodendron trichotomum*. *J Toxicol Environ Health A*. **68**: 389–400 (2005).
5. Chae S., Kim J.S., Kang K.A., Bu H.D., Lee Y., Hyun J.W. and Kang S.S. Antioxidant activity of Jionoside D from *Clerodendron trichotomum*. *Biol Pharm Bull*. **27**: 1504–08 (2004).
6. Rajlakshmi D., Banerjee S.K., Sood S. and Maulik S.K. In-vitro and in-vivo antioxidant activity of different extracts of the leaves of *Clerodendron colebrookianum* Walp in the rat. *J Pharm Pharmacol*. **55**: 1681–86 (2003).
7. Chopra R.N., Nayer S.L. and Chopra I.C. *Glossay of Indian medicinal plant*. (Publication and Information Directorate, CSIR, New Delhi, 1992) 71.
8. Joshi K.C., Prakash L. and Shah R.K. Chemical constituents of *Clerodendrum infortunatum* and *Ficus racemosa*. *J Indian Chem Soc*. **54**: 1104–06 (1977).
9. Akihisa T., Matsubara Y., Ghosh P., Thakur S., Shimizu N., Tamura T. and Matsumoto T. The 24α- and 24β-epimers of 24-ethylcholesta-5,22-dien-3β-ol in two Clerodendrum species. *Phytochemistry*. **27**: 1169–72 (1988).
10. Sinha N.K., Seth K.K., Pandey V.B., Dasgupta B. and Shah A. Flavonoids from the flower of *Clerodendrum infortunatum*. *Planta Med*. **42**: 296–98 (1981).
11. Manzoor-Khuda M. and Sarela S. Constituents of *Clerodendron infortunatum* (bhat)-I. Isolation of clerodolone, clerodone, clerodol and clerosterol. *Tetrahedron*. **21**: 797–02 (1965).
12. Sannigrahi S., Mazumder U.K., Pal D.K. and Parida S. *In vitro* anti oxidant activity of methanol extract of *Clerodendrum infortunatum* Linn. *Oriental Pharm Exp Med*. **9**: 128–34 (2009).
13. Rajakaruna N., Harris C.S. and Towers G.H.N. Antimicrobial activity of plants collected from serpentine outcrops in Srilanka. *Pharm Biol*. **40**: 235–44 (2002).
14. Goswami A., Dixit V.K. and Srivastava B.K. Anti-malarial activity of aqueous extract of *Clerodendrum infortunatum*. *Bionature*. **48**: 45–48 (1998).
15. Pal D.K., Sannigrahi S. and Dutta A. Anthelmintic activity of the leaves of *Clerodendrum infortunatum*. *Indian J Nat Prod*. **23**: 22–25 (2007).

16. Pal D.K., Sannigrahi S. and Mazumder U.K. Analgesic and anticonvulsant effects of saponin isolated from the leaves of *Clerodendrum infortunatum* Linn. in mice. *Indian J Exp Biol.* **47**: 743–47 (2009).
17. Handa S.S., Sharma A. and Chakroborti K.K. Natural products and plants as liver protecting drugs. *Fitoterapia.* **57**: 307–45 (1986).
18. Dhiman R.K. and Chawla Y.K. Herbal Medicines for liver disorders. *Dig Dis Sci.* **50**, 1807–12 (2005).
19. Negi A.S., Kumar J.K., Luqman S., Shanker K., Gupta M.M. and Khanuja S.P.S. Recent advances in plant hepatoprotectives: A chemical and biological profile of some important leads. *Med Res Rev.* **28**: 746–72 (2008).
20. Chatterjee T.K. Medicinal Plants with Hepatoprotective Properties in Herbal Options, Vol **III**. (Books and Allied (P) Ltd., Calcutta, 2000) 135.
21. Strader D.B., Bacon B.R., Lindsay K.L., La Brecque D.R., Morgan T., Wright E.C., Allen J., Khokar M.F., Hoofnagle J.H. and Seeff L.B. Use of complementary and alternative medicine in patients with liver diseases. *Am J Gastroenterol.* **97**: 2391–97 (2002).
22. Khandelwal K.R. Practical Pharmacognosy Techniques and Experiments, 9th ed, (Nirali Prakashan, Pune, 2000) 75.
23. Litchfield J.T. and Wilcoxon F.A. Simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther.* **96**: 99–113 (1949).
24. Okhawa H., Oishi N. and Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem.* **95**: 351–58 (1979).
25. Ellman G.L. Tissue sulphydril groups. *Arch Biochem Biophys.* **82**: 70–77 (1959).
26. Aebi H. Catalase. In: Bergmeyer H.U. ed. *Methods of enzymatic analysis*, vol. **2**. (Chemic Academic Press Inc, Verlag, 1974) 673–85.
27. Karaki Y., Munakata S., Saeki T., Hirota S. and Fujimaki M. Appearance of a carcinoid-like pattern in rat hepatic tumors induced by 3-methyl-4-dimethyl-aminoazobenzene. *Japanese J Cancer Res.* **82**: 397–02 (1991).
28. Niedernhofer L.J., Daniels J.S., Rouzer C.A., Greene R.E. and Marnett L.J. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem.* **278**: 31426–33 (2003).
29. Ray G.M., Shahid M. and Husain A. Effect of nitric oxide and malondialdehyde on sister-chromatid exchanges in breast cancer. *British J Biomed Sci.* **58**: 169–76 (2001).
30. Carmiel-Haggai M., Cederbaum A.I. and Nieto N. Binge ethanol exposure increases liver injury in obese rats. *Gastroenterol.* **125**: 1818–33 (2003).
31. Richie J.P., Lang C.A. and Chen T.S. Acetaminophen-induced depletion of glutathione and cysteine in the aging mouse kidney. *Biochem Pharmacol* **44**: 129–35 (1992).
32. Kluchova Z. and Tkacova R. The role of oxidative stress in lung injury induced by cigarette smoke. *Biologia.* **61**; 643–50 (2006).
33. Margonis K., Fatouros I.G., Jamurtas A.Z., Nikolaidis M.G., Douroudos I., Chatzinikolaou A., Mitrakou A., Mastorakos G., Papassotiriou I., Taxildaris K. and Kouretas D. Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. *Free Rad Biol Med.* **43**: 901–910 (2007).