PharmacologyOnLine
April 30, 2013
Archives • 2013 • vol.1 • 128 - 134

Protective effect of *Heracleum persicum* alcoholic extract against diazinon-induced spatial memory impairment of rat

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Summary

Diazinon is one the most widely used organophosphorous insecticides in agricultural pest control. This insecticide can cause toxic effects in mammals including memory impairment. As some medicinal plants like *Heracleum persicum* contains antioxidant features and influences learning activities and memory, so can be used for protection in poisonings. The aim of this study was to investigate the protective effect of *Heracleum persicum* extract on diazinon-induced spatial memory impairment in rat. Male rats were divided into five groups randomly including a control group, a group receiving corn oil as diazinon solvent, and three experimental groups. Two of three experimental groups received different doses of *Heracleum persicum* (50,150 mg/kg) as pre-treatment for 20 days along with diazinon (100 mg/kg) that injected intraperitoneally in last day of *Heracleum persicum* usage, and one group received only diazinon (100 mg/kg). Spatial memories of rats were studied by using Morris water maze technique. Data were analyzed using one-way ANOVA, Tukey's and Bonferroni test. The results showed that *Heracleum persicum* has protective effect on diazinon-induced impairment in spatial memory of rat and improves the oxidative stress caused by this pesticide.

Key words: Protective effect, Heracleum persicum, diazinon, spatial memory, rat

Introduction

Organophosphorous pesticides are esters of phosphoric or thiophosphoric acids and are highly toxic to mammals because of their capacity to phosphorylate the active site of acetylcholinesterase, leading to accumulation of acetylcholine in synapses. There is wide information about the neurotoxic effects of these pesticides (1, 2).

Due to easy access to organophosphorous pesticides and their higher degree of toxicity, accidental poisonings and also suicides by using them are wide. So, it is one of the toxic materials causing human poisoning and death worldwide annually (3). Toxic effects of poisoning with organophosphors may be chronic or acute. Neuropathy is one of the important side effects (4). Anticholinesterase complexes including suman, sarin, deisopropile and fluorophosphate can cause many disorders in human and animal learning processes and memory. Tissue necrosis was observed in amygdala, hippocampus and thalamus of rats during acute and chronic poisoning with suman (5).

Diazinon is a contact organophosphorous pesticide which is used widely as insecticide, acaricide and nematicide in agriculture and veterinary medicine (6). Toxic effects of diazinon are due to the inhibition of acetylcholinesterase activity, an enzyme needed for proper nervous system function (2). Also, the oxidative stress induction is proposed in poisoning with organophosphors. Increase in oxidative stress can evacuate tissue glutathione and will lead to increase of free radicals in body (7). Oxidative stresses are created as a result of oxidative destruction of biologic macromolecules including nucleic acid, proteins, lipids and carbohydrates by free radicals (8). Brain is a vulnerable organ in oxidative stress. Different studies have shown the role of free radicals in damage of neurons (9). Oxidative stress can also cause oxidative damage induction in hippocampus which is a critical center for memory and learning processes and plays an important role in forming and saving spatial memory (10). Previous studies have shown that oxidative stress is increased in factory workers who are responsible for formulating organophosphorous pesticides (11).

Heracleum (H) persicum (Golpar) which is a member of Apiaceae family that grows in northern altitudes (12). This plant is used frequently as a spice in providing pickles and local medicine. The most important oil found in *H. persicum* is anethole. *H. persicum* extract as an antioxidant, has many beneficial pharmacological effects (13).

In present study, the protective effect of *H. persicum* alcoholic extract has been studied on diazinon-induced spatial memory impairment of rats.

Materials and Methods

Animals

Thirty-five adult male Wistar rats (weighing 200 to 350 g) were provided from Tehran Medical University. All the rats were healthy and not infected with virus or bacteria and the keeping place of them had natural light and darkness. They were sufficiently provided with food and water except in experimentation time. Ethics of working with laboratory animals were considered during all procedures.

Chemicals

Diazinon with 100% purity was purchased from Swiss Fluka company and corn oil (400 mg/ml) used as its solvent (14). Dried fruit of *H. persicum* was prepared as powder in biochemistry laboratory of University of Tabriz. Then 100 g of plant powder was added to one liter of 96% alcohol and placed in a dark place for 72 hours. The solvent was filtered bottom of container was placed in incubator of 38'C till it would be dried (15). Finally *H. persicum* powder obtained and in the next stages dozes of 50, 150 g in kg was provided through solving its powder in distilled water.

Treatment of animals

The 35 rats were divided into 5 groups randomly as: control group, group receiving corn oil as diazi-

non solvent, group receiving diazinon (100 mg/kg, during 24 hours before memory testing), two groups receiving 50 and150 mg/kg *H. persicum* extract (intraperitoneally as pretreatment) for three weeks along with diazinon (100 mg/kg, intraperitoneally) in last day of extract usage.

Morris water maze

Morris water maze technique was used to measure learning degree and spatial memory (16). All rats were trained in a standard Morris water maze task (Morris et al., 1982; Stackman et al., 2002). The rats performed four trials per day for four consecutive days. In the swimming trials, each individual rat was released gently into the water at a randomly chosen quadrant. The rat swam and learned how to find the hidden platform within 60 s. After reaching, the rat was allowed to stay on the platform for 15 s and was then taken back into the cage. The rats were placed on the platform by hand for 15 s if they could not escape to the platform within 60 s by themselves, and their escape latency was accepted as 60 s. During the inter-trial intervals, animals were kept in a dry home cage for 60 s. The time to reach the platform (latency), the length of swim path, and the swim speed were recorded semi-automatically with a video tracking system. Twenty-four hours after the last day of training, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60 s trial.

Statistical analysis

All results were expressed as mean ± SEM. Data was analyzed by using one way ANOVA followed by Tukey's and Bonferroni test. The $p<0.05$ was considered to be statistically significant (17).

Results

Experiment results of Morris water maze test during four consequent days of learning process indicated that compared to group receiving diazinon, there is a significant difference between control group and group receiving *H. persicum* + diazinon (P<0/05), (diagram 1). Age, weight and swimming average speed showed no significant differences between studied groups (P>0/05). But there was a significant difference in parameter of covered distance to find platform in different groups, in a way that rats receiving diazinon compared to control and sham groups covered more distance to find platform.

Diagram 2 indicates average needed time to find platform at first day of training in each five groups. Using one way ANOVA and Tukey's test, statistical analysis indicated no significant difference between control groups and groups, sham and receiving *H. persicum* with dose of 50,150mg/kg + diazinon, this is while the time for rats receiving diazinon (51.91 ± 4.65) compared to control rats increased significantly and the time of finding platform for rats receiving *H. persicum* with dose of 50 mg/kg + diazinon (34.08 ± 5.14) and rats receiving *H. persicum* with dose of 150 mg/kg + diazinon (38.68 ± 3.76) decreased significantly compared to rats receiving diazinon. Diagram 3 indicates needed average time to find platform in second day of training. One way ANOVA and Tukey's tests indicated that compared to rats receiving *H. persicum* with dose of 50 mg/kg + diazinon (19.79 ± 3.86), control groups (26.79 ± 2.64) need significantly more time to find platform (P<0/05). Also no significant difference was observed between group receiving *H. persicum* with dose of 150mg/kg + diazinon, this is while needed time to find platform in rats receiving diazinon (49.89 ± 1.66) was significantly different compared to other groups ($p<0.05$). Results obtained in third day of training has been indicated in diagram 4 and results for fourth day of training has been also presented in figure 5. One way ANOVA and Tukey's test analysis have shown that compared to rats receiving corn oil as diazinon solvent and also control group, rats receiving diazinon spent more time to find platform in 3^{rd} ,4^{th} days of training. A significant difference was observed between groups receiving *H. persicum* with 50,150 mg/kg doses compared to control and sham groups in 3^{rd} and 4^{th} days of training.

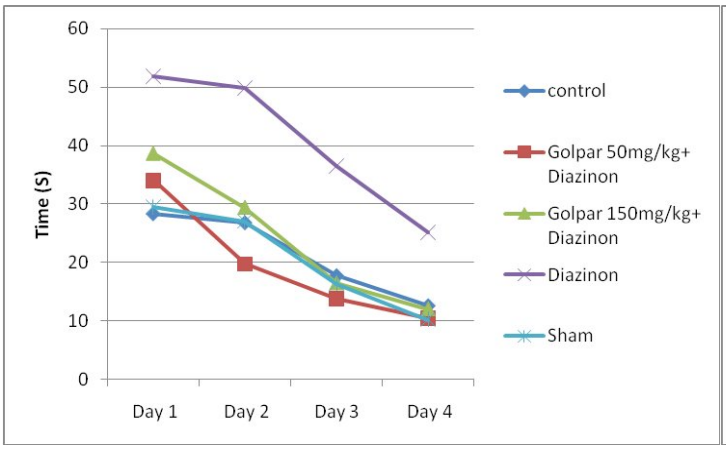

Diagram1: Diazinon (100mg/kg) effect on memory acquisition and positive effects of *H. persicum* essence different doses preventing memory reduction caused by the injection of diazinon.

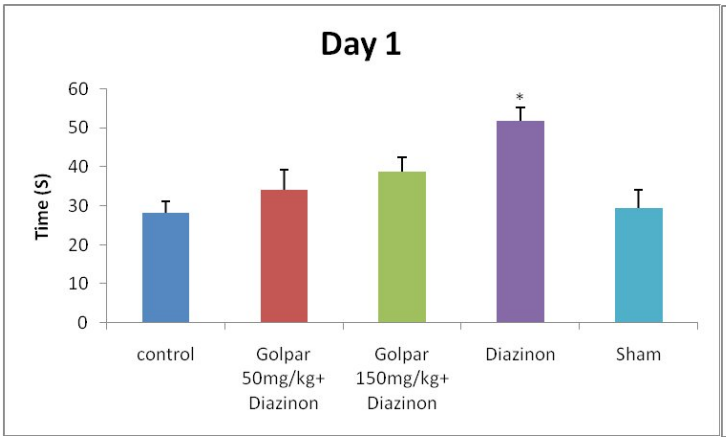

Diagram2: average needed time to find platform at first day of training for each seven groups of rats,
*P<0/05.

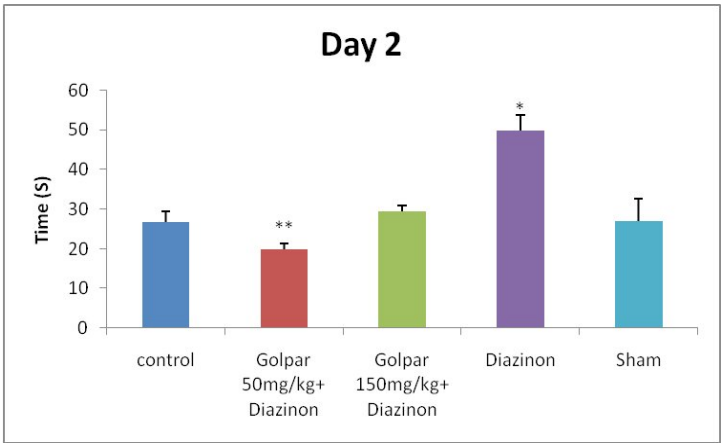

Diagram3: average needed time to find platform at second day of training,
*P<0/05.

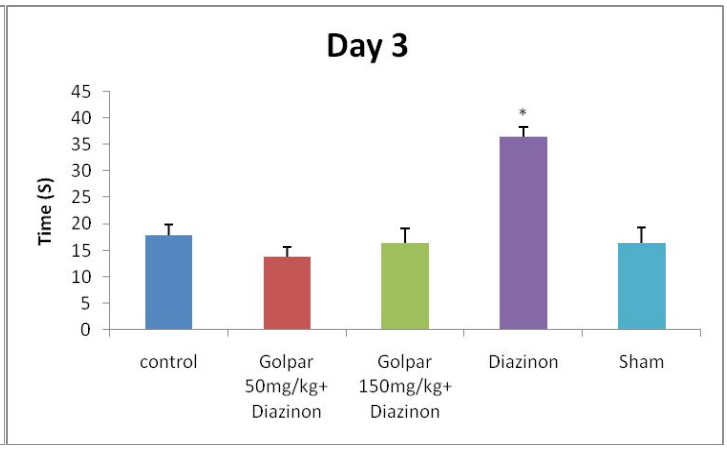

Diagram4: average needed time to find platform at third day of training,
*P<0/05.

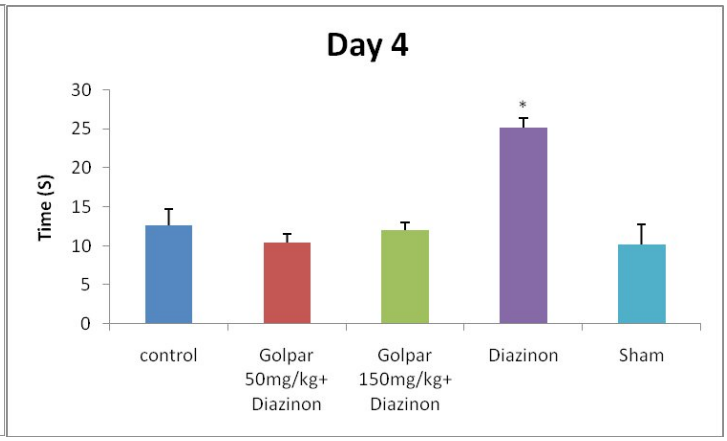

Diagram5: average needed time to find platform at fourth day of training,
*P<0/05.

Discussion

In this investigation, we used Morris water maze to assess spatial memory, since in this system doing needed activities requires correct function of memory (18). According to findings of this study intraperitoneal injection of diazinon with 100mg/kg, causes a significant difference in learning procedure of Morris water maze. In other words, injection of diazinon to rats will result in reducing learning ability and spatial memory acquisition. Consequently we observed an increase in time and covered distance to find platform in rats receiving diazinon with 100mg/kg dose. The reason to choose 100mg/kg dose was that cell damage resulting from diazinon which leads to increase in free radicals

causes body antioxidant defensive system activation (19). In a study by Gholam Ali, et. al. (2002) it was identified that increase in antioxidant enzymes activity and amount of malondialdehyde in diazinon doses higher than 30mg/kg and LDH activity in 100mg/kg dose increases significantly (19). Abbasnejad, et. al. (2009) indicated that intraperitoneal injection of diazinon leads to increase in catalase enzyme and dismutase super oxide activity in doses higher than 30mg/kg and increase in Malondialdehyde in 100mg/kg doses compared to control group (20). Oxidative stress causes to free oxygen radical production which intensifies plaque formation possibility in central neural system. Creating free radical is an aerobic metabolism condition occurring during biochemical interactions and metabolic processes. Oxygen radicals lead to destructive impacts on cell constituent materials including proteins, DNA and lipids. Biochemical and physiological unique characteristics of brain including high density lipid, pressing need for oxygen and energy make brain cells vulnerable against oxygen radicals attacks (21). Diazinon as a oxidative stress factor could change parameters related to memory acquisition. Different studies indicated oxidative factors impact in destructive and memory weakening form (22, 23, 24).

The results of present study showed that intraperitoneal injection of alcoholic extract of *H. persicum* has protective effects against memory acquisition obtained from diazinon injection. Iranian *H. persicum* contains different complexes including: pimpinellin, isopimpinellin, bergaptem, isobergaptem, sphondin (from furanocoumarin family), Hexyl (56.5%), butyrate, octyl acetate (16.5%), hexyl2, methylbutanoate (5.2%) and hexyl and isobutyrate (3.4%), (26, 27). Furanocoumarin complexes existing in Heraclem has antioxidant features and reduces free radicals (28).

Using plant products has been increased in western worlds and also developing countries in recent years. And great deal of natural products have been assessed as treatment factor to treat different cases including weak memory and these plant medicines have been used traditionally to promote cognitive functions, since memory is the most critical aspect human survival and this distinguishes human from animals (25). Medicinal plants and extracted complexes from them are known as intelligent medicines. These plants function in brain is called Nootropic (25).

Previous studies have confirmed that oxidative stress produced by organophosphors including diazinon is related to oxygen free radical formation and also lipid peroxidation in rats (29, 30), and human (31, 32). Hence *H. persicum* plant can balance oxidative stress resulted from diazinon injection and reduce its impact on memory acquisition process through having antioxidant complexes. On the other hand *H. persicum* fruit contains 94% of aliphatic ester, 4% of aliphatic alcohol and 2% of monoterpenes (33). Study of Yousefi et. al. (2011) about lemon balm essence impact on virtual rat spatial memory, showed that terpens are also present in lemon balm and the positive effect of this fruit on memory strengthening is related to this complex (34).

Other studies have shown that terpenes have protective impact on Ach muscarini receptors against free radicals destructive effect and since these receptors are critical in functioning memory and learning processes, so it is possible that *H. persicum* influence memory through it's terpenoid complexes on cholinergic system (35). Several studies have indicated positive impact of cholinergic system on memory promotion (36, 37, 38). It has been confirmed that stimulating muscarini receptors of cholinergic system is effective in memory stabilization (39). More over drugs activating nicotine receivers increase new memory formation (40, 41). Saberi et. al. (2010) obtained dissimilar results regarding diazinon effect on memory from present study (42). Many reasons can be mentioned for difference in this investigation and previous studies including different used doses (43).

The findings of present study indicated that injection of diazinon with 100 mg/kg doses creates oxidative stress in male rats and influences memory acquisition parameters. Also *H. persicum* consump-

tion can reduce diazinon toxic effects due to having antioxidant factors. Its terpenoid complexes also improve memory drastically. It is proposed that *H. persicum* controls produced ROS oxygen radicals by diazinon, so protects brain and consequently lead to memory strengthening.

It is concluded that lower doses of *H. persicum* is protective against diazinon-induced spatial memory impairment in rat.

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