Biosciences, Biotechnology Research Asia Vol. **5**(2), 783-787 (2008)

The antioxidant effect of ethanolic bark extract of *Ougeinia oojeinensis* (Roxb.) Hochr on CCl_4 induced liver damage

R.K. SAHU[1]*, U. SHARMA[1], A. ROY[1], D. DEWANGAN[2] and K.P. NAMDEO[3]

[1]Department of Pharmacognosy, Oriental College of Pharmacy, Bhopal (India).
[2]Aristo Pharmaceutical Pvt. Ltd., Mandideep, Raisen (India).
[3]Smt. S.L.T. Institute of Pharmaceutical Sciences, Bilaspur (India).

(Received: August 04, 2008; Accepted: September 24, 2008)

ABSTRACT

In this paper, the antioxidant activity of ethanolic bark extract of *Ougeinia oojeinensis* (Roxb.) Hochr. on the CCl_4-induced liver damage in rats, is reported. In hepatotoxic rats, liver damage was studied by assessing parameters such as lipid peroxidation levels, catalase activity, glutathione peroxidase activity and superoxide dismutase activities were employed as biomarkers of liver damage, under control condition and after the administration of 100 mg/kg and 200 mg/kg, respectively. Our result suggests that the ethanolic bark extract of *O. oojeinensis* showed a significant antioxidant activity. Superoxide dismutase, catalase and glutathione peroxidase activities were increased, whereas lipid peroxidation is significantly decreased in the ethanolic bark extract-treated group, in comparison to the CCl_4 group. Hence, the ethanolic bark extract, at the aforementioned doses, showed significant protection under CCl_4-induced hepatocellular injury.

Key words: Carbon tetrachloride, marker enzymes, *Ougeinia oojeinensis.*

INTRODUCTION

Antioxidants or inhibitors of oxidation are compounds, which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. The reactive oxygen species (ROS) in the body include superoxide anion, singlet oxygen, hydroxyl radical and hydrogen peroxide. The oxidative damage initiated by these is propagated by lipid peroxidation which may cause further damage to DNA. The body's defence system against the oxidation damage consists of enzymes such as SOD, GPx, CAT and the reducing agent such as glutathions ascorbate and iron. Various theories have been reported to explain the role of antioxidants in treating diseases. In general, circulating ROS tend to react with electrons of another molecule found in the body, affecting several enzyme systems, and cause damage, which may contribute in the development of processes such as cancer, ischemia, aging, adult respiratory distress syndrome, rheumatoid arthritis, among others. Antioxidant nutreaceuticals are those which contain vitamin E, vitamin C, vitamin A and beta – carotene[1].

Ougeinia oojeinensis (Roxb.) Hochr (Fabaceae) known in Hindi as Tinsa and in Sanskrit as Ratha is a deciduous trees, found in the outer Himalayas and sub- Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India into the greater part of Deccan peninsula[2]. The extract of the whole plant *O. oojeinensis* were scientifically evaluated for anti-inflammatory and analgesic activities. A 50% alcoholic extract of stem bark of

O. oojeinensis also showed antispasmodic activity[3]. Further phytochemical investigation showed the presence of lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin, homoferreirin and ougenin (4-6). Naturally-derived antioxidants have gained special interest because of their protection from free radicals. Therefore our aim in this study was to evaluate the free radical scavenging activity of ethanolic bark extract of *O. oojeinensis.*

MATERIAL AND METHODS

Plant material

The barks of O. oojeinensis were collected from the Lamber forest Raipur, Chhattisgarh in the month of May. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The plant material was also compared with a herbarium specimen maintained at Minor Forest Produce (Trading and Development) Co-Op. Fed. Ltd., Shankar Nagar, Raipur, Chhattisgarh, by Expert Medicinal Plant, Mr. S.N.Khotele.

Preparation of extracts

The dried and powdered bark (300 gm) was successively extracted on a Soxhlet apparatus, employing petroleum ether, chloroform, ethyl acetate, ethanol and distilled water respectively. The extracts were further concentrated under reduced pressure with a rotary evaporator. Bark of *O. oojeinensis* yielded 0.62%, 0.45%, 0.57%, 4.50% and 3.7% w/w powdered extract with petroleum ether, chloroform, ethyl acetate, ethanol and distilled water respectively. Suspension of ethanolic extract was prepared using 5% CMC and were divided in two doses 100 mg/kg and 200 mg/kg and subjected for hepatoprotective activity under CCl_4-induced hepatotoxicity.

Animals

For this study, male Wistar albino rats (180-230 g) were employed throughout. They were obtained from the animal facility of Shree Venkateshwara Enterprises, Bangalore and quarantined for 10 days under standard conditions of temperature (27.3°C, 65±10% of relative humidity) and light (12-h light/dark cycle), and fed a standard diet and tape water *ad libitum*. All animals experiments had been approved by our institutional committee (reg. no. CPCSEA/265).

Antioxidant evaluation

Antioxidant activity was evaluated on male Wistar albino rats. Animals were randomly allocated into five groups of six animals each. Group I was regarded as the control group, receiving 5% CMC (10 ml/kg). All other groups received CCl_4 (3 ml/kg, i.p.) mixed with an equal volume of olive oil (50%, by volume). Animals from group III were treated with suspension of the ethanolic extract (100 ml/kg/day, p.o. during seven days), whereas animals from group IV were treated (200 mg/kg/day, p.o. during seven days). Animals from group V received silymarin (25 mg/kg) as the reference drug. After treatment, all animals were sacrificed by cervical dislocation. Further on, the liver was quickly removed and fixed in a 10% buffered neutral formalin solution and stored under refrigeration.

Liver homogenates preparation and biochemical estimation

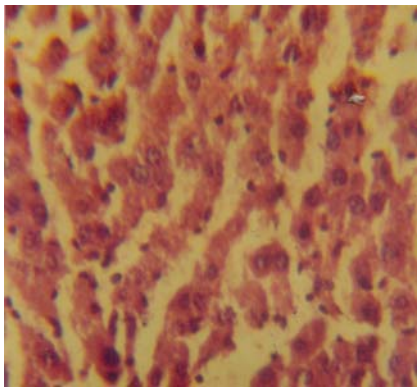
Frozen liver samples were homogenized (with a Potter-Elvehjem homogenizer) in a Tris-HCl buffer or in a phosphate buffer solution (PBS) to give a 20% homogenate. To evaluate levels of lipid peroxidation, the homogenate was centrifuged at 1700 rpm/min for 10 min at 4°C. To assess catalase activity, the homogenate was centrifuged at 3500 rpm/min for 15 min at 4°C and then diluted up to 5%. Further on, the supernatant was again centrifuged either at 10,000 rpm/min for 1 min and diluted to 2% for measurement of glutathione peroxidase activity or at 30,000 rpm/min for 10 min before extraction of tissue superoxide dismutase activity with 20% ethanol[7-11].

Liver histopathology

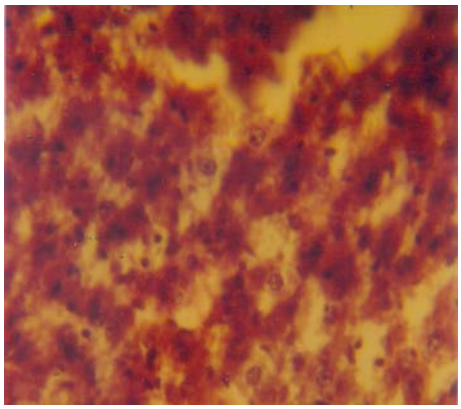
Liver were excised quickly fixed in a 10% buffered neutral formalin and proceeded for histopathology, they were processed for paraffin embedding following the standard microtechnique. Sections of liver stained with alum-haematoxylin and eosin were observed microscopically for histopathological changes. A few photomicrographs of representative types were also taken.

Statistical analysis

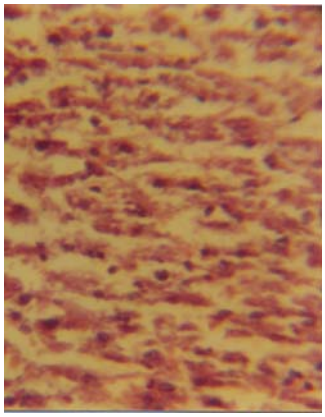
The results are expressed as mean ± SEM



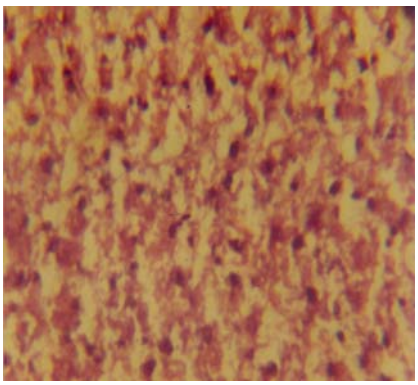
a. Section of Normal Rat Liver showing normal liver architecture



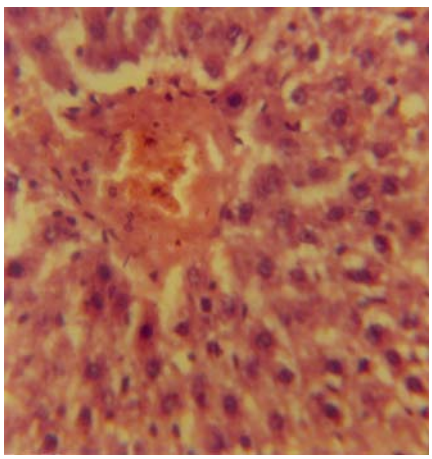
b. Liver section from CCl_4 treated rat showing focal necrosis, portal infiltration, and fatty changes



c. Liver section from CCl_4 + ethanoloic extract (100 mg/kg) treated rats showing more severe form of injury is markedly prevented.



(d) Liver section from CCl_4 + ethanoloic extract (200 mg/kg) treated rats showing the necrosis is markedly prevented



(E) Liver section from CCl_4 + silymarin (25 mg/kg) treated rats showing ballooning degeneration and necrosis is markedly prevented

Fig. 1: Photomicrographs of liver section of rat stained with haematoxylin and eosin.

of six independent experiments. Statistical significance between group was evaluated by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test. A $P < 0.01$ value was considered as statistically significant.

RESULTS

The LD_{50} of the ethanolic bark extract as per OECD guidelines – 420 is greater then 2000 mg/kg. Results from the antioxidant evaluation are shown in Table 1. as it can be observed, administration of the ethanol bark extract of *O. oojeinensis* counteracted the CCl_4-induced free radical activity, which resembles that of silymarin. SOD, GPx and CAT enzyme levels are statistically significant increased, whereas lipid peroxidation is decreased, when compared to CCl_4 condition ($P < 0.01$). These results suggest that the ethanolic extract displays an antioxidant activity. The histopathological study from liver from group I (Fig. 1a) showed a normal hepatic structure. Under CCl_4 conditions (Fig. 1b), it can be observed that the administration of this noxious agent produces focal necrosis, portal infiltration, fatty changes, Kupfer's cells hyperplasia and hydropic changes. After the administration of 100 mg/kg of the ethanol bark extract of *O. oojeinensis* (Fig. 1c), it is observed that necrosis and fatty change are prevented, a situation that is also observed after the administration of an oral dose of 200 mg/kg (Fig. 1d). Under silymarin conditions (Fig. 1e), it is observed that ballooning degeneration and fatty changes associated to hepatocyte necrosis, are prevented.

Table 1: Antioxidant activity of ethanolic bark extract of *Ougeinia oojeinensis*on CCl_4 induced liver damage in rats

Treatment	Catalase(mg liver protein) $^{-1}$	Superoxide dismutase (mg liver protein) $^{-1}$	Glutathione Peroxide (mg liver protein) $^{-1}$	TBA(mg/liver protein)
Normal Control CMC10ml/kg	297.07±18.1	77.91±6.21	0.961±0.030	1.27±0.389
$CCl_4$1ml/kg i.p	165.75*±6.78	32.31*±0.67	0.747*±0.053	1.79*±0.14
Ethanolic Extract (100 mg/kg oral)	230.34**±2.57	73.50**±3.44	0.848**±0.037	1.24**±0.11
Ethanolic Extract (200mg/kg oral)	286.47**±3.51	85.40**±0.94	0.975**±0.062	1.25**±0.08
Silymarin(25 mg/ kg)	271.3**±19.3	74.3**±7.2	0.96**±0.05	1.26**±0.04
One-way F	150.3	152.6	161.48	168.91
ANOVA df	4, 25	4, 25	4, 25	4, 25
P	0.01	0.01	0.01	0.01

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.01 compared to control group, **P<0.01 compared to CCL_4 treated group.

DISCUSSION

Liver participate in several metabolic activities, and in order to fulfill this role, release a wide variety of enzymes. Liver can be injured by many toxicants, as well as by chemicals or drugs. In our model, CCl_4 serves as a toxicant. CCl_4-related hepatotoxicity is associated with elevation in enzyme levels, which may be attributed to the generation of trichloromethyl free radical during metabolism by the hepatic microsomes, which in turn begin lipid peroxidation. Hepatocellular necrosis decreases SOD, CAT and GPx activities, and the increase of such activities into basal values, is a clear indication of plasma membrane stabilization and tissue repair as well. Such an effect is in agreement with the

view that enzyme activities are restored into normal conditions and healing of the hepatic parenchyma, as well as hepatocyte regeneration, are observed. SOD, CAT, and GPx constitute an enzyme defense mechanism against oxidative damage. Under CCl_4 conditions such enzyme activities are decreased, but under plant-treated conditions, a significant increase in their activities is observed, which may serve as a biochemical strategy to reduce lipid peroxidation. The present study revealed that the ethanolic extract under evaluation, at both studies doses, showed a hepatoprotective activity against CCl_4-induced liver damage.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. A. Jaswanth, Department of Pharmacology, Perriyar College of pharmaceutical sciences for girls, Tirchy for providing the entire lab facilities timely and guidance for carrying out pharmacological studies and are thankful to our own college principle for providing necessary facilities to complete this research work successfully in the department of pharmacognocy and pharmacology. And are also thankful Dr. P. Siddheswaran PhD, research facilitator PG studies, for his scientific and invaluable guidance.

REFERENCES

1. Kokate, C.K., Purohit, A.P. and Gokhale, A.P., Pharmacognosy, 18th edition. Nirali Prakashan, 99-100: 540-541 (2002).
2. Kirtikar and Basu, Indian Medicinal Plant, Vol. I, Dehradun, 756 (1998).
3. Khare, C.P., Encyclopedia of Indian Medicinal Plants. Springer, 343 (2004).
4. Mukherjee, D.K., Barua, A.K., and Bose, P.K., "Chemical Investigation of Ougeinia dalbergioides Benth." *Science and Culture*, **29:** 151-152 (1963).
5. Ghosh, A.C. and Dutta, N.L., "Chemical Investigation of Ougeinia dalbergioides Benth." *Journal of Indian Chemical Society,* **42**(12): 831– 835 (1965).
6. Balakrishna, S., Ramanathan, J.D., Seshadri, T.R., and Venkataramani, B., "Special Chemical Components of the Heartwood of Ougeinia dalbergioides Benth." *Proc. Royal Society London,* **1962**: 268A (1965).
7. Draper, H.H., and Hadley, M., "Malondialdehyde Determination as Index of Lipid Peroxidation. Methods." *Enzymol,* **186:** 421-431 (1990).
8. Kakkar, P., Das, B., and Viswanathan, P.N., "A Modified Spectrophometric Assay of Superoxide Dismutase." *Ind. J. Biochem. Biophys*, **21**: 131-132 (1984).
9. Rotruck, J.T., Pope, A.L., Ganther, H.F., Swanson, A.B., Hafeman, D.G., and Heksta, W.G., "Biochemical Role as a Component of Glutathione Peroxidase." *Science,* **179**: 585-95 (1973).
10. Oogawa, M., "Differentiation and Proliferation of Haemopoietic Stem Cell Regulators." *Science,* **18**: 28-84 (1993).
11. Donald, J. Ecobicson., "Fixed Dose Procedure. Guidline 420." The Basis of Toxicity Testing. 2nd edition, *CRC Press* 43 (1997).