

Protective Effects of *Berberis vulgaris* on Diazinon-Induced Brain Damage in Young Male Mice

Azadeh Sonei, Simin Fazelpour, Leila Kanaani, and Mahsa Hadipour Jahromy

Herbal Pharmacology Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran 19168, Iran

ABSTRACT: Diazinon is an imminent and hazardous chemical organophosphate multiplex that is generally used as a pesticide but is toxic for many species particularly vertebrates. Berberry (*Berberis vulgaris* L., family Berberidaceae) is a plant that flourishes in Europe and Asia that has been largely investigated for its therapeutic effects. In the present study, we evaluated the protective effects of *B. vulgaris* on diazinon-induced brain damage in young male mice. Twenty-one young male albino mice weighing 18 ± 2 g were divided in three equal groups of seven mice, and treated orally with either olive oil (control), diazinon 50 mg/kg + *B. vulgaris* extract 200 mg/kg, or diazinon 50 mg/kg. After three weeks, cerebrum and cerebellum samples were collected for antioxidant assays. The results indicated that diazinon increased oxidative stress in the brain of mice. The glutathione content and proceedings of antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and catalase, were significantly reduced in both the cerebellum and cerebrum of diazinon-treated mice, compared with the control group. In addition, acetylcholinesterase (AChE) activity was inhibited by exposure to this pesticide. Administration of 200 mg/kg *B. vulgaris* extract with diazinon significantly decreased oxidative stress indices in all experiments. The results indicated that *B. vulgaris* extract has protective effects against lipid peroxidation of the cerebellum and cerebrum, and in regenerating AChE activity in the brain induced by diazinon.

Keywords: *Berberis vulgaris*, brain damage, diazinon, mice

INTRODUCTION

Pesticides are liquid, solid, or gas chemical substances that are used worldwide in agriculture and health programs to eliminate vegetable and animal pests in agriculture, animal husbandry, industry, and homes (Alavanja et al., 2013). One of the most commonly used pesticides in the world is diazinon (Morteza et al., 2017). Diazinon belongs to a group of chemicals known as organophosphates. Like other organophosphates, diazinon acts by inhibiting the activity of acetylcholine esterase (AChE) via phosphorylation of the serine hydroxyl group in the substrate-binding domain of the enzyme that results in accumulation of acetylcholine (Fulton and Key, 2001).

“Zereshk”, the Persian name for *Berberis vulgaris*, is highly cultivated in Iran, particularly in Birjand and Qaen cities, central and Southern Europe and the Northeastern of the United States (Rounsaville and Ranney, 2010; Mokhber-Dezfuli et al., 2014). Barberry shrubbery is 1 to 5 meters in length and its wood is red, brown, or yellow in color. The tree has oval-shaped leaves with red teeth

(Gopalakrishnan et al., 2006) and its edible fruit has scarlet colored berries that are oblong, slightly curved, and about 0.5 inches long (Arayne et al., 2007).

The presence of pesticides in the environment and food-stuff is a major global concern. Therefore, continuous monitoring of pesticides is one of the most important management strategies (Eskenazi et al., 2007; Pimentel and Burgess, 2014). Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe conditions, such as organ damage, reproductive problems, and even cancer (Resnik and Portier, 2005). Substantial evidence suggests an association between organophosphate insecticide exposure and neurobehavioral alterations (Goldman, 2004; Jurewicz and Hanke, 2008).

Although toxicity of organophosphorus insecticides is mainly due to the inhibition of AChE, oxidative stress is probably involved in their toxicity (Oruç and Usta, 2007).

The aim of this present experimental study was to investigate the protective effects of barberries extract on diazinon toxicity of the central nervous system. One con-

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Correspondence to Mahsa Hadipour Jahromy, Tel: +98-21-2200-8065, E-mail: jahromymh@yahoo.com

Author information: Azadeh Sonei (Researcher), Simin Fazelpour (Professor), Leila Kanaani (Student), Mahsa Hadipour Jahromy (Professor)

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centration of diazinon was considered; diazinon was prepared to mix with pure barberries fruit extract solutions and administered to young male mice orally for three weeks. To better understand brain toxicity induced by diazinon, we assessed the activities of antioxidant enzymes in the cerebellum and cerebrum.

MATERIALS AND METHODS

***B. vulgaris* extract preparation**

Barberry fruits (*B. vulgaris* L.) were collected by colleague from the garden of agriculture, Birjand, Iran. Extracts were prepared using an electric juicer, filtered through no. 42 Whatman filter paper (Whatman, Pleasanton, CA, USA), and concentrated under vacuum at 45°C using a Heidolph rotary evaporator (Laborota 4003; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Finally, the waxy concentrated extracts were dried using desiccators and kept in the dark at 4°C until analysis. The yield of extraction was approximately 12% (w/w).

Pesticide

Diazinon was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Diazinon was administered orally to mice at a concentration of 50 mg/kg for three weeks, with or without 200 mg/kg of *B. vulgaris* extract.

Ethical considerations

All animal procedures were conducted in strict conformation with the local Institute Ethical Committee Guidelines for the care and use of laboratory animals of our institution.

Specific approval/ethics number

Mice treated, in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals and the current law of Medical Sciences Research Center, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran (Ethic Code No. 50855) approved the experimental protocols.

Animals

Young male albino mice weighing 18 ± 2 g were purchased from Pasteur Institute, Karaj Production and Research Center, Iran. Mice were maintained at $22 \pm 3^\circ\text{C}$, with a 12 h light/dark cycle and a minimum relative humidity of 40%. Mice were housed in propylene cages with stainless steel grill tops. A standard pellet diet and water were provided *ad libitum*. The animals acclimatized to the laboratory conditions for 1 week before experiments began.

Experimental protocol

Twenty-one mice were divided into three groups, of seven mice, and treated orally with either olive oil (control group), *B. vulgaris* extract 200 mg/kg+diazinon 50 mg/kg (B+D group), or diazinon 50 mg/kg (D group) for three weeks. The dose of *B. vulgaris* extract was chosen based on our pilot study and the published literature. At the end of the experimental period, mice were anesthetized by intra-abdominal injection with chloral hydrate; cerebrum and cerebellum samples were quickly removed, washed in ice-cold saline solution to remove the blood, and weighed. The cerebrum or cerebellum were collected for antioxidant assays (Ohkawa et al., 1979).

One hundred mg of each cerebrum or cerebellum pool were homogenized in 10% (w/v) of the appropriate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 g for 10 min. Clear supernatants were stored at -80°C for further biochemical assays (antioxidants). Other cerebrum and cerebellum samples were homogenized in phosphate buffer (0.1 M, pH 8.0) with approximately 20 mg of each tissue per mL. Aliquots of the resulting tissue homogenates were stored at -80°C until determination of AChE activity.

Catalase (CAT) assay

The method proposed by Aebi (1984) was used to evaluate CAT activity. This method is used to determine CAT activity based on the reduction of absorbance in the test sample due to hydrogen peroxide (H_2O_2) decomposition in the presence of analytic enzymes. CAT activity was determined by measuring the reduction in absorbance at 240 n/min per 1.5 mL of reaction mixture, which comprised of the cell homogenate (0.1 mL) and H_2O_2 (13.2 mM) in phosphate buffer (50 mM, pH 7.0) for 3 min. A mixture consisting of the cell homogenate (0.1 mL) and phosphate buffer (50 mM, pH 7.0) was also used as the control. CAT activity is presented as micromoles of separated H_2O_2 within 60 s per gram of cellular wet weight.

Superoxide dismutase (SOD) assay

To measure SOD activity, we used the method described by Beauchamp and Fridovich (1971). For this purpose, a mixture with a total volume of 2.6 mL, consisting of the homogenate (0.01 mL), 0.1 M ethylenediaminetetraacetic acid (EDTA) (0.2 mL), nitroblue tetrazolium (0.1 mL), and phosphate buffer (67 mM, pH 7.8), was used. The solution's absorbance was determined at 560 nm against distilled water after addition of riboflavin (0.05 mL). The tubes were illuminated for 15 min, and then the absorbance of the formed blue color was re-evaluated. In addition, the percentage of inhibition was determined after comparing the sample absorbance with that of the control (tube without any enzyme activity). The required sample volume for scavenging 50% of the produced super-

oxide anion was regarded as one unit of enzyme activity.

Glutathione peroxidase (GPx) assay

The method proposed by Flohé and Günzler (1984) was used to measure GPx activity. For this purpose, 2.4 U/mL of a fresh GPx solution in 0.1 M potassium phosphate buffer (pH 7.0) was added to 0.5 mM EDTA, 1 mM H₂O₂ (0.1 mL), potassium phosphate buffer (50 mM, pH 7.0), brain supernatant (0.3 mL), and 1 mM sodium azide. For the reaction, 5% trichloroacetic acid (0.5 mL) was added after incubation for 15 min at 37°C. After centrifuging the tubes for 5 min at 1,500 g, the supernatant was collected. Next, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.7 mL) and 0.1 M phosphate buffer (0.2 mL, pH 7.4) were added to 0.1 mL of the supernatant and mixed; finally, the absorbance was measured at 420 nm.

Reduced glutathione (GSH) assay

The method described by Ellman (1959) and modified by Jollow et al. (1974) was used to determine the total GSH content based on the formation of a yellow color by adding DTNB to compounds consisting of sulfhydryl groups. In short, 4% sulfosalicylic acid (3 mL) was added to the homogenate tissues (500 L) for deproteinisation. The mixture was centrifuged for 15 minutes at 2,500 g, and then Elman's reagent (0.2 mL of DTNB solution) added to 500 L of the supernatant. To determine the total GSH content, the absorbance was recorded at 412 nm. The total GSH content was expressed as µg/g of tissue.

AChE measurement

The colorimetric assay described by Ellman et al. (1961) and Khan et al. (2012) was used to determine AChE activity. The reaction mixture consisted of 0.01 M DTNB and 0.1 M phosphate buffer (pH 8), and the rate of acetylcholine iodide hydrolysis was evaluated at 412 nm with respect to the release of thiol compounds, which produce the color-forming compound, 5-thio-2-nitrobenzoic acid, when they react with DTNB. To initiate the reaction, 0.075 M acetylthiocholine iodide added. AChE activity was expressed as l M of substrate/min/mg protein.

Statistical analysis

Data were analyzed using SPSS software (ver. 24; IBM Corp., Armonk, NY, USA). When applicable, we performed one-way ANOVA followed by Tukey's post-hoc test, whereby $P < 0.05$ was considered significant (asterisks represent significant differences of an experimental group compared with the control, and hashtags represent significant differences between B+D group compared with the D group).

RESULTS

The results revealed that a significant increase in lipid peroxidation occurs in the brain of pesticide treated mice; Table 1 shows the results of the lipid peroxidation assay. The mean values of GPx, SOD, GSH, CAT levels, and AChE activity of all control and experimental groups after 3 weeks are shown in Table 1.

The level of total GSH in the D group decreased by 51.15% in the cerebellum and 42.45% in the cerebrum ($P < 0.01$) compared with those of the control group. We observed a significant decline in the activities of antioxidant enzymes in the cerebellum and cerebrum after diazinon treatment compared with the control group: GPx, 65.84% ($P < 0.01$) and 64.96% ($P < 0.01$), respectively; SOD, 65.79% and 50.95%, respectively ($P < 0.01$); and CAT, 81.17% and 65.91%, respectively ($P < 0.01$) occurred after diazinon treatment. Moreover, significant inhibition of AChE ($P < 0.01$) was found in the cerebellum (62.59%) and cerebrum (64.25%) homogenates of the test mice (Table 1).

In our second analysis, we compared the B+D group with the D group. Table 1 shows the GPx levels in the cerebellum decreased in both the B+D group and the D group compared with the control group ($P < 0.05$ and $P < 0.01$, respectively). However, GPx levels were significantly higher in the B+D group than D group in both the cerebellum and cerebrum ($P < 0.05$).

Further, SOD levels were higher in the B+D group than the D group in both the cerebrum and cerebellum. ($P < 0.05$ and $P < 0.01$, respectively). Interestingly, the difference in SOD levels in cerebrum in the B+D group was not significantly different than the control group, which indicates barberry is protective in the brain.

GSH levels were higher in the cerebellum and cerebrum of mice in the B+D group compared with the D group ($P < 0.01$). However, the difference GSH level in the cerebrum the B+D group was not significantly different from those in the control group, indicating barberry is protective in the brain. Finally, CAT and AChE activity levels were higher in both cerebellum and cerebrum of mice in the B+D group compared with those in the D group ($P < 0.01$).

Overall, the results show that levels of antioxidants and the AChE activity were significantly decreased in the mice receiving only diazinon, but were increased in both the cerebellum and cerebrum of mice in the group also receiving *B. vulgaris* extract.

DISCUSSION

In recent years, there has been increasing concern about the effects of pesticides on the health of humans and an-

Table 1. GPx, SOD, GSH, CAT, and AChE activities in the cerebellum and cerebrum of young albino male mice (n=7) treated orally with either olive oil (control group), diazinon 50 mg/kg, or *B. vulgaris* extract 200 mg/kg, and diazinon 50 mg/kg for three weeks

Parameters and treatments	Cerebellum	Cerebrum
GPx levels in nmol/min/mg protein		
Control group	1.61±0.08	4.88±0.52
Diazinon group	0.55±0.10**	1.72±0.72**
Diazinon + <i>B. vulgaris</i> group	0.98±0.05 [#]	3.12±0.81 [#]
SOD in units/mg protein		
Control group	415.33±82.10	430±50.3
Diazinon group	142.10±15.92**	210.88±40.22**
Diazinon + <i>B. vulgaris</i> group	280.22±28.50 [#]	338.40±60.30 [#]
GSH levels in µg/g organ		
Control group	28.82±2.05	26.15±1.05
Diazinon group	14.08±1.23**	15.05±1.52**
Diazinon + <i>B. vulgaris</i> group	20.05±1.55 ^{###}	22.10±1.72 ^{###}
CAT in µmol of H ₂ O ₂ degraded/min/mg protein		
Control group	11.05±0.22	6.22±0.88
Diazinon group	2.08±0.92**	2.12±0.30**
Diazinon + <i>B. vulgaris</i> group	7.04±0.45 ^{###}	4.75±0.29 ^{###}
AChE activities in nmol/min/mg protein		
Control group	30.08±0.62	25.52±4.05
Diazinon group	11.25±0.35**	9.12±0.81**
Diazinon + <i>B. vulgaris</i> group	22.05±0.22 ^{###}	18.88±3.45 ^{###}

* $P < 0.05$ and ** $P < 0.01$ compared to the control group. [#] $P < 0.05$ and ^{###} $P < 0.01$ in the diazinon + *B. vulgaris* group compared with the diazinon group.

GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase; H₂O₂, hydrogen peroxide; AChE, acetylcholinesterase.

imals (Sánchez-López, 2018). Pesticides increase oxidative stress, leading to an imbalance between oxidants and antioxidants (Ahmed et al., 2000). It has been reported that organophosphorus pesticides cause oxidative stress by inhibiting enzymatic and nonenzymatic antioxidant systems (Soltaninejad and Abdollahi, 2009; Lukaszewicz-Hussain, 2010).

Pesticides act as neurotoxins in the environment by inhibiting AChE activity. Numerous studies have suggested that organophosphorus pesticides caused lipid peroxidation (Oral et al., 2006). Diazinon is an organophosphate insecticide that induces oxidative stress, leading to generation of free radicals and alterations in antioxidant or reactive oxygen species (ROS) scavenging enzymes (Gultekin et al., 2001; Kaur et al., 2007; Rahimi-Madiseh et al., 2017).

The present study revealed that pesticide exposure promotes significant changes to the antioxidant system and induces oxidative damage in the cerebellum and cerebrum of mice. Our results show that diazinon decreases brain AChE activity in mice.

Recently, medicinal plants have attracted much interest for treatment of various diseases. *B. vulgaris* is a fruit that is cultivated in many different countries. The effectiveness of this plant has been largely investigated in recent decades and studies have reported that its alkaloid compounds have antioxidant and anti-inflammatory properties (Rahimi-Madiseh et al., 2017).

Our results indicate that the levels of antioxidants were increased both the cerebellum and cerebrum of mice treated with diazinon + *B. vulgaris* compared with diazinon alone. In addition, brain AChE activity was increased by the presence of *B. vulgaris* extract.

Oxidative stress and the antioxidant system play an important role in pathophysiological cerebral changes. SOD activity is a sensitive index for oxidative damage as it scavenges the superoxide anion to form H₂O₂, leading to diminished toxic effects. CAT enzymatic antioxidants are widely distributed in all animal tissues; CATs decompose H₂O₂ and protect tissues from highly reactive hydroxyl radicals (Nurdiana et al., 2017). GPx is the general name for a family of enzymes with peroxidase activity whose main biological roles are to protect the organism against oxidative damage. The biochemical function of GPx is to reduce lipid hydroperoxides into their corresponding alcohols and to reduce free H₂O₂ (Epp et al., 1983; Shivarajashankara et al., 2002a).

GSH is an antioxidant in plants, animals, fungi, and some bacteria and archaea. GSH is capable of preventing damage to important cellular components caused by ROS such as free radicals, peroxides, lipid peroxides, and heavy metals (Pompella et al., 2003).

One study reported that lipid peroxidation, caused by fluoride, might be one of the important factors in the mechanism of its neurotoxic effects. Because the brain is rich in polyunsaturated lipids and is dependent on aero-

bic metabolism, it is highly vulnerable to oxidative damage. This has been demonstrated in studies that show increased malondialdehyde levels in the brains of fluoride-intoxicated mice (Shivarajashankara et al., 2001; Shao et al., 2002; Yur et al., 2003). In addition, fluoride-induced free radical toxicity has been reported recently in the cerebral hemisphere of female and male mice (Chinoy and Patel, 2000; Memon and Chinoy, 2000; Yur et al., 2003).

The results of our study show that AChE activity is decreased in the brains of mice exposed to pesticides. Thus, a decrease in AChE activity may interfere with synaptic transmission in the brain, which is in agreement with data from Bhatnagar et al. (2006). This decrease could be due to loss of neuron cell bodies in the hippocampus (Bhatnagar et al., 2006), loss of synaptic structures (Shivarajashankara et al., 2002b), or inhibition of enzyme activity (Zhao and Wu, 1998; Bouaziz et al., 2010; Seo et al., 2013). Co-administration of *B. vulgaris* with diazinon induced neuro-protection by decreasing enzymatic activities and processes involved in brain damage.

Therefore, continuously monitoring pesticide residue in dried food is necessary because of the possible health effects, widespread uses and insufficient residue data. Monitoring studies should improve food safety.

To prevent exposure, it is necessary to reduce and control the use of pesticides in these materials by enforcement activities. Nevertheless, monitoring programs are becoming more and more important and essential to minimize levels of pesticide residues in food.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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