Original Article

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An Investigation of the Effects of Optional Exercise and Salvia Officinalis Extracts on Pancreatic Tissue Injuries in Rats Poisoned by Diazinon

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ABSTRACT

BACKGROUND AND OBJECTIVE: Oxidative stress is the most common effect of diazinon on living organisms, which leads to the dysfunction and destruction of pancreatic tissues. In this study, we evaluated the effects of Salvia officinalis extracts and optional exercise on histological changes of the pancreas in rats poisoned by diazinon.

METHODS: In this laboratory study, 35 male Wistar rats were divided into five groups, each consisting of seven rats: control, diazinon, diazinon-extract, exercise-extract and exercise-extract-diazinon groups. The exercise groups practiced on a spinning wheel for four weeks. The rats in the experimental groups received 200 mg of diazinon intraperitoneally once during the intervention. In addition, they received 100 mg of the extract for four weeks (five days a week). The rats were sacrificed 24 hours after the final injection. Pancreatic tissue sections were prepared for microscopic studies.

FINDINGS: The number (4.32±0.67) and diameter (15.84±1.01) of the islets of Langerhans and the number of acinar cells (47.32±4.01) in the diazinon group showed a significant reduction, compared to the control group (6.44±1.38, 16.17±1.2 and 50±7.06, respectively) ($p<0.05$). However, a significant increase was observed in the evaluated parameters in diazinon-extract, exercise-extract and exercise-extract-diazinon groups, compared to the diazinon group ($p<0.05$).

CONCLUSION: The findings of this study indicated that Salvia officinalis extracts and optional exercise could reduce pancreatic tissue damages, induced by diazinon injection.

KEY WORDS: Salvia Officinalis, Diazinon, Pancreas, Optional Exercise..

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Introduction

Considering the population growth in developing countries and increasing demands for food products, agriculture is regarded as one of the major contributors to food production. As a result, many pesticides are being used to reduce or repel agricultural pests in order to increase food production.Organophosphorus insecticides are among agricultural pesticides, which cause

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different irregularities in body tissues by inhibiting acetylcholinesterase and inducing oxidative stress (1). Diazinon is an organophosphorus insecticide, which enters the body through the skin, respiratory tract or digestive system and immediately changes into an active metabolite in the liver and kidneys (2, 3). The extent of destruction induced by diazinon is dependent on the dosage, exposure time, absorption route, cellular structure and the stability of this toxin in the body (4). Diazinon has high stability in water, soil and plants and the induced toxicity may persist for weeks or even months (5). Exposure to this insecticide can occur due to food contamination or occupational/environmental pollution. Therefore, this type of exposure is regarded as one of the most important health issues with significant damages to the human body (6). Studies have showed that diazinon leads to reduced weight in sexual organs, increased sexual disorders and sperm death (7, 8). In addition, this toxin is considered as an important factor for acute pancreatitis (9). Organic phosphorus compounds are able to interact with macromolecules and micromolecules in cells and induce cellular or genetic injuries (10, 11).Some scholars have suggested the increase in lipid peroxidation and production of free radicals, resulting from the metabolism of organic phosphorus pesticides, as the main destruction mechanism in body cells and tissues (12, 13). Therefore, it seems that the pancreas is one of the organs, influenced by these compounds. Considering the harmful effects of diazinon and its widespread use in agriculture, several methods are available for managing its destructive effects. Factors such as exercise and use of natural compounds with antioxidant properties could play an important role in the recovery and mitigation of damages induced by oxidative stress (14). Considering the suspicions surrounding the safety of synthetic antioxidants, use of natural compounds, especially plants, has been recently taken into account due to their active compounds with antioxidant properties (15). Salvia officinalis from the Lamiaceae family is among herbal medicines with an extensive worldwide use. The presence of phenolic compounds in the extracts of this plant induces its antioxidant properties (16, 17). These antioxidant compounds play an important role in tissue protection against the oxidative effects of free radicals such as reactive oxygen species and other active species. The antioxidant effects of secondary metabolites in plants are dependable on their amount (18, 19). As previous studies have indicated, Salvia officinalis could minimize the harmful effects of oxidation through sweeping free radicals (20).There are two viewpoints regarding the effects of exercise. Some experts believe that exercise leads to the formation of reactive oxygen species and an increase in malondialdehydes and similar compounds. As a result, exercise may have harmful effects on cellular function. On the other hand, some scholars claim that regular exercise can adjust the antioxidant system. Research indicates that performing regular sport activities does not increase the activity of anti-oxidative enzymes, and thus, it can reduce cellular damage (21, 22). In addition, regular exercise increases anti-oxidative agents and strengthens the antioxidant system (23).Considering the extensive use of diazinon, its oxidative effects on different body tissues and antioxidant properties of Salvia officinalis extracts and exercise on the body, we aimed to investigate the effects of optional exercise and Salvia officinalis extracts on pancreatic tissues of rats poisoned by diazinon.

Methods

Preparation of lab animals: In this analytical, laboratory study, 35 male Wistar rats (12 weeks of age), weighing 250±20 g, were purchased from Amol Pasteur Institute, Iran. Two weeks before the initiation of the experiment, the rats were maintained in an animal room at Mazandaran University of Medical Sciences to familiarize them with the environment. The conditions of the room were maintained stable throughout the study period (25 °C, 50% humidity and 12:12 light-dark cycle). The rats were divided into five groups, each consisting of seven rats:

Control group: No injections (or exercise) were administered for this group and they were maintained under standard conditions.

Experimental group 1 (diazinon): The rats received 200 mg/kg of diazinon intraperitoneally once (24).

Experimental group 2 (diazinon-extract): The rats received 100 mg/kg of Salvia officinalis extracts (25) intraperitoneally on a daily basis during four weeks (five days a week). Afterwards,

200 mg/kg of diazinon was once injected in rats during the experiment.

Experimental group 3 (exercise-diazinon): The rats exercised on a spinning wheel for four weeks (26). Then, they were injected 200 mg/kg of diazinon once during the experiment.

Experimental group 4 (exercise-diazinon-Salvia officinalis): The rats performed optional exercise for four weeks and received daily doses of the extracts (100 mg/kg). Afterwards, 200 mg/kg of diazinon was injected in the rats.

Preparation of plant extracts: Salvia officinalis plants used in this study were collected from Alborz heights and were approved by an expert botanist. The leaves of Salvia officinalis were dried away from direct sunlight and were powdered by a grinder. Extract preparation was carried out through soaking. In order to prepare the hydroalcoholic extracts from Salvia officinalis leaves, 200 g of the powder was added to 70% ethanol (600 ml volume). The mixture was poured into a shaker with the speed of 325 rpm. Then, it was purified by Whatman filter paper No. 6. The solvent was extracted using a rotary machine (Heidolph WD 2000, Brinkmann, Canada) at a temperature of 50 °C and then dried out.

Preparation of tissue samples: Twenty-four hours after receiving diazinon, the animals were anesthetized by ketamine and xylazine. At this stage, the abdominal cavity was opened up and the pancreatic tissue was removed from the body. The tissue was placed in 10% formalin to be stabilized. After tissue preparation, serial sections (thickness of 5 microns) were made in the tissue. The sections were stained by hematoxylin and eosin (H&E). By using a light microscope, 80 pancreatic islets were randomly selected from the animals.The number and diameter of the islets of Langerhans, acinar cell count and number and diameter of blood vessels were measured, using a graded optical scanning. With a magnification of 40 micrometers, large and small diameters of each islet were measured in micrometers. The mean diameter of each islet was measured. Afterwards, the mean diameter of islets in each group was calculated per area unit.

Data analysis: In this study, data were analyzed, using SPSS version 19. One-way ANOVA test was performed to examine the differences between control and experimental groups. Duncan test was used to investigate the differences between the groups. P-value less than 0.05 was considered statistically significant.

Results

The number and diameter of the islets of Langerhans: The number (4.32±0.67) and diameter (15.84±1.01) of the islets of Langerhans in the diazinon group showed a significant reduction, compared to the control group (6.44±1.38 and 16.17±1.2, respectively) ($p<0.05$). The findings showed that the number and diameter of the islets of Langerhans in the experimental group 2 (6.37±1.01 and 16.09±1.35, respectively), experimental group 3 (5.93±0.83 and 15.84±0.91, respectively) and experimental group 4 (6.28±0.59 and 16.06±2.04, respectively) significantly increased, compared to the diazinon group ($p<0.05$). However, there was no significant difference between the control group and experimental groups 2, 3 and 4 (table 1, fig1).

The number of acinar cells: The number of acinar cells (47.315±4.014) in the diazinon group showed a significant reduction, compared to the control group (50±7.062) ($p<0.05$). The number of these cells in experimental group 2 (49.75±7.38), experimental group 3 (49.36±8.06) and experimental group 4 (49.46±8.96) significantly elevated, compared to the diazinon group ($p<0.05$). However, there was no significant difference between the control group and experimental groups 2, 3 and 4 (table 1, fig 1).

The number and diameter of blood vessels: According to statistical analysis, it was revealed that the number and diameter of blood vessels in experimental group 1 (6.26±0.93 and 5.28±0.57, respectively) significantly reduced, compared to the control group (6.50±1.04 and 5.16±0.69, respectively) ($p<0.05$). However, the number and diameter of blood vessels in experimental group 2 (6.43±0.83 and 5.25±0.78, respectively), experimental group 3 (6.36±2.11 and 5.21±0.32, respectively) and experimental group 4 (6.41±2.04 and 5.24±0.25, respectively) showed a significant increase, compared to the diazinon group, which was statistically significant ($p<0.05$). These parameters in experimental groups 2, 3 and 4 showed a significant reduction, compared to the control group; however, the difference was not statistically significant (table 1).

Table 1. Comparison between the control and experimental groups in terms of the evaluated parameters

Groups \ Paramete	The number of the islets of Langerhans (µm²) Mean±SE	The diameter of the islets of Langerhans (µm²) Mean±SE	Acinar cells (µm²) Mean±SE	Blood vessels (µm²) Mean±SE	The diameter of blood vessels (µm²) Mean±SE
Control group	6.444±1.381	16.166±1.200	50±7.062	6.500±1.043	5.277±0.574
Experimental 1	4.315±0.671	15.842±1.014	47.315±4.014	6.263±0.933	5.157±0.688
Experimental 2	6.37±1.009	16.092±1.348	49.754±7.380	6.429±0.831	5.248±0.873
Experimental 3	5.928±0.828	15.842±0.913	49.357±8.063	6.357±2.107	5.211±0.324
Experimental 4	6.28±0.594	16.061±2.042	49.455±8.90	6.408±2.036	5.242±0.254

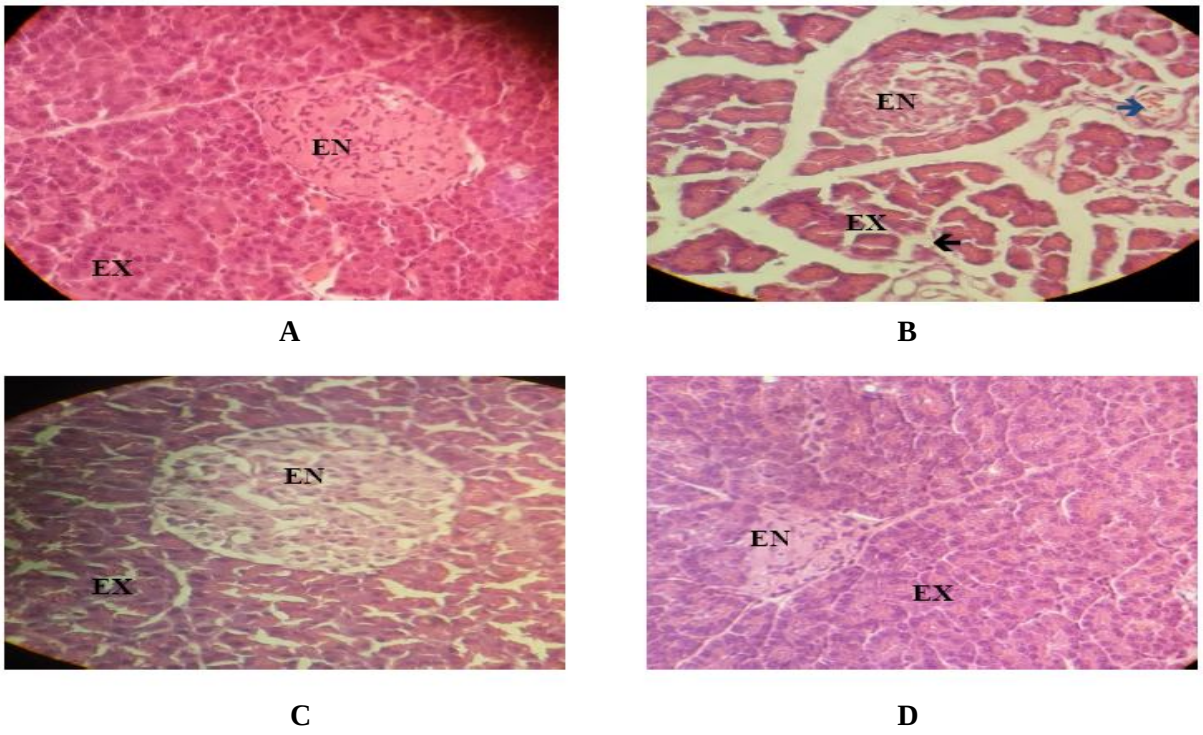

Figure 1. Light microscopic images from rat pancreas, stained with H&E (400x)

A) Control group (normal pancreas): Exocrine (EX) and endocrine portions (EN), B) Diazinon group: Necrosis and cell vacuoles (arrow), cell drainage and vacuoles of the islets of Langerhans (arrowhead) and blood vessels (blue) Severe destruction of acinar cells and loss of the islets of Langerhans are vividly specified, C) Exercise group: Cellular destruction and necrosis in endocrine and exocrine portions are much less evident, D) Exercise-extract-diazinon group: The damage is much reduced.

Discussion

The results showed a significant reduction in the number and diameter of the islets of Langerhans, acinar cell count, as well as the number and diameter of blood vessels in the diazinon group, compared to the control group. However, the evaluated parameters in this study significantly increased in experimental groups 2, 3 and 4, compared to the diazinon group. Our investigations suggest that organophosphate compounds interact with macromolecules and micromolecules in cells and cause genetic and cellular damages (10, 11). Some experts believe that organophosphate compounds by increasing lipid peroxidation, cell apoptosis, production of free radicals and inhibiting the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase lead to destruction in cells and tissues of the body (12, 13).It seems that reduction in the evaluated parameters in the diazinon group is associated with increased

oxidative stress and cell death induction by this toxic compound. Moreover, the results suggest that in groups receiving Salvia officinalis extracts, the number and diameter of the islets of Langerhans, acinar cell count, as well as the number and diameter of blood vessels significantly increased, compared to the diazinon group. Based on the histological results in this study, we can conclude that Salvia officinalis extracts can prevent oxidative stress, induced by diazinon in the islets of Langerhans, acinar cells and blood vessels in rats. In living organisms, in order to prevent the destructive effects of free radicals and oxidative stress, two antioxidant systems including antioxidant enzyme defense (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic defense systems (ascorbic acid, alpha-tocopherol, bilirubin, uric acid, carotene and polyphenols) have been proposed (20, 27).

These compounds by inhibiting the production of free radicals and repairing and reconstructing damaged tissues minimize injuries induced by the activity of free radicals (28). Therefore, consuming natural antioxidants could be one of the proper ways to reduce damages caused by oxidative stress, induced by diazinon intoxication. Researchers consider the presence of phenytoin and camphor cineole in Salvia officinalis as the main factor for its antioxidant properties (29, 30).

It seems that Salvia officinalis extracts in rats treated by diazinon restrain the toxic effect and oxidant property of diazinon and act as a protective agent for body cells due to the presence of phenolic compounds. Another finding of this study was the significant increase in the evaluated factors in the experimental groups, compared to the diazinon group. Some researchers have indicated that exercise can increase plasma uric acid. Given the fact that uric acid is one of the non-enzymatic antioxidant systems in the body, it seems that exercise reduces reactive oxygen species and free radicals by increased production of uric acid (31). A group of experts believe that regular exercise leads to balance and increased antioxidant ability against oxidative stress (23). Therefore, it seems that exercise can play an important role in cell protection against oxidative species such as free radicals. Studies have shown that in some cases, the antioxidant system in the human body is unable to independently neutralize oxidative effects; therefore, in these situations, the role of natural antioxidants gains importance (32, 33). The results of this study showed that exercise along with the use of Salvia officinalis extracts increased the diameter of cells and blood vessels in pancreatic tissues, compared to the diazinon group.

Some researchers believe that secondary metabolites in plants lead to the increased activity of antioxidant enzymes, reduced lipid peroxidation and reduced oxidant activities (34, 35). Therefore, it seems that exercise along with the use of plants with natural antioxidants (such as Salvia officinalis) could have positive effects on the management of oxidative activity in the body. Based on the results of the present study, it seems that Salvia officinalis extracts, exercise or a combination of both could strengthen the antioxidant system of the body by eliminating free radicals and reducing oxidative stress, induced by diazinon.

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