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Radical scavenging activity of *Trianthema triquetra* in male albino rats intoxicated with CCl_4

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Abstract: *Radical scavenging activity of ethanolic extract of Trianthema triquetra root was investigated against CCl_4 in rats. The rats were treated with T. triquetra (100 mg, 200 mg/kg b.w.) for a period of 7 days. Antihepatotoxic effect was studied by assaying the activities of thiobarbituric acid (TBARS), reduced glutathione (GSH), glutathione peroxidase (GPx),catalase (CAT), super oxide dismutase (SOD) and vitamin C (Vit. C). Lipid peroxidation is evidenced by an increase in the value of TBARS and also a distinct diminution in the level of GSH, Vit. C at 200 mg/kg b.w. The activity of antioxidant enzymes, such as GPx, CAT, SOD and Vit. E is significantly recovered towards an almost normal level in animals coadministrated with T. triquetra. The maximum protection against CCl_4 induced hepatic injury was afforded by the dose of 200 mg/kg b.w. of Trianthema triquetra.*

Key words: *Trianthema triquetra, Liver, CCl_4 toxicity, Lipid peroxidation, Antioxidant enzymes*

Introduction

Liver plays a major role in detoxification of many endogenous and exogenous compounds and any injury to it may lead to many complications. Liver injury is a well recognized toxicological problem (Reilly *et al.,* 1991) and it may follow the inhalation, ingestion or parietal administration of a number of chemical agents such as CCl_4. It is a widely used chemical to induce liver damage in experimental animals and its toxicity has been studied extensively. The resulting injury was characterized by leakage of cellular enzymes into the blood stream due to centrilobular necrosis (Recknagel *et al.*, 1989; Muriel *et al.*, 2001). Management of hepatic disorders has become a matter of serious concern world wide and several laboratories are now engaged in identifying an effective hepatoprotective drug from plant sources.

A number of plants have been shown to possess hepatoprotective property. Indian medicinal plants belonging to about 40 families were investigated as liver protective durgs (Handa, 1986).To mention,extracts of *Andrographis paniculata* (Neha and Rawal, 2001*) and Trichopus zeylanicus* (Subramaniam *et al.,*1998) reduce liver injury. However, there is no satisfactory remedy for serious liver diseases and search for effective hepatoprotective drug continues. The present study was undertaken to understand the radical scavenging activity of ethanolic extracts of *Trianthema triquetra* (Family: Azioceal) in the CCl_4 intoxicated swiss albino rats.

Materials and Methods

The root of *T. triquetra* were collected locally, cleaned, dried in shade, pulverized and subjected to soxhlet extraction successively with ethanol. The extracts were concentrated under reduced pressure and controlled temperature (50-60°C). Experimental doses of the extract were prepared by dissolving it in liquid paraffin. Male albino wistar rats (110-130 g) were obtained from the Indian Institute of Science, Bangalore and fed with standard feed and water *ad libitum*. The rats were divided into four groups of six animals each. The first group served as control and was given liquid paraffin (carrier) only. The second group received intraperitonial administration of CCl_4 (1 ml/kg body weight) + liquid paraffin on alternate days for a week. In third group, CCl_4 was administered for 2 consecutive days and 24 hr following the last injection, the treatment was started by the oral administration of ethanol extract of *Trianthema triquetra* at a dose of 100 mg /kg/ b.w. for 7 days. In fourth group, 24 hr following the last injection, treatment was started by the oral administration of ethanolic extract of *T. triquetra* (200 mg/kg b.w.) for 7 days. After the completion of experimental regime, the rats were starved overnight and blood samples were collected by puncturing the retro-orbital pluxes under light ether anesthesia. Serum was prepared by centrifugation at 3000 rpm in cold for further assays.

Results and Discussion

The present study was designed primarily to evaluate the radical scavenging activity of *T.triquetra* in male albino rats intoxicated with CCl_4. In this study, thiobarbuturic acid subtances level, the activities of the antioxidant enzymes namely superoxide dismutase, catalase, reduced glutathione and the non enzymatic antioxidants *viz.* glutathione reductase, Vit. C and Vit. E were determined in the serum sample of CCl_4 and ethanolic extract of *T.triquetra* rats. The results clearly pinpoints the radical scavenging activity of ethanolic extract of *T.triquetra* in male albino rats. Administration of CCl_4 caused significant decrease in the activity of serum enzyme such as SOD,CAT,GSH,GPx, Vit. E and Vit. C (Table 1) and a significant increase was observed in TBARS concentration after CCl_4 administration. CCl_4 induced chronic liver injury was recovered by the cojoint treatment with ethanolic extract of *T. triquetra* orally at 200 mg/kg/b.w. A

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Table - 1: Effects of *T.triquetra* on TBARS, GSH, $GP_{X,}$ SOD ,CAT,Vit E and Vit.C level

S.No	Particulars	Group I	Group II	Group III	Group IV
1	TBARS(µg/dl)	1762.1±157.6	2995.5±1576***	1585.9±157.6***	1879.6±90.98***
2	GSH(µg/dl)	914±108.5	349.8±108.3***	979.5±108.3***	914.5±108.5***
3	GPx(µmol/min)	55.9±3.09	15.9±9.58***	57.9±3.09***	59.96±5.77***
4	SOD(NBT/min)	627.3±11.03	106.4±10.4***	510.6±10.4***	625.3±11.01***
5	CAT(µmol/min)	55111.1±689.0	38666.6±1192.5***	46222.2±1821.6***	4711.1±1821.6***
6	Vit E(mg/dl)	2320.2±132.94	1241.8±133.9***	1896.4±50.6***	2058.8±87.6***
7	Vit C(mg/dl)	4375±559.01	2083.3±322.7***	5625±559.01***	4583.8±559.0***

Values are expressed as mean ± S.D for 6 rats in each group (Group I, Group II, Group III and Group IV). Significantly different from normal (Student 't' followed). ***p<0.001

remarkable recovery was seen in the decreased level of TBARS and increased level of GSH,GPx, SOD, CAT, Vit. C and Vit. E after *T.triquetra* treated rats.

Liver disease is known to be associated with impaired hepatic drug metabolizing capacity and impaired activity of various hepatic enzymes (Joglekar *et al*., 1963; Srinivasan *et al*., 1968). Free radicals are constantly formed in the human body and are removed by the antioxidant defense system (Hallivell, 1999). In any hepatic disease and injury, biochemical alterations are the immediate responses in the body. The antioxidant system is of vital importance under this environment in an organism's defense against oxidative stress. Hence variations in antioxidant system provide the basis of liver damage and thus this study relies on TBARS, GSH, SOD,CAT, GPx, Vit. E and Vit. C in CCl_4 toxicity.

Melnodialdehyde is the major reactive aldehyde resulting from the per oxidation of biological membrane polyunsaturated fatty acids (Vaca *et a*l., 1988) and it is a secondary product of lipid peroxidation (LPO) which is used as an indicator of tissue damage by a series of chain reactions (Ohkawa *et al.,*1979). Liver TBARS level is considered as valuable indicator of toxicant induced hepatic damage from production of free radicals. Elevated level of TBARS in liver of group II (CCl_4 treated) rats is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system. The significant decline in the concentration of TBARS in *T. triquetra* administrated rats unveils antioxidant efficacy of it.

GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of GSH has been shown to be associated with an enhanced toxicity to chemicals including CCl_4. In this study, decline in activities of GSH and glutathione peroxidase levels in CCl_4 administrated rats and recovery to near normality in *T. triquetra* treated rats revealed that oxidative stress elicited by CCl_4 intoxication has been nullified due to antioxidant the effect of *T. triquetra.*

SOD act as scanvengers of free radicals and reduce the toxicity of oxygen. Tissues are protected from superoxides by the specific enzyme superoxide dismutase (Flescher *et al.,*1998). Catalase catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by $H_2O_2$and OH (Tolbert, 1981). Decreased activity of SOD and catalase in CCl_4 treated (group II) rat serum may increase their susceptibility to oxidative injury. However, the over expression of the antioxidant molecules with ethanol extract of *T. triquetra* is indicative of their ability to reactive hepatocellular antioxidant defense in the liver.

Vit. E and Vit. C are the major free radical scavengers of the hepatic cells and Vit. E is a major lipid soluble antioxidant, a critical component of antioxidant system in all body tissues. Currently however, Vit. E is recognized as potent chain breaking antioxidant and prevent lipid peroxidation (Gey, 1987). In our study, CCl_4 treated rats showed significant decrease in the activity of Vit. E and Vit. C as compared to group I, which indicates defect in glutathione dependent systems. The restoration of Vit. E level in *T. triquetra* treated rats may be due to the increase in the level of GSH which in turn help in the regeneration of Vit. E and Vit. C inactive metabolites.

The present study indicates that administration of *T. triquetra* extract to CCl_4 treated rats reduced the enhanced levels of lipid peroxidation, superoxide and hydroxyl radicals and reduced the harmful effects caused by free radical mediated toxicity.

In conclusion, the results of the present study indicate that ethanolic extract of *T. triquetra* have radical scavenging effect against CCl_4 induced liver damage and the mechanism against its action can be worked out in the future studies.

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