

**EFFECT OF THE STEROL DEMETHYLATION-INHIBITING FUNGICIDE FENARIMOL ON
SELECTED BIOCHEMICAL PARAMETERS IN RATS**

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Fenarimol, a 2,4'-dichloro- α -(pyrimidin-5-yl) benzhydryl alcohol fungicide and steroid demethylation inhibitor, has been reported to affect the actions of multiple CYP450 isoforms, including key enzymes. The aim of this study was to evaluate the effects of fenarimol on a set of biochemical parameters in rats. Rats were intraperitoneally injected with a 200 mg kg⁻¹ dose of fenarimol. Blood samples were collected for measurements of serum alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), creatine kinase (CK) activity, and levels of glucose, urea, sodium, and potassium. Serum was analysed at 0, 2, 4, 8, 16, 32, 64 and 72 h following injection. Our results demonstrated a statistically significant increase in ALP, ALT, AST, CK activities and levels of urea from time to time ($p < 0.05$). Our results demonstrate that treatments with fenarimol can alter the activity and levels of selected serum enzymes and biochemical parameters in rats.

Key words: fenarimol, lactate dehydrogenase, alkaline phosphatase, alanine amino transferase, aspartate amino transferase

INTRODUCTION

Although the use of pesticides over the last few decades has had a number of benefits, their toxicological effects on humans and environment need to be considered. Fenarimol is a 2,4'-dichloro- α -(pyrimidin-5-yl) benzhydryl alcohol fungicide and steroid demethylation inhibitor (Tomlin *et al.*, 2006) that is widely used in horticulture and agriculture on a wide range of fruits, vegetables, hops, and wheat to effectively control the incidence of apple scab and powdery mildew of peas, cherries, grapes, and so on. Fenarimol is rapidly absorbed by the soil and sediments and is highly persistent (WHO, 1995).

In mammalian cells, fenarimol induces various CYP450 isoforms from all of the inducible families including key enzymes involved in the biosynthesis and metabolism of steroids (Paolini *et al.*, 1996; Hrelia *et al.*, 1994; Cantelli-Forti *et al.*, 1993). Fenarimol has also been shown to have reproductive, teratogenic, and oncogenic effects in experimental animals. It inhibits aromatase activity, which

may result in irreversible infertility in male rats (Andersen *et al.*, 2002; Vinggaard *et al.*, 2005).

Fenarimol may cause toxic effects by binding to cell macromolecules. Di Ilio *et al.* (1995) demonstrated the electrophilic nature of this fungicide and suggested its possible reactivity with DNA. When tested *in vivo* on rats, fenarimol was capable of inducing DNA damage in hepatocytes with a significant increase in DNA unwinding (Grilli *et al.*, 1991). Fenarimol also induced prenatal and perinatal genotoxicity in leukocytes of *in vivo* treated rats (Castro *et al.*, 2005). Long-term bioassays analysing the carcinogenesis of fenarimol reported a significant increase in hepatic lesions (adenomas and hyperplastic nodules) in Wistar rats compared to untreated controls (Flodstrom *et al.*, 1990).

The activity of serum enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma glutamyltransferase (GGT), can be used as a diagnostic tool in the evaluation of hepatic damage. Although these enzymes are not completely specific, an increase in their activity reflects active liver damage (Nemcsok *et al.*, 1987; Asztalos *et al.*, 1990). Palut *et al.* (1997) investigated the effects of fenarimol on marker enzymes in rat liver and demonstrated a marked increase in GGT activity and an increase in the number of GGT positive hepatocytes.

Given the observed impact of fenarimol on energy metabolism, the aim of this study was to analyze the effects of fenarimol on various enzymes and selected biochemical parameters in the blood serum of rats.

MATERIALS AND METHOD

Animals

Wistar rats (*Rattus norvegicus*) weighing 200-250 g were used in this study. Rats were purchased from the Experimental Animals Feeding and Research Centre of Uludag University Medical Science Faculty in Bursa, Turkey. Animals were acclimatised in a 12 h light/dark cycle at 21–23°C. Animal care was conducted according to institutional guidelines.

Animal treatment

For each trial period, two rats from the control group and four from the experimental group were used (totalling 16 animals for the controls and 32 for the experimental groups). Control groups were treated with corn oil while experimental groups were injected intraperitoneally with a 200 mg kg⁻¹ (LD₅₀) dose of fenarimol. The rats were left without food and water for 24 h prior to injection, ensuring the simultaneous initiation of metabolism of animals in both groups at the same time. Following injection, food and water were regularly given to the animals until the trial periods were completed. Treated and control rats were kept in plastic metabolic cages. Animals were killed via cervical dislocation at each time point (0, 2, 4, 8, 16, 32, 64 and 72 h post-injection).

Analysis of Biochemical Parameters

Blood samples were collected by cardiac puncture. To prepare the serum samples, blood samples from rats were immediately centrifuged in a Nüve NT 201 at 12000 rpm for 15 min at 4°C. The supernatant serums were separated from the pellets and used for biochemical analyses. Biochemical assays were performed using a Hitachi 911 autoanalyser. The following biochemical parameters were assayed: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) using commercially purchased biochemical analysis kits (Hitachi kits).

Statistical analysis

Data were analysed using SPSS 13.0 for Microsoft Windows. Independent t-tests were applied between the data of control and treated animals at each time point. The significance was calculated using one-way analysis of variance (ANOVA) and Student's t-tests. A value of $p < 0.05$ was interpreted as being statistically significant.

RESULTS

The effects of fenarimol administration were evaluated by monitoring marker enzymes in serum samples from control and fenarimol-treated rats. The results showed that fenarimol caused increases in ALP, AST, ALT and CK activities in all experimental periods (Table 1).

While increases in ALT activity were significant in all experimental periods except in the initial period, ALP activities were significant only in the 8th, 16th, and 32nd hours compared with control groups ($p < 0.05$). When AST activity in serum was examined, it was observed that AST activity was significantly increased in all experiment periods except for the initial period ($p < 0.05$) (Table 1).

The glucose, urea, sodium, and potassium levels were increased following treatment with fenarimol in rats. While the increase in glucose, sodium, and potassium levels were statistically significant in all experiment periods, the levels of urea were significant at all periods for the initial period ($p < 0.05$) (Table 1).

DISCUSSION

Severe poisoning from organophosphate insecticides is common and widely recognised. The uncontrolled use of pesticides in agriculture can cause changes in the local ecological balance of treated area, adversely affecting or killing many non-target organisms (Das and Mukherjee, 2000). Because of the potential environmental impacts of pesticides, and the large population potentially exposed by their use, the effects of pesticide exposure need to be determined.

To the best of our knowledge, there are only two reports of intoxication by fenarimol and no reports of severe toxicity with recovery (Salameh *et al.*, 2008; Proença *et al.*, 2003). In addition, there has been a limited amount of research

Table 1. The change in some enzymes activities in serum of control and fenarimol-treated group animal

Enzyme (U/L)	Time (Hours)	0	2	4	8	16	32	64	72
ALP	Control	155.4±38.9	153.1±33.3	161.3±41.2	155.0±38.7	160.2±39.3	166.4±32.7	159.5±39.2	164.8±35.5
	Fenarimol	159.8±35.3	185.5±44.8	188.8±45.4	222.1±63.8*	234.9±54.7*	241.6±44.8*	197.3±48.3	175.4±46.2
ALT	Control	71.7±19.1	77.3±20.2	81.4±24.2	74.4±22.9	78.2±30.4	79.2±25.7	78.8±26.6	73.6±23.3
	Fenarimol	74.9±20.7	108.5±30.9*	147.6±41.2*	167.5±33.3*	178.9±48.1*	170.5±36.7*	169.4±38.8*	165.1±39.4*
AST	Control	144.7±44.5	145.2±48.8	165.3±33.8	157.7±42.5	155.7±32.4	163.9±44.9	166.7±40.4	160.4±33.3
	Fenarimol	150.6±42.1	210.5±449.4*	233.2±32.7*	255.5±49.1*	229.4±55.1*	238.4±57.4*	267.5±46.7*	254.8±44.4*
CK	Control	1562.8±125	1689.7±182	1528.4±198	1596.4±166	1602.5±175	1645.9±173	1775.4±195	1705.9±188
	Fenarimol	1687.5±188	1721.1±201	1796.3±169*	1869.4±155*	1777.4±163	1806.9±188	1822.3±171	1799.7±154
Glucose	Control	77.4±5.7	142.8±8.5	141.8±9.7	142.4±8.5	148.7±8.1	140.7±9.4	148.9±8.2	139.3±6.3
	Fenarimol	78.7±5.5	148.4±9.7	152.1±8.9	155.5±9.4	154.9±8.7	159.4±8.8	148.7±8.8	144.7±7.8
Urea	Control	41.1±2.6	50.8±3.3	48.6±2.7	42.7±2.1	45.4±3.2	43.5±2.8	49.2±3.4	47.5±2.6
	Fenarimol	50.8±2.2	60.2±3.1*	64.3±3.3*	67.6±4.1*	64.4±2.2*	58.8±3.8*	67.6±4.1*	60.2±4.5*
Sodium	Control	146.4±9.2	146.8±5.4	148.4±6.9	150.1±5.5	149.5±8.9	148.3±9.4	150.4±8.8	149.5±8.7
	Fenarimol	147.1±7.9	150.2±8.8	154.1±7.3	156.2±7.8	159.4±8.7	155.5±7.2	153.1±8.1	158.6±8.3
Potassium	Control	8.81±0.41	9.04±0.52	9.05±0.81	9.81±0.92	9.74±0.56	8.43±0.75	8.54±0.44	8.38±0.53
	Fenarimol	8.91±0.82	9.21±0.53	9.15±0.85	9.91±0.46	9.42±0.77	9.08±0.57	8.93±0.65	8.75±0.45

* All means are significantly different ($p < 0.05$)
 Results are expressed as mean ± SD.

carried out on the effects of fenarimol on biochemical parameters in mammals. Therefore, we wanted to evaluate the effects of fenarimol on select biochemical parameters. We showed that fenarimol affected important hepatic marker enzyme activities (ALP, ALT, AST, and CK) and some biochemical parameters in the serum. Serum enzymes have long been considered reliable indicators of hepatic damage. Dehydrogenases and phosphatases are important enzymes in many biological processes including detoxification, metabolism, and the biosynthesis of energetic macromolecules for different essential functions. Any interference in the normal functioning of these enzymes leads to biochemical impairment and lesions in tissue and cellular function. They are considered as specific indicators for hepatic dysfunction and damage (Khan *et al.*, 2001). However, the toxicological study showed concentrations of fenarimol higher in the liver than in the other tissues in postmortem samples (Salameh *et al.*, 2008).

In vitro studies have found that glyphosate and paraquat are able to inhibit certain enzyme activities, including those of ALT, AST, LDH, and AChE (El-Demerdash *et al.*, 2001). Experimental studies in rats have reported significant changes in the activity of these enzymes following chronic administration of mancozeb in a dose-dependent manner (Kackar *et al.*, 1999). Borges *et al.* (2007) reported that the observed increase in ALT, AST, and LDH enzyme activities in diphenyl ditelluride-treated rats were regarded as the biochemical manifestation of the toxic action of the diphenyl ditelluride. In addition, chronic exposure of rats and mice to pesticide led to increased levels of serum ALT and AST (Gomes *et al.*, 1999). We found that fenarimol caused a significant increase in AST, ALT, ALP, and CK activities and that these increases were statistically significantly ($p < 0.005$). Our present data, in agreement with those from a previous study, found that dinitro-*o*-cresol, dichlorvos, and methyl parathion, which are all used as pesticides, caused increases and decreases in serum enzyme activities and some biochemical parameters in female and male rats (Dere *et al.* 2007; Ozdikicioglu *et al.*, 2008; Ari and Dere, 2008).

In our study, the underlying cause for serum enzyme activation with treatment may be due to the effect of fenarimol on cellular organelles in the rat tissues. Any alterations in cell organelle function will indirectly influence enzyme activities. The increase in serum enzyme activity in fenarimol-treated rats is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. In other words, increases in serum enzyme activity result in response to cellular degeneration or destruction of the liver.

Grabarczyk *et al.* (1989) found that fenarimol caused functional and structural changes of blood cells and organs with a dosage of 25 mg/mL intragastrically over a period of 5 days. They found that the osmotic fragility of erythrocytes, the phagocytic activity of neutrophils, and the number of blood cells decreased while the percentage of lymphocytes increased. An additional study showed that carbendazim, another fungicide, affected the liver and caused specific changes in haematological and biochemical parameters in the rat (Muthuviveganandavel *et al.*, 2008). Long-term carcinogenesis bioassays on fenarimol reported a significant increase in hepatic lesions (adenomas and hyperplastic nodules) in Wistar rats (Flodstrom *et al.*, 1990).

The direct activity of fenarimol, or its metabolites, may involve the binding of fenarimol to protein, DNA, or RNA. Di Ilio *et al.* (1995) has demonstrated the electrophilic nature of the fungicide and suggested its possible reactivity with DNA. When tested *in vivo* in rats, fenarimol was capable of inducing DNA damage in hepatocytes with a significant increase in DNA unwinding (Grilli *et al.*, 1991) and a reduction in mitotic index at higher doses in mice (Aydemir and Bilaloglu, 2004). Fenarimol also induced genotoxicity in leukocytes of *in vivo* treated rats (Castro *et al.*, 2005).

Studies of reproductive toxicity in rats have shown that fenarimol reduces male fertility, both in exposed adult males and in the male offspring of exposed females. This effect is manifested as an absence of male sexual behaviour that might result from altered perinatal development of male patterns of sexual behaviour (Grünfeld and Bonefeld-Jorgensen, 2004; Hirsch *et al.* 1987; Hirsch *et al.*, 1986). It was shown that oral administration of fenarimol considerably reduced the weight of the ventral prostate, seminal vesicles, and bulbourethral glands of castrated and testosterone-treated male rats (Vinggaard *et al.*, 2005). In addition, fenarimol inhibited 5 α reductase (one of the key enzymes in human androgen metabolism) in prostate homogenates (Lo *et al.*, 2007).

Our results show that glucose, urea, sodium, and potassium levels are increased in fenarimol-treated rats. Borges *et al.* (2007) demonstrated that plasma creatinine and urea levels increased after diphenyl ditelluride exposure. Another study showed that Ca²⁺ concentration decreased in rat serum following intragastric administration of fenarimol over 5 days (Wozniak and Kopeć-Szlezak, 1991).

In conclusion, based on the experimental evidence obtained here, we suggest that AST, ALT, ALP, and CK enzymes and biochemical parameters can serve as useful biomarkers for the field monitoring of pesticide exposure effects on wildlife. Further studies are necessary, however, to investigate other important biochemical parameters in order to explain the effects of fenarimol in mammals. Fenarimol, when employed excessively and unconsciously in agriculture, pollutes the natural world and indirectly results in negative changes to all exposed living organism. Hence, it is of critical importance to public health that these chemicals are used carefully and selectively, and that consumers and producers are informed of the possible and real damage caused by these agents.

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UTICAJ FUNGICIDA FENARIMOLA – INHIBITORA DEMETILACIJE STEROLA NA ODABRANE BIOHEMIJSKE PARAMETRE KOD PACOVA

ARI F i DERE E

SADRŽAJ

Fenarimol je fungicid koji deluje inhibitorno na demetilaciju steroida i utiče na funkcije brojnih enzima. Cilj ovih oglada je bio da se ispita uticaj fenarimola na vrednosti odabranih biohemijskih parametara u serumu pacova kojima je intraperitonealno aplikovano 200 mg kg⁻¹ ovog preparata. Uzorci krvi su prikupljeni 0, 2, 4, 8, 16, 32, 64 i 72 sata posle aplikacije i u serumu je određivana aktivnost alkalne fosfataze (ALP), alanin-amino transferaze (ALT), spartat-amino transferaze (AST) i kreatin kinaze (CK) kao i koncentracije glukoze, uree, natrijuma i kalijuma. Naši rezultati ukazuju da postoji statistički značajan porast aktivnosti ALP, ALT, AST i CK i koncentracije uree između vrednosti registrovanih u posmatranim vremenskim intervalima (p<0,05).