Effect of aqueous extract of *Crocus sativus* L. (saffron) stigma against subacute effect of diazinon on specific biomarkers in rats

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Seyed Adel Moallem[1], Alireza Timcheh Hariri[2], Mahmoud Mahmoudi[3] and Hossein Hosseinzadeh[4]

Abstract

In this study, the effect of aqueous extract of *Crocus sativus* L. (saffron) stigma was studied against subacute toxicity of diazinon (DZN) on specific biochemical markers in rats. Vitamin E (200 IU/kg) and the aqueous extract of saffron 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. Red blood cell (RBC) cholinesterase activity was inhibited by DZN and this effect was not affected by vitamin E or saffron plus DZN. The levels of serum tumor necrosis factor-α (inflammation marker), direct 8-iso-prostaglandin $F_{2\alpha}$ (oxidative stress marker) and soluble protein-100 β (S100β, neuronal damage marker) were increased significantly by DZN. The saffron extract inhibited the effect of DZN on these biomarkers levels. However, vitamin E was able to only reduce 8-iso-prostaglandin $F_{2\alpha}$ and S100β levels. This study showed that the aqueous extract of saffron prevents DZN-induced rise of several specific inflammation, oxidative stress and neuronal damage biomarkers.

Keywords

Saffron, diazinon, vitamin E, TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$, S100β

Introduction

Organophosphorus compounds (OPs) are primarily used as pesticides (Jokanović and Kosanović, 2010). Some OPs are used as drugs and the most toxic compounds as nerve agents. Acute toxicity of OPs is due to the inhibition of acetylcholinesterase, the critical enzyme in neurotransmission (Bosak, 2006). Exposure to OPs can cause several side effects such as nausea, vomiting, diarrhea, salivation, lacrimation, bradycardia, arrhythmias, bronchoconstriction, bronchosecretion, mitosis, involuntary defecation and urination through muscarinic receptors. They also have nicotinic effects and induce twitching of fine skeletal muscles, hyperreflexia, fasciculations, muscle weakness and paralysis that can affect diaphragm and respiratory muscles (Jokanović and Kosanović, 2010). Central nervous system (CNS) toxicity including anxiety, restlessness, ataxia, convulsions, respiratory depression and coma as well as the atypical neurological manifestations of an intermediate syndrome and delayed peripheral polyneuropathy have been observed (Hsieh et al., 2001). Diazinon (DZN), an OP insecticide is an irreversible cholinesterase inhibitior which has been used worldwide in agriculture and domestically for several years. DZN reduces

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the activities of antioxidant compounds and induces oxidative stress (Shah and Iqbal, 2010).

Saffron, the dried stigmas of *Crocus sativus* L. is well known for its aromatic and coloring power (Gresta1 et al., 2008). A variety of pharmacological activities such as anticancer (Abdullaev and Espinosa-Aguirre, 2004), antidepressant (Hosseinzadeh et al., 2004) and anticonvulsant effects (Hosseinzadeh and Sadeghnia, 2007; Hosseinzadeh and Talebzadeh, 2005) have been reported for saffron or its constituents such as crocin or safranal. There are several reports detailing the antioxidant activity of saffron and its constituents (Assimopoulou et al., 2005; Hosseinzadeh et al., 2009b). Saffron or its constituents have shown some protective effects on different markers of oxidative damage in ischemic tissues from rats (Hosseinzadeh and Sadeghnia, 2005).

Therefore, the use of antioxidants and free radical scavenger agents may be rational in counteracting DZN including its subacute toxicity which is partly induced by oxidative stress. The aim of this study was to investigate the protective effect of saffron extract on subacute effects of DZN as assessed by evaluating three specific biomarkers in rats. These biomarkers are tumor necrosis factor-α (TNF-α; inflammation marker, direct 8-iso-prostaglandin $F_{2\alpha}$ (oxidative stress marker) and soluble protein-100 β (S100β, neuronal damage marker).

Materials and methods

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 2 days prior to testing. All animal experiments were approved by the Animal Care Committee of Mashhad University of Medical Sciences.

Chemicals

DZN (Merck Co, 99% purity) was a gift from the Agricultural Research, Education and Development Organization (AREEO; Tehran, Iran). Vitamin E (DL-a tocopherol acetate) was obtained from OSVE Pharmaceutical Co. (Tehran, Iran).

Sandwich Elisa kits for quantization of rat TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$ and rat soluble protein-100B were obtained from Koma Biotech (Korea), assay designs (USA) and USCN LIFE (China), respectively.

Animal treatment schedule

Rats were divided into 10 groups (n = 6). The compounds were administrated in the morning (between 9:00 and 11:00 AM) to nonfasting rats. All rats were treated for 4 weeks.

Group 1: control group. The control group received sweet almond oil at doses of 20 mg/kg/day through gavages once a day.

Group 2: DZN-treated group. DZN at a dose of 20 mg/kg/day in sweet almond oil was given through gavage to rats once a day

Group 3: vitamin E-treated group. Vitamin E (200 IU/kg) was administered intraperitoneally (IP) three times per week.

Group 4: vitamin E- + DZN-treated group. Vitamin E (200 IU/kg three times per week) was administered IP and DZN was administered orally (20 mg/kg/day once a day in sweet almond oil) via gavage needle.

Groups 5, 6 and 7: saffron- + DZN-treated group. Saffron was administered at doses 50, 100 and 200 mg/kg/day IP to rats 3 days per week before oral administration of DZN (20 mg/kg/day).

Groups 8, 9 and 10: saffron-treated groups. Saffron was administered at doses 50, 100 and 200 mg/kg/day IP to rats 3 days per week.

Blood sampling

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

Specific biomarker evaluation

The biomarkers TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$ and S100β levels were measured following the

protocols of the kit manufacturers: Koma Biotech, Assay Designs and USCN LIFE, respectively.

Statistical analysis

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

Results

Evaluation of erythrocyte (RBC) cholinesterase activity

A significant decrease was observed in RBC cholinesterase activity in the DZN-treated rats compared to the control group ($p < 0.05$). At all doses saffron and vitamin E were not able to significantly inhibit the effect of DZN on RBC cholinesterase activity (Figure 1).

Changes in biomarkers

The levels of serum TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$, and S100β were increased significantly by DZN in comparison to the control group (Figures 2 to 4). Saffron at the highest dose (200 mg/kg) inhibited the rise of direct 8-iso-prostaglandin $F_{2\alpha}$, and TNF-α levels induced by DZN. This extract at the 100 and 200 mg/kg doses also prevented the increase in S100β caused by DZN treatment.

Vitamin E also inhibited the increasing effect of DZN on direct 8-iso-prostaglandin $F_{2\alpha}$ and S100β levels. However, it could not prevent the effect of DZN on TNF-α level.

Discussion

This study showed that the aqueous extract of saffron inhibited the effect of DZN on specific biomarker such as TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$ and S100β.

Vitamin E and the aqueous extract did not change the cholinesterase activity. This implies that the inhibitory effect of these agents was not mediated via cholinesterase enzyme. As some of the toxicity of DZN is mediated through the reduction of antioxidant activity and induction of oxidative stress (Shah and Iqbal, 2010), it is possible that saffron could counteract these effects. There are reports that suggest saffron and its constituents have antioxidant and radical-*scavenging* activities (Assimopoulou et al., 2005; Hosseinzadeh et al., 2009b).

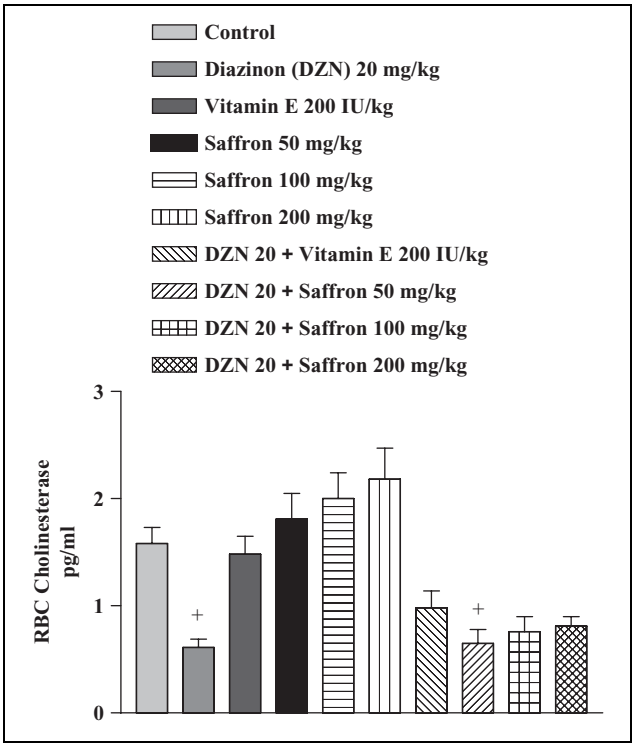

Figure 1. Effect of DZN and DZN plus vitamin E or saffron on RBC cholinesterase levels in rats. Vitamin E, saffron and control were administered intraperitoneally three times per week. DZN in sweet almond oil was given through gavage to rats once a day. Data are shown as mean ± SEM; $^{+}$comparison with control, $p < 0.05$, Tukey Kramer test, $n = 6$. DZN: diazinon; RBC: red blood cell.

We have shown in this study that DZN can significantly increase serum TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$ and S100β. TNF-α performs a key role in inflammatory responses. Several human inflammatory, infectious and autoimmune CNS disorders might be mediated by TNF-α (Probert et al., 1997). In our study, vitamin E decreased TNF-α level, thought not significantly. The aqueous extract of saffron at the highest dose (200 mg/kg) reduced this inflammatory biomarker. We have shown previously that crocin, an active ingredient of saffron, can reduce the rise of TNF-α by DZN (Hariri et al., 2010). Crocin and crocetin, the constituents of saffron, reduced the lipopolysaccharide (LPS)-stimulated productions of TNF-α, interleukin-1β and intracellular reactive oxygen species. In fact, these compounds showed neuroprotective effects by reducing the production of several neurotoxic molecules from activated microglia

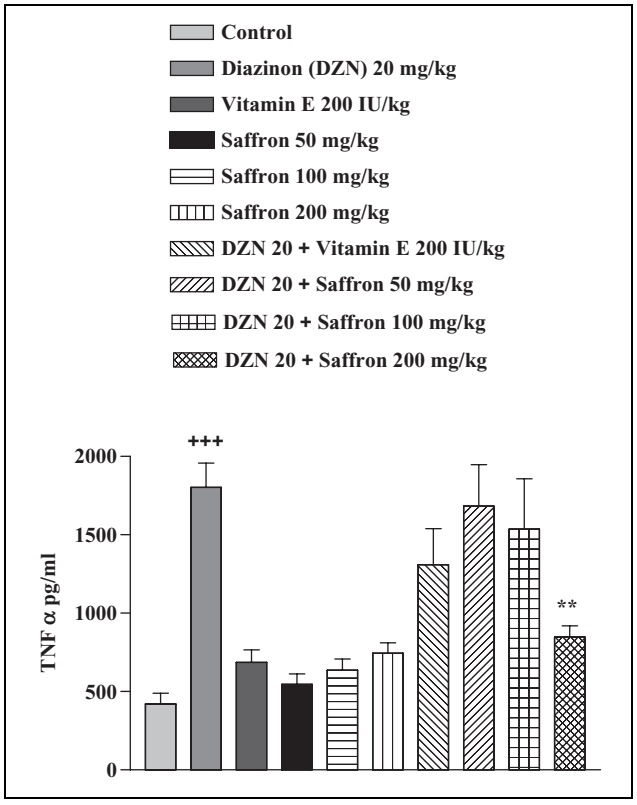

Figure 2. Effect of DZN and DZN plus vitamin E or saffron on TNF-α levels in rats. Vitamin E, saffron and control were administered intraperitoneally three times per week. DZN in sweet almond oil was given through gavage to rats once a day. Data are shown as mean ± SEM; +++p < 0.001 comparison with control; **p < 0.01 comparison with diazinon treated group, Tukey Kramer test, $n = 6$. DZN: diazinon; TNF-α: tumor necrosis factor-α.

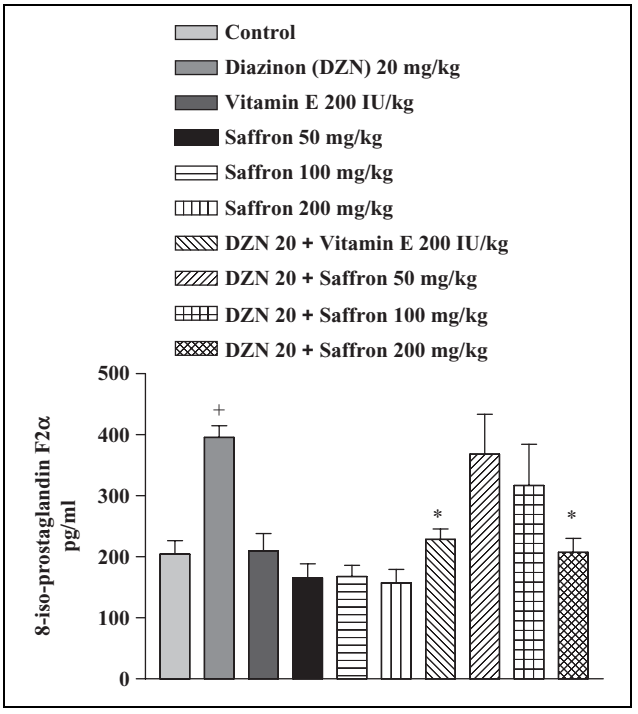

Figure 3. Effect of DZN and DZN plus vitamin E or saffron on direct 8-iso-prostaglandin $F_{2\alpha}$ levels in rats. Vitamin E, saffron and control were administered intraperitoneally three times per week. DZN in sweet almond oil was given through gavage to rats once a day. Data are shown as mean ± SEM; + p < 0.05 comparison with control; *p < 0.05 comparison with diazinon treated group, Tukey Kramer test, $n = 6$. DZN: diazinon.

(Nam et al., 2010). The aqueous and ethanolic extracts of saffron stigma and petal also showed antinociceptive and anti-inflammatory effects in mice (Hosseinzadeh and Younesi, 2002).

TNF-α and leptin were enhanced in epididymal white adipose tissue in fructose-fed rats. Crocetin effectively restored TNF-α to its normal level and improved insulin sensitivity (Xi et al., 2007).

In one study, it has been shown that crocin modulated the expression of bcl-2 family proteins and thus reduced a TNF-α-induced release of cytochrome c from the mitochondria. Crocin also inhibited the effect of TNF-α on neuronally differentiated PC-12 cells (Soeda et al., 2001). DZN induced neurotoxicity in cortical culture by mechanisms other than acetylcholine involvement. DZN also induced apoptotic neuronal death (Rush et al., 2010).

S100 proteins are a group of closely related, small, acidic Ca^{2+}-binding proteins which are rich in the brain. In the mammalian brain, S100α and S100β are confined to glial cells (Donato, 1986). The S100β protein has been evaluated in blood as a biochemical marker for brain injury and acute neurological disorders such as global hypoxia, ischemic or hemorrhagic stroke, traumatic brain injury and disability after head trauma (Stroick et al., 2006; Townend and Ingebrigtsen, 2006). It seems S100β protein is an encouraging early predictor of brain damage (Wojtczak-Soska and Lelonek, 2010). DZN induced neurotoxicity and significantly increased the level of serum malondialdehyde (MDA) in rat brain (Yilmaz et al., 2011). In our study, while S100β levels were enhanced in the DZN-treated group, this disorder was effectively normalized in saffron-treated rats. In this regard, it has been demonstrated that saffron and its constituents reduced *ischemia–reperfusion injury in brain* (Hosseinzadeh and Sadeghnia, 2005).

Saffron at the highest dose (200 mg/kg) decreased the augmentation of direct 8-iso-prostaglandin $F_{2\alpha}$ level induced by DZN. Isoprostanes, specifically 8-iso-prostaglandin $F_{2\alpha}$, are reliable biomarkers of lipid

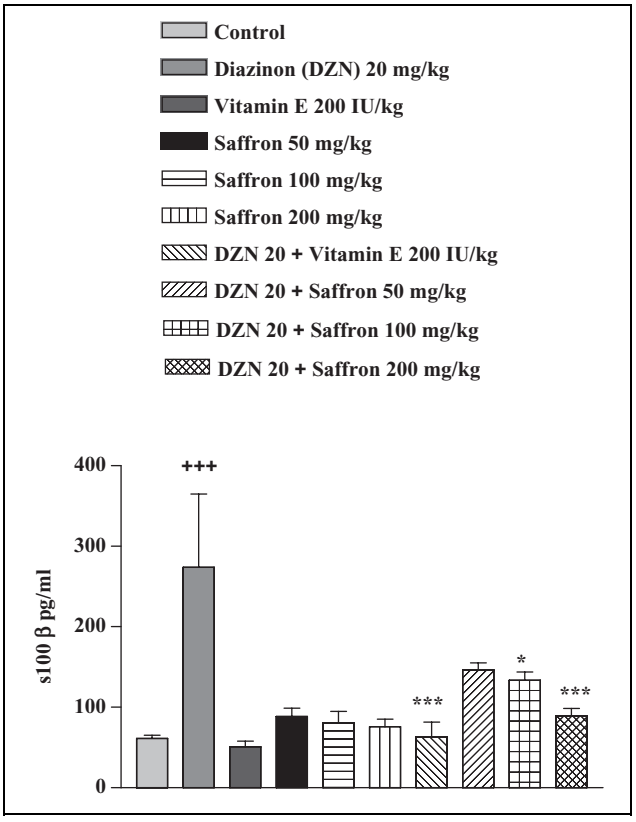

Figure 4. Effect of DZN and DZN plus vitamin E or saffron on soluble protein-100 β (S100β) levels in rats. Vitamin E, saffron and control were administered intraperitoneally three times per week. DZN in sweet almond oil was given through gavage to rats once a day. Data are shown as mean ± SEM; +comparison with control; *comparison with diazinon treated group $^{+++}p < 0.001$ and $^{*}p < 0.05$, $^{***}p < 0.001$, Tukey Kramer test, $n = 6$. DZN: diazinon.

peroxidation which provide a proper tool for assessment of oxidative stress. The reliability of isoprostanes as *in vivo* markers of lipid peroxidation makes them a highly valuable tool for finding effective antioxidant agents (Wood et al., 2003). In different studies, saffron and its components, crocin and safranal showed antioxidant activity (Hosseinzadeh et al, 2009b) or inhibited lipid peroxidation in a variety of tissues such as the brain (Hosseinzadeh and Sadeghnia 2005), kidneys (Hosseinzadeh et al., 2005) or skeletal muscles (Hosseinzadeh et al., 2009a).

In conclusion, we have shown that DZN induced lipid peroxidation, inflammatory and neurotoxicity effects which was normalized with the administration of the aqueous extract of saffron. The effect of this spice most likely is not mediated by the action on acetylcholinesterase enzyme.

Authors' note

The results described in this article are part of a PhD thesis.

Conflict of interest

The authors declare no conflicts of interest.

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