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Fenvalerate induced Hepatotoxicity and its amelioration by Quercetin

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Abstract: Fenvalerate is a synthetic pyrethroid pesticide used in agriculture to protect a variety of crops. Fenvalerate exposure is associated with serious health consequences in mammals and other non target species. It has been reported to cause liver damage in rats. Fenvalerate is an oxidative stress inducer in liver and Quercetin is a potential antioxidant and is known for its hepatoprotective properties. Therefore, the aim of the present investigation was to assess the ability of Quercetin to protect liver from Fenvalerate induced toxicity. In rats treated with Fenvalerate enzymatic and non enzymatic hepatic function markers along with MDA were significantly elevated while antioxidant enzyme activities were significantly reduced compared to control group, thereby indicating oxidative stress and liver damage while Quercetin pretreatment followed by Fenvalerate administration showed better recovery as evidenced by the significant reduction of liver specific markers in serum and elevated levels of antioxidants with the depletion of MDA compared to Fenvalerate treated group.
Key words: Quercetin, Fenvalerate, Oxidative stress, Liver.

Abbreviations: Fenvalerate (Fen), Quercetin (QE), Aspartate transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Lactate Dehydrogenase (LDH), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSD-Px), Reduced Glutathione (GSH), Melonaldehyde (MDA).

INTRODUCTION

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and are widely used over organochlorine and organophosphate pesticides. Synthetic pyrethroids account for more than 30% of insecticide use worldwide in household, agricultural, and veterinary applications (1, 2, 3, 4).

Fenvalerate is the most widely used compound of the cyanophenoxy-benzyl group of the synthetic pyrethroid pesticides and it is used in agriculture to protect a wide variety of crops including cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts from insect pests (5). In India, it is used primarily to control pests of cotton and vegetables (6). Fenvalerate is one of the most persistent synthetic pyrethroids in soils. Fenvalerate is highly toxic for fish (7) and bees, while for birds and mammals its toxicity is low. Pyrethroid pesticides such as cypermethrin and Fenvalerate show high toxicity to a wide range of insects, including some pesticide resistant strains (8) and low toxicity to mammals and birds (9) but when administered at a high dose, pyrethroids produce evident neurotoxicity in mammals. However, the current information is not sufficient to adequately assess the risk posed by fenvalerate to non-target

organisms, though some work has been done to assess its toxicity to non target species (10, 11).

High doses of Fenvalerate has been reported to be associated with reduction of body mass, increase in liver mass, and proliferation of the smooth endoplasmic reticulum in hepatic cells, and induction of the activity of microsomal enzymes (12, 13, 14). According to available reports, oral administration of Fenvalerate cause degenerative changes in the liver (15). Histopathological studies carried out in rats treated with Fenvalerate showed centrilobular necrosis, nuclear degeneration, fatty change, bile duct proliferation and round cell infiltration (16, 17). Liver is the predominant site of Pesticide metabolism through hydrolysis and it is susceptible to injury by pyrethroids.

The use of natural antioxidants, including flavonoids for curing pesticide induced liver toxicity or injury is being studied extensively (18). Even though newly developed drugs have been used to treat chronic liver diseases, they have often side effects hence there is a need for hepatoprotective compounds (19).

Flavonoids are phenolic phytochemicals present in human diet and promote optimal health partly via their antioxidant effects (20). Antioxidants have been reported to provide protection from the toxicity of pesticides (21). **Quercetin** (3, 5, 7, 3, 4-pentahydroxy flavon) is one of the most distributed flavonoid, semi essential food components, in certain species of plants. Quercetin is the most common flavonol in the diet and its antioxidant properties has been extensively studied in vitro. Quercetin's strong antioxidative effect and its ability to reduce oxidative damage have been documented (22, 23).

Quercetin is believed to play beneficial role on liver (24) and therefore, the aim of the present study was to investigate the ability of Quercetin to protect liver from Fenvalerate induced toxicity.

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.

At the end of the experiment, rats were sacrificed by cervical dislocation. Livers were dissected out and washed in physiological saline. The liver homogenate was utilized for the estimation of catalase, superoxide dismutase, glutathione peroxidase reduced glutathione and Malonaldehyde. Blood samples were collected in dry and clean glass test tubes. Blood samples were kept at 4^{o}C and centrifuged at 1000 g for 30 min. The sera obtained were used for the biochemical investigation of enzymatic and non enzymatic hepatic markers.

Liver function tests:

Aspartate Aminotransferase (AST) (E.C.2.6.1.1) and Alanine Aminotransferase (ALT) (E.C.2.6.1.2) were assayed spectrophotometrically by the method of Bergmeyer and Bernt, 1974 (25, 26). Alkaline Phosphatase (AST) (E.C.3.1.3.1) activity was assayed by the method of Andersch and Szczypinski, 1947 (27). Gamma Glutamyl Transferase(GGT) (E.C.2.3.2.2) activity was assayed according to the method of Rosalki et al. 1970 (28) using gama glutamyl-p-nitroanilide as substrate. Lactate Dehydrogenase (LDH) (1.1.2.7) activity was assayed following the method of King, 1965 (29).The method of Lowry et al., 1951 was used to estimate the amount of Total Protein (30). Serum Albumin was determined following the method of Doumas et al. (1971), modified by Spencer and Price (1977) as detailed in Varley et al.,1980 (31).

Preparation of Liver Homogenate

10% homogenate of liver tissue was prepared in 100 mM KH2PO4 buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following experiments as described below. Concentration of protein in supernatant was estimated by using crystalline BSA as standard by using the method of Lowry et al., 1951 (30).

Estimation of Antioxidants and TBARS:

Catalase was estimated by the method of Chance & Maehly, 1955 (32). Superoxide dismutase was estimated following the method of Kakkar et al., 1984 (33). Glutathione peroxidase was estimated using the method of Mohandas et al., 1984 (34). Reduced Glutathione was estimated following the method of Beutler et al., 1963 (35). Malonaldehyde was estimated following the method of Buege & Aust, 1978 (36).

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 AND DISCUSSION

In the present study, the protective role of Quercetin has been carried out against Fenvalerate induced liver toxicity in rats. Groups treated with Quercetin showed no differences in hepatic markers compared to control group. Administration of Fenvalerate to rats resulted in induction of toxicity to the liver as reflected by elevation of liver damage marker enzymes like alkaline phosphatase (Fig.3), aspartate transaminase (Fig.1), alanine transaminase (Fig.2), Gamma Glutamyl Transferase (Fig.4) and lactate dehydrogenase (Fig.5). Groups treated with Fenvalerate plus Quercetin showed significant reduction in the activities of AST, ALT, ALP, GGT and LDH.

The enhanced activities of these enzymes may be due to lysis/damage of hepatocytes resulting in the permeation of these enzymes into serum. Enzymes like ALP, AST, ALT and GGT have been commonly associated with liver dysfunction/ damage and released into the circulation after cellular damage leading to their elevation in serum (37, 38, 39, 40). Alkaline phosphatase mainly reaches the liver from bone, excreted into the bile; therefore its elevation in serum can be associated with hepatobiliary disease (41). Lactate dehydrogenase (LDH) is a cellular enzyme that contributes to carbohydrate metabolism. It is released during tissue injury (42). Increased activity of LDH in serum can be used as an indicator of cellular damage and cytotoxicity of pesticides (43). Therefore, elevation in the activity of transaminases, GGT, ALP and LDH in Fenvalerate treated group could be due to the liver damage induced by Fenvalerate.

Our results are in resemblance with Prasanthi et al., who demonstrated that fenvalerate administration can cause significant increases in activities of hepatic transaminases, ALP and LDH associated with oxidative damage in liver of rats (44). Foldstrom and Mani et al. also demonstrated elevated levels of SGOT and SGPT in rats treated with Fenvalerate (45, 17).

In our experiment, a reduction in the serum Total Protein (Fig.6) and Albumin (Fig.7) was noted in the Fenvalerate treated group. Bradbury et al. pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cellular function, and consequent impairment in protein synthetic machinery (46). The reduction in serum protein, particularly albumin, in fenvalerate treated group could be due to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein depression may be due to loss of protein either by reduction in protein synthesis or increase in proteolytic activity or degradation (47).

In this experiment, the Quercetin plus Fenvalerate treated group showed significantly reduced level of AST, ALT, GGT, ALP, LDH, Total Protein and Albumin in serum compared to Fenvalerate alone group suggesting that Quercetin could have prevented the leakage of these marker enzymes, Protein and Albumin from hepatocytes by protecting hepatocytes from Fenvalerate mediated damage.

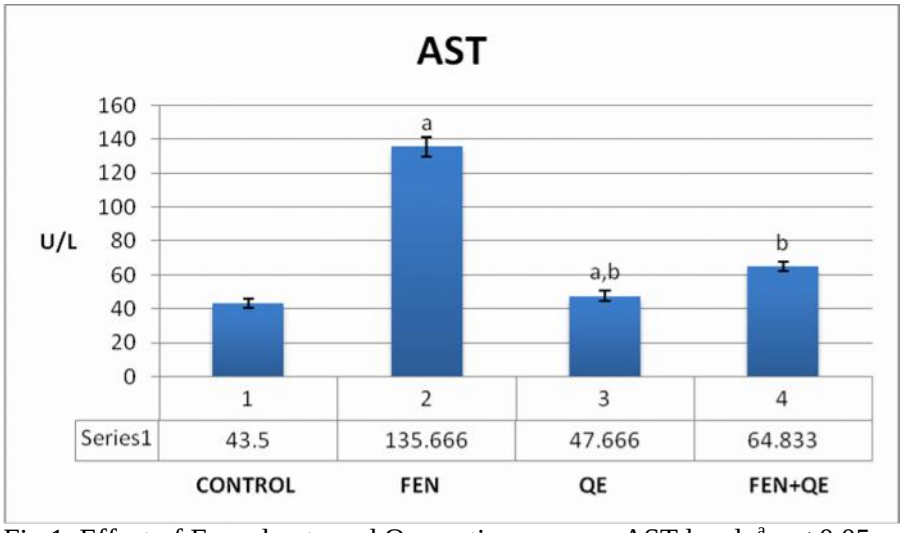

Fig.1. Effect of Fenvalerate and Quercetin on serum AST level. [a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate

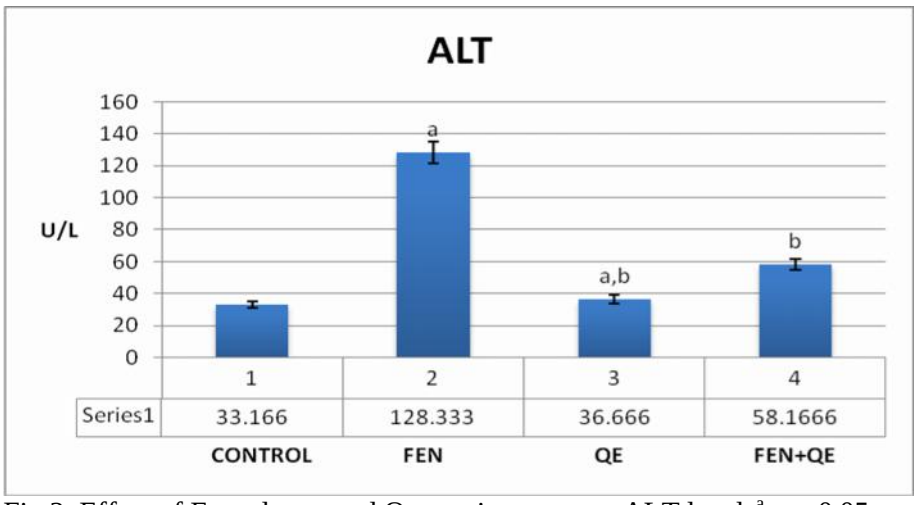

Fig.2. Effect of Fenvalerate and Quercetin on serum ALT level. [a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate

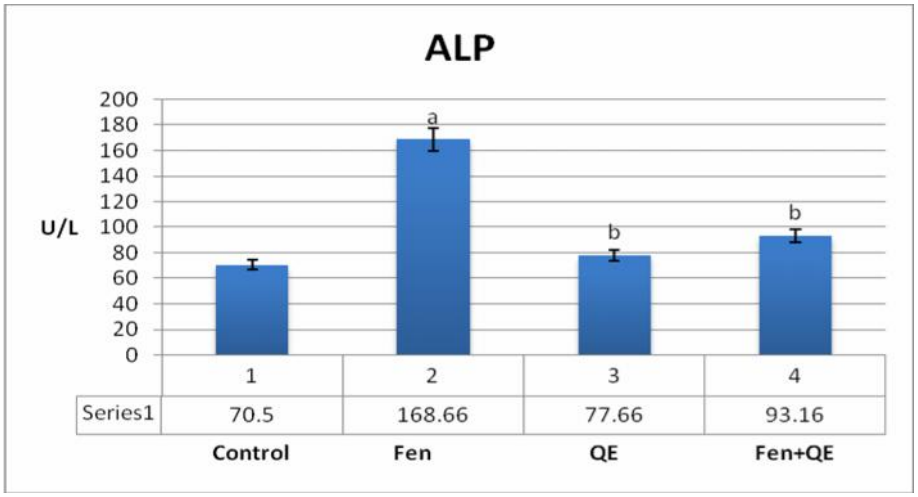

Fig.3. Effect of Fenvalerate and Quercetin on serum ALP level. [a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate.

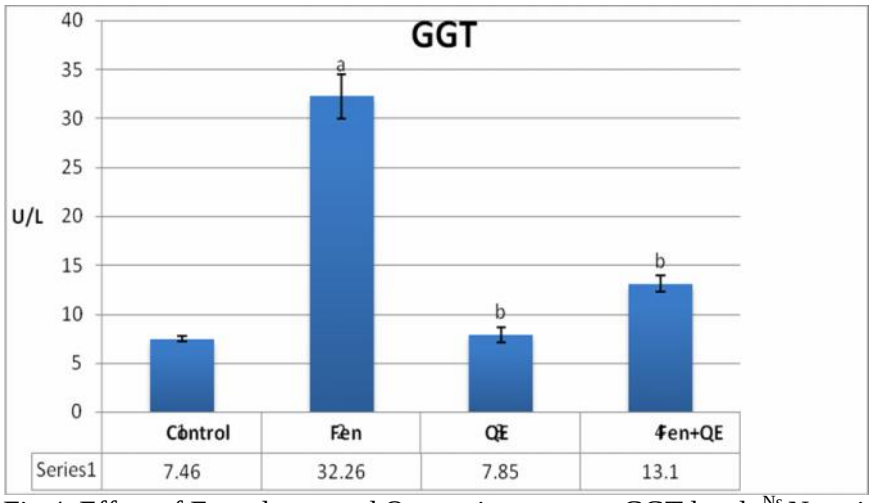

Fig.4 .Effect of Fenvalerate and Quercetin on serum GGT level. [Ns] Non significant *vs*. control; [a] $p < 0.05$ *vs*. control; [b] $p < 0.05$ *vs*. Fenvalerate.

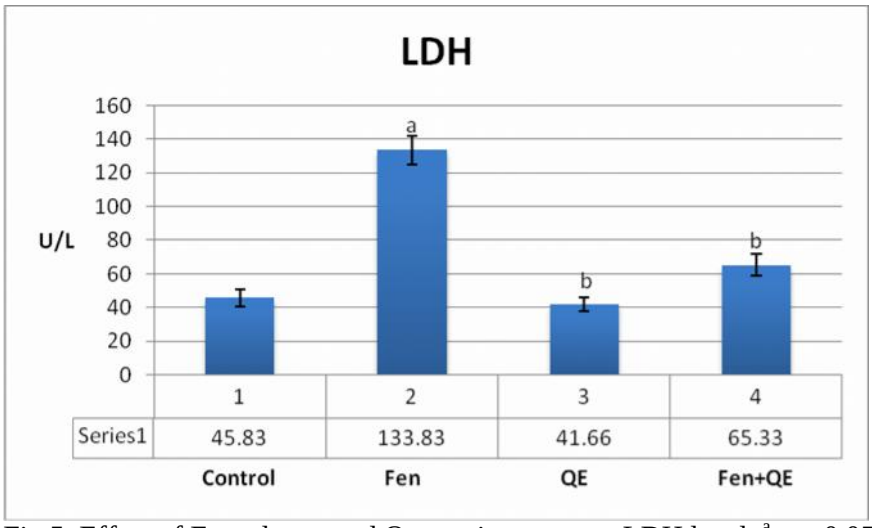

Fig.5. Effect of Fenvalerate and Quercetin on serum LDH level. [a] $p < 0.05$ *vs*. control; [b] $p < 0.05$ *vs*. Fenvalerate

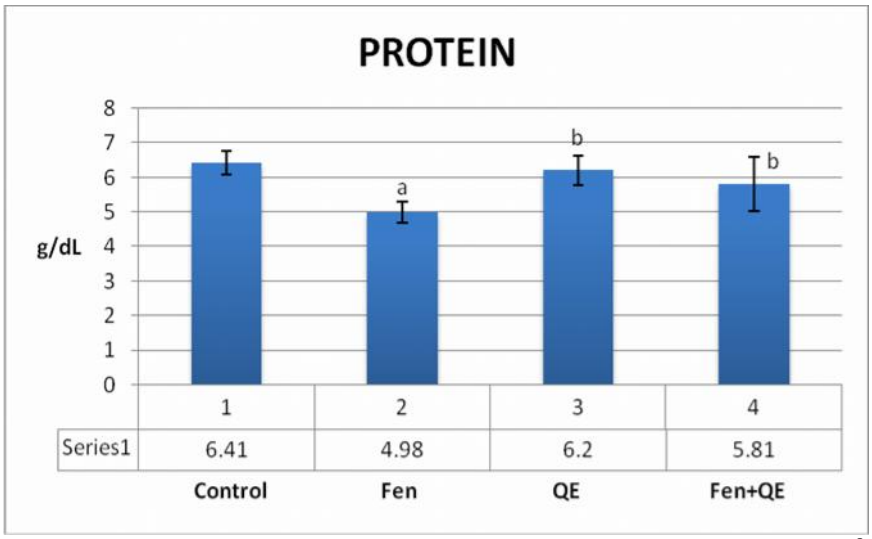

Fig.6. Effect of Fenvalerate and Quercetin on serum Total Protein level. [a] $p < 0.05$ *vs*. control; [b] $p < 0.05$ *vs*. Fenvalerate.

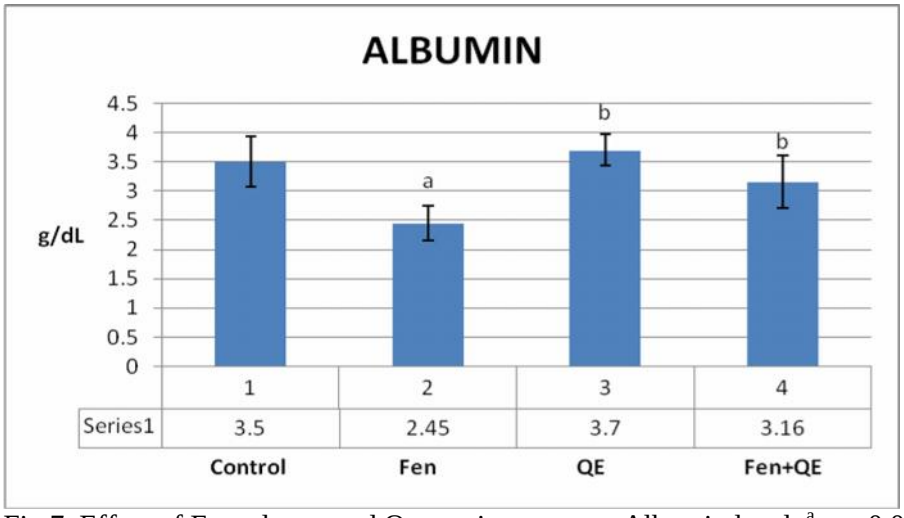

Fig.7. Effect of Fenvalerate and Quercetin on serum Albumin level. [a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate.

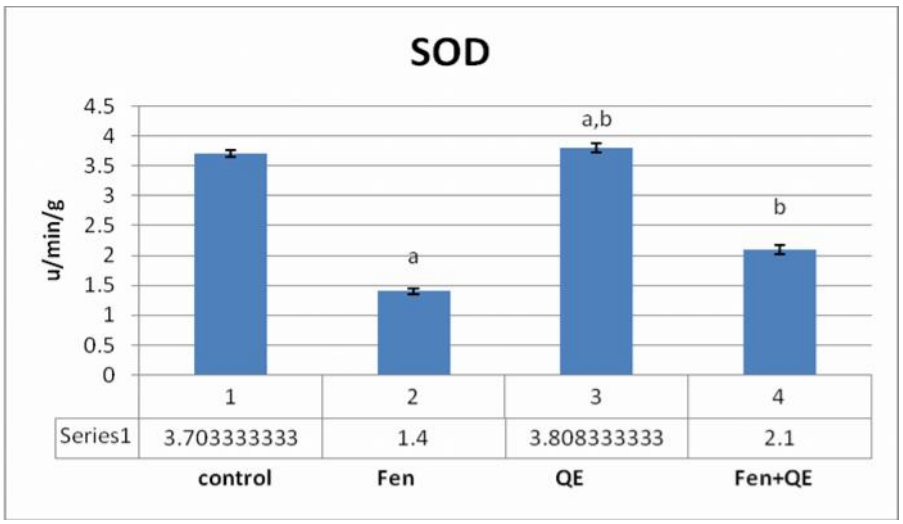

Fig.8. Effect of Fenvalerate and Quercetin on Superoxide dismutase activity in liver. $p < 0.05$ *vs.* Control; [b] $p < 0.05$ *vs.* Fenvalerate.

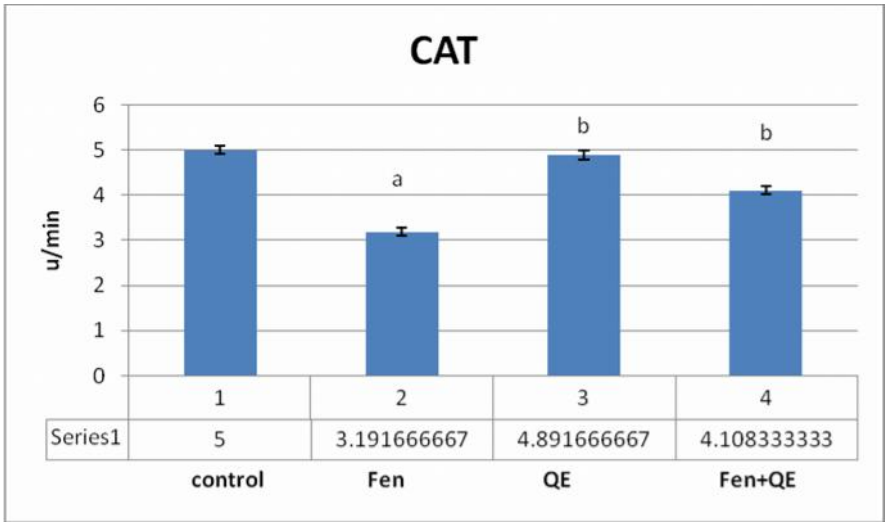

Fig.9. Effect of Fenvalerate and Quercetin on Catalase activity in liver. [a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate.

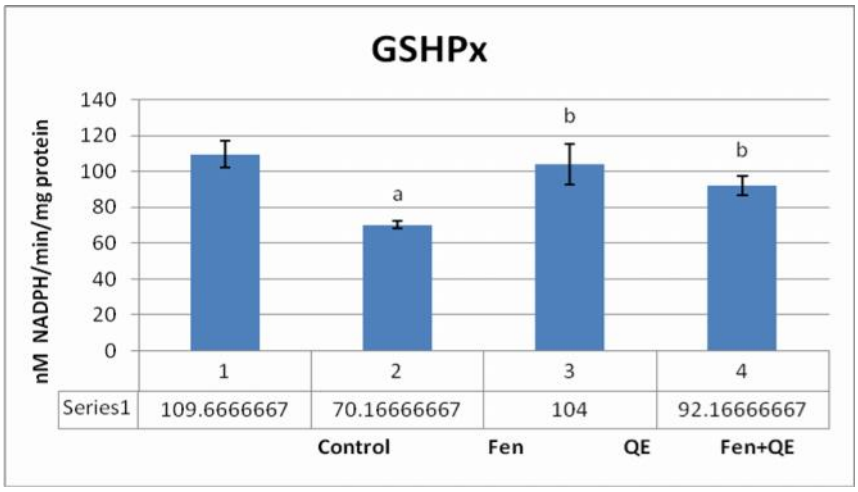

Fig.10. Effect of Fenvalerate and Quercetin on Glutathione peroxidase activity in liver.
[a] $p < 0.05$ *vs.* control; [b] $p < 0.05$ *vs.* Fenvalerate

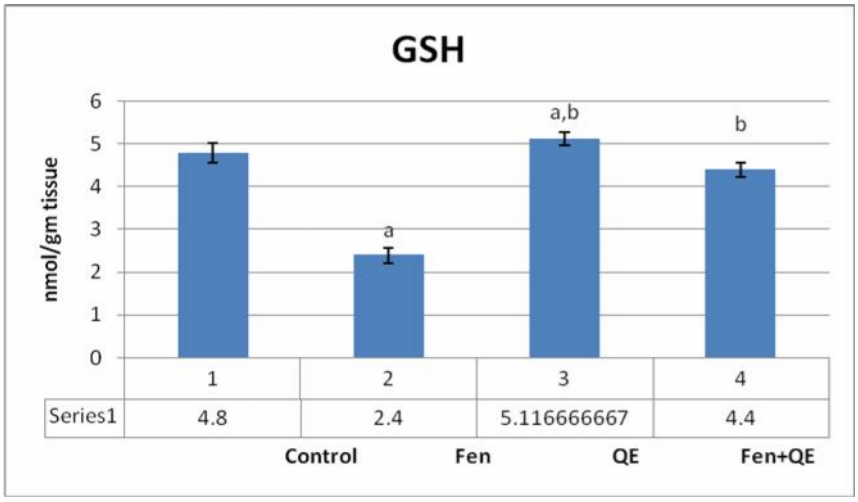

Fig.11. Effect of Fenvalerate and Quercetin on Glutathione content in liver. [a] $p < 0.05$ *vs.* control;
[b] $p < 0.05$ *vs.* Fenvalerate

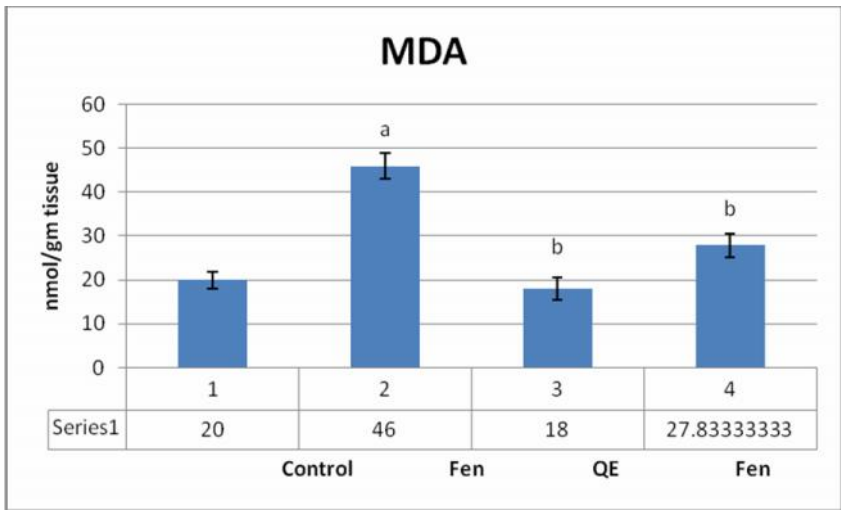

Fig.12. Effect of Fenvalerate and Quercetin on Malonaldehyde level in liver. [a] $p < 0.05$ *vs.* control;
[b] $p < 0.05$ *vs.* Fenvalerate

In our study, the fenvalerate treated groups showed decreased activity levels of SOD (Fig.8), CAT (Fig.9), GSHPx (Fig.10) and GSH (Fig.11) in the liver homogenate while the amount of lipid peroxidation was high as evidenced by increase in the level of MDA (Fig.12).

SOD dismutates superoxide radicals into hydrogen peroxide and O_2 (48). Catalase further detoxifies hydrogen peroxide into H_2O and O_2. GPx also functions in detoxifying hydrogen peroxide similar to catalase. Thus these enzymes act mutually and protect the biological systems from oxidative stress (49).Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species generated exceeds the antioxidant capability of the cell (50). It is possible that during the pyrethroid metabolism, there is generation of reactive oxygen species (ROS) and pyrethroids may produce oxidative stress in intoxicated rats. (51).Therefore, the decreased activity levels of these enzymes after Fenvalerate treatment indicates oxidative stress in hepatocytes.

The level of GSH was reduced in liver of rats treated with Fenvalerate. GSH is a an antioxidant, which offers protections against free radicals and toxic compounds GSH redox cycles play crucial role in cellular antioxidant defenses and are essential for the tissues to protect themselves against the ROS damage. They participate in the elimination of ROS, acting both as a non-enzymatic oxygen radical scavenger and as a substrate for various enzymes such as GSHPx. Increase in GSH levels in turn contribute to the recycling of other antioxidants such as vitamin E and C (52, 53). In our study, decreased GSH content might have contributed to the decrease in the activity of GSH-Px. Hence, depletion in the content of GSH may be related to the oxidative stress generated in hepatocytes of rats treated with Fenvalerate.

According to the results obtained in our study, Fenvalerate elevates the MDA content in liver tissue of rats. Increase of MDA in liver could be attributed to the excessive production of ROS. The status of lipid peroxidation as well as altered level of certain endogenous radical scavengers is taken as direct evidence for oxidative stress (18).

Fenvalerate plus Quercetin group showed significant increase in the activities of SOD, CAT, GSH-Px, GSH (24, 54,), while the MDA content in the liver was significantly reduced compared to Fenvalerate alone group.

We conclude that Fenvalerate toxicity in liver could be attributed to the oxidative stress in the liver, which led to the depletion of the antioxidant enzymes, leading to increased lipid peroxidation. Cotreatment of rats with Quercetin led to the recovery of some of the enzymatic activity lost due to fenvalerate administration, thereby decreasing the oxidative stress in hepatocytes and therefore, Quercetin treatment could be useful in preventing fenvalerate induced hepatotoxicity by ameliorating oxidative stress.

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