



# Protective Effects of Vitamin E on Liver and Pancreas tissues of Male Rats After Exposed to Spirotetramat Poison

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## Abstract

**Background:** This study was to evaluate the effects of vitamin E on histopathology of liver and pancreas following Spirotetramat (Movento) pesticide on rats.

**Methods:** Rats were divided into 5 group. Movento and vitamin E was performed every two days for 10 days. Treatment with vitamin E was performed 30 minutes after Movento consumption.

**Results:** Chemical analysis showed that the level of AST enzyme was significantly higher in Movento toxin group, whereas the Movento-vitamin E group did not show a significant difference compared to the sham group. LDL lipoprotein was significantly higher in Movento group, while LDL lipoprotein were no significant differences in vitamin E and Movento-vitamin E groups compared with sham group. Hepatocyte diameter decreased significantly in Movento compared to sham group. The number of lymphocytes and Kupffer cells was significantly increased in the Movento group compared to the shams group. There was significant difference in the area of Langerhans islands in the Movento groups.

**Conclusion:** It can be deduced that high dose of Movento toxin causes cellular changes and inducing tissue damages particularly in liver than pancreas tissue, Also, vitamin E consumption may reduce the effects of Movento toxin to some extent.

**Keywords:** Vitamin E; Movento; Spirotetramat; restorative effects; poison expose

**Abbreviations:** ROS: Reactive Oxygen Species; LDL: low density lipoprotein; HDL, high density lipoprotein; AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; MV: Movento

## Introduction

Nowadays the usage of the varieties of pesticides are increasing dramatically in order to control population of plant pest in agricultural fields. There are some reports showed these toxic chemicals may affect health of people who are directly or indirectly exposed to these chemicals [1]. Spirotetramat is a keto-

enol insecticide developed by Bayer Crop Science under the brand names Movento and Ultor, derived from tetramic acid is the most used toxin, nowadays [2]. Spirotetramat absorbs through the digestive tract, and prevent the production of acetyl coenzyme carboxylase [3]. Spirotetramat works by inhibiting lipogenesis,

resulting in fat content diminishing, and growth inhibition of young insects [4]. Previous studies showed that Spirotetramat causes anemia and skin tumors, as well as inducing carboxylic acid stasis and oxidase inhibition in rat. This toxin also effects on reproductive organs [5]. Biosecurity concerns are increased about abusing Spirotetramat. Spirotetramat had effects on testicular parameters, sperm count and motility in men, and also on number and size of healthy follicles in women [6]. Also, the toxic effect of movento in nervous system, particularly in hippocampal neurons, has been shown. [7]. Because of organophosphates are more lipophilic, they can bind to the phospholipid bilayer cell membrane and damage that and then increase the glycogen activity in the brain and liver [8]. Organophosphates with serine amino acid phosphorylation at the active site of acetylcholine enzyme inhibit the enzyme, leading to increased acetylcholinesterase and cholinergic crisis, seizures and in acute cases of brain injury and death. Another mechanism of action is the production of free radicals and disrupting the body's antioxidant system [9]. Antioxidants are cleaners that poison excess ROS (Reactive Oxygen Species) and play an important role in balancing the body's oxidant-antioxidant system. There are two types of antioxidants: enzymatic and non-enzymatic. Vitamin E (alpha-tocopherol), a non-enzymatic antioxidant and a fat-soluble vitamin in the cell, acts as an antioxidant known to protect cell membranes and lipoproteins from peroxidation and support the immune system [10]. Although there have been numerous studies on the adverse effects of organophosphates on liver and pancreas tissue and the protective effect of vitamin E, the effects of vitamin E have received little attention. However, in the search, no study was found that showed the effects of the Movento pesticide on the microscopic structure of the liver and pancreas. As a result, in this study, we first evaluated the effects of Movento poison on the liver and pancreas tissue, and then we examined the effects of vitamin E following the administration of Movento in male rats.

## Materials and Methods

### Animals Experiments

In the present study, male Wistar rats (250-300 g) were kept under standard conditions; free access to water and food, 12 h dark and light, and 22 °C and then they were divided into 5 experimental groups (n=8 per group), randomly [11]. All procedures were approved by the Medical Ethics Committee of Kerman University of Medical Sciences (Ethics Code: IR.KMU.REC.1397.293.) and conducted following the guidelines of the National institute of Health for the care. All methods used in our experiment were in accordance with the ARRIVE guidelines and performed in accordance with the relevant guidelines and regulations. In the sham group only the gavage process was performed, and the animals received equal volume of normal saline (1 ml). In the Movento group, the animals were gavaged once every two days for ten days at a dose of 1000 mg/kg Movento. In the Movento-vitamin E group, the animals were gavaged at a dose of 1000 and 200 mg/kg, respectively. It should be noted that gavage of vitamin E was performed half an hour after gavage of Movento [6]. In the Vitamin E group, the animals were

gavaged at a dose of 200 mg/kg [12]. The rats were weighed in the first and 10<sup>th</sup> day.

### Biochemical Analysis

Finally, at the end of the 10th day, the animals were anesthetized by using chloral hydrate (200 mg/kg), approximately 3 ml of blood was collected from the animal's heart. EDTA blood samples were added to ethylene diamine tetra-acetic acid, which is an anticoagulant after centrifugation at 3000 rpm for 10 min and stored at -20 °C. Blood glucose and lipoprotein levels including low density lipoprotein (LDL), high density lipoprotein (HDL), aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) was measured by using the diagnostic kits.

### Histological Studies

The anterior abdominal area was opened and after excision of the peritoneum and stomach, liver and pancreas tissues (separated from the spleen) were removed and fixed in 10% formalin for 24 h. Sections of 5-7 µm were stained by H&E and studied by light microscopy [13]. To measure hepatocyte diameter, 5 fields were selected from each slide and 20 hepatocytes were randomly selected and their diameter was measure by X40 magnified optical microscopy and image-j software [14]. In each group, 800 cells were measured, and the mean diameter was calculated. In this experiment, the numbers of Kupffer and lymphocyte cells were counted in the liver slides and the mean was obtained. The average was compared between all groups [15]. Seven-micron thick slides of the Langerhans islands of the pancreas were provided and the images of the islets in all slides were taken by X40 magnified light microscopy. Then, the diameter of each island was measured, and the area of each island was calculated by using the circle area formula [16]. The mean area in each group was calculated and compared statistically analyzed between different groups.

### Count of Pancreatic Islets of Langerhans

The total number of islets of Langerhans was counted in all slices with X40 magnification.

### Statistical Analysis

The data of the present study were statistically analyzed by Prism (8<sup>th</sup> version) software. After confirming the normality of the data with Kolomogorov- Smirnov and shapiro-wilk tests, parametric tests including one-way ANOVA following Tukey's tests and non-parametric test (Kruskal-Walli's test followed by Dunn's multiple comparisons test) were used to compare the data. Data were shown as mean ± SE and P ≤ 0.05 was considered as a significant level.

## Results

### Evaluation of the weight changes

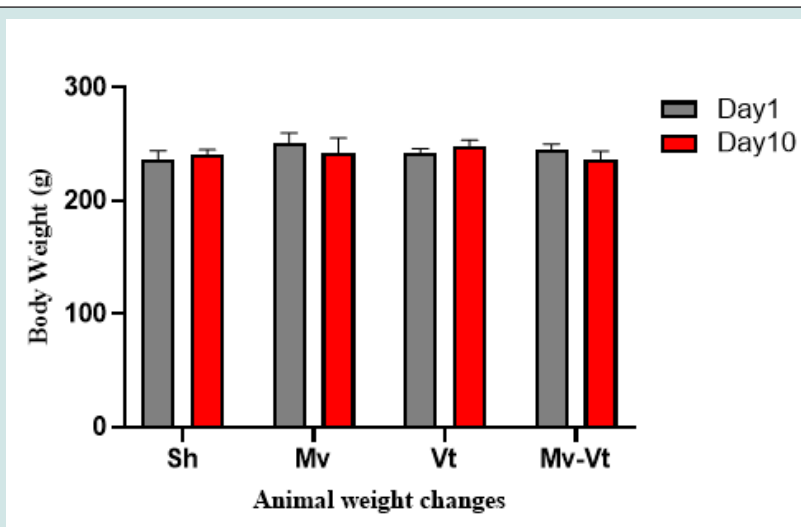
Animal weight changes were investigated intragroup during day 1 to 10 (Figure 1). There was no significant difference between groups in the animal weight changes (P ≤ 0.05). A little increase in the weight was observed in the sham and vitamin E groups at the

10th day compared to the first day. Also, a little weight loss was seen in the Movento and Movento-vitamin E groups at the 10th day compared to the first day (Figure 1). Animal weight changes in different groups on the first (Day1) and 10<sup>th</sup> (Day10) day of experiment.

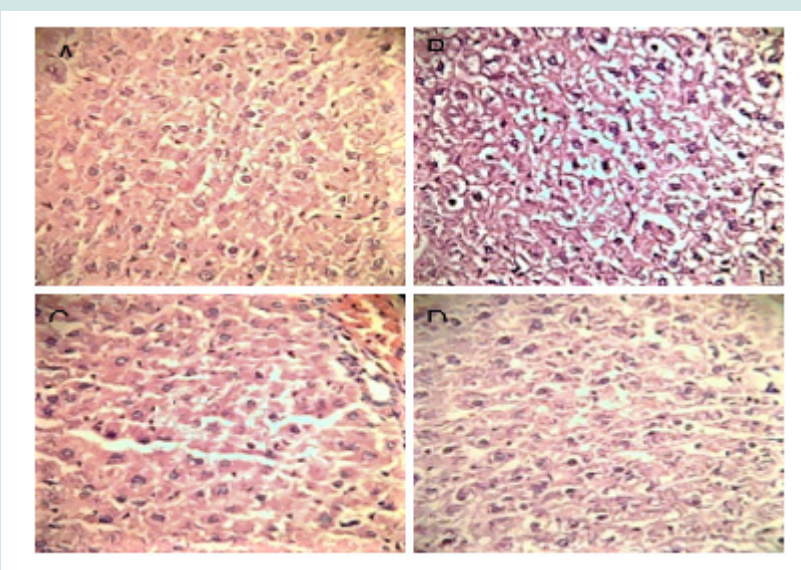
**Histological Findings**

Figure 2 shows histopathological section of liver in all groups (X40 magnification). The damaged hepatocyte cells with a dense and condensed nucleus of clear and diffuse cytoplasm with discrete cell membranes can be detected, which can be a cell with necrosis status (Figure 2B). Hepatocyte cell diameter also was decreased significantly in the Movento group compared to the sham group (P < 0.0001). Results showed that hepatocyte cell diameter in Movento-Vitamin E group increased significantly compared to the

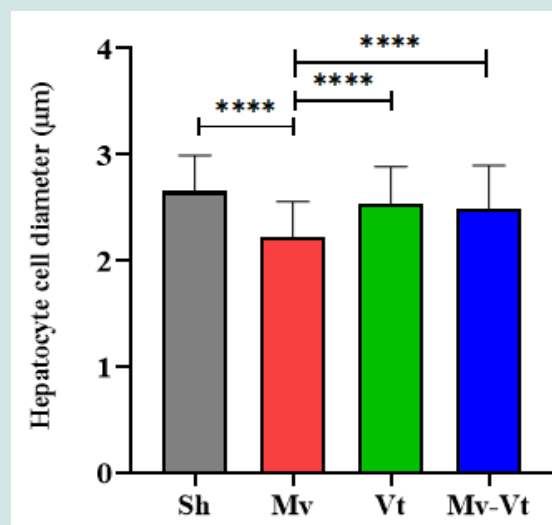
Movento group (P < 0.0001) (Figure 3). Figure 2. sections of liver tissue in H&E staining in all groups (X40 magnification). A: Sham, B: Movento, C: vitamin E, D: Movento-vitamin E (Figure 3). Protective effect of the vitamin E followed by gavage of Movento poison on the hepatocyte’s cells of liver tissue in H&E staining. Hepatocyte cell diameter was compared in different groups on the 10<sup>th</sup> day. The asterisk sign indicates a significant difference between the groups. (P < 0.0001). Number of lymphocytes was increased in the Movento, compared to sham group (P < 0.0001). Results showed that there were significant differences between vitamin E and Movento-vitamin E groups compared to sham group. While the number of lymphocytes was decreased in vitamin E (P < 0.0001) and Movento-vitamin E (P = 0.0002) groups compared to Movento group (Figure 4).



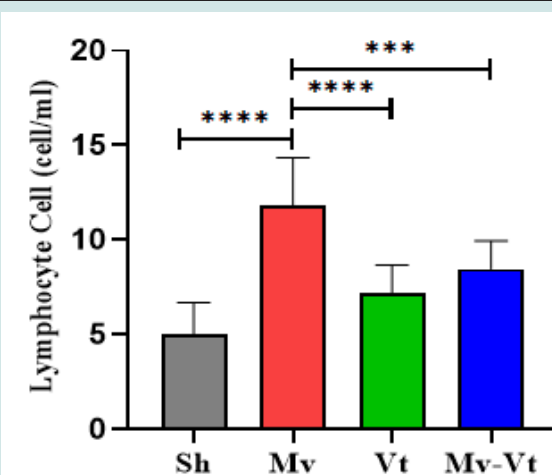
**Figure 1.** Animal weight changes in different groups on the first (Day1) and 10<sup>th</sup> (Day10) day of experiment



**Figure 2.** sections of liver tissue in H&E staining in all groups (X40 magnification). A: Sham, B: Movento, C: vitamin E, D: Movento-vitamin E.



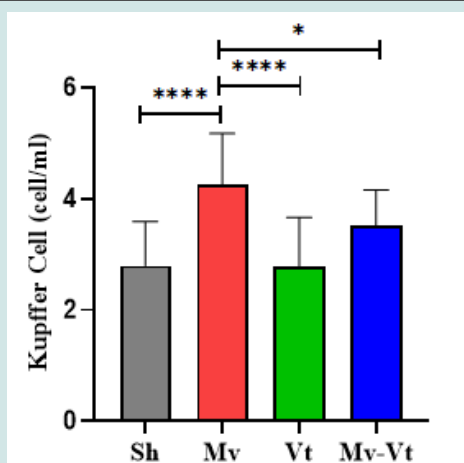
**Figure 3.** Protective effect of the vitamin E followed by gavage of Movento poison on the hepatocyte's cells of liver tissue in H&E staining. Hepatocyte cell diameter was compared in different groups on the 10th day. The asterisk sign indicates a significant difference between the groups. ( $P < 0.0001$ ).



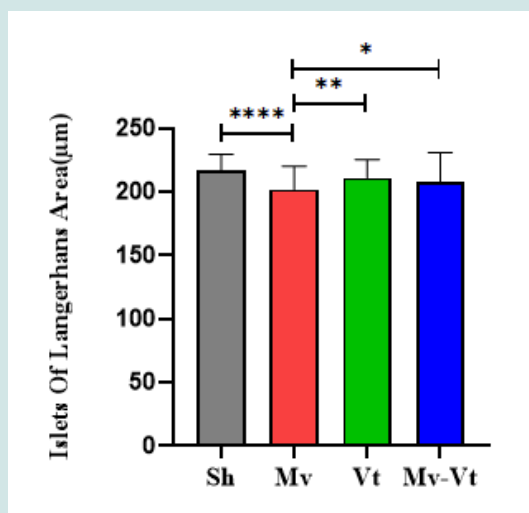
**Figure 4.** Protective effect of the vitamin E followed by gavage of Movento poison on the lymphocyte cells of liver tissue. Comparison of lymphocyte cells number in different groups on the 10th day. The asterisk sign indicates a significant difference between groups ( $***P < 0.001$ , and  $****P < 0.0001$ ).

Figure 4. Protective effect of the vitamin E followed by gavage of Movento poison on the lymphocyte cells of liver tissue. Comparison of lymphocyte cells number in different groups on the 10th day. The asterisk sign indicates a significant difference between groups ( $***P < 0.001$ , and  $****P < 0.0001$ ). The number of Kupffer cells was significantly different in Movento group compared to sham, vitamin E and Movento-vitamin E groups. This difference was significant in Movento-vitamin E group compared to sham group ( $P = 0.003$ ). Kupffer cells were increased in Movento-vitamin E compared to vitamin E group, whereas those were increased significantly in Movento group compared to vitamin E group ( $P < 0.0001$ ) (Figure 5). Figure 5. Protective effect of the vitamin E followed by gavage of Movento poison on the Kupffer cells of liver tissue. Comparison

of Kupffer cells number in different groups on the 10th day. The asterisk sign indicates a significant difference between groups ( $*P < 0.05$ , and  $****P < 0.0001$ ). The average area of the islets of Langerhans in all groups was decreased compared to the sham group (mean: Sh: 175.3, Mv: 98.99, Vt: 142, Mv-Vt: 137.7). A Kruskal-Wallis test followed by Dunn's multiple comparisons test showed Movento (Mv group) reduced significantly compared with the sham, Vitamin and Movento-vitamin groups in following oral administration of Movento (1000 mg/kg) ( $P < 0.0001$ ,  $P < 0.009$ ,  $P < 0.02$ , respectively) (Figure 6). The area of the islets of Langerhans on the 10<sup>th</sup> day in different groups was compared. The asterisk sign shows a significant difference between the Movento (1000 mg/kg) group and the Sham, Vitamin and Movento-Vitamin groups ( $*P < 0.05$ ,  $**P < 0.01$  and  $****P < 0.0001$ ).



**Figure 5.** Protective effect of the vitamin E followed by gavage of Movento poison on the Kupffer cells of liver tissue. Comparison of Kupffer cells number in different groups on the 10<sup>th</sup> day. The asterisk sign indicates a significant difference between groups (\*P < 0.05, and \*\*\*\*P < 0.0001)

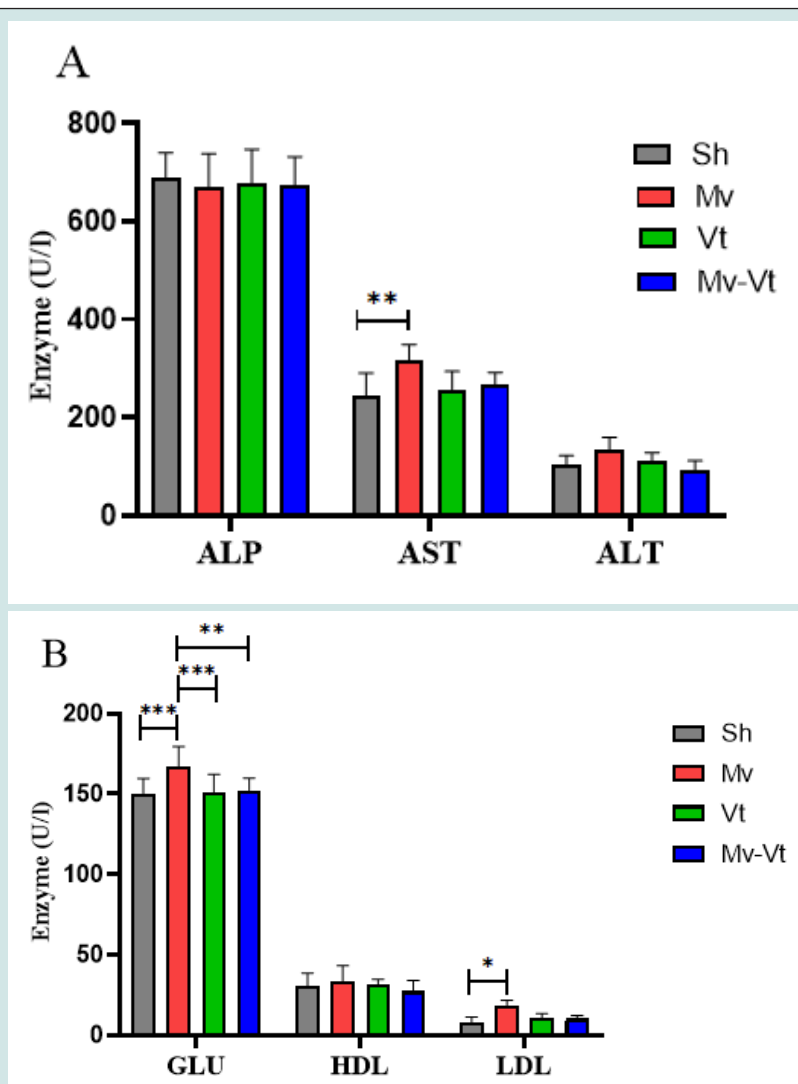


**Figure 6.** The area of the islets of Langerhans on the 10th day in different groups was compared. The asterisk sign shows a significant difference between the Movento (1000 mg/kg) group and the Sham, Vitamin and Movento-Vitamin groups (\*P < 0.05, \*\*P < 0.01 and \*\*\*\*P < 0.0001).

### Blood Test Result

Study of the ALP, ALT, and HDL did not show any differences between groups significantly. The level of AST enzyme in Movento group was higher compared to sham group and it was statistically significant with two-way ANOVA following Tukey's tests (P = 0.00). Also, AST enzyme did not show any significant difference in Movento-vitamin E group compared to the movento group (P = 0.11) (Figure 7A). It is clear from the diagram that LDL lipoprotein

and blood glucose showed a significant increase in Movento group compared to other experimental groups (Figure 7). The assessment of rats' blood parameters in different groups. The effects of Movento (1000 mg/kg) on the ALP, AST, ALT (A) and GLU, HDL, LDL (B) within the four groups (n = 8). Data are shown as mean ± SEM. The asterisk sign shows a significant difference between the Movento (1000 mg/kg) group and the other group in AST, GLU and LDL parameter. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001



**Figure 7:** The assessment of rats' blood parameters in different groups. The effects of Movento (1000 mg/kg) on the ALP, AST, ALT (A) and GLU, HDL, LDL (B) within the four groups (n = 8). Data are shown as mean  $\pm$  SEM. The asterisk sign shows a significant difference between the Movento (1000 mg/kg) group and the other group in AST, GLU and LDL parameter. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

## Discussion

In the previous study, we evaluated Movento doses higher than 1000 mg/kg that increased mortality [7]. The results of the present study showed that Movento poison at a dose of 1000 mg/kg on the tenth day of gavage slightly reduced the weight of animals compared to the first day whereas in the sham and vitamin E groups the animal weight was increased on the 10th day. The liver is the first organ that is exposed to chemical compounds, drugs, and poisons and is capable of neutralizing these compounds. But if the amount of these compounds in the body increases, it can damage the liver tissue. The effects of various poisons on animals have been evaluated, including mancozeb, diazinon, movento and etc. Unfortunately, few studies have investigated the effects of Movento on body tissues.

Mancozeb, similar to Movento is also a pesticide. A study showed that mancozeb gavage at doses of 500, 1000, 1500 mg/kg for 30, 90, 180, 360 days causes mortality and body weight loss in a dose-dependent way [17]. Other studies have shown that mancozeb toxin does not affect body weight. In our study, which used Movento poison, the effects of weight loss were observed in the animals, although this weight loss was not significant in any of the studied groups. These differences between toxins can be related to the type of toxin or different doses and exposure time [18]. It seems that by receiving the poison, the absorption of water and nutrients in the animal's body decreases. A study showed that diazinon poison by inducing behavioral disorders and reducing the daily intake of water and nutrients in animals has a major contribution to reducing the weight of animals at the end of the poison administration period. [9]. In the present study, vitamin E caused changes in appetite, and

food intake, and improved animal body weight. These findings are consistent with other study's findings [9,19,20]. In the Movento group, the mean diameter of hepatocytes cells was decreased compared to the sham group. The results showed that vitamin E reduces the number of damaged hepatocyte cells compared to Movento group. A study on diazinon pesticide showed that many structural changes in the liver of wistar rats, including, hepatocyte degeneration, necrotic cell formation and decreased nucleus diameter [21]. Also, Propiconazole toxin induces hepatocyte hypertrophy [22]. The toxicity of endosulfan on the liver tissue causes liver necrosis, decreased hepatocyte diameter, and central vein dilation [23]. In the present study, in the Movento group due to inflammation and immune response, number of lymphocyte cells was increased compared to the sham group, whereas in the animals that received vitamin E (Mv-Vt), number of lymphocyte cells was decreased significantly. In general, the results showed that vitamin E can decrease inflammation and consequently decrease lymphocyte cells by decreasing the immune response. It has been proven that diazinon can also induce inflammation in rats [24]. Kupffer cells are an important component of the phagocytosis system and play an essential role in the systemic response to pathogens and toxins [25]. Kupffer cells were significantly increased in Movento group in this study. Coadministered vitamin E and poison (Mv-Vt) to the animals showed that the number of Kupffer cells in this group decreased. This indicates a positive effect of vitamin E on reduce of inflammation and improve the pathological condition of the area. Kupffer cells, as the most abundant innate immune cells in the liver, has an essential role in the pathogenesis of many liver disorders acting these cells in liver damage, results in the release of oxygen species, which increases the hepatic injury [26]. Histological results of the present study indicate that Kupffer cells proliferation may playing a role in hepatotoxicity induced by treated Movento poison. It is also showed that due to the use of the plant pesticide, there is a significant increase in the number of Kupffer cells, with their alienating role in digesting those cells. The result of the liver tissue that has been injured due to diazinon has been correlated with our results [20]. Another study in India showed that endosulfan over a 15-day period, causes the reduce in body weight and increase the number of Kupffer cells and also changes in the liver tissue [27]. Also, mancozeb causes liver tissue necrosis, liver lobes to deform and increase Kupffer cells [28].

In the present study, if it is assumed that the increase in the number of Kupffer cells is caused by the effect of Movento toxin, it seems that the increase of these cells is related to the damage of hepatocytes and the role of phagocytosis of Kupffer cells. In the present study, examining the area of Langerhans islands showed that there is a significant difference between the Movento group and the other groups, and the area has decreased in this group, which is according with the report of the function of other toxins. In an experimental study, animals were injected with diazinon at doses of 100 and 200 mg/kg for 4 weeks. Results showed that the diameter and number of pancreatic islets were significantly

reduced [29]. Also, in the present study, there was significant difference in blood glucose (GLU), LDL and AST in biochemical assays with other groups. Results of the histological pancreatic evaluation showed significant changes in the area of the pancreatic islets between Movento and other groups which could possibly be a reason for changing in the blood sugar level. The results showed that the ALP and ALT enzymes was no Significant changes in Movento group (1000 mg/kg) compared to the other groups. It is clear from the diagram 7A that the level of ALT in the MV group has increased compared to the other groups but is not significant. It has been well considered that serum activity of hepatic enzymes is an appropriate indicator for assessing the extent of damage to the liver and hepatocytes. increased secretion of ALT, ALP enzymes and AST enters the blood [10]. Previous studies have shown that pesticide toxins cause cell membrane damage and cell death through the production of free radicals and reactive oxygen species, and the levels of alanine aminotransferase and aspartate aminotransferase enzymes increase [21,30]. Vitamin E supplement was able to alleviate the toxic effect of diazinon through potent free radical and antioxidant neutralizing activities [31]. For 4 weeks, the level of biochemical parameters associated with liver injury, including the levels of liver enzymes AST, ALT, ALP and gamma glutamine transferase, cholesterol and triglyceride were significantly increased [9,31,32]. Endosulfan in the different doses over a 30-day period intraperitoneally increased ALT, AST and ALP enzymes as biomarkers to assessing the damage to their liver function in tissue [33]. In other study, it was found that organophosphorus pesticides induce an oxidative stress process that involves erythrocyte membrane damage, DNA damage and alterations in the genome, as well as increased levels of AST, ALT and ALP in human [34]. However, it has been reported that reactive oxygen species (ROS) that cause oxidative damage may be inhibited by antioxidant supplements including vitamin E, and reduce oxidative damage.

## Conclusion

According to the results of this study exposure to the Movento (1000 mg/kg) caused damage to the hepatocyte cells, increased the number of lymphocytes and Kupffer cells, and increased blood enzymes e.g., AST and LDL and blood glucose levels. Also, it was conducted that vitamin E could improve the caused damage and reduce the number of lymphocytes and Kupffer cells, and blood enzymes. It seems that the antioxidant status of the animal improves after vitamin consumption and has an inhibitory effect on the damage caused by Movento.

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The authors confirm contribution to the paper as follows: study conception and design: Ali Shamsara; data collection: Ali Shamsara and Sanaz balooch; analysis and interpretation of results: Mohsen Basiri, Reza Malekpour Afshar; draft manuscript preparation: Hasan Pahang and Touba Eslaminejad. All authors reviewed the results and approved the final version of the manuscript.

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