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Research article

INFLUENCE OF FENVALERATE AND QUERCETIN ON HEPATIC ANTIOXIDANT ENZYMES

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ABSTRACT: Pyrethroids are a class of neurotoxic pesticides with high selectivity for insects. Fenvalerate is a synthetic pyrethroid pesticide used to protect a variety of crops. Fenvalerate has been reported to exert deleterious effects on non target organisms including mammals. Recently, Fenvalerate was reported to cause liver damage in rats probably by generating oxidative stress while Quercetin, a potential antioxidant, has been reported to posses hepatoprotective activity. Therefore, the aim of the present investigation was to assess the ability of Quercetin to protect liver from Fenvalerate induced toxicity. In the present investigation an effort was made to evaluate the effect of Quercetin and Fenvalerate on hepatic G 6PD, GST and GR. Fenvalerate administration demonstrated significant reduction in the activities of hepatic G-6-PD, GST and GR while rats co treated with Quercetin showed significant recovery in the activities of these antioxidant enzymes.
Key Words: Quercetin, Fenvalerate, Hepatotoxicity, Oxidative stress, Antioxidant enzymes
Abbreviations: Glutathione reductase; GR, Glutathione *S*-transferase; GST, Glucose-6-Phosphate Dehydrogenase; G-6-PDH

INTRODUCTION

Because of impending bans on many chlorinated hydrocarbon, organophosphorous and carbamate pesticides there has been a rapid rise in the use of Fenvalerate in recent years and the current information is not sufficient to adequately assess the risk posed by fenvalerate to non-target organisms (Sanchez- Fortun and Barahona 2005). Pesticides help control agricultural pests and plant, human and livestock disease vectors insure increased food production, a safe and secure food supply along with other secondary benefits. However, pesticides have been found to be harmful to the non target organisms. The signs of poisoning in nontarget species are similar in some respects to the severe signs of poisoning observed in insects (R. Edwards et al., 1986, D.C. Dorman et al., 1990).

Synthetic pyrethroids, including Fenvalerate are the major class of synthetic organic insecticides. Pyrethroids are structural derivatives of naturally occurring pyrethrins, which are present in pyrethrum, an extract from the flowers Chrysanthemum cinerarifolium (J.E. Casida, 1980, J.E. Casida and G.B. Quistad, 1998). Fenvalerate show high toxicity to a wide range of insects, including some pesticide resistant strains (Elliot et al., 1978) and low toxicity to mammals and birds (Parker et al., 1984). Fenvalerate has been reported for its deleterious effects on humans (Kolmodin Hedman et al. 1982). Morphological changes were observed in nerve fibres in rats and mice which were administered lethal or sublethal oral doses of fenvalerate (WHO (Ed); 1990).

The hepatotoxic potential of formulated Fenvalerate in rats exposed by nose only inhalation was reported in a study and the results indicated hepatomegaly, along with pronounced histopathological damage of liver (Mani et al., 2004). Okuno et al. observed the presence of numerous microgranulomas in the liver of mice and rats administered with Fenvalerate (Okuno et al. 1986). Oral administration of Fenvalerate caused degenerative parenchymatous changes of hepatocytes in the liver (Tos luty et al. 2001). Flavonoids are phenolic phytochemicals that represent substantial constituents of the non energetic part of the human diet and are thought to promote optimal health, partly via their antioxidant effects in protecting cellular components against reactive oxygen species (Poli & Parola (1997).

Quercetin is a potential antioxidant (Hollman et al., 1997; Terao, 1999) commonly found in food products (16) and is one of the most abundant natural flavonoids. Quercetin has been reported to prevent liver cirrhosis probably by inducing antioxidant enzyme system (Amalia et al., 2007).

In the present study an effort has been made to evaluate the role of Quercetin on liver antioxidant enzymes such as G 6 PD, GR and GST of rats administered with Fenvalerate.

MATERIALS AND METHODS

Chemicals

Quercetin and Fenvalerate were purchased from Sigma Aldrich & co. All other used chemicals including substrates, enzymes and coenzymes in the present study were of pure analytical grade.

Experimental design:

After one week acclimatization period, 24 adult male albino rats, weighing 180 to 200g randomly distributed to the different experimental groups (i.e. n=6 per group). They were kept on standard rat chow with free access to water *ad libitum*, in rooms with controlled temperature and humidity, under a 12-hr light-dark cycle. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institutional Animal Ethics Committee. One group served as control and received corn oil intragastrically for 21 days. Second group received Quercetin at a daily dose of 50 mg/kg b.wt for 21 days. The third and fourth groups were injected with sub lethal doses of Fenvalerate i.e.50 mg/kg b.wt. i.p. for alternate days(i.e. for 10 days) but the fourth group animals were simultanously treated with Quercetin at a dose of 50mg /kg b.wt for 21 days.

Assessment of liver antioxidant enzymes

10% homogenate of liver tissue was prepared in 100 mM KH_2PO4 buffer containing 1 mM EDTA (pH 7.4). The homogenate was centrifuged and the supernatant was collected and used for the following experiments as described below. Concentration of protein in liver homogenate was estimated by using Lowry's method (Lowry et al. 1951).

Glutathione reductase (GR) (E.C.1.6.4.2)

Glutathione reductase was assayed according to the method of Carlberg & Mannervik (1955). The assay was carried out by using NADPH as the substrate. Briefly, 0.1 ml of liver supernatant was mixed with 1.65 ml phosphate buffer: (0.1 M; pH 7.6), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1 mM) and 0.1 ml of NADPH (0.1 mM) and the mixture was measured at 340 nm. Enzyme activity was calculated as nM NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22 × 103 M-1cm-1.

Glutathione *S*-transferase (GST) (E.C.1.11.1.6)

Glutathione *S*-transferase (GST) activity toward CDNB was determined spectrophotometrically at 340 nm by the method of Habig et al. (1975) with slight modifications.

Briefly, the assay was performed at 25◦C using 100 mM potassium phosphate buffer, pH 6.5, with GSH and CDNB (dissolved in ethanol) at a final concentration of 1 mM each. The reaction was followed for 4 min and the activity was calculated from the changes in absorbance at 340 nm using the extinction coefficient of 9.6 mmol−1 cm−1 for the reaction product, dinitrophenyl-*S*-glutathione (DNP-SG). Nonenzymatic conjugation was subtracted using a blank containing buffer and the substrate, but no enzyme. One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of 1 μmol of CDNB with GSH per minute at 25°C.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH) (E.C.1.1.1.49)

The enzyme was assayed following the method of Lohr and Waller (1974).

The reaction mixture was prepared by adding 2.4ml of triethanolamine buffer, 0.05 ml of glucose-6-phosphate and 0.5ml of tissue extract. After the addition of 0.05 ml of NADP solution, the reaction mixture was incubated for 5 minutes at 25°C. The contents were mixed thoroughly after each addition.

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A blank was prepared in the same manner containing 0.05 ml of triethanoamine buffer instead of NADP. The increase in optical density was recorded at 340 nm in a spectrophotometer, at one minute interval for 10 minutes. One unit of G-6-PDH activity is defined as the amount of enzyme which converts one micromole of NADP to its reduced form NADPH per minute, under the above mentioned assay conditions.

Statistical Analysis:

Data collected were summarized as mean ± SD for six observations and results were considered significantly different if $p < 0.05$. All the data were analyzed using SPSS/10 student software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD.

RESULTS

In our experiment, Groups treated with Quercetin showed no significant differences in hepatic markers compared to control group except for GST which was found to be significantly reduced. (Table 1). In groups treated with Fenvalerate significant reduction in the activities of G-6-PD, GST and GR was observed while group co treated with Quercetin showed significant elevation in the activities of G-6-PD, GST and GR (Table 1).

Table.1. Effect of Fenvalerate and Quercetin on G-6-PD, GST and GR. ^{Ns} Non significant *vs*. control; ^{a} $p < 0.05$ *vs.* control; ^{b} $p < 0.05$ *vs.* Fenvalerate.

GROUPS	G 6 PD (μ moles of NADPH /min/mg protein)	GST (μ moles /min/mg protein)	GR (nM/min/mg protein)
I (CONTROL)	485.33 ± 8.52	1.4 ± 0.08	139 ± 6.60
II (FENVALERATE)	289.83 ± 9.41^{a}	0.7 ± 0.08^{a}	83 ± 4.42^{a}
III (QUERCETIN)	476.66 ± 9.17^{NS}	1.0 ± 0.12^{a}	135.83 ± 7.62^{NS}
IV (FENVALERATE + QUERCETIN)	344.83 ± 12.13^{b}	0.98 ± 0.06^{b}	110.5 ± 8.11^{b}

DISCUSSION

As mentioned above, the hepatotoxic effect of Fenvalerate in rats have been documented in several studies (Mani et al. 2004, Okuno et al. 1986, Tos luty et al. 2001). Pyrethroids may produce oxidative stress in intoxicated rats. (M. Kale et al.1999). Exposure to fenvalerate was reported to gradually decrease the activity of glucose 6-phosphate dehydrogenase (G6-PDH) in the liver of cat fish (Tripathi and Priyanka, 2004). According to Khan, treatment with pyrethoid pesticide resulted in decreased activities of glutathione reductase and glutathione-S-transferase (Mahboob Khan, 2006). Depletion of antioxidant enzyme activity could be caused by a direct effect on the enzyme by Fenvalerate induced ROS generation followed by the depletion of the enzyme substrates. On the other hand, Quercetin was reported to protect liver from ethanol induced injury (Xi Chen, 2010). In animals treated with quercetin, a reduction in oxidative damage was observed, with regeneration of GSH and an increase in the activity of antioxidant enzymes (Maria Amalia et al. 2007). Quercetin treatment was also reported to restore the activities of GST and GR in the liver of rats administered with CCL4 (Kuntal Maiti et al. 2005, Maria Amalia et al. 2007). Our study is in tune with above findings.

G 6 PD is an important enzyme of hexose monophosphate shunt and it generates NADPH, which is required for the conversion of oxidized glutathione to reduced glutathione which inturn is required for the maintenance of membrane integrity (Marks PA, 1961).GR, a flavoprotein enzyme, catalyses the reduction of oxidized glutathione to reduced glutathione. GST is the phase II metabolic enzyme that favors protection against lipid peroxides and promotes conjugation of toxic radicals with electrophilic characteristics with glutathione (Jakoby WB, 1988). According to a previous study, feeding the quercetin-supplemented diet significantly lowered cytosolic GST activity in rat liver (H.Wiegand et al. 2009), which is in tune with our results. The capacity of the liver to detoxify xenobiotics can possibly be influenced by high dose Quercetin (H.Wiegand et al. 2009) but under stress condition Quercetin can restore the level of GST thereby facilitating the detoxification mechanism as evidenced by the level of GST in the Fenvalerate plus Quercetin administered group.

In our study, significant reduction in the activities of G-6-PD, GST and GR was observed in groups treated with Fenvalerate and co treatment with Quercetin showed significant elevation in the activities of G-6-PD, GST and GR (Table 1). Therefore, it can be concluded that, Quercetin can restore hepatic antioxidant and phase II metabolic enzymes during Fenvalerate induced oxidative stress and may provide protection against Fenvalerate induced toxicity in hepatocytes.

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