




# Comparison of the protective effects of *Scrophularia striata* extract with vitamin E on cognitive function, anxiety and pain sensitivity in diazinon-exposed male rats

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## ABSTRACT

**Introduction:** *Scrophularia striata* is used in traditional medicine to treat various disorders and has neuroprotective effects. There are no studies about the effects of *S. striata* on cognitive functions in diazinon (DZN)-exposed rats. According to the results of previous studies, vitamin E (Vit. E) can also act as a protective agent against cognitive impairments. Therefore, the present study was designed to compare the effects of Vit. E and *S. striata* on DZN-induced behavioral impairments in male rats.

**Methods:** Neuroprotective effects of *S. striata* (30mg/kg, 5 days/week for 8 weeks) and Vit. E (200mg/kg, 5 days/week for 8 weeks, IP) were assessed through changes in memory, anxiety-like behaviors and pain threshold following DZN exposure. Open field, shuttle box and hot plate were used to examine anxiety-like behaviors, passive avoidance learning and memory as well as pain sensitivity, respectively.

**Results:** Our findings indicated that exposure to DZN caused a significant decrease in memory retention and an increase in anxiety-like behaviors. *S. striata* and Vit. E administration compensated memory and emotional impairments induced by DZN. As well as, *S. striata* alone decreased reaction time against thermal stimulus in the hot plate test. The findings of the present study also indicated that exposure to DZN significantly decreased body weight, while *S. striata* and Vit. E consumption restored it.

**Conclusion:** Results of our study indicated the protective effects of *S. striata* consumption like Vit. E against DZN-induced disruptions in anxiety, cognitive function and body weight loss.

## Keywords:

Vitamin E

*Scrophularia striata*

Diazinon

Cognitive functions

Anxiety-like behaviors

Nociception

## Introduction

Exposure to environmental contaminations such as diazinon (DZN) and other organophosphorus insecticides in commercial pesticides, agricultural,

home gardening and indoor pest control leads to a variety of acute and chronic effects in different organs of the body and can cause nausea, depression, learning and memory impairment and long term neurological deficits

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(Androutsopoulos et al., 2011; Huen et al., 2012; Poirier et al., 2018). There is a lot of information about the high potential of toxicity and neurotoxic effects of DZN (Naseh et al., 2013). This toxin induces accumulation of acetylcholine in synapses, because of their capacity of phosphorylating the active zone of acetylcholine-esterase. Organophosphates such as DZN can inhibit acetylcholine-esterase. This ability has brought up the hypothesis that the application of neurotoxic effects of organophosphates by rising acetylcholine concentrations can cause overactivity of cholinergic receptors. Besides, the excitotoxic effects of organophosphates may lead to induce seizure activity and neuronal death (Abdel-Diam et al., 2019; Bali et al., 2019; Rush et al., 2010). However, DZN has harmful effects on the brain and neurodevelopment in children at critical periods (Sapbamrer and Hongsihsong, 2019) by different mechanisms. Also, DZN can change the expression of neurotrophic factors (Slotkin et al., 2008), induce oxidative stress and impair cognitive function in animals (Timofeeva et al., 2008). Additionally, another effect of organophosphorus pesticides is increased oxidative stress. Oxidative stress can also cause oxidative destruction in the hippocampus which is the main area for learning and memory processes and plays an important role in producing and saving spatial long-term memory (Nili-Ahmadabadi et al., 2018). Therefore, hippocampus-related learning and memory, NMDA receptor and neurotrophin levels in the brain are also diminished following the neonatal exposure to DZN (Win-Shwe et al., 2013).

Vitamin E (Vit. E,  $\alpha$ -tocopherol) is considered as a fat-soluble antioxidant that disturbs lipid peroxidation release in the plasma membrane, protects the integrity of the cell membranes from free radical attack and decreases the concentration of these oxidants. This vitamin inhibits oxidative stress induced by pesticides in animals (El-Shenawy et al., 2010; Shokrzadeh et al., 2012). Vit. E can also act as a protective agent against cognitive impairments and learning disability in aging (Takatsu et al., 2009), especially after traumatic brain injury (Wu et al., 2010) and diabetes (Hasanein and Shahidi, 2010).

Many classes of the genus *Scrophularia* (family of Scrophulariaceae) have been used traditionally for medical care to treat illnesses (such as different types of dermatosis, tumors, inflammatory disorders, neuritis,

etc) (Diaz et al., 2004). Neuroprotective effects of some of these species have been also considered in traditional medicine in Iran and other countries. Findings of experimental studies have shown neuroprotective (Azadmehr et al., 2013), cognitive-improving and anti-amnesic properties of this plant (Kim et al., 2003). According to the reported data from *in vitro* experimental studies, some components prepared from *Scrophularia* roots have neuroprotective potential against glutamate excitotoxicity (Salavati et al., 2013). *S. striata* Boiss has some pharmacological effects such as analgesic, anti-microbial, nephron-protective and nitric oxide suppressive properties (Alqasoumi, 2014; Zengin et al., 2019).

Although this plant is commonly used in Iranian traditional medicine due to its neuroprotective effect, no research has been done to investigate its effects on DZN-induced behavioral impairments. On the other hand, the protective effects of Vit. E were demonstrated in the previous studies. Therefore, the present study aimed to compare the neurobehavioral consequences of *S. striata* with Vit. E in DZN -exposed male rats.

## Material and methods

### Animals

Forty-nine male Wistar rats (3 months old), weighing 200-260g were used in the present study. The animals obtained from Pasteur Institute, Tehran, Iran, were maintained in five in the standard cages (40×26×15cm) and were housed in a temperature-controlled situation (temperature, 22±2°C, the humidity, 40-45% and 12h light-dark cycle, light from 7am). Standard food and tap water were accessible. After two weeks of adaptation to the environment and measuring the weight of each animal, the animals were randomly allocated. The rats were randomly divided to seven groups (7 rats in each group); control, vehicle (dimethyl sulfoxide, 0.1%), DZN (diazinon; 30mg/kg/day for 8 weeks, IP), SS (*S. striata*; 200mg/kg/day for 8 weeks, IP), DZN+SS (diazinon and *S. striata*), Vit. E (vitamin E; 200mg/kg/day for 8 weeks, IP) and DZN+Vit.E (diazinon and vitamin E) groups (Mamiya et al., 2008; Nili-Ahmadabadi et al., 2018). All experimental procedures were accepted by the Ardabil University of Medical Sciences Ethics Committee (Ethics code: IR.ARUMS.REC.1397.122). We tried to decrease animals suffering at all stages of the experiment.

### Preparation of extract

An extract was obtained from a native plant called *S. striata*. Plant specimens of *S. striata* were bought from a reliable medicinal herb supplier in the Parsabad region, Ardabil (Northwest of IRAN, end of July). The plant was identified and verified by Dr. Ali Teimourzadeh, a botanist from the School of Agriculture of Mohaghegh Ardabili. A voucher specimen (No.: 043-2019) was deposited at the herbarium of the School of Pharmacy, Ardabil University of Medical Sciences. The 100g aerial parts of the plant were separated and were air-dried at 22°C, powdered and dissolved in a 70% ethanol solution and distilled water, then this mixture was kept at laboratory temperature (22°C) for 72h on a shaker. Then, the mixture was filtered and purified with Whatman filter paper No. 1 and was placed inside a boiling water bath (50°C) and the extract was concentrated in a sterile plate and dried in the incubator (50°C).

Twenty grams of the powder was dissolved in the DMSO and effective concentration was obtained. Therefore, for the preparation of non-toxic DMSO, 1.5 µl of this solvent was diluted in distilled water to reach the desired volume. Using this dilution method, the final concentration of obtained DMSO was 0.1% which was not toxic for the cells. This concentration was added to the extract until the desired volume was reached 10 ml. Finally, 0.2 ml of this solution was injected into the animals (vehicle group). In the present study, to provide fast and complete absorption, DZN (Polychem Co., Tehran, IRAN) and Vit. E (Sigma) were dissolved in DMSO. Prepared solutions were kept in the refrigerator (2-8°C) to prevent contamination. The time interval between administration of DZN and *S. striata* or Vit. E in the DZN+SS and DZN+Vit.E groups was 4 hours. Also, the possible side effects such as weight changes, tremors, sweating, diarrhea or constipation, mobility, etc were investigated by daily visual monitoring.

### Passive avoidance apparatus

To evaluate passive avoidance learning and memory, a shuttle box was used. This device consists of a light and a dark chamber of a similar size (20×20×30cm) divided by a guillotine door. Stainless steel rods (3mm diameter and separated by 1cm apart) were used to make the floor of both compartments. Electrical shocks (50Hz, 3s) by an isolated stimulator were designed on the floor of the dark chamber.

At first, two trials were given to all experimental groups to habituate them to the shuttle box. For conducting these habituation trials, each rat was located in the lightened chamber of the shuttle box. Then the rat was facing away from the door of the compartment and after 10s the dividing guillotine door was elevated. The animal prefers the dark compartment naturally. After the entrance of the rat to the dark compartment, the door was closed. Thirty seconds later, the rat was placed in the home cage from the dark compartment. The second habituation trial was repeated after 30min and the same interval by the primary acquisition trial was followed. The latency of entrance to the dark chamber verification was recorded when all four paws of the rat have placed in the dark chamber. In the acquisition trial, the closure of the guillotine door and application of an electrical shock (50Hz square wave, 1mA for 3s) was conducted after the spontaneous pass of the animal in the dark compartment. The rat was taken and located in its home cage after the 30s. The repeated trial was conducted after 2min. In each reentrance of the rat to the dark compartment, it received a foot-shock. The end of the trial was the time when the rat stayed in the light compartment for 120 successive seconds. The task acquisition index and short term memory were recorded by the use of the trial numbers or entries into the dark compartment. The retention test was conducted 24h after the end of the acquisition trial. Each animal was located in the lighted compartment and after 10s, the guillotine door was elevated. The latency of entrance to the dark chamber was recorded for up to 300s. In the condition that the rat didn't enter the dark compartment during 300s, the retention test was finished and the assignment of the maximum score of 300s was verified. The electrical shock wasn't used during the retention phase (Saadati et al., 2010; Sakhaie et al., 2020a).

### Hot plate test

In order to evaluate the reaction time to thermal stimulus, we conducted the hot plate test (Borj Sanat Azma, M.H9500). This apparatus contained a plate that was pre-heated and adjusted at a temperature of 50.8±0.5. The diameter of the plate and the height Plexiglas wall of this apparatus are 19cm and 30cm respectively. The time between test onset and whipping front paw or jumping was considered as the reaction time to the thermal pain. Also, the maximum cut-off was

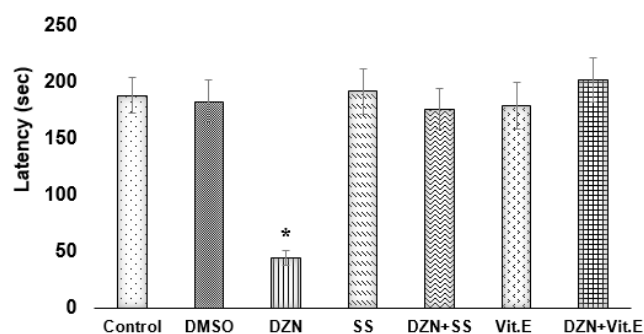
considered the 60s (Sadegzadeh et al., 2020; Sakhaie et al., 2020a).

### Open field test

The open-field test was done after 24h of eight weeks of intervention. The open-field task was 60×60cm enclosed by 40 cm high walled Plexiglas chambers in controlled room light circumstances. In this experiment, each rat was located in the center of the apparatus for 5min and was allowed to explore the compartment freely. A video camera was used for recording the activity of animals. Additionally, the acquired data were analyzed by a motion recognition software system (Noldus Ethovision® system, version 5, USA). In the open field test, the following behavioral parameters were evaluated: the total time spent in the center of the arena; the number of rearing (as the vertical activity index) and the frequency of grooming (rubbing the body, mouth and head with paws). Feces were calculated for 5min for each rat. The open-field arena was cleaned with alcohol after each recording. Temperature, noise and light were controlled during the task (Saadati and Sheibani, 2017; Sakhaie et al., 2020b).

### Statistical analysis

Data are presented as mean±SEM (n=7). Data of passive avoidance and anxiety tests were analyzed using one-way analysis of variance (ANOVA) and the expression of the differences among the groups was conducted with Tukey's post-hoc test. The body weight results were analyzed by two-way repeated-measures ANOVA with treatment as between-subject factors and age as a within-subject (repeated measure) factor.



**FIGURE 1.** Effect of *S. striata* and Vit. E on long-term memory in the passive avoidance test in DZN- exposed rats. Results are presented as mean±SEM (n=7). \* $P<0.05$  indicates DZN group in comparison with the other groups (SS: *Scrophularia striata*, DZN: Diazinon, Vit. E, Vitamin E).

SPSS software was used to perform all of the analyses and calculations.  $P<0.05$  was considered statistically significant.

## Results

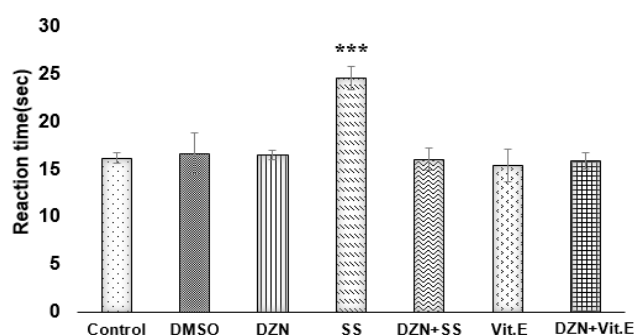
### *Vit. E and S. striata prevent memory alterations induced by DZN*

According to the results of the present study, before the training test, no significant difference in the latency of entrance to the dark compartment was measured in groups, representing their similarity. Besides, during the acquisition phase in the passive avoidance test, a statistically significant difference among groups wasn't observed. Twenty-four hours after training, the retention test was carried out. The results of the retention test indicated that the latency to the entrance to the dark compartment significantly ( $F(6,42)= 3.69$ ,  $P=0.013$ ) decreased in the DZN- exposed group compared to the corresponding control group.

One way analysis also indicated that memory impairment caused by DZN was alleviated by using *S. striata* ( $P=0.022$ ) and Vit. E ( $P=0.005$ ). Therefore, administration of *S. striata* or Vit. E combined with DZN resulted in longer latency in passive avoidance test (Figure 1).

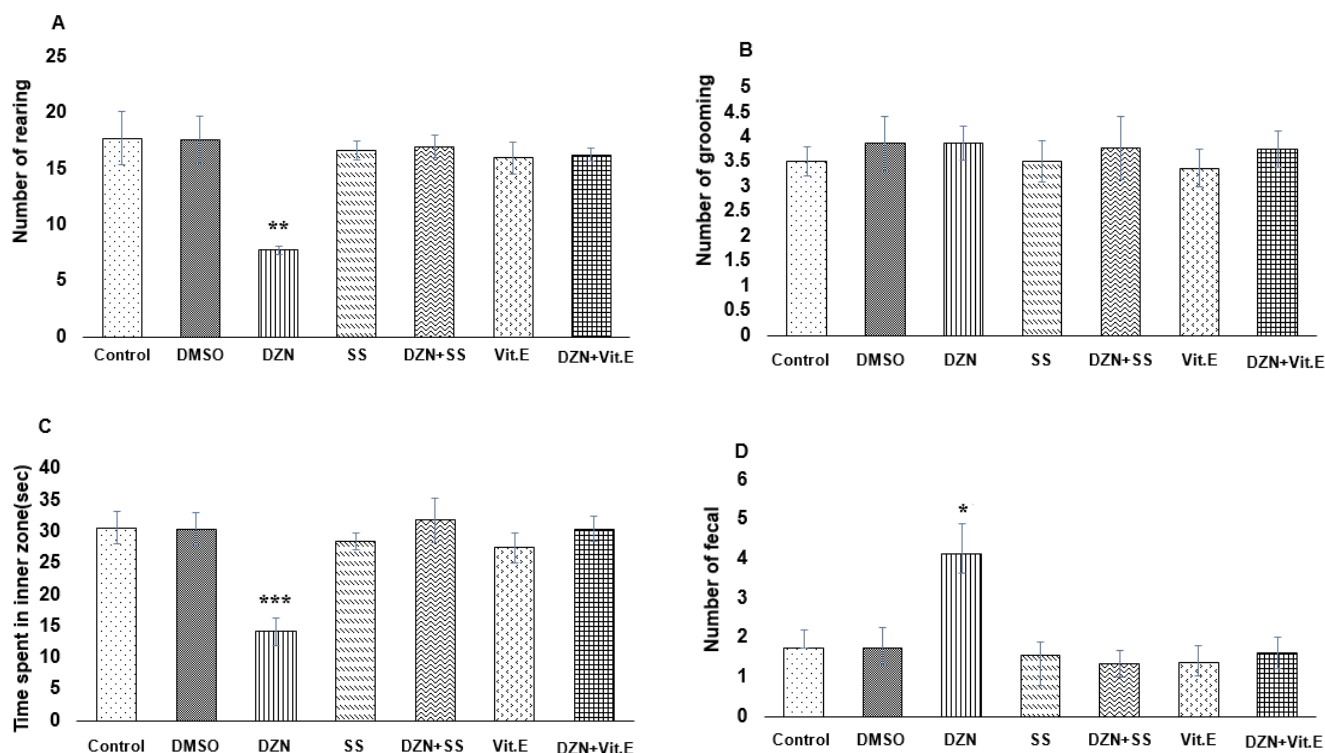
### *Effects of DZN, Vit. E and S. striata on pain sensitivity*

To examine the sensitivity to the pain stimulus, the hot plate test was used. There was no significant difference between the DZN group compared to other groups on the threshold of response to thermal stimulus in the hot-plate test. While increased reaction time was seen in the *S. striata* group compared to the control group ( $F(6,42)=$



**FIGURE 2.** The effect of *S. striata* and Vit. E on reaction time to the thermal stimulus in DZN- exposed rats. \*\*\* $P<0.001$  indicates SS group vs control group. (SS: *Scrophularia striata*, DZN: Diazinon, Vit.E: Vitamin E). Data are expressed as mean±SEM (n=7).





**FIGURE 3.** Effects of *S. striata* and Vit. E on anxiety-like behaviors in the DZN- exposed rats. These behaviors include rearing number (A), the number of grooming (B), the time spent in inner zone (C) and the number of fecal boli (D) in the open field test. (SS: *Scrophularia striata*, DZN: Diazinon, Vit.E: Vitamin E). \* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$  indicates DZN group compared to the other groups. Data are expressed as mean $\pm$ SEM (n=7).

6.7,  $P<0.05$ ). Therefore, *S. striata* -treated rats have increased reaction time in the hot plate compared to the other groups (Figure 2).

#### Effects of DZN, Vit. E and *S. striata* on anxiety-related behaviors

An assessment of anxiety-like behaviors was conducted through the open-field test. Our findings indicated that the administration of DZN (30mg/kg for 8 weeks) altered some of the parameters examined in this test. Vit. E and *S. striata* had protective effects on changes induced by DZN. The results of our study showed that the rearing ( $P<0.001$ ; Figure 3A) decreased in DZN- exposed group compared to the control group. However, co-administration of *S. striata* ( $P<0.001$ ) and Vit. E ( $P=0.002$ ) with DZN significantly improved these changes.

One way analysis also indicated that the time spent in the inner zone ( $F(6,42)=5.5$ ,  $P<0.001$ ; Figure 3C) was decreased in DZN -exposed group in comparison with the control group. Treatment with *S. striata* ( $P<0.001$ ) and Vit. E ( $P<0.001$ ) combined with DZN significantly increased time spent in the inner zone. Additionally, number of fecal boli was increased in DZN- exposed

group compared to the control group ( $F(6,42)=4.324$ ,  $P=0.014$ ; Figure 3D). Administration of *S. striata* ( $P=0.002$ ) and Vit. E ( $P=0.008$ ) significantly decreased the number of fecal boli in DZN -treated rats. The number of grooming was similar among the groups ( $F(6,42)=1.7$ ,  $P=0.12$ ; Figure 3B). Our findings also showed that anxiety-like behaviors did not change through alone administration of *S. striata* and Vit. E compared to the control and DMSO groups.

#### The effect of *S. striata* and Vit. E on body weight in DZN- exposed rats

Our findings indicated that DZN- treated rats had a significant decrease ( $F(6,42)=7.8$ ,  $P=0.02$ ) in their body weight after six weeks. The reduction of body weight in the DZN -treated group, occurred in the 7th ( $P<0.001$ ) and 8th weeks ( $P=0.005$ ) compared to the control group. *S. striata* and Vit. E compensated body weight loss in the DZN- exposed rats in 7th ( $P=0.017$  and  $P=0.001$ , respectively) and 8th weeks ( $P=0.011$  and  $P=0.001$ , respectively). Additionally, Vit. E alone obviously changed the body weight until the 8th week ( $P=0.04$ ), while *S. striata* alone didn't change the body weight of the experimental animals (Table.1). Apart from weight

**TABLE 1:** Effect of *S. striata* and Vit. E on body weight (g) in diazinon- exposed rats.

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Control	277.85±8.5	283.57±8.6	304.14±10.1	294.43±8.7	301.14±8.8	315.57±10.9	316.57±8.4	316.579±8.4
DMSO	296±10.95	300.2±10.1	305±11.11	310.4±10.92	315.4±10.69	354.8±17.22	325.2±10.77	329.8±10.93
DZN	263.67±12.34	267.67±12.56	272±12.67	286.43±6.22	280±12.79	283.83±12.96	271±12.3***	274.67±12.37**
SS	269.42±5.93	274.8571	281.14±6.12	286.43±6.22	290.57±5.86	297.57±6.29	302.86±6.19	308.43±6.22
DZN+SS	268/6±12/01	274.2±12.11	281.5±10.35	287.67±10.51	293.67±10.85	300.33±10.91	306.67±10.76 <sup>#</sup>	313.5±10.94 <sup>#</sup>
Vit.E	287.33±6.84	295.33±6.84	302.67±6.67	311.67±6.67	321.67±6.67	331.33±6.84	341.67±6.69	361.33±16.34 <sup>\$</sup>
DZN+Vit.E	289±10.66	295.5±10.9	301.75±11.53	307.5±11.57	313.75±12.11	319.75±12.58	326±12.98 <sup>##</sup>	334.25±14 <sup>##</sup>

The effects of diazinon, *S. striata*, and vitamin E on body weight of the rats. (SS: Scrophularia striata, DZN: Diazinon, Vit.E: Vitamin E). \*\*\* $P<0.001$  and \*\* $P<0.01$  indicates DZN group vs control group in 7th and 8th weeks, respectively. <sup>#</sup> $P<0.05$ , DZN+SS group vs DZN group in 7th

loss, no other side effects such as weight changes, tremor, sweating, diarrhea or constipation, mobility, etc were observed in DZN and *S. striata* treated groups.

## Discussion

Exposure to DZN, a type of organophosphorus pesticide leads to toxic effects on different organs of the

body. Utilizing DZN and the other organophosphorus compounds also cause an increase in oxidative stress and distraction of the antioxidant defense mechanisms. Also, the nervous system is extremely vulnerable to oxidative damage (Altuntas et al., 2004; Bali et al., 2019; Naseh et al., 2013). In the present experimental study, DZN showed angiogenic activity and caused

cognitive impairment which was observed as decreased the latency time for entering the dark compartment in the passive avoidance test. In our study, exposure to DZN also induced anxiety in rats that were revealed as decreased time spent in the inner zone and the number of rearing, and enhanced amount of fecal boli in the open-field test. Our results of the open field test were consistent with previous findings indicate that exposure to organophosphorus pesticides leads to emotional and mental disorders (López-Crespo et al., 2009). Besides, the consumption of *S. striata* and Vit. E in the DZN-exposed rats significantly reduced the anxiety in the open-field test. Organophosphorous pesticides inhibit the acetylcholinesterase enzyme, induce the accumulation of acetylcholine and result in anxiety (Maxwell et al., 2006). As well as, cholinergic neuronal overstimulation, accumulation of enormous free radicals and cytotoxicity may cause neuronal and neuropsychiatric disorders such as anxiety and cognitive impairments (Chen, 2012). In the present study, the administration of *S. striata* and Vit. E compensated the harmful effects of DZN on cognitive functions in the rats. Vitamin C and E are antioxidants that can defend against the deleterious influences of DZN on cognition by collecting free radicals, according to the results of previous studies (Yu et al., 2008). Therefore, in the present study, we compared the protective effects of *S. striata* with Vit. E in DZN- exposed rats. Our results indicated that *S. striata* similar Vit. E can be used for protective effects.

In western areas of Iran, a plant of *S. striata* has traditional medical usage. Various extracts of this plant have anti-inflammatory, antioxidant, immunomodulatory, antimicrobial and neuroprotective effects (Ardehshiri-Lagimi et al., 2009; de Santos Galíndez et al., 2002; Kim et al., 2002). Also, several *S. striata* have been investigated and found to comprise some classes of secondary metabolites including phenolic, flavonoid, flavonol, cinnamic acid, phenylpropanoid, nepitrin, flavonoid glycoside, acteoside and phenylpropanoid glycoside, iridoids, phenolic acids and saponins compounds (Kim and Kim, 2000; Kim et al., 2002). Some of these compounds were indicated to have various pharmacological and therapeutic effects including neuroprotective and antioxidative effects (Kim et al., 2002).

However, no experimental investigation has been accomplished yet to evaluate the neuroprotective

effects of *S. striata* on DZN- induced neurotoxicity. The initial purpose of the current study was to assess the neuroprotective impacts of *S. striata* extract against DZN-induced neurobehavioral impairments. We observed a significant protective effect of this plant against DZN neurotoxicity. *S. striata* extract can decrease the anxiety and depression behaviors in rodents (Babri et al., 2012). Additionally, the learning and memory deficiencies that are induced by scopolamine can be improved significantly through the usage of *S. striata* extract (Kim et al., 2003). The findings of our study indicated that *S. striata* and Vit. E can improve memory impairment in the DZN- exposed rats. Results obtained from a previous study suggest that Vit. E defends the nervous system against oxidative stress during aging and therefore, averts the deterioration of cognitive functions (Fukui et al., 2002). Therefore, there are no differences between the effects of *S. striata* and Vit. E on the improvement of cognitive functions. Results of the previous study showed that pretreatment of the PC12 cell line with *S. striata* extracts significantly raised survival and reduced cell death ratio against H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity (Azadmehr et al., 2013). Cinnamic acid, flavonoids and other related compounds of *S. striata* have been reported to attenuate glutamate-induced neurotoxicity and oxidative stress. Specifically, flavonoids found in this plant have been shown to reduce glutamate-induced neurotoxicity (Salavati et al., 2013). Also, neonatal DZN exposure impairs recognition memory through the modulation of the NMDA receptor and expression of the genes which are associated with it in the hippocampi of young and adult mice (Win-Shwe et al., 2013). We also investigated the effect of *S. striata* extract and Vit. E on the pain threshold in the DZN- exposed rats. Our results indicated that *S. striata* increased the reaction time in the hot plate test, but DZN and Vit. E did not have a significant effect on pain sensitivity. This plant has been used as a treatment for different diseases including rheumatics and inflammatory impairments. According to the results of *in vitro* studies, it also can suppress pro-inflammatory mediators such as nitric oxide, TNF- $\alpha$ , IL-1 $\beta$  and the production of prostaglandins by macrophages (Azadmehr et al., 2011; Azadmehr et al., 2012). *S. striata* produces anti-inflammatory compounds that inhibit the production of nitric oxide. Accordingly, it can have an analgesic effect, especially its inflammatory type. Intraperitoneal injection of the ethanolic extract of

*S. striata* has a significant analgesic effect (Sofiabadi et al., 2012).

The results of the present examination also indicated that only those rats treated with DZN exhibited a highly significant decrease in their body weights after administration for six weeks and continued until the eighth week; while, administration of *S. striata* and Vit. E compensated the decreasing effects of DZN on body weight of rats. Besides, short and long-term administration of DZN caused a rise in blood serum glucose, triiodothyronine, thyroxin and reduced the body weight of rats (AL-Shinnawy and Ab, 2008). Therefore, this decline of body weight in the DZN exposed rats may be associated with appetite losing, reduced food consumption and/or metabolic disorders caused by DZN exposure, but these disorders can be improved by *S. striata* and Vit. E.

## Conclusion

In conclusion, our results demonstrated that exposure to DZN impairs passive avoidance memory and increases anxiety-like behaviors. However, the administration of *S. striata* or Vit. E combined with DZN compensates impairments caused by DZN. Also, DZN exposure decreases body weight, and co-treatment of DZN with *S. striata* or Vit. E leads to a significant improvement in weight gain of rats. In this study, we were unable to find the active ingredients of the *Scrophularia striata*.

## Conflict of interest

The manuscript doesn't contain any conflict of interest.

## Acknowledgment

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