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ORIGINAL ARTICLE

Toxicological effects of methyl eugenol and fenitrothion on hematological, hepatic, renal, and oxidative stress-related biochemical characteristics of white albino rats

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ABSTRACT

The present study aimed to investigate the possible adverse effects of methyl eugenol (ME), a potent kairomone, and fenitrothion insecticide that are used extensively in male annihilation programs worldwide during the management of fruit flies. Therefore, the side effects of ME, fenitrothion (FEN) and their mix (ME+FEN) were tested in a 28 days-inhalation study on white albino rats. Rats were grouped and exposed to 1/20 LC_{50} of ME, FEN, and their mixture. Then hematological components, hepatic and renal parameters, and oxidative stress enzymes were measured. Results revealed significant decrease effects on mean cell hemoglobin and mean cell hemoglobin concentration (up to 18% decrease of control), red blood cell (28%), acetylcholinesterase (39%), and total lipids (36%) levels. On the other hand, lactate dehydrogenase (increased to 29.5% of control), uric acid (109%), creatinine (140%), γ-glutamyl transferase (159%), and oxidative stress-related enzymes (glutathione peroxidase (149%), glutathione reductase (170%), glutathione-S-transferase (34%), and superoxide dismutase (88%)) values were increased compared to results of control group. Adverse effects of ME, FEN, and their mixture might trigger an alarm for farmers who directly or indirectly inhale these chemicals continuously in the field. More importantly, safe alternatives to agricultural chemicals, including pesticides, should be enforced.

KEY WORDS: occupational exposure; fenitrothion; methyl eugenol; inhalation toxicity

Introduction

Worldwide and during pest management practices tons of pesticides are being applied. According to FAOSTAT, 4,088,167.77 tons of pesticides were applied on agricultural crops worldwide (FAOSTAT, 2019). Among these pesticides, the pheromone and kairomones are used in traps to deter or attract insects. This approach is coined as environmentally-safe (Bhagat *et al.,* 2013). The kairomones such as methyl eugenol (ME) have been used extensively in many male annihilation programs (Ghanim *et al.,* 2010; Keng & Nishida, 2012). The ME, 3,4-dimethoxyallylbenzene is the main constituent of many plant species including basil, tarragon, fennel, marjoram, mace, allspice, star anise, and anise (Keng & Nishida, 2012; NTP, 2000; Smith *et al.,* 2002). Also, it is used as a synthetic flavoring substance in non-alcoholic beverages, condiments, and hard and soft candy (Smith *et al.,* 2002). In agriculture, it is used solely or in combination with the organophosphate insecticides as an insect attractant (Benford *et al.,* 2010; IARC, 2013; Vargas *et al.,* 2010).

Although ME was declared as generally safe (GRAS) by the Food and Drug Administration as a food additive (NTP, 2000). Several studies described the adverse effects of the alkoxyallylbenzenes group of chemicals including ME, where it was reported as a DNA-reactive compound that induces cancer (neoplastic lesions) in the livers of rodents and mice (Burkey *et al.,* 2000; Thompson *et al.,* 1990; Williams, 2010; Williams *et al.,* 2008). It was reported as cytotoxic to cultured hepatocytes isolated from rats and mice *via* causing unscheduled DNA synthesis (UDS) in both species in a dose-dependent manner (Burkey *et al.,* 2000; Martelli *et al.,* 1994). Also, the glutathione-S-transferase isozymes (GSTs) were inhibited by ME as illustrated by studies on rats and humans (Rompelberg *et*

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al., 1996). After a 14-weeks study, liver and testis weight were increased significantly when rats were given daily doses from 300 to 1000 mg/kg of ME. Also, the authors reported a decreased mean packed red cell volume and increased platelet counts, alanine aminotransferase, and sorbitol dehydrogenase activities after the administration of ME (Luo & Guenthner 1995; Rompelberg *et al.* 1996). In another 2-years study, ME induced a high incidence of atrial thrombosis in female mice treated with 75 mg/kg or higher doses (Yoshizawa *et al.,* 2005).

Moreover, oral administration of ME induced erythrocyte microcytosis and thrombocytosis, and caused an increase in the activities of alanine aminotransferase and sorbitol dehydrogenase and bile acid concentration in male and female rats, suggesting hepatocellular injury, cholestasis or altered hepatic function (Abdo *et al.,* 2001). Additionally, ME triggered hypoproteinemia and hypoalbuminemia in both male and female rats. In mice, it increased incidences of adrenal gland cortical hypertrophy and/or cytoplasmic alteration in the submandibular salivary glands, adrenal glands, testis, and uterus were considered secondary to the chemical-related effects observed in the liver and glandular stomach of rats (Abdo *et al.,* 2001). But it was reported that ME might elicit DNA repair synthesis in cultured rat and mouse hepatocytes (Burkey *et al.,* 2000).

In parallel to the abovementioned, fenitrothion (FEN), *O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl) phosphorothioate is an organophosphorus insecticide that is widely used as a mixture with ME in traps to control the Bactrocera insects (Ghanim *et al.,* 2010; Keng & Nishida, 2012). It is a broad-spectrum non-systemic insecticide with high selective toxicity to insects compared to mammals and moderate toxicity, with an oral LD_{50} of 330 mg/kg, to rats (Briggs, 1992; Vásquez-Castro *et al.,* 2012). FEN is being sprayed worldwide against a wide range of insects on rice, cereals, fruits, vegetables, stored grains, cotton and forests, and public health disease vectors (Wang *et al.,* 2012; WHO, 2010). Therefore, it might be considered as a hazard compound to mammals (Khan *et al.,* 1990). FEN was reported to have lower toxicity to mammals relative to insects because of the enhanced detoxification system (NRCC, 1975). It's well documented that FEN acts by inhibiting the activity of the acetylcholinesterase enzyme, which highlights acute neurotoxicity (Wang *et al.,* 2012). In addition, several studies reported toxicity of FEN to mammals via different routes of administration with a limited number of studies that investigated its inhalation toxicity. For example, FEN at 6.7, 20, and 60 pg/l of air for 2 hr/day for 30 consecutive days reduced body weight, food intake, muscle fasciculations, erythrocyte content and brain cholinesterase activity (Breckenridge *et al.,* 1982).

When young Japanese children inhaled FEN at 0–44 ng/kg/day, they suffered severe health effects (Kawahara *et al.,* 2005). In another study, it was reported that single inhalation exposure of rats to 500 mg/m^3 of FEN showed no serious pulmonary toxicity but minor modifications of lung alveolar tissues (Chevalier *et al.,* 1984). Exposure of rabbits to fenitrothion as thermal fog at 10 l/ha for 30 days significantly reduced the body weight (13.34%) but increased weights of liver, heart, and lungs by 23.37, 20.23 and 14.76%, respectively and reduced the activity of brain, blood, plasma, and erythrocytes cholinesterases (Al-Sarar *et al.,* 2011).

To the best of our knowledge, the inhalation toxicity of the mixture of ME and FEN was not tested. Therefore, the current study aimed to investigate the inhalation toxicity of sublethal doses of methyl eugenol (1/20 LC_{50}), fenitrothion (1/20 LC_{50}), and their mixture on hematology and biochemical parameters of white albino rats after 28 days of exposure.

Materials and methods

Chemicals

Fenitrothion, O,O-dimethyl-O-(4-nitrophenyl) phosphorothioate, (CAS 122–14–5, 99% purity) was obtained from Supelco Analytical (USA). It was diluted in corn oil for the final test concentration. Methyl eugenol (purity 99%) was obtained from Ministry of Agriculture and Land Reclamation, National Pesticides Committee, Egypt. Acetylthiocholine iodide (ATChI; Sigma order #A5751-250MG), 5,5-dithiobis-2-nitrobenzoic acid (DTNB-Ellman's reagent: Sigma order #D218200), brilliant (Coomassie) blue G-250 (Sigma order #B0770-5G), and bovine serum albumin (BSA: Sigma order #A2153-10G) were provided from Sigma-Aldrich, USA.

Animals and treatments

Young, 60-days old, male white albino Sprague Dawley rats (*Rattus norvegicus*) weighing 120±5 g were purchased from the Animal Health Research Center, Cairo, Egypt. Rats were housed in plastic cages in an animal room maintained at 25±2°C and in an alternate 12 hr cycle of light and darkness. They fed on a pellet diet and tap water was provided *ad libitum*. After 2-week acclimatization period, the rats were randomly divided into four groups (6 animals in each group): control (corn oil), FEN (0.25 mg/l air: 1/20 LC_{50} inhalation (EPA, 1996)), ME (240 mg/l air: 1/20 LC_{50} (Beroza *et al.,* 1975)), and FEN+ME at 1:1 ratio. The rats inhaled treatments for 28 days from chipboard blocks (cotton rolls No. 2, medium-sized, with 6×2×2 cm dimensions, Defend, MyDent International, Hauppauge, NY, 11788, USA) that were impregnated by dipping in corn oil, FEN, ME, and FEN+ME concentrations. Blocks were hanged in 45 l plastic cages using metal wire in a way to avoid any direct contact with rats for 8 hr daily. Cages of each group were separated completely from the other groups. Rats in each group were anesthetized with diethyl ether and sacrificed by decapitation at the end of treatment. Initial and final body weights of individual rats in each group were recorded to calculate relative weight changes (Chapman *et al.,* 1959). All rats were handled in accordance with the standard guide for the care and use of laboratory animals of the National Research Council (NRCNA, 2011) and the animal ethics guidelines of Damanhour University.

Hematological parameters

After killing rats, trunk blood was collected gently in sterilized EDTA tubes containing an anticoagulant (ethylenediamine tetra-acetic acid, EDTA) to give a final concentration of 5 mg EDTA/ml blood. Samples were mixed gently and discarded if any clots were seen in the vial. Total red blood cell (RBC) count ($\times 10^6$/µl), hemoglobin (Hb) content (g/dl), hematocrit (HCT) value (%), mean corpuscular volume (MCV; fl), mean cell hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC) values (g/dl), total WBC count ($\times 10^3$/µl) and platelet count ($\times 10^3$/µl) were assessed using Sysmex KX-21 hematological analyzer (Sysmex KX21, Japan) in a certified clinical laboratory.

Biochemical parameters

Blood samples were collected in test tubes without an anticoagulant, allowed to clot at room temperature for 15 min, and then centrifuged at 1390 g (Universal 32R, Hettich Zentrifugen model D-78532, Germany) for 10 min for serum separation. Serum was used for the following assays. The AChE activity was quantified following a modified method of Ellman *et al.* (Ellman *et al.,* 1961). Blood glucose (Trinder, 1969), uric acid (Barham & Trinder, 1972), total protein (Gornall *et al.,* 1949), total lipids (Zöllner & Kirsch, 1962), alkaline phosphatase (ALP) (Belfield & Goldberg, 1971), glutathione-S-transferase (GST) (Habig *et al.,* 1974), glutathione peroxidase (GP) (Paglia & Valentine, 1967), glutathione reduced (GR) (Beutler *et al.,* 1963), superoxide dismutase (SOD) (Nishikimi *et al.,* 1972), and creatinine (Bartels *et al.,* 1972) were measured using BioDiagnostic kits (Diagnostic research and Reagents, Giza, Egypt). Lactate dehydrogenase (LDH) activity (Burtis *et al.,* 2005) was determined using BioSystems Kit (BioSystems S.A., Costa Brava, Barcelona, Spain). Determination of γ-glutamyl transferase (GGT) activity was done using Spectrum Kit (MDSS, GmbH, Schiffgraben, Germany) (Heersink *et al.,* 1980). The replicates were measured in triplicates.

Statistical analysis

Toxicological data were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) (Version 9.3, Cary, NC, USA, 2016). Means were compared using Scheffe's *post-hoc* Multiple Comparison ($p \leq 0.05$) (SAS, 2016).

Results and discussion

Mortality and relative bodyweight

During the study period, there was no mortality or severe clinical signs of toxicity in treated rats. Body weight changes were reported, and relative weights of internal organs liver, kidney, heart, brain, spleen, lungs, and testes were reported (Table 1). Weekly percentages of change of body weight were significantly different between the control group and treated groups. FEN, ME, and ME+FEN showed an increased body weight compared to the control, but they had similar % of change. For the relative organ weight, kidney and spleen had similar percentages to the control group. The ME+FEN treatment increased the liver relative weight compared to the other treatments. The FEN reduced the heart and brain relative weights compared to control, ME, and ME+FEN groups that were similar. Lung relative weights were reduced after treatments of ME+FEN, ME, and FEN compared to the control. The ME significantly reduced the relative weights of testes of treated rats compared to control, ME+FEN, and FEN groups that had similar results.

Hematology

Application of pheromones mixed with insecticides would reduce the amounts of pesticides that are required to control plant pests and were coined as a safe approach. The ME, an alkenylbenzene pheromone, is used as a mixture with organophosphate insecticides in the annihilation programs against fruit flies. Possible adverse effects on hematological parameters of rats that inhaled ME, FEN and their mix were reported herein. Results in Table 2 showed that the numbers of white blood cells (WBCs) were increased significantly in rats that inhaled ME (mean WBC's equal to 11.70×10^3/µl), FEN (15.22×10^3/µl), and ME+FEN (15.35×10^3/µl) compared to the control group (9.25×10^3/µl). Moreover, both FEN and FEN+ME treatments revealed increased numbers of WBCs compared to the ME group. Similarly, platelets numbers were increased in blood samples of the three treated groups of rats after the treatment with ME (667.8×10^3/µl), FEN (677.3×10^3/µl), and ME+FEN (650.3×10^3/µl) compared to control (452.5×10^3/µl), but they were not different from each other.

On the other hand, numbers of red blood cells (RBCs), hemoglobin (HGB), and hematocrit (HCT) values were

Table 1. Mean relative organ weights ± SE of liver, lung, kidney, heart, brain, spleen, and testes of white albino rats inhaled methyl eugenol (ME), fenitrothion (FEN), and their mixture (ME+FEN) for 28 days.

Treatments	% of Change / Week	Liver	Kidney	Heart	Brain	Spleen	Lung	Testes
Control	$12.89^{c}\pm0.58$	$3.16^{bc}\pm0.14$	$0.74^{a}\pm0.06$	$0.39^{a}\pm0.05$	$0.72^{a}\pm0.05$	$0.26^{a}\pm0.05$	$0.86^{a}\pm0.08$	$1.15^{a}\pm0.10$
FEN	$19.05^{a}\pm0.82$	$3.59^{ab}\pm0.16$	$0.76^{a}\pm0.07$	$0.33^{b}\pm0.02$	$0.62^{b}\pm0.04$	$0.26^{a}\pm0.04$	$0.73^{b}\pm0.06$	$1.08^{a}\pm0.12$
ME	$15.27^{ab}\pm0.58$	$3.28^{b}\pm0.14$	$0.72^{a}\pm0.06$	$0.39^{a}\pm0.05$	$0.77^{a}\pm0.05$	$0.28^{a}\pm0.05$	$0.70^{bc}\pm0.08$	$0.63^{b}\pm0.10$
ME+FEN	$15.16^{ab}\pm0.58$	$3.71^{a}\pm0.14$	$0.78^{a}\pm0.06$	$0.38^{a}\pm0.05$	$0.72^{a}\pm0.05$	$0.30^{a}\pm0.05$	$0.67^{c}\pm0.08$	$1.17^{a}\pm0.10$

n=6 rats, SE; standard error, means with the same superscript letter were not significantly different, $p<0.05$, % of body weight change/week = ((final body weight-initial body weight)/ initial body weight)/no of weeks × 100, and relative organ weight = (organ weight/final body weight) × 100

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Table 2. Mean ± SE values of total white blood cell (WBC) counts (×10³/µl), platelets (PLT No.) number (×10³/µl), total red blood cell (RBC) count (×10⁶/µl), hemoglobin (HGB) content (g/dl), hematocrit (HCT) value (%), mean cell hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC) values (g/dl), and mean corpuscular volume (MCV; fl) of white albino rats inhaled methyl eugenol (ME), fenitrothion (FEN), and their mixture (ME+FEN) for 28 days.

Treatments	WBC (×10³/µl)	PLT No. (×10³/µl)	RBC (×10⁶/µl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)
Control	9.25^{c}±0.44	452.5^{b}±31.52	5.09^{a}±0.23	13.05^{a}±0.32	35.43^{a}±0.80	26.02^{a}±1.34	36.99^{a}±1.66	70.49^{a}±4.81
ME	11.70^{b}±0.39	667.8^{a}±28.20	3.78^{b}±0.20	9.52^{b}±0.29	31.20^{b}±0.72	25.30^{a}±1.20	30.59^{ab}±1.48	83.55^{a}±4.31
FEN	15.22^{a}±0.44	677.3^{a}±31.52	3.74^{b}±0.23	7.85^{c}±0.32	28.75^{b}±0.80	21.25^{a}±1.34	27.42^{b}±1.66	77.68^{a}±4.81
ME+FEN	15.35^{a}±0.44	650.3^{a}±31.52	3.96^{b}±0.23	8.45^{bc}±0.32	29.05^{b}±0.80	21.39^{a} ±134	29.22^{b}±1.66	73.58^{a}±4.81

Means were statistically compared using the Scheffe's *post-hoc* multiple comparison method at $p \leq 0.05\%$. Means with the same superscript letter are not significantly different. Each treatment was replicated 6 times and each replicate was measured 3 times.

Table 3. Mean ± SE values of glucose, uric acid, creatinine, total protein, and total lipids contents and acetylcholinesterase (AChE; nM ATChI/min), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), and lactate dehydrogenase (LDH) enzyme activities of serum of white albino rats inhaled methyl eugenol (ME), fenitrothion (FEN), and their mixture (ME+FEN) for 28 days.

Treatments	AChE	Glucose (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)	ALP (IU/l)	γ-GT (U/l)	LDH (U/l)	Total Lipids (mg/dl)
Control	1.22^{a}±0.03	63.81^{c}±2.95	1.69^{c}±0.20	1.07^{c}±0.121	8.30^{a}±0.36	184.40^{c}±5.90	64.56^{c}±6.72	132.90^{a}±11.74	770.20^{a}±17.37
ME	0.97^{b}±0.03	117.13^{b}±2.64	2.42^{bc}±0.18	1.38^{c}±0.109	6.05^{b}±0.32	278.65^{b}±5.27	113.25^{b}±6.01	149.05^{a}±10.50	408.08^{d}±15.53
FEN	0.83^{b}±0.03	149.84^{a}±2.95	3.54^{a}±0.20	2.57^{a}±0.121	5.34^{b}±0.36	313.69^{a}±5.90	167.05^{a}±6.72	172.02^{a}±11.74	500.00^{c}±17.37
ME+FEN	0.75^{b}±0.03	149.96^{a}±2.95	3.07^{ab}±0.20	1.91^{b}±0.121	6.32^{b}±0.36	336.96^{a}±5.96	134.04^{b}±6.72	163.12^{a}±11.74	623.74^{b}±17.37

Means were statistically compared using the Scheffe's *post-hoc* multiple comparison method at $p \leq 0.05\%$. Means with the same superscript letter are not significantly different. Each treatment was replicated 6 times and each replicate was measured 3 times

decreased relative to the control group (Table 2). In addition, inhalation of sub-lethal doses of FEN and ME+FEN caused a reduction in mean corpuscular hemoglobin concentration (MCHC) compared to control. However, their effects were not different from the ME group that was not different from the control group. Results also revealed no difference between treated groups and the control group in the mean corpuscular hemoglobin (MCH) and mean cell volume (MCV).

The results reported herein showed that exposure to ME and fenitrothion as separate treatments or mixed caused hematological adverse effects to rats. Inhalation of ME, FEN, and ME+FEN significantly increased numbers of WBCs and platelets but numbers of RBCs and HGB, HCT, and MCHC values were decreased significantly relative to the control group. In previous studies, ME significantly reduced the size of RBCs (erythrocyte microcytosis) and elevated numbers of platelets (thrombocytosis) in male and female rats (Abdo *et al.,* 2001). In a 90-days study, rats fed ME daily at 18 mg/kg b.w. showed a decrease in mean packed red cell volume and increased platelet counts (Gardner *et al.,* 1996; Luo & Guenthner, 1995; Rompelberg *et al.,* 1996). Also hematocrit value, WBCs, and lymphocytes percentages were increased in blood samples of rats orally given FEN at ¼ of LD_{50} (60 mg/kg bw) for 12 days (Al-Sahhaf, 2006).

Effects on acetylcholinesterase (AChE)

The activity of acetylcholinesterase enzyme (AChE) was depleted significantly after rats have been inhaling ME, FEN, and ME+FEN (Table 3). Results showed a significant reduction in the enzyme activity compared to the control group, but it was not different from each other. Inhalation of ME, FEN, and ME+FEN reduced AChE activity by 21, 32, and 39% of the control, respectively (Figure 1). The organophosphate insecticides are known as inhibitors of acetylcholinesterase (Farghaly *et al.,* 2010). In addition, ME was reported in the current study as an inhibitor of AChE. However, rare studies were conducted on the inhalation of fenitrothion and/or methyl eugenol. Current results might be compared to that of the study of exposure of rabbits to thermal fogging of FEN at 10 l/ha for 30 days, which showed a significant reduction of cholinesterase activity of brain, blood, plasma, and erythrocytes (Al-Sarar *et al.,* 2011). To highlight the suppressive effect of FEN on AChE activity, some results of other studies with different routes of administration were reported. The oral doses between 2.5 and 10 mg/kg b.w. of FEN for 30 days caused a decrease in tissue esterase activity (Trottier *et al.,* 1980). It was noticed that the hepatic ChE was the most inhibited enzyme compared to other esterases (Trottier *et al.,* 1980). After a 13-week study, administering oral doses of 30 mg/kg of FEN to male and female rats inhibited the plasma, erythrocyte, and brain ChE activity (Ecobichon *et al.,* 1980; Yoshida *et al.,* 1997). Similar inhibition of plasma cholinesterase (ChE) after administering of 1/30 and 1/60 LD_{50} of FEN doses to rats for 28 days was reported (Elhalwagy *et al.,* 2008).

Effects on glucose content

Glucose contents were increased significantly after the treatment with ME, FEN, and ME+FEN compared to control. The mean values were 63.81±2.95, 117.13±2.64, 149.84±2.95, and 149.96±2.95 mg/dl, respectively (Table 3). However, there was no difference between both FEN and ME+FEN, but both treatments were significantly different

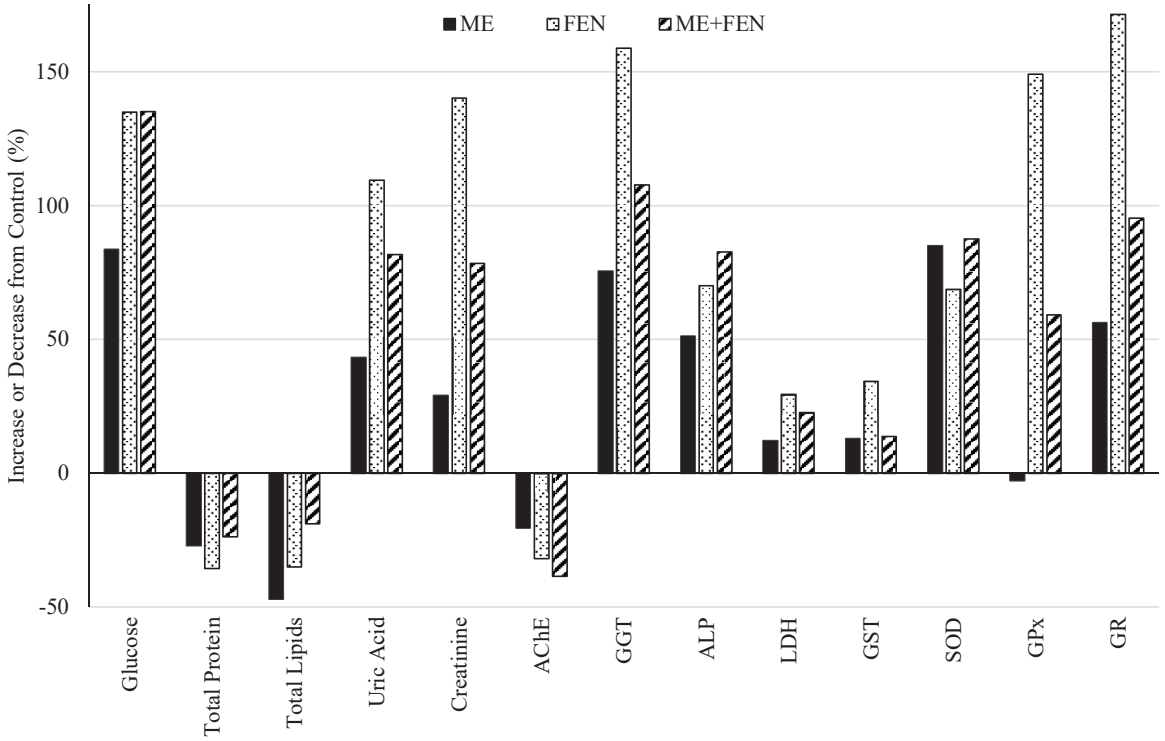

Figure 1. Average percentages of increase or decrease of biochemical parameters of control group after the inhalation study for 28 days. Relative decrease or increase from control = (Treatment Reading – Control Reading)/Control Reading) × 100

from ME. Glucose contents were severely increased by 84, 135, and 135% of control after rats have been inhaling ME, FEN, and ME+FEN (Figure 1), respectively.

Effects on renal characteristics

Kidney functions were impaired as revealed by the elevated levels of creatinine in serum (Table 3 and Figure 1). Creatinine results were affected significantly by ME, FEN treatments, and their mixture. Levels of creatinine were 1.07±0.121, 1.38±0.109, 2.57±0.121, and 1.91±0.121 mg/dl for control, ME, FEN, and ME+FEN, respectively. FEN treatment showed the greatest increase in creatinine followed by ME+FEN, while ME was not different from the control. Creatinine levels were increased by ME, FEN treatments, and their mixture by 29, 140, and 78% of control, respectively (Figure 1). It was reported that oral doses of FEN at ¼ of LD_{50} (60 mg/kg b.w.) for 12 d increased cholesterol and creatinine causing renal damage (Al-Sahhaf, 2006).

In addition, levels of uric acid were increased from 1.69±0.20 (control) to 2.42±0.18, 3.54±0.20, and 3.07±0.20 mg/dl after ME, FEN, and ME+FEN treatments, respectively (Table 3). The ME, FEN, and ME+FEN treatments increased uric acid contents by 43, 109, and 78% of control, respectively (Figure 1). The FEN treatment increased uric acid content compared to control and ME groups, which were not different from each other and ME was not different from the mixture treatment (ME+FEN). Yet, the effect of ME on uric acid was similar to that of ME+FEN.

Effects on hepatic biochemical parameters

The liver was the most affected organ by repeated oral administration of FEN (Trottier *et al.,* 1980). As well, ME caused tumors in liver cells of rats and mice (Burkey *et al.,* 2000; Thompson *et al.,* 1990). Besides, ME was minimally cytotoxic to hepatocytes isolated from rats and mice and caused UDS in both species in a dose-dependent manner (Burkey *et al.,* 2000; Martelli *et al.,* 1994). Current results showed that total protein contents were significantly different between control and ME, FEN, and ME+FEN treatments from 8.30±0.36 g/dl to 6.05±0.32, 5.34±0.36, and 6.32±0.36, respectively (Table 3). However, there were no differences between ME, FEN, and ME+FEN inhalation treatments. Total protein contents were decreased relative to control by 36% as a result of FEN treatment (Figure 1). Additionally, ME induced hypoproteinemia and hypoalbuminemia evidenced by the decreased concentrations of total protein in both male and female rats due to the induced liver and glandular stomach toxicity (Abdo *et al.,* 2001).

Alkaline phosphatase (ALP) activity was increased significantly after rats had inhaled ME, FEN, and ME+FEN compared to the control group. Both FEN and ME+FEN doses revealed elevated adverse effects on ALP compared to ME. The ALP activity was increased significantly after rats inhaled ME, FEN, and ME+FEN by 51, 70, and 83% of control, respectively (Figure 1). γ-Glutamyl transferase (γ-GT) activity values were 64.56±6.72, 113.25±6.01, 167.05±6.72, and 134.04±6.72 U/l for control, ME, FEN, and ME+FEN, respectively (Table 3). All three treatments

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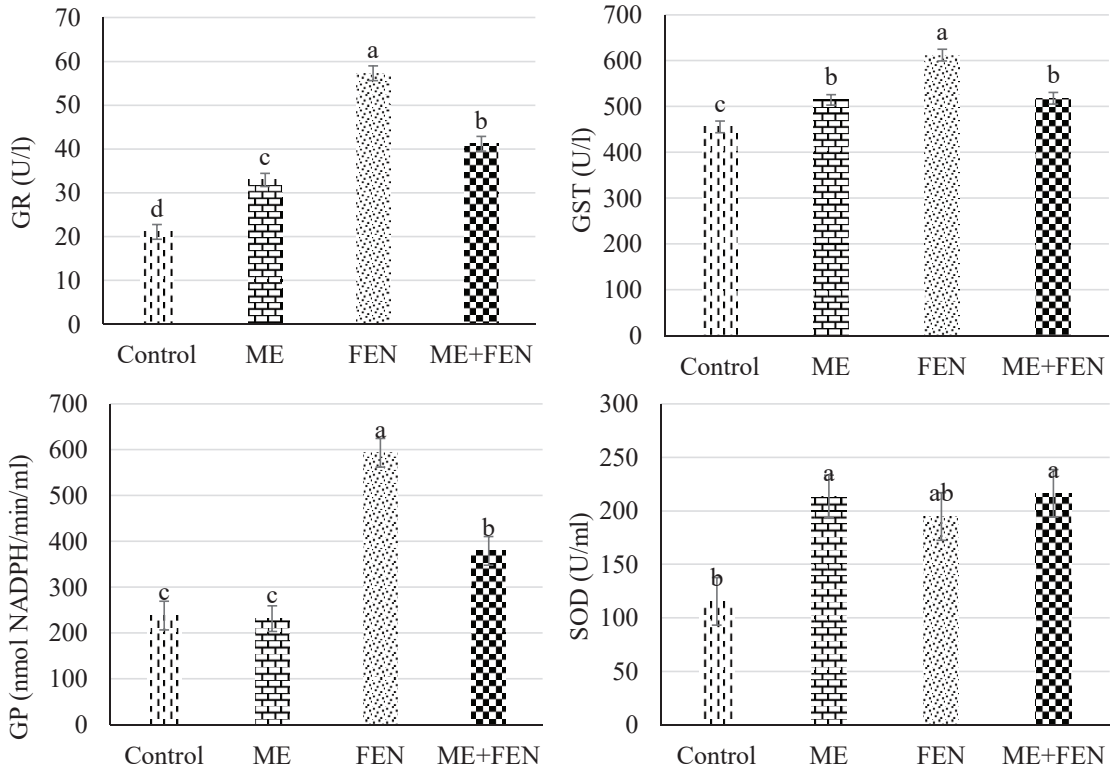

Figure 2. Mean ± SE activity values of glutathione-S-transferase (GST; U/l), superoxide dismutase (SOD; U/ml), glutathione peroxidase (GP; nM NADPH/min/ml), and glutathione reductase (GR; U/l) of serum of white albino rats inhaled methyl eugenol (ME), fenitrothion (FEN), and their mixture (ME+FEN) for 28 days. Means were statistically compared using the Scheffe's *post-hoc* multiple comparison method at $P \leq 0.05\%$. Columns with the same superscript letter are not significantly different. Each treatment was replicated 6 times and each replicate was measured 3 times.

significantly increase γ-GT activity compared to control. Rats that inhaled FEN showed the greatest activity of γ-GT followed by ME and ME+FEN treatments.

Inhalation of ME, FEN, and ME+FEN significantly decreased total lipid contents with mean values of 408.08, 500, and 623.74 mg/dl, respectively, compared to the control (770.20 mg/dl) (Table 3). The ME group showed the least amount of total lipids followed by FEN and ME+FEN groups. The ME, FEN, and ME+FEN treatments caused a significant decrease in the total lipids content by 47.02, 35.08, and 19.02% of control, respectively (Figure 1). In literature, it was reported that FEN, in a dose-dependent manner from 5 to 20 mg/100 g, caused lipid accumulation with the alteration of the ratio of various components of phospholipids and neutral lipids in various organs of rats (Roy *et al.,* 2004). Lactate dehydrogenase (LDH) activity tended to increase after inhalation of ME, FEN, and ME+FEN, but there was no significant difference between treatments and control group (Table 3). The elevated activity of LDH by FEN could be due to cell lysis or membrane damage and indicates significant pulmonary injury (Khan *et al.,* 1990), but the current study reported a tendency of increase in the activity of LDH.

Effects on oxidative stress enzymes

Oxidative stress enzymes glutathione-S-transferase (GST), glutathione peroxidases (GP), glutathione reductase (GR), and superoxide dismutase (SOD) activities were increased after the ME, FEN, ME+FEN treatments (Figure 2). FEN caused a significant increase in the GST activity compared to ME and ME+FEN treatments that showed similar effects but had greater GST activity compared to the control group. Similarly, FEN insecticide caused the greatest increase in GP activity (593.33 nM NADPH/min/ml) and followed by ME+FEN (379.34) and ME (231.5) treatments, respectively, compared to the control group (238.2 nM NADPH/min/ml). The GR activity (U/l) was significantly increased in the serum of rats that inhaled FEN (57.28), ME+FEN (41.20), and ME (32.95) in an ascending order compared to the control (21.10). In addition, SOD activity (U/l) was increased significantly after ME+FEN (216.35), ME (213.46), and FEN (194.72) in ascending order but not different from each other. FEN treatment tended to increase the SOD activity compared to the control group but not significantly different.

It was noticed that the FEN insecticide caused the highest increase in GR activity where it was increased in the serum of rats by 170% of control (Figure 1). Additionally, the GP activity values were increased up to 150% of control. The SOD activity was increased significantly after ME+FEN, ME, and FEN inhaling by 85, 69, 88% of control, respectively. In previous studies, the GST isozymes in rat and human samples were inhibited by ME (Rompelberg *et al.,* 1996). Fenitrothion was reported to has lower toxicity to mammals relative to insects due to the enhanced detoxification system through dealkylation by glutathione

dealkyltransferase (NRCNA, 2011). It was suggested that GSH, GP, SOD, and GST play a key role in the defense against induced oxidative stress in isolated hepatocytes when incubated with FEN (El-Shenawy, 2010).

Conclusions

Annihilation systems as part of the integrated pest management practices might cause inhalation toxicity to farmers. The ME and FEN are used extensively in the pheromone-baited traps in agricultural fields. Therefore, the inhalation of ME, FEN, and their mix (ME+FEN) might incite hematological and biochemical parameters (AChE, glucose, lipids, hepatic, renal, and oxidative stress enzymes) in rats. The studied parameters were altered significantly compared to the control group. Significant decreases in mean cell hemoglobin and mean cell hemoglobin concentration, red blood cell, AChE, and total lipids levels were reported. On the other hand, lactate dehydrogenase, uric acid, creatinine, γ-glutamyltransferase, and oxidative stress-related enzymes (glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and superoxide dismutase values were increased compared to the results of the control group. Therefore, handling safe-labeled chemicals should be carefully done.

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