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Full Length Research Paper

Evaluation of antioxidant potential of *Ficus religiosa* (Linn.) roots against carbon tetrachloride-induced liver injury

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Antioxidant activity of the aqueous (FRWE) and alcoholic extract (FRAE) of *Ficus religiosa* roots was evaluated in rats by inducing liver injury with carbon tetrachloride:olive oil (1:1). The extract possessed remarkable antioxidant activity showing increased levels of glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GRD), superoxide dismutase (SOD) and catalase (CAT) and decreased level of lipid peroxidation (LPO). *F. religiosa* root extracts, FRWE and FRAE, at a dose level of 500 mg/kg showed significant antioxidant activity against carbon tetrachloride-induced liver injury in rats.

Key words: Superoxide dismutase, catalase, marker enzymes in liver, antioxidant activity.

INTRODUCTION

Ficus religiosa (Family: Moraceae) is a large glabrous tree found throughout India including, Sub-Himalayan forests, Bengal and Central India. All parts are used in diseases of the blood, vagina and uterus. Root-exude cures sex-debility, fruit in diseases of the blood and heart, root in gout, root-bark in leucorrhoea, aphrodisiac, seed in urinary discharges, leaves as antiemetic, fruits as purgative (Kirtikar and Basu, 2005; Guha and Sensharma, 2001; Asolkar et al., 1992; Anonymous, 1956). So, based on these reports, the present study has been undertaken to evaluate the antioxidant potential of aqueous (FRWE) and alcoholic extract (FRAE) of the roots of *F. religiosa* in carbon tetrachloride intoxicated liver injury.

MATERIALS AND METHODS

Drugs and chemicals

Reduced glutathione (GSH), nicotine adenine dinucleotide phosphate, thiobarbituric acid (Sisco Research Lab., Mumbai), 5,5“-dithio-bis-2-nitrobenzoic acid, epinephrine (Sigma-Aldrich USA), ethylenediamine-tetra-acetic acid, hydrogen peroxide (Qualigens Fine Chemicals, Mumbai), 1-Chloro-2,4-dinitrobenzene (S.D. Fine Chemicals Ltd, Mumbai) were used for the study. All other reagents used were of analytical grade.

Plant material

The fresh roots of *F. religiosa* were collected from District Sirsa, Haryana (India) during April, 2008 and the plant was identified and authenticated by Dr. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard (Hamdard University), New Delhi (India). The voucher specimen (VFR/8) has been retained in the pharmacognosy Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana (India).

Plant extraction

The freshly collected roots of the plant were cut into small pieces, shade dried and coarsely powdered. The dried powder was then successively extracted with alcohol using soxhlet extractor and distilled water by maceration. The alcoholic and aqueous extracts were dried under reduced pressure using a rotary evaporator and were kept under refrigeration. Both the extracts were administered to the animals as a suspension in propylene glycol.

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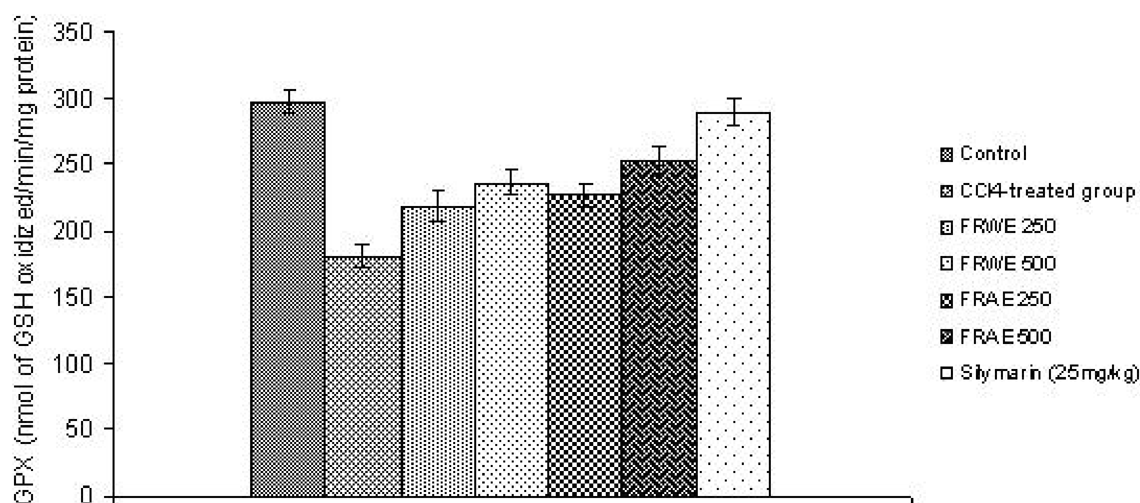

Figure 1. Effects of FRWE and FRAE on hepatic gluthione peroxidise levels in CCL_4 treated rat.

Experimental animals

Adult Wistar rats (120 to 200 g) of either sex (CCL Haryana Agriculture University, Hisar) were housed in polypropylene cages in well ventilated and maintained room temperature with natural day-night cycle. The animals were allowed free access to food (standard pellet feed) and water. They were allowed an acclimation period of seven days before the study. The study protocol was approved by the IAEC (Institutional animal ethics committee) of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India.

Pharmacological activities

Antioxidant studies

The adult Wistar rats were divided into 7 groups of six animals each. Group I received only propyleneglycol (5 ml/kg per day p.o.) for seven days and served as control. Group II animals received single dose of equal mixture of carbon tetrachloride and olive oil (50% v/v, 5 ml/kg i.p.) on the seventh day. Group III and IV animals were treated with FRWE at a dose level 250 and 500 mg/kg per day p.o., respectively, for seven days. On the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil was administered (50% v/v, 5 ml/kg i.p.). Group V and VI animals were treated with FRAE at a dose level 250 and 500 mg/kg per day p.o., respectively, for seven days. On the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil was given (50% *v/v*, 5 ml/kg i.p.). Group VII animals were treated with solitarian (25 mg/kg per day *p.o.*) for seven days and on the seventh day, a single dose of equal mixture of carbon tetrachloride and olive oil (50% v/v, 5ml/kg i.p.) was given.

All animals were sacrificed by cervical decapitation under light ether anesthesia on the eighth day. Immediately after sacrifice, the liver was dissected out, washed in the ice-cold saline, and the homogenate was prepared in 0.1 M Tris-Hcl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes namely glutathione peroxidase (GPX) (Necheles et al., 1968), glutathione S-transferase (GST) (Habig et al., 1974), glutathione reductase (GRD) (Dubler and Anderson, 1981), superoxide dismutase (SOD) (Mishra and Fridovich, 1972), catalase (CAT) (Bergmeyer et al., 1994), lipid peroxidation (LPO) (Ohkawa et al., 1979), total protein content (Doumas et al., 1971).

Statistical analysis

The statistical analysis was carried out using Oneway Analysis of Variance (ANOVA) followed by Dunnet's 't' test. P value <0.05 were considered as significant.

RESULTS

Glutathione peroxidase (GPX)

The effect of FRWE and FRAE on GPX content in the liver is shown in Figure 1. GPX level in normal group (297.18 n mol of GSH oxidized/min/mg protein) was measured to be higher than in CCl_4 control group (180.1 n mol of GSH oxidized/min/mg protein). GPX level of FRWE 250 and 500 mg/kg groups (218.47 and 236.35 n mol of GSH oxidized/min/mg protein, $P < 0.05$) were increased by (32.77 and 48.04% respectively) as compare to CCl_4 control group. GPX level of FRAE 250 and 500 mg/kg groups (227.02 and 252.42 n mol of GSH oxidized/min/mg protein, $P < 0.05$) were increased by (40.07 and 61.76% respectively) as compare to CCl_4 control group. Silymarin 25 mg/kg also restored the GPX level in CCl_4 treated groups to the normal level.

Glutathione transferase (GST)

The effect of FRWE and FRAE on GST content in the liver is shown in Figure 2. GST level in normal group

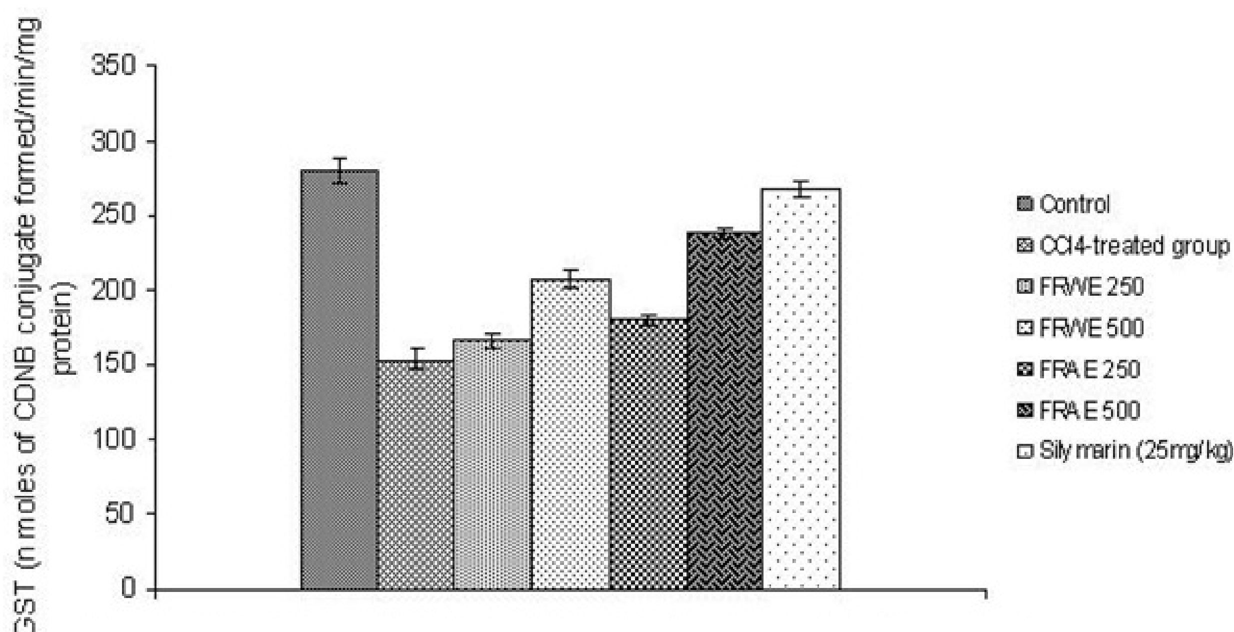

Figure 2. Effects of FRWE and FRAE on hepatic gluthione transferase levels in CCL_4 treated rat.z

(280.06 n mol of CDNB conjugate formed/min/mg protein) was measured to be higher than in CCl_4 control group (154.01 n mol of CDNB conjugate formed/min/mg protein). GST level of FRWE 250 and 500 mg/kg groups (165.6 and 207.1 n mol of CDNB conjugate formed/min/mg protein, $P < 0.05$) were increased by (9.19 and 42.12% respectively) as compare to CCl_4 control group. GST level of FRAE 250 and 500 mg/kg groups (180.13 and 238.06 n mol of CDNB conjugate formed/min/mg protein, $P < 0.05$) were increased by (20.72 and 66.68% respectively) as compare to CCl_4 control group. Silymarin 25 mg/kg also restored the GST level in CCl_4 treated groups to the normal level.

Glutathione reductase (GRD)

The effect of FRWE and FRAE on GRD content in the liver is shown in Figure 3. GRD level in normal group (27.2 n mol of GSSG utilized /min/mg protein) was measured to be higher than in CCl_4 control group (10.3 n mol of GSSG utilized /min/mg protein). GRD level of FRWE 250 and 500 mg/kg groups (16.21 and 18.08 n mol of GSSG utilized /min/mg protein, $P < 0.05$) were increased by (34.97 and 46.04% respectively) as compare to CCl_4 control group. GRD level of FRAE 250 and 500 mg/kg groups (17.15 and 20.5 n mol of GSSG utilized /min/mg protein, $P < 0.05$) were increased by (40.53 and 60.36% respectively) as compare to CCl_4 control group. Silymarin 25 mg/kg also restored the GRD level in CCl_4 treated groups to the normal level.

Superoxide dismutase (SOD)

The effect of FRWE and FRAE on SOD content in the liver is shown in Figure 4. SOD level in normal group (92.55 Kat/g protein) was measured to be higher than in CCl_4 control group (45.5 Kat/g protein). SOD level of FRWE 250 and 500 mg/kg groups (52.9 and 62.9 Kat/g protein, $P < 0.05$) were increased by (15.73 and 36.98% respectively) as compare to CCl_4 control group. SOD level of FRAE 250 and 500 mg/kg groups (59.2 and 71.9 Kat/g protein, $P < 0.05$) were increased by (29.11 and 56.11% respectively) as compare to CCl_4 control group. Silymarin 25 mg/kg also restored the SOD level in CCl_4 treated groups to the normal level.

Catalase (CAT)

The effect of FRWE and FRAE on superoxide dismutase content in the liver is shown in Figure 5. CAT level in normal group (185.4 n mol of H_2O_2 decomposed/min/mg protein) was measured to be higher than in CCl_4 control group (53.15 n mol of H_2O_2 decomposed/min/mg protein). CAT level of FRWE 250 and 500 mg/kg groups (70.46 and 100.9 n mol of H_2O_2 decomposed/min/mg protein, $P < 0.05$) were increased by (14.74 and 35.88%, respectively) as compare to CCl_4 control group. CAT level of FRAE 250 and 500 mg/kg groups (87.6 and 120.6 n mol of H_2O_2 decomposed/min/mg protein, $P < 0.05$) were increased by (26.05 and 51.0% respectively) as compare to CCl_4 control group. Silymarin 25 mg/kg also restored

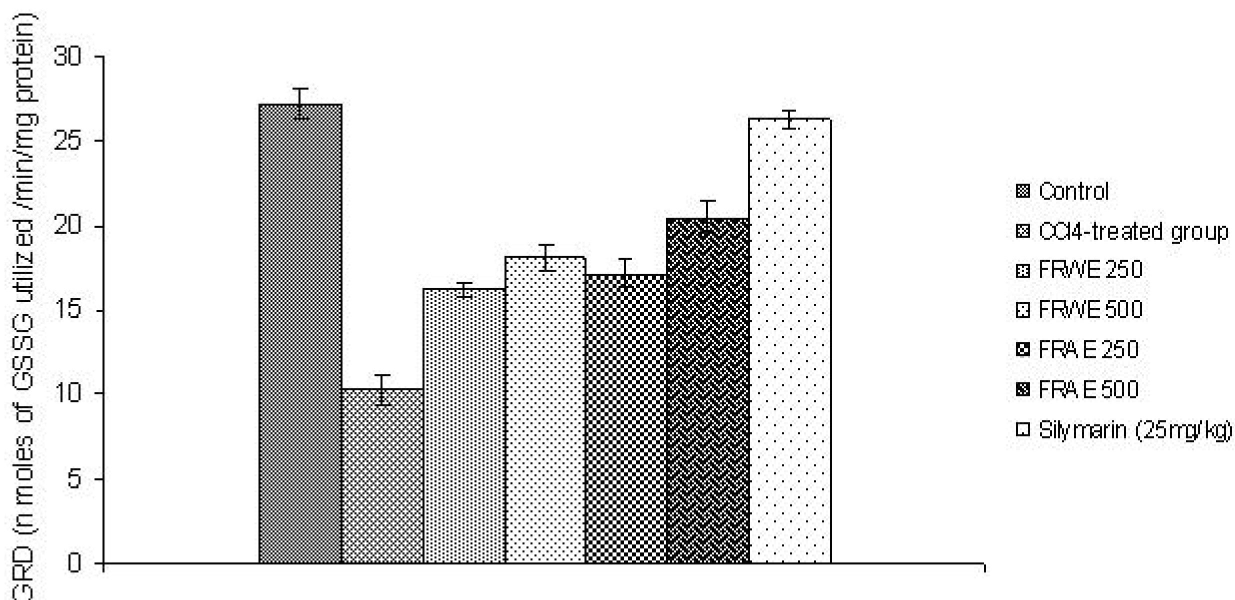

Figure 3. Effects of FRWE and FRAE on hepatic gluthione reductase levels in CCL_4 treated rat.

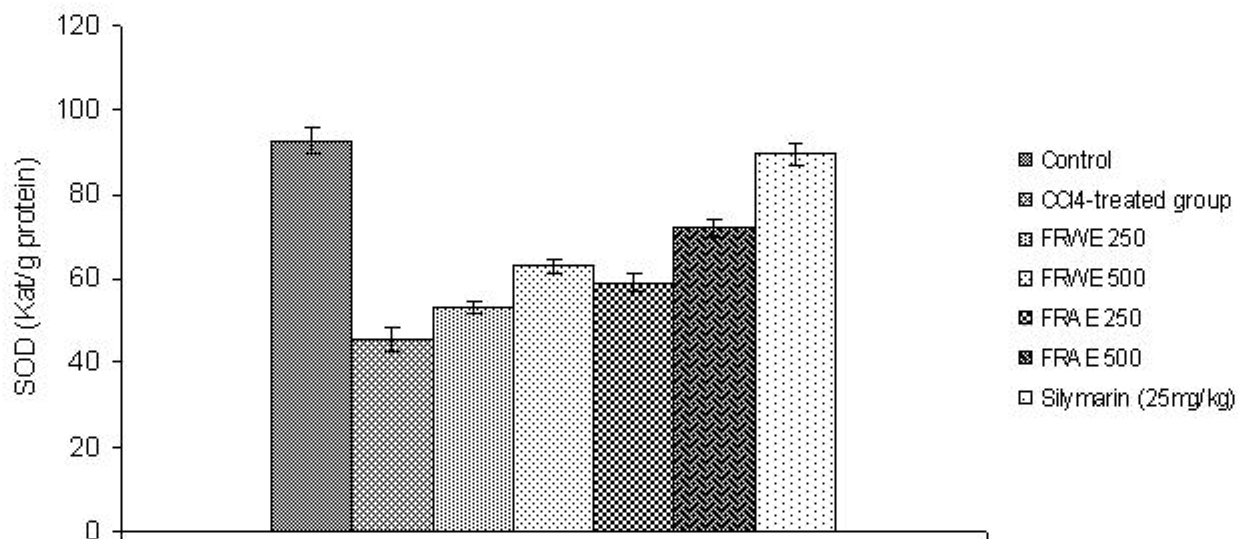

Figure 4. Effects of FRWE and FRAE on hepatic superoxide dismutase levels in CCL_4 treated rat.

the CAT level in CCl_4 treated groups to the normal level.

In vivo lipid peroxidation

The localization of radical formation resulting in lipid peroxidation, measured as malondialdehyde (MDA) in rat liver homogenate, is shown in Figure 6. MDA content in the liver homogenate was increased in CCl_4 control group (15.8 n mol of MDA/mg protein) compared to normal group (7.7 n mol of MDA/mg protein). MDA level of FRWE 250 and 500 mg/kg group (14.1 and 13.1 n mol of MDA/mg protein, $P < 0.05$) were inhibited by 21 and 33.3% compared to CCl_4 control. MDA level of FRAE 250 and 500 mg/kg group (13.6 and 12.2 n mol of MDA/mg protein, $P < 0.05$) were inhibited by 27.16 and 44.4% compared to CCl_4 control. At the same time, the effect of silymarin 25 mg/kg on MDA levels in CCl_4 were inhibited

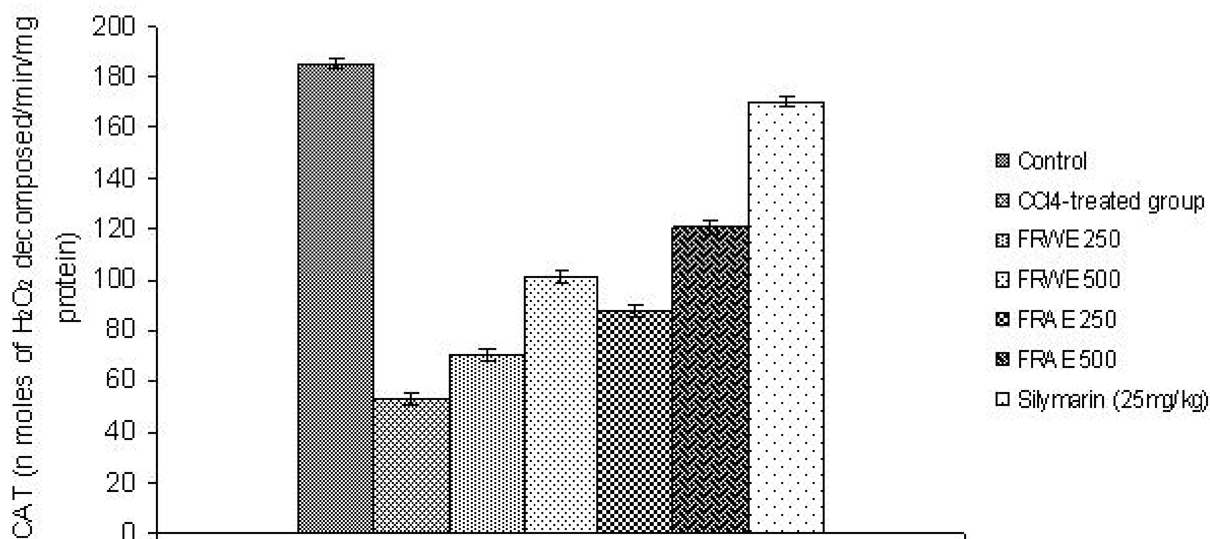

Figure 5. Effects of FRWE and FRAE on hepatic catalase levels in CCL_4 treated rat.

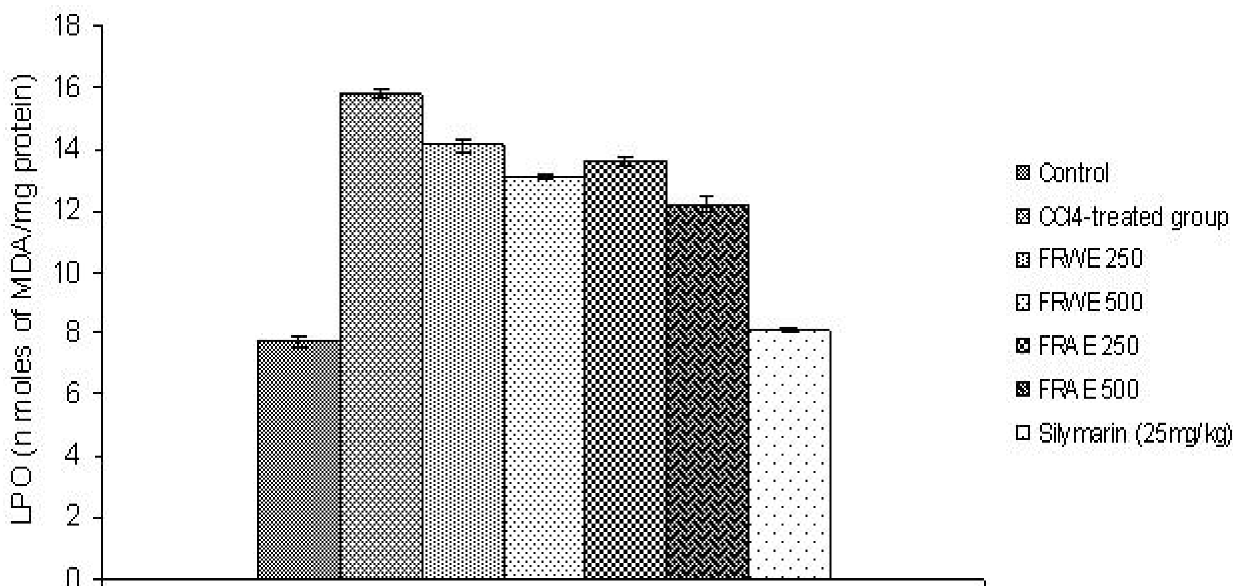

Figure 6. Effects of FRWE and FRAE on hepatic lipid peroxidation levels in CCL_4 treated rat.

by 95.06%.

DISCUSSION

In the liver, CCl_4 is metabolized by the cytochrome P450-dependent monooxygenase system followed by its conversion to more chemically active form, trichloromethyl radical (CCl_3) (Noguchi et al., 1982). When O_2 tension rises, a greater fraction of CCl_3 present in the system reacts very rapidly with O_2 and more reactive free radical, CCl_3OO has been generated from CCl_3 (Packer et al., 1978). These free radicals initiate the peroxidation of membrane poly-unsaturated fatty acids (PUFA) (Recknagel et al., 1989), generates PUFA and covalently bind to microsomal lipids and proteins (Tom et al., 1984). This results in the generation of ROS. Various enzymatic and non-enzymatic systems developed by the cell to cope up with ROS and other free radicals. However, when oxidative stress results, the defense

capabilities against ROS becomes insufficient (Halliwell and Gutteridge, 2000). ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH and other marker enzymes like SOD, CAT (Yamamoto and Yamashita, 1999). The oxidative stress leads organ injury and carcinogenesis (Stal and Olson, 2000).

GPX plays a crucial role in H_2O_2 catabolism (Eaton, 1991) and the detoxification of endogenous metabolism peroxides and hydroperoxides which catalyses GSH (Floka, 1971). GPX activity was markedly reduced after CCl_4 treatment when compared to control. The enhancing of GPX activity after pretreatment with FRWE and FRAE is due to antioxidant activity by scavenging/detoxifying the endogenous metabolic peroxides generated after CCl_4 injury in the hepatic tissue.

It has been suggested that GST offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH (Jakoby, 1988). GST plays a physiological role in initiating the detoxification of potential alkylating agents. Chemicals like chloroform and CCl_4 alter the liver GST activity (Aniya and Anders, 1985). GST level was significantly reduced in CCl_4-treatment animals and upward reversal was observed after the treatment with extracts. An increase in GRD activity implies that FRWE and FRAE protect the liver tissue from oxidative damage by GSH regeneration from its oxidized form (GSSG).

It has been reported that SOD, CAT and GST constitute a mutually supportive team of defense against ROS (Bandhopadhy et al., 1999; Tabatabaie and Floyd, 1994). The decrease activity of SOD in liver in CCl_4 treated rat may be due to the enhance lipid peroxidation or inactivation of the antioxidative enzymes. This would cause an increase accumulation of superoxide radicals, which could further stimulate LPO. GST binds to liophilic compounds and acts as an enzyme for GSH conjugation reaction (Anandan et al., 1999). Decrease in GSH activity during CCl_4 toxicity might be due to the decreased availability of GSH resulted during the enhanced LPO. Administration of extracts FRWE and FRAE prior to CCl_4-intoxication protect the antioxidant system of liver by enhancing the level of GPX, GST, GRD, SOD, CAT and decreasing level of LPO.

The present study concluded that FRWE and FRAE protects liver tissue against oxidative damage against CCl_4 induced hepatic damage.

Further work is needed to fully characterize the responsible active principle(s) present in the plant and to elucidate mode of action through which it acts as an antioxidant.

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