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ORIGINAL ARTICLE

The Effect of *Rosa Damascena* Extract on Diazinon Toxicity in Mice

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KEYWORDS

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ABSTRACT: Diazinon (DNZ) is one of the most widely used organophosphorus poisons, which plays an important role in chemical pest control by controlling a wide range of chewing and sucking pests in gardens and fields. Diazinon causes death in living organisms by reducing cholinesterase and disrupting nerve cells. In this research, the effect of *Rosa damascena* extract against DNZ toxicity and oxidative damage induced by DZN in mice was studied. The mice toxified with DNZ (32.5 mg kg^{-1}, intraperitoneally) and treated by pralidoxime (PM) (20 mg kg^{-1}, intraperitoneally) or *R. Damascena* extract (50, 100, 200 mg kg^{-1}, orally) daily for two weeks. In the end, the acetylcholinesterase (AchE), ferric-reducing antioxidant power (FRAP), Malondialdehyde (MDA), ALT, AST, ALP, and total bilirubin were assayed. DZN administration significantly lowered the AchE, and FRAP and increased the MDA ($P < 0.05$). However, *R. Damascena* extracts treatment caused a significant reduction in MDA level and restored the levels of AchE and FRAP as well as significantly prevented the DZN-induced increase in liver aminotransferases, ALP, and total bilirubin. We found that *R. Damascena* administration nearly eliminated DZN-induced toxicity by preventing oxidative stress in mice.

INTRODUCTION

Medicinal plants with several biological activities can be a suitable alternative in medicine [1]. Previously, the effect of herbal medicine on some chemical toxicity was approved [2]. *Rosa Damascene* (*R. Damascene*), is one of the popular medicinal plants in Iran, which is mainly cultivated in Kashan, Shiraz and Qazvin regions [3]. In traditional Iranian medicine, many properties have been attributed to *R. Damascena* and it is effective in treatment of some diseases [4]. The rose genus has 200 species, of which *Rosa Damascene* is one of the best and most famous species that is cultivated all over the world to extract essential oil. Essential oil is usually used in cosmetics, perfumery and food industries [4, 5].

Various alkane, alcohol, phenol, terpene and terpenoid compounds are present in the *Rosa Damascene*. Quercetin, kaempferol, gallic acid and syringic acid are the most phenolic compounds in *Rosa Damascene.* The medical activity of *Rosa Damascene* may relate to phenolic content

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[5, 6] and play a main role in health as antioxidant, antimicrobial, anti-inflammatory, pain reliever, anticancer, anticonvulsant and sedative, memory booster and heart booster, and used as a mild laxative in traditional medicine [6-8]. The flavonoid glycoside in this plant inhibits the activity of the acetylcholinesterase (AchE) enzyme [8]. Therefore, it seems that the plant will be able to improve the failures caused by acetylcholinesterase [3, 5, and 8]. Organophosphates are the most common chemical compounds used to control agricultural pests and are considered the most toxic insecticides for vertebrates [9]. These compounds are used extensively in the environment due to their low accumulation and short-term shelf life and also, are the most important source of environmental pollution [10]. The effect of organophosphorus toxins on the nerves may be due to the prevention of the AchE enzyme, which is the destruction of the acetylcholine in the synaptic space, which causes the continuation of transmission of stimulation of the nerves and continuous stimulation and excessive nicotine and muscarinic receptors [11]. Diazinon (DZN) is an artificial chemical with extensive pesticide property that utilized in agricultural, veterinary, and public health aims [12, 13]. Animals and humans in polluted environments may be accidentally exposed to DZN through inhalation or digestion [14, 15]. The most significant feature of DZN toxicity is the irrevocable inactivity of acetylcholinesterase, which leads to death in high doses. DZN toxicity occurs by binding of its oxygen analogue to the AchE enzyme. In these conditions, acetylcholine accumulates in nerve tissues and effector organs. [16, 17]. Following the over-utilization of organophosphates, it is important to examine new compounds to neutralize the effects of DZN. Hence, the study achieved for evaluating the effect of *R. Damascena* extract on Diazinon toxicity in mice.

MATERIAL AND METHODS

Animals

Male albino mice (35–40 g) were prepared from the Pastur institute (Iran). The mice were reared in a ventilated animal house with an intermittent light-dark cycle (12 L- 12 D, in 24 h) and proper temperature (24 ± 3°C) with free water and food. Animals were quarantined for 6 days before the beginning of the experiments.

Chemicals

Diazinon 60 EC (O, O-diethyl-o-[2-isopropyl-6-methyl-4-pyrimidinyl]-phosphorothioate, 97% purity) was obtained from partonar company (Tehran, Iran) and it was diluted in water for preparation of suitable concentration. Pralidoxime (PM) and DTNB reagent were purchased from Sigma-Aldrich (Steinheim, Germany).

Plant Extract Preparation

Fresh leaves of *R. Damascena* were collected from Kashan, Iran, and identified by botanists (Professor Naghi-Nezhad) at the University of Mazandaran. The petal of the plant under study was extracted using the Soxhlet Extraction Method. The soxhlet extraction of petals was achieved by using the following procedure; Methanolic extract was obtained from 20 grams of washed and dried leaves of *R. Damascena* using Soxhlet machine. After extraction, the solvent was removed using a rotary evaporator and the extracted compound was collected [18].

Animal treatment schedule

Seventy mice were grouped in seven treatment pens, randomly. The first (C) group received NaCl 0.9%. The second (positive control) group received diazinon (32.5 mg kg^{-1}) intraperitoneally (IP) for 14 days [19]. The third group receives pralidoxime (20 mg kg^{-1}, IP) for 14 days. The fourth group (E) received R. *Damascena* extract (200 mg kg^{-1}) orally for 14 days. (Treatment groups): The fifth (T1), sixth (T2) and seventh (T3) groups received 50, 100 and 200 mg kg^{-1} (respectively) *R. Damascena* extract orally and diazinon (32.5 mg kg^{-1}, IP) for 14 days. After 24 hours their blood was obtained from their heart. After centrifugation (at 1,800 × g for 10 min at 4°C), the acetylcholinesterase (AchE), ferric-reducing antioxidant power (FRAP), Malondialdehyde (MDA), ALT, AST, ALP, and total bilirubin were determined. All doses and the duration of

treatment were used according to the previous study [20-22].

Measurement of plasma cholinesterase activity (AchE)

AchE level was determined in plasma according to Ellman et al. [22] The K2 HPO3 -K H2 PO3 (3 ml, pH = 7.8, and 75 mmol L^{-1}) and 5,5′-dithio-bis-2-nitrobenzoic acid (0.25 mmol L^{-1}) was added to samples (10 µl). The addition of acetyl thiocholine iodide (10 µl, 3 mmol L^{-1}) can induce enzyme activity. The result was read at 412 nm by spectrophotometer [23].

Measurement of ferric-reducing antioxidant power (FRAP) assay

(FRAP) level was determined with Benzie and Strain (1996) method. The blue color can be seen by complexing of Fe^{2+} and TPTZ. The absorbance at 593 nm was determined by spectrophotometer. $FeSO_4$-$7H_2O$ was used as a standard FRAP assay at 100 to 1000 µM [24].

Measurement of Malondialdehyde (MDA)

MDA activity was measured by MDA assay kit (ZellBio GmbH). The MDA-thiobarbituric acid (TBA) complex formed at elevated temperatures. MDA was determined at 535 nm in the acidic condition at 100 °C, colorimetrically. The sensitivity of the MDA mearsurement was 0.1 µM. The coefficient of variation in intraassay and interassay were 5.8 and 7.6%, respectively.

Biochemical analysis of liver enzymes

The amount of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin were determined by spectrophotometer (Auto analyzer machine, Alfa classic), using Pars Azmon company kits (Iran).

Statistical analysis

The data analyzed using the statistical SPSS software. One-away analysis of variance was selected followed by Tukey test. The data was significant at P<0.05 level.

RESULTS

Cholinesterase activity (AchE)

Figure 1 shows that the activity of AchE in the DZN received group was significantly lower than the C group ($P < 0.05$). As shown in Figure 1, treatment with *R. Damascena* increased AchE level in treatment groups compared with the DZN group. However, these differences were statistically significant only for T2 (100 mg kg^{-1}) and T3 (200 mg kg^{-1}) groups ($P < 0.05$). No significant difference in AchE level was seen in groups of E, PM and C (Figure 1).

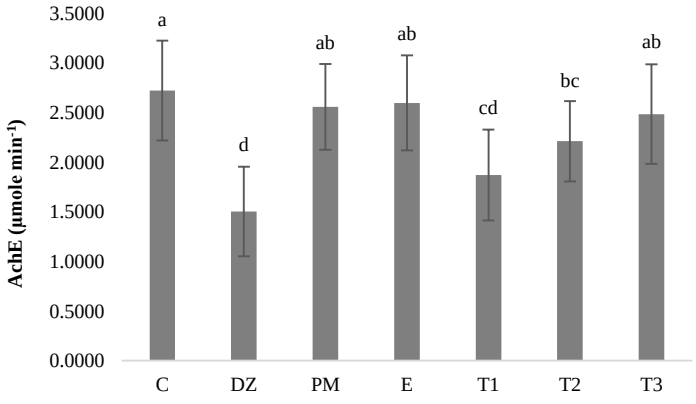

Figure 1. AchE activity in the mice (24 h after DZN injection and *R. Damascena* extract treatment). In this figure C: Control, DZN: Diazinon, PM: pralidoxime, E: *R. Damascena* extract (200 mg kg^{-1}), T1: *R. Damascena* extract (50 mg kg^{-1}) + Diazinon, T2: *R. Damascena* extract (100 mg kg^{-1}) + Diazinon, and T3: *R. Damascena* extract (200 mg kg^{-1}) + Diazinon.

Ferric-reducing antioxidant power activity (FRAP)

FRAP activity in the DZN received group was lower than the C group ($P < 0.05$). After administration of *R. damascena* extract the level of FRAP increased in T1-T3 compared with the DZN group. Although, these differences were statistically significant only for T2 (100 mg kg^{-1}) and T3 (200 mg kg^{-1}) groups ($P < 0.05$). No significant difference in FRAP level was seen in groups of E, PM and C. (Figure 2).

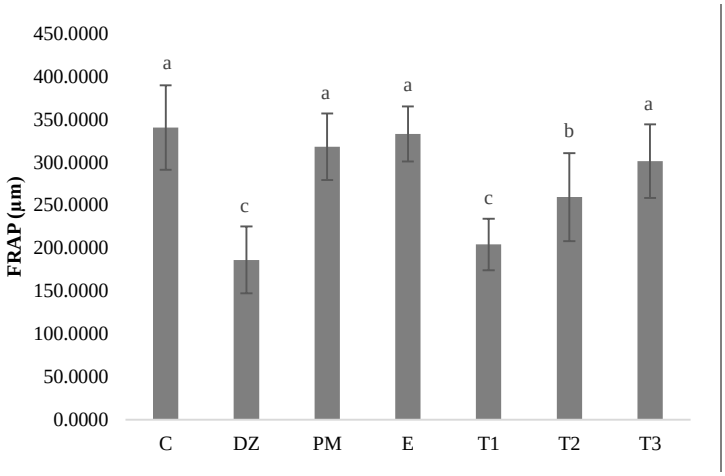

Figure 2. FRAP activity in the mice (24 h after DZN injection and *R. Damascena* extract treatment). In this figure C: Control, DZN: Diazinon, PM: pralidoxime, E: *R. Damascena* extract (200 mg kg^{-1}), T1: *R. Damascena* extract (50 mg kg^{-1}) + Diazinon, T2: *R. Damascena* extract (100 mg kg^{-1}) + Diazinon, and T3: *R. Damascena* extract (200 mg kg^{-1}) + Diazinon.

MDA activity

In Figure 3, the MDA activity in the DZN received group was significantly higher than the C group ($P < 0.05$). The decrease of MDA activity in the groups treated with *R. Damascena* demonstrates its healing effect. However, these differences were statistically significant only for T2 (100 mg kg^{-1}) and T3 (200 mg kg^{-1}) groups in comparison with the DZN-received group ($P < 0.05$). No significant difference was found in the MDA level in groups E and PM compared to the C group.

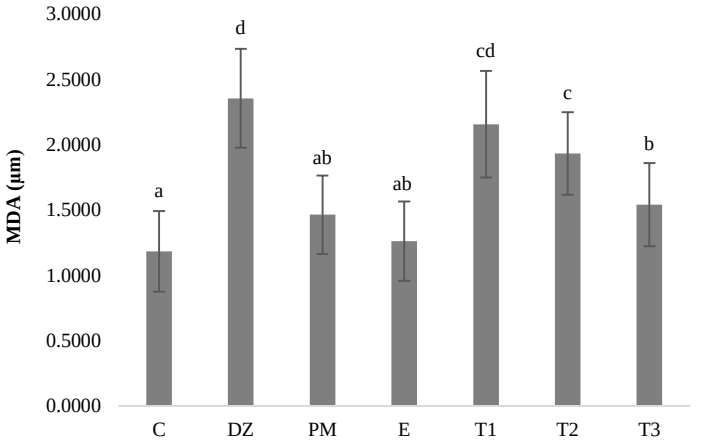

Figure 3. MDA level in the mice (24 h after DZN injection and *R. Damascena* extract treatment). In this figure C: Control, DZN: Diazinon, PM: pralidoxime, E: *R. Damascena* extract (200 mg kg^{-1}), T1: *R. Damascena* extract (50 mg kg^{-1}) + Diazinon, T2: *R. Damascena* extract (100 mg kg^{-1}) + Diazinon, and T3: *R. Damascena* extract (200 mg kg^{-1}) + Diazinon.

Liver enzymes (ALT, AST, and ALP) and total bilirubin

The levels of ALT, AST, and ALP in DZN-received group was significantly higher than the C group ($P < 0.05$). Intraperitoneal injection of DZN induced renal injury. In DZN-received group, the administration of *R. Damascena* extracts in doses of 100, and 200 mg kg^{-1}, caused the decrease in level of ALT and AST significantly ($P < 0.05$). A significant reduction in ALP level was seen in groups T2 and T3 in comparison with the DZN-received group. Furthermore, no significant difference was found in groups T2 and T3 compared to the C group. The groups showed no significant difference for total bilirubin level. According to the levels of ALT, AST, and ALP as the main factor in liver function, the group which received 200 mg kg^{-1} of *R. Damascena* extract showed lower ALT, AST, and ALP level than control group (Table 1).

Table 1. The effect of different doses of *R. Damascena* extract (50, 100, and 200 mg kg^{-1}) on serum ALT, AST, ALP, and total bilirubin level

Groups	Number	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Total bilirubin(mg dl^{-1})
C	10	78.00±10.00^{a}	104.4 ± 17.00^{a}	187.00±17.00^{a}	0.39 ±0.3145^{a}
DZN	10	420.3±35.00^{f}	417.2±27.00^{e}	375.1± 11.00^{e}	0.45 ±0.4773^{a}
PM	10	146.0±23.00^{bc}	164.3± 23.00^{b}	212.5± 16.02^{ab}	0.39 ±0.2333^{a}
E	10	108.4±23.01^{b}	121.1±24.00^{a}	194.4± 17.00^{a}	0.40 ±0.2981^{a}
T1	10	352.6±19.00^{e}	320.4±18.00^{d}	349.7± 17.00^{d}	0.38 ±0.3266^{a}
T2	10	202.9±15.00^{d}	286.5± 28.00^{c}	275.7± 19.00^{c}	0.39 ±0.3266^{a}
T3	10	152.2±13.00^{c}	176.1± 30.00^{b}	221.5±18.08^{b}	0.37 ±0.3266^{a}
	(p-value)	<0.001	<0.001	<0.001	0.078

* C: Control, DZN: Diazinon, PM: pralidoxime, E: *R. Damascena* extract (200 mg kg^{-1}), T1: *R. Damascena* extract (50 mg kg^{-1}) + Diazinon, T2: *R. Damascena* extract (100 mg kg^{-1}) + Diazinon, and T3: *R. Damascena* extract (200 mg kg^{-1}) + Diazinon; Significant difference was manifested by different letters ($P < 0.05$).

DISCUSSION

The present work was achieved to evaluate the effects of *R. Damascena* on DZN-induced toxicity in mice. DZN is one of the most commonly used organophosphate pesticides which, inhibits the activity of acetylcholine esterase. Furthermore, it can induce oxidative stress after exposure [25, 26]. Ache is an important neurotransmitter that plays role in the regulation of cognitive functions. As observed in our results, DZN reduced the level of Ache, and treatment with R. *Damascena* significantly attenuated these levels. Zeinali et al [27] reported DZN induced sub-acute toxicity in mice. These investigators reported that 20 mg kg^{-1} of DZN given orally reduced the AchE level and caused hematological and genotoxicity in mice [12]. In another experimental model. El-Mazoudy and Attia [19] investigated diazinon induced-toxicity in male mice. These investigators observed that 2, 4.1, and 8.2 mg kg^{-1} BW per day for 4 weeks given orally decreased levels of acetylcholinesterase activities and induced toxicity in mice [7, 19], which is in agreement with our findings.

R. Damascena is an herbal medicine that is used for treating of different diseases in the world, effectively [28]. It seems that the main biological properties of the *R. Damascena* extract are usually related to phenolic compounds. *R. Damascena* is rich of minerals such as calcium, phosphorus, potassium, sodium, iron, magnesium, boron, zinc, and manganese. Phenolic compounds can induce antioxidants, anti-inflammatory, anticancer, and antidepressant effects [28, 29]. Treatment with *R. Damascena* (100, and 200 mg kg^{-1}) was able to modulate the liver enzymes and protect the liver against DZN toxicity.

Antioxidant activity is a complex method that usually occurs through various mechanisms and is affected by many factors [30]. The FRAP test was used for estimating

the antioxidant activity of *R. Damascena*. The findings of the present study indicate that after administration of *R. Damascena* the levels of FRAP in groups T1-T3 increased compared to the DZN group which represents the antioxidant power of *R. Damascena*.

Free radicals induce the lipid peroxidation in a cell. The elevation of free radical content in cells causes increasing production of MDA. MDA is an indicator of oxidation and it is the main products of fatty acids peroxidation in a cell [31]. In the present study, MDA levels in groups T1-T3 reduced with *R. Damascena* treatment compared to DZN-received group.

CONCLUSIONS

The *R. Damascena* exhibits antioxidant effects owing to its free-radical scavenging properties. In the present study, we found that *R. Damascena* confers protection against DZN-induced oxidative stress and toxicity in mice, which could improve significantly AchE, MDA, and FRAP activity levels and also, can reduce the DZN-induced oxidative stress and liver damage. Our findings indicate that *R. Damascena* (200 mg kg^{-1}) produced a marginally protective effect against DZN-induced toxicity.

ETHICAL CONSIDERATION

Animal care and research has been carried out in accordance with the ethical principles of working with laboratory animals approved by the Islamic Azad University, Babol Branch (213-98).

Conflict of interests

The authors declared no competing interests.

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