



Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: Protective effects of crocin and safranal

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ABSTRACT

In this study, the effects of crocin and safranal were studied against sub-acute toxicity of diazinon (DZN) on specific biomarkers, biochemical indices and enzymes levels in rats. Vitamin E (200 IU/kg), safranal at doses 0.025, 0.05 and 0.1 ml/kg and crocin at doses 50, 100 and 200 mg/kg were injected intraperitoneally three times per week alone or with DZN (20 mg/kg/day, orally) for 4 weeks. The parameters were evaluated at the end of 4 weeks. Diazinon did not change serum urea, creatinine, cholesterol, triglyceride, total and direct bilirubin levels. Total protein and albumin concentrations were decreased by diazinon. Crocin, safranal and vitamin E prevented the effect of diazinon on some biochemical indices and enzymes levels. The levels of serum TNF- α , direct 8-iso-prostaglandin F_{2 α} and soluble protein-100 β (S100 β) were increased significantly by diazinon. The augmentation of direct 8-iso-prostaglandin F_{2 α} and S100 β levels by diazinon was significantly decreased by crocin, safranal and vitamin E. TNF- α level was significantly decreased in diazinon plus crocin 50 and 100 mg/kg treated groups compared to the diazinon group. This study showed that vitamin E, safranal and crocin could prevent diazinon induced enzymes elevation and augmentation of some specific biomarkers.

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1. Introduction

Organophosphorus compounds (OPCs) such as diazinon are neurotoxic chemical agents that inhibit acetylcholinesterase (AChE) activity. OPCs and carbamates are the most widely used AChE inhibitors as insecticides (Delfino et al., 2009). These agents or their active metabolites inhibit acetylcholinesterase (AChE). This causes accumulation of acetylcholine at cholinergic synapses leading to increased activation of nicotinic and muscarinic receptors.

Recent studies indicate that pesticide intoxication produce oxidative stress by generation of free radicals and induce tissue lipid peroxidation in mammals and other organisms (Shadnia et al., 2005; Kovacic, 2003). Thus, oxidative stress is another mechanism that has been proposed for the toxicity of OPCs in animals and human. Diazinon after malathion is one of the most commonly used OPCs in the world (Ghafour-Rashidi et al., 2007). In addition to its inhibition of AChE, it can induce oxidative stress that is important in its toxicity (Amirkabirian et al., 2007; Shadnia et al., 2007).

Saffron, the stigma of *Crocus sativus* L. (Iridaceae), is a very delicate spice, flavor and a golden color. It contains many constituents such as picrocrocin and volatile compounds including safranal as well as crocins (glycosyl esters of crocetin) are unusual water soluble carotenoids and are responsible for its characteristic color (Kanakis et al., 2009; Schmidt et al., 2007). Saffron as highly valued medicinal plant and its constituents are widely evaluated for their pharmacological activities such as anticancer (Abdullaev and Espinosa-Aguirre, 2004), antidepressant (Hosseinzadeh et al., 2004; Akhondzadeh et al., 2005), anticonvulsant (Hosseinzadeh and Talebzadeh, 2005) and treatment of memory impairment (Abe and Saito, 2000). Saffron and its constituents showed antioxidant activity and reduced oxidative damages in different organs such as muscle skeletal (Hosseinzadeh et al., 2009a), kidney (Hosseinzadeh et al., 2005) and hippocampus (Hosseinzadeh and Sadeghnia, 2005). It has been suggested that the antioxidant activity of saffron compounds can protect DNA and RNA from harmful chemicals (Kanakis et al., 2009). Saffron, crocin (Hosseinzadeh and Sadeghnia, 2007a), and safranal (Hosseinzadeh et al., 2008) showed protective effects against methyl methanesulfonate (MMS)-induced DNA damage in mouse

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organs as demonstrated with an alkaline single-cell gel electrophoresis (comet) assay method.

Therefore, the aim of this study was to investigate the sub-acute effects of diazinon on biochemical indices and specific biomarkers as well as the protective effect of safranal and crocin on this toxicity in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (weighing approximately 220–270 g) were obtained from the animal house of the Pharmaceutical Sciences Research Center of Mashhad University of Medical Sciences. The animals were fed a standard laboratory diet and water *ad libitum*. Rats were kept at 12 h light 12 h dark cycles at a room temperature of 18–22 °C at least two days prior to testing. All animal experiments were approved by the Animal Care Committee of Mashhad University of Medical Sciences.

2.2. Chemicals

Diazinon (Merck Co., 99% purity) was a gift from the Agricultural Research, Education and Development Organization (AREDO) (Tehran, Iran). Crocin and safranal were purchased from Fluka (Germany), vitamin E (DL- α Tocopherol acetate) from OSVE Pharmaceutical Co. (Tehran, Iran), acridine orange from Merck and reticulocyte stain from Sigma–Aldrich.

Urea, creatinin (Creat), uric acid (U.A), total and direct bilirubin (T&D bil), total protein (Pr), albumin (Alb), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (Chol), triglyceride (Tg), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), CPK-MB, gamma glutamyl transferase (GGT) and amylase commercial colorimetric kits were obtained from Pars Azmun (Tehran, Iran). Lipase kit was purchased from Randox (UK).

Sandwich Elisa kits for quantitation of rat TNF- α , Direct 8-iso-prostaglandin F_{2 α} and rat Soluble protein-100B were obtained from Koma Biotech (Korea), Assay Designs (USA) and USCN LIFE (China), respectively.

2.3. Animal treatment schedule

Rats were divided into 16 groups ($n = 6$). The compounds were administered in the morning (between 9:00 and 11:00 AM) to non-fasted rats. All rats were treated for 4 weeks.

2.3.1. Group 1: control group

The control group received sweet almond oil at doses of 20 mg/kg per day through gavages once a day.

2.3.2. Group 2: diazinon-treated group

Diazinon at a dose of 20 mg/kg/day in sweet almond oil was given through gavage to rats once a day.

2.3.3. Group 3: vitamin E treated group

Vitamin E (200 IU/kg) was administered intraperitoneally three times per week.

2.3.4. Group 4: vitamin E + diazinon-treated group

Vitamin E (200 IU/kg three times per week) was administered intraperitoneally and diazinon was administered orally (20 mg/kg per day once a day in sweet almond oil) via gavage needle.

2.3.5. Group 5, 6, and 7: crocin + diazinon-treated group

Crocin was dissolved in saline and administered at doses 50, 100 and 200 mg/kg/day intraperitoneally to rats three days per week before oral administration of diazinon (20 mg/kg per day).

2.3.6. Group 8, 9, and 10: crocin treated groups

Crocin was administered at doses 50, 100 and 200 mg/kg/day intraperitoneally to rats three days per week.

2.3.7. Group 11, 12, and 13: safranal + diazinon-treated groups

Safranal dissolved in paraffin and was administered at doses 0.025, 0.05, and 0.1 ml/kg/day intraperitoneally three days per week before oral administration of diazinon (20 mg/kg per days).

2.3.8. Group 14, 15 and 16: safranal treated groups

Safranal dissolved in paraffin and was administered at doses 0.025, 0.05, 0.1 ml/kg/day intraperitoneally three days per week.

2.4. Blood sampling

After 28 days, animals were anaesthetized by chloroform. Blood samples were collected by cardiac puncture into sterile tubes with anticoagulant (EDTA) for evaluation of RBC cholinesterase activity and non anticoagulant tubes for others tests. Blood samples in non anticoagulant tubes were centrifuged at 5000 rpm for 15 min and serum was discarded.

2.5. Biochemical and specific biomarker evaluation

Urea, creatinine, uric acid (U.A), total and direct bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase (LDH), creatine phosphokinase (CPK), myocardial band enzymes of CPK (CPK-MB), Gamma-Glutamyl Transpeptidase (GGT), total cholesterol, triglyceride, total protein, albumin, and amylase were measured using commercial colorimetric kits.

The biomarkers, TNF- α , direct 8-iso-prostaglandin F_{2 α} and soluble protein-100B levels were measured using Koma Biotech, Assay Designs and USCN LIFE Elisa kits manufacturer's protocols, respectively.

2.6. Statistical analysis

The mean \pm SEM were determined for each study group. Data were analyzed by one-way ANOVA and Tukey multiple comparison procedure to calculate the significance. $P < 0.05$ value between study groups was taken as statistically significant.

3. Results

3.1. Changes in enzymes levels

AST (SGOT), ALT (SGPT), ALP, LDH, CPK, CPK-MB and GGT levels were increased significantly in the diazinon-treated group when compared to the control group. Crocin (all doses) and vitamin E prevented this effect of diazinon on ALP, LDH, CPK, CPK-MB levels (Table 1).

AST, LDH, CPK and CPK-MB levels were significantly decreased in the diazinon plus safranal 0.1 ml/kg group when compared to the diazinon-treated group. AST, ALT and GGT activities were significantly decreased in diazinon plus safranal 0.025 ml/kg group and ALT significantly decreased in diazinon plus safranal 0.05 ml/kg group when compared to the diazinon-treated group (Table 2).

Table 1

Effect of diazinon (DZN) and DZN plus vitamin E or crocin on serum enzymes levels in rats. Vitamin E, crocin, and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	G.G.T IU/L	Lipase IU/L	Amylase IU/L	CPK-MB IU/L	CPK IU/L	LDH IU/L	ALP IU/L	SGPT IU/L	SGOT IU/L
Control	24.6 ± 1.0	54.8 ± 5.4	642.6 ± 37.8	108.8 ± 9.3	546.8 ± 72.8	793.5 ± 27.1	455.8 ± 22.4	85.5 ± 4.3	182 ± 11.8
Diazinon(DZN) 20 mg/kg	46.6 ± 6.4+	49.4 ± 7.1	686.1 ± 34.3	193 ± 28.7++	1029.5 ± 104.1+++	1770.2 ± 242.3++	685.2 ± 48.7++	164.7 ± 40.7+	372.3 ± 34.6+++
Vitamin E 200 IU/kg	34.4 ± 2.5	51.5 ± 8.6	664.3 ± 29.9	111.7 ± 12.3**	565.7 ± 75.7***	835.5 ± 82.0**	555 ± 27.9	85.8 ± 4.7**	213.7 ± 11.3**
DZN + Vitamin E	36.5 ± 3.8	39.7 ± 4.4	700.2 ± 16.2	116.8 ± 11.2*	522.3 ± 38.8**	808.3 ± 50.7**	420.3 ± 62.8**	85.2 ± 3.6**	234 ± 29.2**
Crocin 50 mg/kg	24.5 ± 3.2*	38.4 ± 4.4	668.3 ± 45.4	90.5 ± 6.7***	485 ± 64.3***	755 ± 87.4***	456.7 ± 24.1**	80.5 ± 7.5**	233.3 ± 24.0**
Crocin 100 mg/kg	24.8 ± 3.2*	45.3 ± 4.9	643.3 ± 48.5	90.8 ± 8.3***	550 ± 67.7***	720 ± 61.6***	461.2 ± 39***	75 ± 7.6***	240 ± 24.9**
Crocin 200 mg/kg	44.4 ± 6.8	38.5 ± 1.7	633.3 ± 37.8	101.8 ± 14.3**	573.3 ± 71.6***	678.2 ± 52.4***	411.4 ± 27.6*	72.7 ± 6.1***	225 ± 20**
DZN + crocin 50 mg/kg	23.4 ± 3.2*	47.5 ± 2.0	675.0 ± 45.6	112.2 ± 8.3**	658.3 ± 28.8*	708.3 ± 63.0**	490.2 ± 63.3++	147.5 ± 20.1+	356.7 ± 27.4+++
DZN + crocin 100 mg/kg	22.5 ± 3.4**	44.7 ± 2.3	680.0 ± 48.9	114.5 ± 19.9*	705 ± 66.4*	868.3 ± 68.8**	466.0 ± 27.3**	160 ± 33.4+	251.7 ± 25.2**
DZN + crocin 200 mg/kg	33.0 ± 6.3	41.8 ± 5.5	700.0 ± 46.5	98.0 ± 12.9**	663.3 ± 71.9*	961.7 ± 76.6**	422.4 ± 42.7**	177.2 ± 22.3+	235 ± 20.6**

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group, + or ***P* < 0.05, ++ or ****P* < 0.01 and +++ or *****P* < 0.001, Tukey Kramer test, *n* = 6.

Table 2

Effect of diazinon (DZN) and DZN plus vitamin E or safranal on serum enzymes levels in rats. Vitamin E, safranal and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	G.G.T IU/L	Lipase IU/L	Amylase IU/L	CPK-MB IU/L	CPK IU/L	LDH IU/L	ALP IU/L	SGPT IU/L	SGOT IU/L
Control	24.6 ± 1.0	54.8 ± 5.4	642.6 ± 37.8	108.8 ± 9.3	546.8 ± 72.8	793.5 ± 27.1	455.8 ± 22.4	85.5 ± 4.3	182 ± 11.8
Diazinon(DZN) 20 mg/kg	46.6 ± 6.4+	49.4 ± 7.1	686.1 ± 34.3	193.0 ± 28.7++	1029.5 ± 104.1+++	1770.2 ± 242.3++	685.2 ± 48.7++	164.7 ± 40.7+	372.3 ± 34.6+++
Vitamin E 200 IU/kg	34.4 ± 2.5	51.5 ± 8.6	664.3 ± 29.9	111.7 ± 12.3**	565.7 ± 75.7***	835.5 ± 102.8**	555 ± 27.9	85.8 ± 4.7**	213.7 ± 11.3**
DZN + Vitamin E	36.5 ± 3.8	39.7 ± 4.4	700.2 ± 16.2	116.8 ± 11.2*	522.3 ± 38.8*	808.3 ± 50.7**	420.3 ± 62.8**	85.2 ± 3.6**	234.0 ± 29.2**
Safranal 0.025 ml/kg	28.3 ± 2.6*	48.1 ± 5.9	673.6 ± 12.4	77.1 ± 9.4***	621.5 ± 71.8	1159.8 ± 97.8	539.6 ± 32.8	84.0 ± 2.9*	212.3 ± 19.5*
Safranal 0.05 ml/kg	31.5 ± 2.9	60.3 ± 6.3	623.3 ± 80.1	79.1 ± 9.5***	643.2 ± 84.2	1365.6 ± 118.7	435 ± 29.5*	90.5 ± 6.5	245.5 ± 22.5*
Safranal 0.1 ml/kg	42.6 ± 2.6	+84.7 ± 11.7	560.0 ± 25.5	87.8 ± 9.4***	368.6 ± 42.7**	696.3 ± 98.8*	386.7 ± 32.4*	94.0 ± 5.9	228.6 ± 20.8*
DZN + Safranal 0.025 ml/kg	28.5 ± 3.4*	47.0 ± 6.5	632.3 ± 29.6	188.8 ± 22.9+	589.8 ± 61.0	1159.8 ± 190.1	611.3 ± 25.0+	86.6 ± 5.9*	210.5 ± 20.26**
DZN + Safranal 0.05 ml/kg	43.5 ± 5.7	79.3 ± 9.3+	543.8 ± 98.9	228.3 ± 29.9++	963.6 ± 218.44+	1963.3 ± 431.0+	547.8 ± 42.6	78.1 ± 2.48**	309.8 ± 28.5++
DZN + Safranal 0.1 ml/kg	41.2 ± 5.8	77.7 ± 4.5	551.0 ± 74.2	107.2 ± 12.2**	344.2 ± 23.4*	715.2 ± 88.6*	580.4 ± 48.8	108.0 ± 8.6	201.2 ± 25.8***

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group, + or ***P* < 0.05, ++ or ****P* < 0.01 and +++ or *****P* < 0.001, Tukey Kramer test, *n* = 6.

Table 3

Effect of diazinon (DZN) and DZN plus vitamin E or crocin on biochemical indices levels in rats. Vitamin E, crocin, and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	Total bilirubin mg/dl	direct bilirubin mg/dl	ALB g/dl	Protein g/dl	TG mg/dl	Cholesterol mg/dl	Uric acid mg/dl	Creatinine mg/dl	Urea mg/dl
Control	0.60 ± 0.01	0.30 ± 0.02	4.20 ± 0.15	7.20 ± 0.15	70.5 ± 2.7	69.8 ± 6.8	2.4 ± 0.2	0.95 ± 0.08	53.2 ± 2.7
Diazinon(DZN) 20 mg/kg	0.50 ± 0.10	0.30 ± 0.02	3.50 ± 0.20++	5.60 ± 0.30++	70.8 ± 6.2	70.7 ± 6.2	5.2 ± 0.7+++	0.95 ± 0.08	49.2 ± 3.7
Vitamin E 200 IU/kg	0.50 ± 0.10	0.30 ± 0.03	4.20 ± 0.30	6.40 ± 0.30	73.7 ± 3.0	70 ± 6.4	2.9 ± 0.1***	1.05 ± 0.08	46.3 ± 2.3
DZN + Vitamin E	0.60 ± 0.10	0.20 ± 0.02	3.40 ± 0.20++	6.00 ± 0.40	88.2 ± 9.5	75.3 ± 3.8	4.7 ± 0.5+++	1.00 ± 0.07	46.2 ± 2.1
Crocin 50 mg/dl mg/kg	0.60 ± 0.10	0.30 ± 0.05	3.90 ± 0.30	6.75 ± 0.3	80.8 ± 4.2	74.7 ± 5.0	4.5 ± 0.3++	1.05 ± 0.08	44.3 ± 2.7
Crocin 100	0.70 ± 0.10	0.30 ± 0.05	4.00 ± 0.10	7.00 ± 0.1	77 ± 5.7	83.5 ± 2.3	3.9 ± 0.4	1.08 ± 0.1	49.7 ± 3.9
Crocin 200 mg/dl	0.60 ± 0.10	0.30 ± 0.06	4.00 ± 0.20	6.70 ± 0.3	82 ± 5.4	82.8 ± 6.4	4.1 ± 0.3+	1.02 ± 0.06	47.2 ± 4.0
DZN + crocin 50 mg/kg	0.70 ± 0.10	0.30 ± 0.1	3.05 ± 0.15++	5.28 ± 0.25++	82.5 ± 2.2	74.5 ± 4.8	4.5 ± 0.5+++	0.98 ± 0.08	48.5 ± 3.5
DZN + crocin 100 mg/kg	0.60 ± 0.10	0.30 ± 0.06	3.20 ± 0.30++	6.60 ± 0.2	80.5 ± 4.8	72.3 ± 5.1	4.3 ± 0.4++	0.96 ± 0.08	43.7 ± 1.5
DZN + crocin 200 mg/kg	0.60 ± 0.10	0.30 ± 0.06	3.70 ± 0.20+	6.70 ± 0.3	86 ± 3.9	71.7 ± 5.8	4.8 ± 0.4+++	1.10 ± 0.15	48.2 ± 2.9

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group, +*P* < 0.05, ++*P* < 0.01 and +++ or *****P* < 0.001 Tukey Kramer test, *n* = 6.

No statically significant changes in levels of serum lipase and amylase were observed among almost all groups including diazinon, crocin and safranal (Tables 1 and 2).

3.2. Changes in biochemical indices

Diazinon, vitamin E and safranal did not change serum urea, creatinine, cholesterol, triglyceride, total and direct bilirubin levels significantly (Table 4). Total protein and albumin concentrations were decreased significantly in the diazinon-treated group compared to the control group (Table 3).

A significant increase was observed in uric acid in the diazinon-treated group, safranal (0.05, 0.1 ml/kg) and crocin (50 and 200 mg/kg) when compared to the control (Tables 3 and 4). An in-

creased uric acid level by diazinon was restored by vitamin E and a low dose of safranal (0.025 ml/kg) (Table 4).

3.3. Changes in specific biomarkers

The levels of serum TNF- α , direct 8-iso-prostaglandin F_{2 α} and soluble protein-100 β (S100 β) were increased significantly in the diazinon-treated group compared with the control group (Tables 5 and 6). The augmentation of direct 8-iso-prostaglandin F_{2 α} level by diazinon was significantly decreased by crocin (all doses), safranal (all doses) and vitamin E (Tables 5 and 6).

Crocin, safranal and vitamin E also inhibited the increment effect of diazinon on S100 β level (Tables 5 and 6).

Table 4
Effect of diazinon (DZN) and DZN plus vitamin E or safranal on serum biochemical indices levels in rats. Vitamin E, safranal and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	Total bilirubin mg/dl	Direct bilirubin mg/dl	ALB g/dl	Protein g/dl	TG mg/dl	Cholesterol mg/dl	Uric acid mg/dl	Creatinine mg/dl	Urea mg/dl
Control	0.60 ± 0.01	0.30 ± 0.02	4.20 ± 0.15	7.20 ± 0.15	70.5 ± 2.7	69.8 ± 6.8	2.4 ± 0.2	0.95 ± 0.08	53.2 ± 2.7
Diazinon(DZN) 20 mg/kg	0.50 ± 0.10	0.30 ± 0.02	3.50 ± 0.20++	5.60 ± 0.30++	70.8 ± 6.2	70.7 ± 6.2	5.2 ± 0.7+++	0.95 ± 0.08	49.2 ± 3.7
Vitamin E 200 IU/kg	0.50 ± 0.10	0.30 ± 0.03	4.20 ± 0.30	6.40 ± 0.30	73.7 ± 3.0	70 ± 6.4	2.9 ± 0.1***	1.05 ± 0.08	46.3 ± 2.3
DZN + Vitamin E	0.60 ± 0.10	0.20 ± 0.02	3.40 ± 0.20++	6.00 ± 0.40	88.2 ± 9.5	75.3 ± 3.8	4.7 ± 0.5++	1.00 ± 0.07	46.2 ± 2.1
Safranal 0.025 ml/kg	0.50 ± 0.03	0.20 ± 0.03	4.16 ± 0.12	7.06 ± 0.26*	78.8 ± 16.2	92.7 ± 4.1	1.6 ± 0.1*	0.80 ± 0.02	48.1 ± 3.5
Safranal 0.05 ml/kg	0.60 ± 0.04	0.30 ± 0.04	3.70 ± 0.80	6.80 ± 0.80*	83.6 ± 17.1	94.0 ± 4.8	5.4 ± 0.4+	0.75 ± 0.03	43.0 ± 2.1
Safranal 0.1 ml/kg	0.70 ± 0.03	0.30 ± 0.03	3.90 ± 0.13	7.10 ± 0.35*	79.0 ± 12.5	88.0 ± 6.1	6.1 ± 0.6+	0.82 ± 0.03	40.1 ± 4.4
DZN + Safranal 0.025 ml/kg	0.50 ± 0.04	0.20 ± 0.03	3.96 ± 0.08	5.50 ± 0.30++	92.8 ± 4.5	89.3 ± 4.5	3.1 ± 0.3*	0.80 ± 0.06	52.7 ± 4.8
DZN + Safranal 0.05 ml/kg	0.60 ± 0.04	0.20 ± 0.02	3.86 ± 0.07	5.75 ± 0.30+	72.6 ± 8.7	71.3 ± 5.4	6.0 ± 0.9+++	0.71 ± 0.05	45.0 ± 1.9
DZN + Safranal 0.1 ml/kg	0.60 ± 0.04	0.20 ± 0.02	3.60 ± 0.21	6.15 ± 0.3	87.0 ± 4.1	91.5 ± 10.9	5.2 ± 0.5+	0.67 ± 0.06	38.9 ± 11.8

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group.+ or *P < 0.05, ++P < 0.01 and +++ or ***P < 0.001, Tukey Kramer test, n = 6.

TNF- α level was significantly decreased in diazinon plus crocin 50 and 100 mg/kg treated groups compared to the diazinon group. However, vitamin E and safranal could not prevent the effect of diazinon on TNF- α level. Also this biomarker significantly increased in safranal 0.1 ml/kg treated group compared with the control group (Table 6).

Table 5
Effect of diazinon (DZN) and DZN plus vitamin E or crocin on serum specific biomarkers levels in rats. Vitamin E, crocin, and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	8-Iso-prostaglandin F _{2α} pg/ml	TNF α pg/ml	S100 β pg/ml
Control	197.8 ± 15.7	387.4 ± 68.9	51.0 ± 12.2
Diazinon (DZN) 20 mg/kg	369.0 ± 2.9+++	774.8 ± 181.6+	264.0 ± 91.8+
Vitamin E 200 IU/kg	200.0 ± 26.5***	693.3 ± 74.1***	53.0 ± 6.7**
DZN + Vitamin E	225.2 ± 13.0***	1321.2 ± 262.5+	61.7 ± 14.3*
DZN + crocin 50 mg/kg	229.6 ± 16.3***	830.0 ± 181.5**	95.2 ± 22.4
DZN + crocin 100 mg/kg	209.2 ± 36.97***	831.3 ± 137.7***	50.3 ± 3.3*
DZN + crocin 200 mg/kg	219.6 ± 19.5**	1177.2 ± 136.8	73.8 ± 55.3*
Crocin 50 mg/kg	213.6 ± 19.3***	618.5 ± 35.4***	35.9 ± 8.7**
Crocin 100 mg/kg	217.2 ± 23.1***	889.6 ± 177.9**	21.5 ± 8.9**
Crocin 200 mg/kg	258.7 ± 8.6*	1253.5 ± 121.6	11.3 ± 2.9**

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group.+ or *P < 0.05, **P < 0.01 and +++ or ***P < 0.001, Tukey Kramer test, n = 6.

Table 6
Effect of diazinon (DZN) and DZN plus vitamin E or safranal on serum specific biomarkers levels in rats. Vitamin E, safranal and control were administered intraperitoneally three times per week. Diazinon in sweet almond oil was given through gavage to rats once a day.

Parameter	8-Iso-prostaglandin F _{2α} pg/ml	TNF α pg/ml	S100 β pg/ml
Control	197.8 ± 15.7	387.4 ± 68.9	51.0 ± 12.2
Diazinon 20 mg/kg	369.0 ± 2.9+++	1774.8 ± 181.6+	264.0 ± 91.8+
Vitamin E 200 IU/kg	200.0 ± 26.5***	693.3 ± 74.1***	53.0 ± 6.7**
Diazinon + Vitamin E	225.2 ± 13.0***	1321.2 ± 262.5+	61.7 ± 14.3*
Safranal 0.025 ml/kg	211.7 ± 43.4***	1414.0 ± 116.2	34.2 ± 8.2**
Safranal 0.05 ml/kg	236.6 ± 15.1***	1297.5 ± 149.3	35.6 ± 8.3**
Safranal 0.1 ml/kg	228.5 ± 13.1***	1968.5 ± 288.7+	62.4 ± 12.5*
Diazinon + Safranal 0.025 ml/kg	261.0 ± 6.5**	1876.5 ± 140.2	50.2 ± 17.9**
Diazinon + Safranal 0.05 ml/kg	142.6 ± 14.4***	2372.2 ± 626.6	112.3 ± 31.5
Diazinon + Safranal 0.1 ml/kg	252.0 ± 6.4**	3618.3 ± 238.8+	11.2 ± 2.7***

Data showed as mean ± SEM, +Comparison with control, *Comparison with diazinon-treated group.+ or *P < 0.05 **P < 0.01 and +++ or ***P < 0.001, Tukey Kramer test, n = 6.

4. Discussion

The major findings of our study were: diazinon increased some serum enzymes such as AST, ALT, ALP, LDH, CPK, CPK-MB and GGT levels and also the levels of serum TNF- α , direct 8-iso-prostaglandin F_{2 α} and soluble protein-100 β (S100 β) biomarkers were increased significantly by diazinon-treated group. Vitamin E, crocin and safranal inhibited most of these effects.

OPCs such as diazinon have been shown that in addition to their inhibition of cholinesterase can induce oxidative stress and produce free radicals in biological systems (Abdollahi et al., 2004).

It has been reported that diazinon alters some biochemical and hematological indices and some other biomarkers in *in vivo* and *in vitro* experimental studies (Jacobsen et al., 2004; Kalender et al., 2005). In another study, saffron and its constituents such as crocin and safranal showed significant antioxidant activity and allow free radicals to attract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids (PUFA), thus breaking the chain of free radical reactions, the resulting antioxidant radical being a relatively non reactive species (Pascoe et al., 1987; Assimopoulou et al., 2005). Recently, we showed that the ethanolic and aqueous extracts of saffron have antioxidant activity in different *in vitro* methods using three experimental approaches i.e. deoxyribose assay, erythrocyte membrane peroxidation and rat liver microsomal lipid peroxidation induced by Fe²⁺/ascorbate (Hosseinzadeh et al., 2009b).

Treated rats by diazinon showed significant increase in AST, ALT, ALP, LDH, CPK, CPK-MB and GGT levels. Serum enzymes including ALT, AST, ALP and LDH are mainly monitored for the evaluation of liver damage. Although these enzymes are not necessarily specific, increase in enzyme activities reflects active liver destruction. Organophosphate insecticides like diazinon may cause increase in ALP, ALT and AST enzyme activities (Gokcimen et al., 2007). These enzyme level alterations might differ dependent on exposure time and dose. Diazinon treatment has led to hepatic degeneration in fish, as indicated by the increased ALT, AST and LDH levels (Asztalos et al., 1990). In our study, diazinon caused prominent increment in ALT, AST, ALP and LDH enzyme activities in rats. These results suggest that increased serum enzyme activities are associated with hepatic degeneration. It is likely that diazinon induced biochemical changes in liver (Nakagawa and Moore, 1999).

The pre-treatment of orally aqueous extract of saffron (20, 40 and 80 mg/kg) for five consecutive days prior to the administration of some genotoxins (cisplatin, cyclophosphamide, mitomycin-C and urethane) reduced the extent of lipid peroxidation with a concomitant increase in liver enzymatic (SOD, CAT, GST, GPx) and non-enzymatic antioxidants (reduced glutathione) (Premkumar et al., 2003). Pre-treatment of female Sprague–Dawley rats with crocin

50 mg/kg daily for three consecutive days prevented the elevations of levels of serum marker enzymes such as ALT, AST, ALP and LDH induced by the hepatotoxic agents, aflatoxin B₁ and dimethylnitrosamine (Lin and Wang, 1986).

Creatine phosphokinase-MB (CPK-MB) is the most sensitive and specific indicator available for the diagnosis of damage to the heart. In this study, the level of this marker was increased by diazinon. Crocin (all doses) and safranal (at the highest dose) prevented the diazinon induced damage on heart muscles.

Recently, the protective effect of saffron and its constituent, crocin, was shown on rat's heart with isoproterenol-induced myocardial injury (Joukar et al., 2010, Goyal et al., 2010). Saffron plus isoproterenol group showed significantly decreased intensity of tissue destruction and decreased serum levels of heart troponin I (Joukar et al., 2010). In another study, crocin pre-treatment (20 mg/kg/day) showed cardioprotective effect in isoproterenol-induced toxicity through modulation of oxidative stress. Crocin restored the endogenous antioxidants, controlling lipid peroxide formation and preserved activities of CK-MB, LDH enzymes (Goyal et al., 2010). Vitamin E prevented the rise of CPK-MB and CPK levels due to doxorubicin-induced myocardial damage (Puri et al., 2005). It seems that crocin and safranal effects on these enzymes are partially similar to Vitamin E. As crocin was effective at all doses, it is possible that other mechanisms of actions such as suppression of elevation of intracellular calcium (He et al., 2004) may be involved in this effect.

In the present study, crocin and safranal decreased lipid peroxidation as they decreased the level of direct 8-iso-prostaglandin F_{2α} that was elevated by diazinon. This biomarker is a specific product of non-enzymatic lipid peroxidation and is a more accurate marker of oxidative stress *in vivo* in humans than other available methods (Morrow and Roberts, 1997). Our previously reports have also demonstrated saffron or its constituents inhibited lipid peroxidation in renal (Hosseinzadeh et al., 2005), hippocampal (Hosseinzadeh and Sadeghnia 2005) and muscle skeletal homogenates during ischemia-reperfusion-induced oxidative damage in rats (Hosseinzadeh et al., 2009a). The ethanolic and aqueous extracts of saffron and its constituents have shown antioxidant activity in different *in vitro* methods (Hosseinzadeh et al., 2009b). *C. sativus* L. extract and its bioactive constituents, safranal and crocin have shown radical scavenging activity (Chen et al., 2008). Similar to vitamin E effect, these data revalidate that saffron and its constituents, crocin and safranal, have a significant potential to reduce lipid peroxidation in pathological conditions such as the toxicity of diazinon.

Tumor necrosis factor-alpha (TNF- α) is a central regulator of inflammation, and TNF- α antagonists may be effective in treating inflammatory disorders in which TNF- α plays an important pathogenetic role (Esposito and Cuzzocrea, 2009). After acute organophosphorus pesticide poisoning, the mRNA expression of TNF- α increased in mice liver and spleen (Ouyang et al., 2009). Sub-lethal and sub-acute concentrations of diazinon specifically targets neurite outgrowth in neuronal cells by differentiating cells of neuronal origin and this effect is associated with disruption of axonal cytoskeleton proteins (Flaskos et al., 2007). Diazinon induced neurotoxicity in cortical culture which was independent from its effect on cholinesterase inhibition activity. This effect was inhibited by the caspase inhibitor Z-VAD. This suggests that diazinon induced apoptotic neuronal death (Rush et al., 2010). In our study, crocin with lower doses (50 and 100 mg/kg) reduced the TNF- α increment induced by diazinon. However, vitamin E and safranal did not prevent this effect of diazinon. In Langerhans islets, also diazinon significantly increased TNF- α , thiobarbituric acid reactant substances (TBARs), and NO levels (Ghafour-Rashidi et al., 2007). Safranal and vitamin E could not prevent the augmentation effect of diazinon on TNF- α , it seems the antagonistic effect of crocin on TNF- α may not

be mediated by its antioxidant activity. Crocin suppressed the effect of tumor necrosis factor (TNF)- α on neuronally differentiated PC-12 cells and also inhibited the TNF- α -induced expression of Bcl-X_s and LICE mRNAs and simultaneously restored the cytokine-induced reduction of Bcl-X_L mRNA expression (Soeda et al., 2001). PC-12 cells exposed to TNF- α showed apoptotic morphological changes and DNA fragmentation. Crocin also blocked the cytochrome c-induced activation of caspase-3. Crocin inhibited neuronal PC-12 cell death induced by both internal and external apoptotic stimuli (Soeda et al., 2001). Depriving the neuronally differentiated pheochromocytoma (PC-12) cells of serum/glucose caused peroxidation of their cell membrane lipids and decreased intercellular superoxide dismutase (SOD) activity. Treating these cells with 10 μ M crocin inhibited the formation of peroxidized lipids, partly restored the SOD activity, and maintained their neuronal morphology. These antioxidant effects of crocin were more effective than those of α -tocopherol at the same molar concentrations (Ochiai et al., 2004).

The S100 family of calcium binding proteins contains about 16 members each of which displays a unique pattern of tissue/cell type specific expression (Zimmer et al., 1995). S100 beta is a multifunctional protein that is found in large amounts in astrocytes and a number of other tissues (Marshak, 1995). The concentration of the protein S100 in serum is used as a brain damage marker in various conditions. This protein is expressed in large quantities in astrocytes, and found intracellularly and extracellularly in the brain at subnanomolar–nanomolar concentrations. As a serum marker, it reflects the severity of brain damage and has been proposed for the assessment of condition such as neurological outcome after traumatic and damaged brain (Donato, 2003; Piazza et al., 2007). OP-induced brain injury is characterized by rapid loss of consciousness, seizures, central respiratory inhibition as well as long-term behavioral changes in sub-lethal injuries (Shrot et al., 2009). *In vitro* experiments have shown that TNF increased the release of S100 from astrocyte cultures without damaging the cells (Piazza et al., 2007). Organophosphate-induced delayed polyneuropathy (OPIDP) is a rare toxicity resulting from exposure to certain organophosphorus esters. It is characterised by distal degeneration of some axons of both the peripheral and central nervous systems occurring 1–4 weeks after single or short-term exposures. Neuropathy target esterase (NTE) is thought to be the target of OPIDP initiation (Lotti and Moretto, 2005). However, diazinon does not inhibit NTE and cannot induce OPIDN (De Blaquiére et al., 2000). Safranal showed protective effect against pentylentetrazol-induced seizures (Hosseinzadeh and Sadeghnia, 2007b) and on different markers of oxidative damage in hippocampal tissue from ischemic rats (Hosseinzadeh and Sadeghnia, 2005). In this study, diazinon increased S100 β protein level five folds. All of the pre-treatment compounds i.e. vitamin E, safranal and crocin effectively inhibited this effect of diazinon.

Safranal and crocin at lower doses prevented diazinon induced toxicity on other parameters such as GGT (by crocin and safranal) and TNF- α , (by crocin). The lack of these protective effects at higher doses might be partially related to some toxic effects at these doses.

In conclusion, it might be stated that sub-acute doses of diazinon induced biochemical enzymatic changes, inflammatory and neurotoxicity effects which were partially prevented by crocin and safranal. The efficacy of crocin was higher than safranal in this regard.

Conflict of interest

The authors declare that there are no conflicts of interest.

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