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## Effect of Oral Administration of Fenitrothion on Biochemical and Hematological Parameters in Rats

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**Abstract:** The aim of this study was to evaluate the effects of acute exposure to various doses of fenitrothion (FNT) on level of serum glucose, total protein, triglycerides (TG), total cholesterol (TC) and some hematological parameters. The study was conducted on 8-week old male Wistar rats that divided into four groups (three experimental groups and one control group), were treated orally with different doses (25, 50 and 100 mg kg<sup>-1</sup>) of fenitrothion for 28 consecutive day. After treatment, blood samples were collected for biochemical and hematological studies. Present results demonstrated that exposed groups led to significant dose-dependent increase in serum glucose and cholesterol levels. Significant decrease was observed in some hematological parameters [Red Blood Cell (RBC) counts, Hemoglobin (Hb), Haematocrite (Ht) and Mean Corpuscular Hemoglobin (MCH) values]. Serum total protein and triglycerides were also decreased not significantly in exposed groups when compared with control. Generally, the degrees of observed variations were found to be dose dependent.

**Key words:** Fenitrothion, hematology, rat, total protein, cholesterol, triglycerides

### INTRODUCTION

There have been increasing concerns about the effects of various organophosphate insecticides in humans and experimental animals. All organophosphorous pesticides (OPs) exert neurotoxicity via inhibition of acetyl cholinesterase. In fact inhibition of blood cholinesterase activity is known as a marker of toxicity of OPs (Shadnia *et al.*, 2005). Toxicities of OP pesticides cause adverse effects on many organs and blood factors (Gupta, 2006). These compounds show strong insecticidal properties accompanied by low toxicity for vertebrates (Tos-Luty *et al.*, 2003). Fenitrothion [O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate] is one of the organophosphate insecticides that mainly used in agriculture for controlling chewing and sucking insects on rice, cereals, fruits, vegetables, stored grains and cotton. After oral administration, fenitrothion is rapidly and extensively absorbed from the mammalian intestinal tract (about 90-100% of the dose) and eliminated, predominantly in the urine (up to about 93% of the dose) and faeces (6-15% of the dose), within 24 h. It is mainly distributed to the liver, blood and carcass. Elimination is very rapid. The main metabolites are the phosphate fenitrothion (FNO), the mono methyl analogues of FNT and FNO (DM-FNT and DM-FNO, respectively) and 3-methyl-4-nitrophenol (NMC) and its corresponding

sulphate and glucuronide. The oral LD<sub>50</sub> for technical fenitrothion in rats is 240-1700 mg kg<sup>-1</sup> b.wt. Some studies have demonstrated that fenitrothion is toxic to the mammalian central nervous system and biochemical factors. Groups of 36 male, CD rats received technical fenitrothion by oral gavage (2.5, 5.0, 10.0 or 20.0 mg kg<sup>-1</sup> day<sup>-1</sup>) for 30 consecutive days. A dose-dependent decrease was noted in the brain, plasma and erythrocyte ChEs as well as tissue esterase activities (Trottier *et al.*, 1980). Oral administration of fenitrothion at 7.25 or 14.5 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> to male Wistar rats (5/group) for 28 days resulted in increases in plasma corticosterone and glucose levels, 7 days after the start of treatment and in the relative adrenal weight, 2 weeks after the start of treatment (Yamamoto *et al.*, 1982). However, there is limited research investigating the effects of fenitrothion on biochemical and some hematological parameters in the literature. The aim of this study was to evaluate the oral toxicity of various doses of fenitrothion in adult male Wistar rats based on the results of hematological and biochemical investigation.

### MATERIALS AND METHODS

**Chemical substances:** Fenitrothion 50 EC was applied as commercial emulsifiable concentrate formulation containing 50% active ingredient. It was diluted in olive

oil for the final test concentration. The test concentration of fenitrothion was calculated from the percentage of the active ingredients. The acute oral LD<sub>50</sub> for fenitrothion in male albino rats was 800 mg kg<sup>-1</sup> body weight. Therefore 25 mg kg<sup>-1</sup> (1/32 LD<sub>50</sub>) as low dose, 50 mg kg<sup>-1</sup> (1/16 LD<sub>50</sub>) as medium dose and 100 mg kg<sup>-1</sup> (1/8 LD<sub>50</sub>) as high dose were selected in the study. Solutions were freshly made before usage.

**Animals and treatment:** Twenty four male Wistar albino rats of initial body weight of 200-240 g. were obtained from the animal house of Science Faculty, Urmia University. All animals were acclimatized for 10 days before the start of the experimental procedure. After 10 days of acclimation, the animals were randomly assigned to either the experimental groups (25 mg kg<sup>-1</sup>; low dose group, 50 mg kg<sup>-1</sup>; medium dose group and 100 mg kg<sup>-1</sup>; high dose group) or the control group, each containing 6 rats. The animals were housed in labeled cages with solid plastic sides and stainless-steel grid tops and floors, in a room designed for control of temperature (approximately 21±1°C), humidity (45-75%) and light cycle (12 h light, 12 h dark). Animals were orally fed daily a normal diet in standard laboratory pellets (10 g/day/rat). The first group (control group) of animals received the olive oil only and the other groups received fenitrothion dissolved in olive oil at doses of 25, 50 and 100 mg kg<sup>-1</sup> by gavage. 0.4 mL of solution was administered daily for 28 days. Tap water was also available. At the end of experiment, in Physiology Laboratory of Urmia University, the animals of all groups were anaesthetized with ether and 4 mL of blood samples were drawn from the heart of each animal. Two blood samples were taken with or without EDTA. The one with EDTA was used for hematological analysis and the other for preparation of serum for biochemical assays.

**Hematological analysis:** The blood sample with EDTA was used for hematological analysis. Serum was separated and analysed for blood parameters namely Red Blood Cell (RBC) counts, White Blood Cell (WBC) counts, Hemoglobin (Hb), Haematocrite (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) by using MS,CELL counter in Ghavam Laboratory in Urmia, Iran.

**Biochemical studies:** The blood sample without EDTA was used for preparation of serum for the biochemical assays. After centrifugation of these samples at 5000 rpm for 20 min at 4°C, serum was separated and stored at -70°C until the biochemical analysis. Total protein was assayed

using the method of Lowry. In this method, serum protein was measured with folin phenol reagent (Lowry *et al.*, 1951). Serum glucose level was measured in the presence of glucose oxidase and peroxidase using o-dianisidine-HCL as a chromogen. The amount of glucose formed is related to the amount of o-dianisidine oxidation products that were measured spectrophotometrically at 436 nm (Teimouri *et al.*, 2006). Total Cholesterol (TC) was determined by the enzymatic colorimetric method of Flegg (Ibrahim and El-Gamal, 2003; Emeis, 1977). Triglycerides (TG) determination was carried out by the enzymatic colorimetric method of Wahlefeld (Ibrahim and El-Gamal, 2003; Santillo, 1995). These biochemical analysis were performed in Ghavam Laboratory in Urmia, Iran.

**Statistical analysis:** The results of hematological and biochemical analysis were presented as the mean ± SEM. Comparisons were made between control and treatment groups using one-way Analysis of Variance (ANOVA) and the post hoc Tukey multicomparison tests. Values of p = 0.05 were regarded as statistically significant.

## RESULTS AND DISCUSSION

In this study the hematological parameters like RBC counts, Hb, Hct and MCH were significantly decreased in exposed groups as compared to control (Table 1). In rats treated with high dose fenitrothion, RBC counts, Hb, MCH values show a more significant decrease. In addition, 100 mg kg<sup>-1</sup> fenitrothion treatment caused a significant decrease in Ht value. Data of serum glucose, total protein, TC and TG of exposure groups are shown in Table 2. Administration of fenitrothion at doses of 25, 50 and 100 mg kg<sup>-1</sup> showed a significant increase (p<0.05) in

Table 1: Results of hematological analysis of rats in control and fenitrothion treatment groups (n = 6)

| Parameters                 | Control    | 25 50 100<br>----- (mg kg <sup>-1</sup> ) ----- |             |               |
|----------------------------|------------|---|-------------|---------------|
|                            |            | RBC   | 8.62±0.05   | 7.78±0.19     |
| Hb (g dL <sup>-1</sup> )   | 14.23±0.13 | 13.35±0.36                                      | 13.60±0.18  | 12.500±0.33** |
| Ht (%)                     | 52.02±0.49 | 45.65±1.16                                      | 47.02±0.73  | 44.880±1.37** |
| WBC                        | 3.56±0.43  | 2.86±0.53                                       | 2.91±0.58   | 0.865±4.50    |
| MCV (fl)                   | 59.62±0.16 | 58.07±0.55                                      | 60.20±2.03  | 56.830±0.38   |
| MCH (pg)                   | 17.15±0.09 | 16.82±0.12                                      | 17.18±0.31  | 16.500±0.10*  |
| MCHC (g dL <sup>-1</sup> ) | 28.78±0.14 | 28.95±0.19                                      | 921.17±79.6 | 29.030±0.12   |

Each value presents the mean±SE, n = 6. \*p<0.05, \*\*p<0.01

Table 2: Results of biochemical assays of rats in control and fenitrothion treatment groups

| Parameters    | Control    | Low dose    | Medium dose | High dose     |
|---------------|------------|-------------|-------------|---------------|
| Glucose       | 66.17±15.8 | 67.50±14.5  | 71.17±1.60  | 111.30±1.30** |
| Total protein | 7.48±0.26  | 7.66±0.18   | 6.66±0.33   | 7.31±0.16     |
| Cholesterol   | 95.12±2.40 | 111.70±6.56 | 103.30±7.30 | 138.80±8.80** |
| Triglycerides | 48.83±4.60 | 29.17±4.30  | 33.67±3.60  | 35.00±2.90    |

Values are given as mean±SEM, n = 6. \*p<0.05, \*\*p<0.01

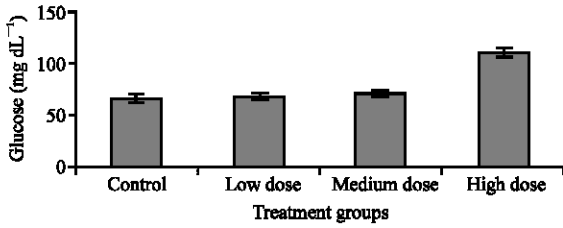


Fig. 1: Serum blood glucose levels of rats in both control and all doses of treatment groups. Values are given as mean±SEM. n = 6 for each group

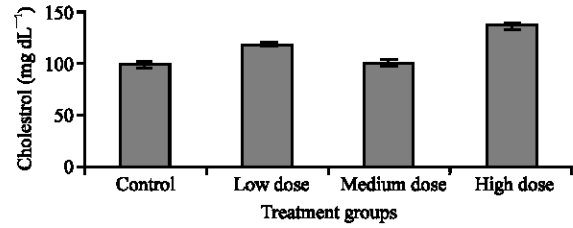


Fig. 3: Serum blood cholesterol levels of rats in both control and all doses of treatment groups. Values are given as mean±SEM. n = 6 for each group

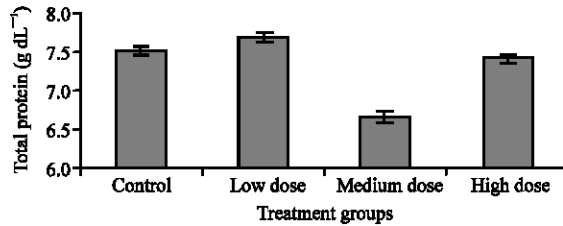


Fig. 2: Serum blood total protein values of rats in both control and all doses of treatment groups. Values are given as mean±SEM. n = 6 for each group

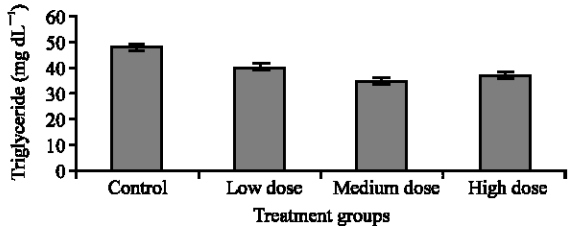


Fig. 4: Serum blood triglyceride values of rats in both control and all doses of treatment groups. Values are given as mean±SEM. n = 6 for each group

blood glucose concentrations. These levels were high and more significant in rats that were treated with the 1/8LD<sub>50</sub> dose level (Fig. 1). Data of total protein of exposure groups are shown in Fig. 2. Serum total protein in the fenitrothion treated animals showed a non significant reduction in comparison to the value in the control animals. The highest decrease value was recorded in rats that were treated with the medium dose level. The data show a significant increase ( $p < 0.01$ ) in serum level of cholesterol in rats treated with different doses of fenitrothion compared to control (Fig. 3). Figure 4 shows that the daily oral fenitrothion administration at all of applied dose levels in to rats caused a not-significant reduction in the plasma TG levels.

The results of this study showed that fenitrothion caused a significant decrease in some hematological parameters of the rats such as RBC counts, Hb, Hct, MCH values. Especially, high dose fenitrothion treatment in this experiment caused a significant decrease in these hematological parameters. A similar decrease of these hematological factors was previously recorded in the serum of cypermethrin-treated rats. The decrease in Hb value is due to an increase in the rate at which Hb is destroyed. Present results are in accordance with the results indicating a disruption of erythropoiesis or an increase in destruction of blood cells (Sayim *et al.*, 2005). We think that the decrease in the Hb along with the decrease in the RBC might be due to the effect of pesticides on blood forming organ in rats. Many steps in heme biosynthesis are inhibited by pesticidal residues and this might be a possible physiological reason for the

inverse proportion of the result obtained (Wolfe *et al.*, 1967). Fenitrothion had no significant effect in other hematological factors.

The results of the present study confirmed that acute exposure to fenitrothion increases blood glucose significantly ( $p < 0.05$ ). We think that fenitrothion increases glucose release from liver into blood through activation of glycogenolysis and gluconeogenesis as a detoxication mechanism to overwhelm fenitrothion-induced toxic stress (Teimouri *et al.*, 2006). It was observed that the serum total protein level was reduced not significantly in exposed groups as compared to control. Total protein decreased as a result of chronic disease when large number of parenchyma liver cells has been destroyed. Furthermore, the decrease of total serum protein may caused by the reduction of serum globulin level which markedly declined at the same time (Baron, 1984). Proteins play an important role in the life of all living organism. Pesticides disturb protein synthesis. A similar decrease of total protein concentration was previously recorded in the serum of the rats that were treated with pesticides: Diazinon (Ibrahim and El-Gamal, 2003), cypermethrin (Sayim *et al.*, 2005; Yousef *et al.*, 2003), quinalphos (Dikshith *et al.*, 1982). In the present study, serum TC was significantly increased in treatment groups. Similar results were also reported in rats treated with benzene hexachloride (BHC). Pesticides inhibit hepatic cyt-p-450 enzymes. The increase in cholesterol level indicates inhibitory action of pesticide on cyt-p-450 enzymes. Also, increased cholesterol concentration indicates liver disorders and cholestasis (Shivanandappa

and Krishnakumari, 1981; Zarn *et al.*, 2003). Other insecticide members have been reported to produce a rise in serum TC. These included methomyl (Antal *et al.*, 1979), ronnel (Rumsey *et al.*, 1983), dieldrin (Shakoori *et al.*, 1984) and furadan (Gupta *et al.*, 1986). As in the present study associated with the earlier studies, mean cholesterol concentration after treatment with fenitrothion were detected to increase significantly in contrast to control group ( $p < 0.01$ ). It is generally accepted that high total cholesterol level is linked with a greater risk for development of coronary artery disease and other organ complication. The data in the present study showed that the oral administration of fenitrothion decreased the level of serum TG. A similar decrease of TG was previously recorded in the serum of acephate-treated rats (Choudhari and Chakraharti, 1984). The decrease of serum TG may be a reflection of the insecticide-induced reduction of this lipid fraction in all lipoproteins classes, particularly Low-Density Lipoprotein (LDL) (Ibrahim and El-Gamal, 2003).

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