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Full Length Research Paper

Protective role of Vitamin C against hepatorenal toxicity of fenvalerate in male rats

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There is increasing evidence that complications related to insecticides are associated with increased oxidative stress. Fenvalerate is causing different toxicities and Vitamin C has been reported to be an effective antioxidant. Therefore, the present study is aimed to elucidate the possible protective effects of Vitamin C in alleviating the toxicity of fenvalerate on liver and kidney performance, enzyme activities and lipids profile in serum of male rats. Adult male albino Wister rats (8 weeks), weighing 195 to 225 g were used in this study. The acute toxicity (LD50) of fenvalerate insecticide and its effects on male rats were carried out. Fenvalerate was given orally to male rats daily for 30 successive days (2.8 mg kg_1 b.wt. corresponding to 1/10 LD50) alone and in combination with Vitamin C (20 mg kg_1 b.wt. corresponding to acceptable daily intake. After this period the levels of oxidative stress parameters and activity of antioxidant enzymes were determined in various tissues. Fenvalerate altered antioxidant enzyme activities such as AST and ALT. These elevated enzymatic activities induced by oxidative stress were significantly restored to near normal by oral administration of Vitamin C as compared to untreated rats. There was a significant elevation in the level of liver and kidney malondialdhyde (MDA), while the activities of antioxidant enzymes superoxide dismutase and catalase (SOD and CAT) were significantly decreased in fenvalerate treated rats which also restored to normal after Vitamin C treatment. These biochemical observations showing that feeding Vitamin C may control oxidative stress by inhibiting the increase in TBARS and protein carbonyls and reversing altered antioxidant enzyme activities.

Keywords: Fenvalerate, Vitamin C, antioxidant, enzyme activities, Rats.

INTRODUCTION

Pesticides have brought about the green revolution in the world and are being widely used to control agricultural pests and insects causing public health hazards. Problems and/or outbreaks are reported to occur among animals and human from insecticide toxicity, which usually occurs either from direct exposure to insecticides or indirectly from contaminated feeds or water by such chemicals. Prolonged exposure to insecticides causes chronic neurological syndrome, malignant tumors, immunosuppressive action, teratogenic effect, abortion and decreased male fertility in experimental animals (Nafstad et al., 1983; El-Rahman, 1988; Al- Qwari et al., 1999; Meeker et al., 2006; Youssef, 2010). Pyrethroides, derivatives of carbamic acid, represent a large variety of compounds which have some field applications as insecticides, herbicides and fungicides. Many of these chemicals are potential neurotoxicants, particularly

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following occupational, accidental or intentional exposure. These compounds cause reversible carbamaylation of the acetylcholinesterase enzyme, allowing accumulation of acetylcholine, the neuromediator at parasympathetic neuro-effector junctions (muscarinic effect) and autonomic ganglia (nicotinic effect) and in the brain (Baron, 1991).

Fenvalerate is an insecticide of moderate mammalian toxicity. In laboratory animals, central nervous system toxicity is observed following acute or short-term exposure. Fenvalerate has applications against a wide range of pests. Residue levels are minimized by low application rates. Fenvalerate is most toxic to bees and fish. It is found in some emulsifiable concentrates, ULV, wettable powders, slow release formulations, insecticidal fogs, and granules. It is most commonly used to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables. Fenvalerate does not affect plants, but is active for an extended period of time. Fenvalerate acts as an insecticide against lepiodopterous, suppresses coleopterous and some hemipterous insects. In fact, it acts as an ovicide against cotton bollworms and tobacco budworms (Mahgoub and Mednay, 2001). In Saudi Arabia, the use of insecticides represents a great risk because of the huge amounts of insecticides used in the field and the lack of proper protective measures against pollution by these chemicals (El-Rahman, 1988).

Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress. It is also a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy. In animals these reactions are especially important in wound-healing and in preventing bleeding from capillaries (Kotze et al., 1974).

Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protects against adverse effects of free radical production in vivo (Halliwell and Gutteridge, 1989). Oxidative stress results from an imbalance between radical production and reduced activity of antioxidant defenses or both these phenomena. Insecticides causes release of tissue damaging reactive oxygen species (ROS) balance between radical production and protective antioxidant defense (Signorini *et al.*, 2002; Halliwell and Gudtteridge, 1990).

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The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems, the levels of which are altered in toxicities (Wohaieb and Godin, 1987).

Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protects against adverse effects of free radical production in vivo (Halliwell and Gutteridge, 1989). Oxidative stress results from an imbalance between radical production and reduced activity of antioxidant defenses or both these phenomena. The present study aimed to determine the acute oral LD50 of fenvalerate insecticide and to examine its toxic effects alone and in combination with vitamin C, on liver and kidney of male rats.

MATERIAL AND METHODS

Insecticide

Fenvalerate is an insecticide. It is a mixture of four optical isomers which have different insecticidal activities. The 2-S *alpha* (or SS) configuration is the most insecticidal active isomer. Fenvalerate consists of about 23% of this isomer. It was obtained from the stores of Agricultural Pesticides, Jeddah, Kilo 2 road, Saudi Arabia in the form of a pure white crystal powder.

Vitamin C

Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress. It was obtained from El Nahdi Company, Jeddah, Saudi Arabia.

Animals

Eight week male albino Wister rats weighing (195- 225g) were used in this study. The animals were selected from inbred colony maintained in the Animal House of King Fahd center of medical research. The animals were kept under controlled hygienic conditions and maintained at a temperature of 25 ± 2 _C, relative humidity of 50 ± 5% and photoperiod at 12-h dark/ light. Rats were fed on rat pellets which composed of wheat bran 10%, soya bean powder 44%, net protein 22%, fats 4.7%, fibers 3.3%, fish meal, and molasses, salts (sodium chloride, calcium carbonate, and calcium phosphate). The diet was offered and water was allowed *ad libitum* during the experiment period. Rats were allowed to acclimatize to the laboratory environment for 7 days before start of the experiment Animals were provided with standard commercial chow and water *ad libitum*. The care and use of all experiment-

Table 1. Antioxidant enzyme activities of liver and kidney of rat; superoxide dismutase (SOD), catalase (CAT), lipid peroxide product or Malendialdlyde (MDA) and serum aminotransferase enzymes (ALT and AST) of all studied groups male albino Wister rats (Mean ± SD).

Animal group parameter	Control group		Fenvalerate		Fenvalerate + VC		VC	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Serum ALT(IU/ml)	37.9 ± 4.49	38.1± 4.44	78.8±7.91	77.2±8.88	46.8±6.99	47.0±7.09	40.9±4.99	41.2±4.67
P_1 Value	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001
P_2 Value	-	-	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.05	P ≤ 0.05
P_3 Value	-	-	-	-	-	-	P ≤ 0.05	P ≤ 0.05
Serum AST(IU/ml)	41.8±4.55	42.1±4.49	87.7±8.01	86.2±7.99	49.8±6.69	50.1±7.007	45.8±4.72	44.9±4.32
P_1 Value	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001
P_2 Value	-	-	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.05	P ≤ 0.05
P_3 Value	-	-	-	-	-	-	P ≤ 0.05	P ≤ 0.05
MDA (m mol/mg protein)	2.34±0.16	2.36±0.18	6.22±0.55	6.29±0.49	2.99±0.23	3.02±0.31	2.57±0.32	2.62±0.47
P_1 Value	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001
P_2 Value	-	-	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.01	P ≤ 0.01
P_3 Value	-	-	-	-	-	-	P ≤ 0.05	P ≤ 0.05
SOD (MU/mg protein)	222.3±14.1	225.1±13.8	107.9±21.7	111.2±2704	214.3±23.8	208.1±27.7	188.7±23.3	189.9±24.
P_1 Value	-	-	P ≤ 0.001	P ≤ 0.001	N.S.	N.S.	P ≤ 0.001	P ≤ 0.001
P_2 Value	-	-	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.01	P ≤ 0.01
P_3 Value	-	-	-	-	-	-	P ≤ 0.01	P ≤ 0.01
CAT(n mol/min/mg protein)	9779.1±122.1	9769.9±142.1	2196.8±139.1	2322.4±144,8	8322.4±144.7	8401.7±137.7	4011.1±177.6	4032.7±146.4
P_1 Value	-	-	P ≤ 0.001	P ≤ 0.001	N.S.	N.S.	P ≤ 0.001	P ≤ 0.001
P_2 Value	-	-	-	-	P ≤ 0.001	P ≤ 0.001	P ≤ 0.01	P ≤ 0.01
P_3 Value	-	-	-	-	-	-	P ≤ 0.05	P ≤ 0.05

VC: Vitamin C, **P1:** Comparison to control, **P2:** Comparison to Fenvalerate group, **P3:** Vitamin C versus Fenvalerate, **N.S**= Not Significant.

tal animals complied with relevant animal welfare laws.

Acute toxicity experiment

For estimating the LD50 of Fenvalerate 40 male albino Wister rats were distributed into four groups each containing 10 animals. Rats were given orally, by stomach tube, the tested insecticide in graded doses. Toxic symptoms and the number of rats that died in each group after 48 h observation were recorded. The LD50 of Fenvalerate was then calculated according to the method described in Gad and Weil (1982).

Experimental design

Effects of Fenvalerate at (1/10 of the LD50) which equal 2.8 mg kg_1 b.wt and Vitamin C at acceptable daily intake (20 mg kg_1 b.wt.). For estimating the effect of Fenvalerate, Vitamin C and their combination on male fertility, 40 mature male mice were allocated into for equal groups. The 1st group was given orally 1.0 ml distilled water/day (vehicle) and kept as normal control. The 2nd group were given orally 1/10 (2.8 mg kg_1 b.wt.) of the LD50 of Fenvalerate. The 3rd group of male rats was orally given 2.8 mg kg_1 b.wt. combined with 20 mg kg_1 b.wt of Vitamin C calculated from acceptable daily intake (ADI) in man according to Paget and Barnes (1964). The

4th group was given 20 mg kg_1 b.wt of Vitamin C alone. Oral administration of the tested compounds continued for 30 consecutive days. At the end of experimental period, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose, hemoglobin and glycosylated hemoglobin. Plasma was separated for the assay of insulin. The liver and kidney were also dissected out and were kept at -20°C in ice-cold containers for biochemical analysis.

Serum biochemical assay

Serum enzymes aspartate aminotransferase (AST) and serum glutamate pyruvate transaminase (ALT) were determined according to (Reitman and Frankel, 1957).

Estimation of MDA, SOD, CAT in liver and kidney tissues

Liver and kidney samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Each tested tissue homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using glass homogenizer in ice. The cell debris was removed by centrifugation at 5000 rpm for 15 at 40°C using refrigerated centrifuge. The supernatant was used for the estimation of malondialdehyde (MDA) (Yagi and Rastogi, 1979), superoxide dismutase (SOD) (Kakkar *et al*., 1972) and catalase (CAT) (Smna, 1972) levels.

Statistical analysis

Statistical analysis was performed on a PC using SPSS, V.13, (special package for social sciences). Data are presented as arithmetic mean ± S.D., The difference among means has been analyzed by one way ANOVA followed by Student "*t*" test. A value of $P < 0.05$ was considered as statistically significant (Snedecor and Cochran, 1986).

RESULTS

The results of protective effect of vitamin C on Fenvalerate-induced liver and kidney injures in rats are shown in Table 1. In the Fenvalerate group, serum AST and ALT were significantly increased as compared to control group ($p<0.001$). The elevated activities of serum AST and ALT were significantly reduced in the animal groups treated with control or vitamin C. Results obtained revealed an increase in the level of liver and kidney MDA in Fevalerate treated rats compared to control group. Treatment with vitamin C significantly prevented this raise in levels. The activities of SOD and CAT were significantly reduced in the vitamin C treated group and were significantly elevated near the normal values in this groups pretreated with vitamin.

The levels of blood glucose, total hemoglobin, glycosylated hemoglobin and plasma insulin of different experimental groups were shown in Figure 1. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in Fenvalerate treated rats (F groups), compared with normal rats. Administration of Vitamin C tended to bring blood glucose and plasma insulin towards normal. The control rats showed a significant decrease in the level of total hemoglobin and significant increase in the level of glycosylated hemoglobin. No significant difference between liver and kidney parameters were found in all studied groups. Oral administration of vitamin C to Fenvalerate treated rats significantly restored total hemoglobin and glycosylated hemoglobin levels.

DISCUSSION

Pyrethroides are among the most widely used synthetic insect pesticides. The widespread use of pyeothroides has stimulated research into the possible existence of effects related with their hepatorenal toxic activity (Joshi *et al*., 2007). The transaminases and phosphatase in different organs play an important role in transamination and phosphorylation processes in tissue metabolism (El-Kashoury and Tag El-Din, 2010). The present results revealed a significant ($P < 0.05$) decrease in the activities of serum AST, ALT of rats treated with fenvalerate. El-Kashoury and Tag El-Din (2010) reported that pyrothroides in different local manufactures (cyhalothrin, pyrifos and cypermithrin) at doses of 23.43, 21.40 and 17.43 mg/kg b.w., respectively causes significant decrease in the activities of AST and ALT. This fact is a conventional indicator of liver injury due to fenvalerate - treatment (El-Banna et al, 2009).

Moreover, fenvalerate (2.8 mg/kg b.w./ day) was determined to cause harmful effects in rats by increasing the levels of glucose. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in fenvalerate treated rats which come in agreement with the studies of Cetin, et al. (2010) who stated that administration of propolis insecticide (100 mg/kg b.w./day) rats alleviated the harmful effects of enzyme activities.

Glycosylated hemoglobin was significantly increased in fenvalerate control rats and this increase is directly proportional to fasting blood glucose (Koenig *et al*., 1976). Anemia is much more common disease toxicant patients, contributing to the pathogenesis of meta-bolic complications. In the present study, the decreased

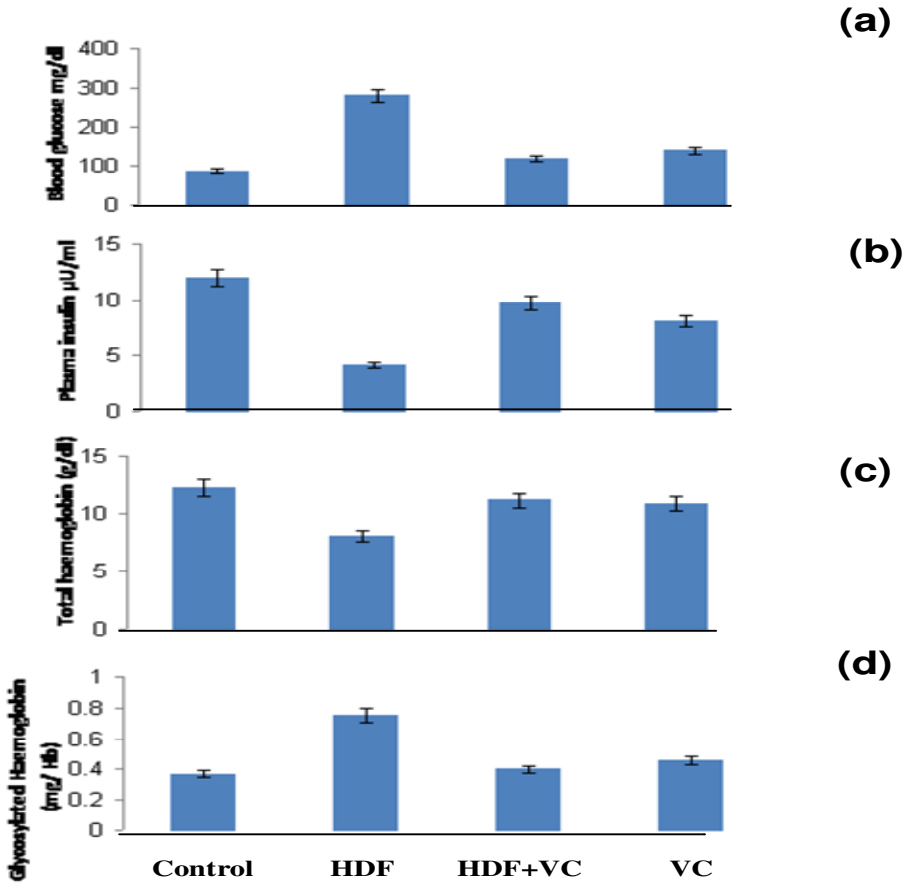

Figure 1. Effect of vitamin C on (a): The levels of blood glucose, (b): Plasma insulin, (c): Total haemoglobin and (d): Glycosylated haemoglobin in normal and experimental rats: High dose Fevnalerate (HDF); High dose Fenvalerate combined with Vitamin C (HDF+VC), and vitamin C only (VC). Values are given as means for 10 rats in each group ± SD.

concentration of hemoglobin indicates the anemia in fenvalerate treated rats, in as much as during toxicity; the excess glucose transport in the blood reacts with hemoglobin to form glycosylated hemoglobin. Free radicals react with lipids and causes per-oxidative changes that result in enhanced lipid peroxidation (Girotti, 1985). In our study, the lipid peroxidation markers (TBARS) may elevate in erythrocytes of fenvalerate treated rats as reported earlier (Zhang and Swaan, 1999). The increase in lipid peroxidation might be a reflection of decrease in enzymatic and nonenzymatic antioxidants of defense systems.

Serum AST and ALT activities were used as a marker of tissue damage. An insecticide produces an experimental damage due to its toxic metabolite (Zhang and Swaan, 1999). The toxic metabolite free radical is produced by cytochrome p450 which further reacts with oxygen to produce trichloromethyl peroxy radicals. These radicals bind covalently with the macromolecule and cause per-oxidative degradation of lipid membranes of the liver and kidney. The reduction of AST and ALT activities by Vitamin C as an antioxidant is an indication of repair of tissue damage induced by insecticide complications. This is in agreement with Rekka *et al*. (1992), who found that serum transaminases returned to normal activities with the healing of tissue parenchyma and regeneration of hepatocytes and renal tissues. Vitamin C induced suppression of increased ALT and AST activities. Thus, administration of this vitamin revealed protective activity against the toxic metabolites of diabetes.

In fact the increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Associated with the changes in lipid peroxidation the toxicant tissues showed decreased activities of key antioxidants SOD and CAT and increase MDA which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals in vivo. Previous studies have reported that the activity of SOD is low in insecticides treated rats (Feillet-Coudray *et al*., 1999). A decrease in the activity of these antioxidants can lead to an excess availability of

superoxide anion O2 •– (free radical anion) and hydrogen peroxide in biological systems, which intern generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation (Kumuhekar and Katyane, 1992). The result of increased activities of SOD and CAT suggest that vitamin C contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of O2•– and OH•. The increased activity of SOD accelerates dismutaion of O2•– to hydrogen peroxide (H_2O_2) which is removed by CAT (Aebi, 1984). This action could involve mechanisms related to scavenging activity of vitamin. Lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. The free radical scavenging activity of vitamin was evaluated.

Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation. Reduced lipid peroxidation was revealed by a significant decrease in MDA level in groups pretreated with vitamin, simultaneously with a significant elevation in SOD and CAT activities. The present study revealed that SOD and CAT activities decreased in insecticide treated animals, which may be due to altered antioxidant status. This is in accordance with results that indicated a decreased CAT in STZ animals may be due to the utilization of antioxidant enzymes in the removal of released H_2O_2 released (Blum and Fridovich, 1985; Cerutti *et al*., 1994). SOD and CAT activities increased significantly in the treated group versus the untreated animals. In conclusion, the present study showed that the administration of vitamin C alone caused significant improvements in enzyme characteristics of liver and kidney of male rats. Therefore, the present study elucidated the therapeutic effects of vitamin C administered in combination with fenvalerate to minimize its tissue toxicity.

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