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# Protective Effects of Red Onion (Allium cepa) Ethanolic Extract on Learning and Memory Impairments in Animal Model of Diabetes

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## Abstract

**Background:** Onion (*Allium cepa*) is a plant with anti-diabetic and antioxidant properties. The aim of this study was to evaluate the protective effects of red onion ethanolic extract on learning and memory impairments in animal model of diabetes. **Material and Methods:** For induction of diabetes, streptozotocin (55 mg/kg) was injected intraperitoneally to male Wistar rats. Thirty two male Wistar rats were randomly divided to 4 groups (n=8). Diabetic rats located to 3 groups including 2 treatment groups with onion ethanolic extract (125 and 250 mg/kg/day) for 4 weeks and one diabetic control group. In addition, the fifth group was considered as health control group. Finally, learning and memory changes were evaluated in the Morris water maze and passive avoidance tests. **Results:** Our finding showed that escape latency and traveled distance was significantly increased in diabetic control compared with health control. The administration of onion extracts at 125 and 250 mg/kg significantly decreased the escape latency and traveled distance. In addition, the induction of diabetes caused a significant impairment in memory consolidation compared with health rats. However, animals received extract at 125 mg/kg spent longer time and traveled greater distance in target quarter compared with diabetic control. Our data in passive avoidance test also showed that diabetes reduced step-through latency in animals. However, administration of onion ethanolic extract onion ethanolic extract led to increased step-through latency in diabetic rats. **Conclusions:** Our finding showed that oral administration of red onion ethanolic extract improves learning and memory performances impaired by streptozotocin in diabetic rats.**[GMJ.2017;6(3):249-57] DOI:10.22086/gmj.v0i0.909**

**Keywords**: Onion; Memory; Learning; Streptozotocin; Rats

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## Introduction

Diabetes mellitus is a metabolic disease with different complication such as cardiovascular disease and impairment of neural system such as neuropathic pain, Parkinson's, Alzheimer's diseases [1-3]. It is also associated with memory and learning disorders [4]. Wang et.al showed that diabetes leads to other aspects of cognitive such as dysfunction in language, attention and executive reasoning [5]. Memory impairment has a direct relationship with age, diabetes duration and control of blood glucose levels [6, 7]. One of the mechanisms involved in this disorder is imbalance between oxidant and antioxidant capacity and oxidative stress in diabetes [8]. There are several strategies for treatment of memory impairment with emphasis on improvement of antioxidant capacity such as, chemical substances including, superoxide dismutase mimetics [9], metformin [10] and natural source substances including, flavonoids [11, 12], polyphenol [13] and alkaloids compounds [14]. Onion (Allium cepa L.) is a plant belongs to the Alliaceae family [15]. In previous studies it has been demonstrated that the active ingredients of this plant are mainly attributed to its polyphenols and flavonoids compounds such as quercetin and rutin [16]. The levels of these compounds in red onion are more than white onion [17]. According to our finding, no study is available on evaluating the effect of onion on memory deficit. However, this plant showed anti-diabetic and antioxidant effects. It has been shown that onion was effective in the treatment of cardiac ischemia through antioxidant activity [18]. Onion improves brain ischemia in mice with inhibition of oxidative stress biomarkers [19]. In a meta-analysis [20] demonstrated that onion has beneficial effects on control of blood glucose in animal model of diabetes.According to anti-diabetic and anti-oxidant properties of onion, we hypothesized that administration of onion ethanolic extract might influence learning and memory in animal model of diabetes. Therefore, this study was performed to evaluate the protective effects of red onion ethanolic extract on learning and memory impairments in animal model of diabetes.

## Material and Methods

*1.Preparation of Ethanolic Extract of Onion*

In this study, we used red onion because it has been reported that the levels of flavonoid compounds in red onion are more than white onion [17]. The red onion was prepared from market of Kashan city on September 2013 and was identified by Department of Agriculture, Research and Development Center of Barij Essence Company, Iran. After washing with water; cut into small pieces, and they were dried under room temperature. Then, the dried onion was crushed. The obtained powder was extracted with ethanol 96 % by maceration method for 72 h.

*2.Analysis of Extracts*

*2.1.Determination of Total Flavonoid Content*

Total flavonoid content was determined by Five microliter of aluminum trichloride (AlCl3) (2% in methanol) was added to 5 mL of extract (0.4 mg/mL). After 10 min, the absorbance of the mixture was measured at 415 nm. Blank sample consists of 5 mL extract and 5 mL methanol without AlCl3. Total flavonoid content was measured by using a standard curve with quercetin (0–100 mg/L). Total flavonoid content was expressed as mg of quercetin as equivalents (QE)/g of extract.

2.2.*Determination of Total Phenolics Content*

Folin Ciocalteu reagent was used for analysis of total phenolics content . Briefly, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent. The solution was kept at 25oC for 5-8 min before adding 2 ml of sodium carbonate solution 7.5 % and adjusting the volume to 8 ml with water. After 2 h, the absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g).

*3.Animal*

The 32 male Wistar rats were randomly divided into 4 groups (n=8): health control group, diabetic control group and 2 treatment groups including: diabetic rats treated with ethano-

lic extract of red onion (125 and 250 mg/kg/day).The animals were located under standard conditions of temperature (25°C ± 5°C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. Also, the animals received standard food and water ad libitum. This study was performed according to the guidelines expressed in the Declaration of Helsinki and approved by the Ethics Committee of Kashan University of Medical Sciences, Kashan, Iran.

*4.The Induced Diabetes Model*

For induction of diabetes, streptozotocin (Sigma, USA) was injected intraperitoneally (55 mg/kg in a 0.1 mol/l citrate buffer, pH= 4.5) to overnight fasting rats. In the fifth day, the blood glucose of rats was measured by a kit to confirm the induction of diabetes. Rats with blood glucose higher than 250 mg/dl were regarded as diabetic.

*5.Fasting Blood Glucose Levels*

At the baseline and end of the experiment, all rats were decapitated under ketamine anesthesia (50 mg/kg, i.p.) and blood samples were drawn from the cut tip of the tail. A fasting blood glucose was measured using kit (Pars Azmoon, Iran) [21].

*6.Morris Water Maze*

The spatial learning and memory was assessed by Morris water maze as described previously [22]. Morris water maze is a water circular pool (140 cm in diameter × 60 cm in depth) that was divided into four imaginary quadrants and a platform located in one of the quadrants. Water was added to the maze up to 20 cm below the rim. Water temperature was maintained at approximately 22 °C. This test was including acquisition phase and probe test stage. In acquisition phase, the learning process was evaluated during 4 nights and each night for 4 trials. The escape latency and traveled distance was examined as a criterion for spatial learning ability. In probe test, consolidation of spatial memory was evaluated by removing the hidden platform in fifth night. In this stage time and distance spent in the target quadrant of the maze was measured as a criterion for consolidation of spatial memory. In both stages, rat's movements in the water were recorded by camera (in above the center of the water maze) and data collected by a computer equipped with water maze software (Radiab 7, IR Iran) for behavioral analysis.

*7.Passive Avoidance*

The passive avoidance test, the apparatus for assessment of the short-term memory, had illuminated compartments of the light and dark parts. The two compartments were separated by a guillotine door. Five s after placement of rat in the lighted part, the door was raised and habitually it entered into the dark part. The door was closed which then the animal was returned to its cage. Thirty minutes after placement of rat in the lighted part and allowing it to enter the dark part and a shock (0.8 mA) in 3 s was delivered to the feet of the animal. This process was repeated after 2 min and not entering to the dark part was considered as a successful acquisition. This experiment was continued in 24 h after the acquisition trial as a testing stage. No shock was used and the step-through latency was measured for 300 s.

*8.Statistical Analysis*

The results of acquisition phase in Morris water maze were analyzed by Two-way repeated measures analysis of variance (ANOVA). Also, multivariate ANOVA was used for analysis of the probe trial phase. Step-through latency and fasting blood glucose data were analyzed by one-way ANOVA. Bonferroni post hoc test was also used to the significant data. The threshold of significance was regarded to ($P<0.05$).

**Results**

*1.Preliminary Phytochemical Test*

Our finding in preliminary phytochemical test showed that total polyphenol content and total flavonoid content in red onion ethanolic extract were 8.36% and 4%, respectively.

*2.Morris Water Maze*

*2.1.Acquisition Phase*

Analysis of variance indicated that there are statistical differences between function of the groups in the Morris water maze ($F_{3, 124} = 8.905$; $P < 0.0001$). Bonferroni post hoc

showed that the animals of diabetic control group longer to find the hidden platform compared to the health control group (P = 0.014). Administration of ethanolic extract of red onion reduced escape latency compared to the diabetic control group (at 125 mg/kg: P = 0.0001and at 250 mg/kg: P = 0.003) (Figure-1). Also, a two-way analysis of variance showed that there are significant effects in traveled distance between groups (F3, 124 = 17.480; P < 0.0001). Our result showed that there are significant difference between control groups (P = 0.01). Groups received this extract at 125 and 250 mg/kg traveled less distance to find the hidden platform than the diabetic control group (P < 0.0001). (Figure-2)

*2.2.Probe Trial Phase*

Analysis of variance showed that there are statistical difference between five testing group in probe trial phase ($F_{3, 28}$ = 7.259; P = 0.001). Our finding showed that the diabetic control group spent less time in target quadrant than health control groups (P = 0.009). Also, the animals received ethanolic extract of red onion at 125 mg/kg spent more time compared diabetic control group (P = 0.001). But, this extracts at 250 mg/kg had not significant effect on consolidation of spatial memory (P = 0.353) (Figure-3). The traveled distance also was significant between all groups ($F_{3, 28}$ = 6.185; P = 0.002). The traveled distance in the correct quadrant was decreased significantly in diabetic control group compared with health control group (P = 0.041) and the animals received this extract at 125 mg/kg traveled more distance in correct quadrant compared with diabetic control animals (P = 0.001). But, No effect was observed in the animals received ethanolic extract of red onion at 250 mg/kg (P = 0.071). (Figure-4)

*3.Passive Avoidance*

Analysis of variance showed that there are significant differences in mean step-through latency between all groups ($F_{3, 28}$ = 8.705; P<0.0001). Bonferroni post hoc showed that mean step-through latency reduced in diabetic control group compared with health control group (P<0.0001). However, administration of red onion ethanolic extract led to increased step-through latency in animals compared with diabetic control group (at 125 mg/kg: P=0.004, at 250 mg/kg:P=0.019) (Figure-5).

*4.Fasting Blood Glucose Levels*

Baseline levels of fasting blood glucose were higher than 250 mg/dl (Data not shown). ANOVA analysis showed significant differences in mean fasting blood glucose in studied groups ($F_{3, 28}$ = 17.714; P<0.0001). Injection of STZ led to significant increase in fasting blood glucose in diabetic control groups compared with health control group (374.5±21.89 vs. 117.25±9.19, P<0.0001). There was significant differences in the fasting blood glucose of diabetic control groups compared with treatment groups received extract at 125 and 250 mg/kg (374.5±21.89 vs. 217.87±25.1 and 209.5± 37.11, P<0.0001).

**Discussion**

In present study, we evaluated the effect of red onion ethanolic extract on alteration of learning and memory abilities in animal model of diabetes by Morris water maze and passive avoidance. Our finding showed that administration of onion improves diabetes-induced learning and memory deficits in Morris water maze and passive avoidance. Limited studies were performed about protective effects of onion on nervous system dysfunction. These studies have been reported the benefit properties of this plant on brain and nervous system dysfunction by inhibition of oxidative stress. Hyun *et al*. reported that onion improves brain ischemia in mice with control of oxidative stress biomarkers such as glutathione and catalase [19]. Also in another study showed that administration of onion at 100 mg/kg has a significant protective effect in cerebral ischemia [23]. In addition, Shir *et al*. reported that onion extract improves memory impairment by abatement of oxidative stress in animal model of ischemia [24]. One of the mechanisms involved in this disorder is imbalance between oxidant and antioxidant capacity and oxidative stress in diabetes [8]. It has been reported that hyperglycemia in diabetes mellitus leads to production of oxidative stress in neuron cells [25, 26] that is one most of factors for impairment of learning and

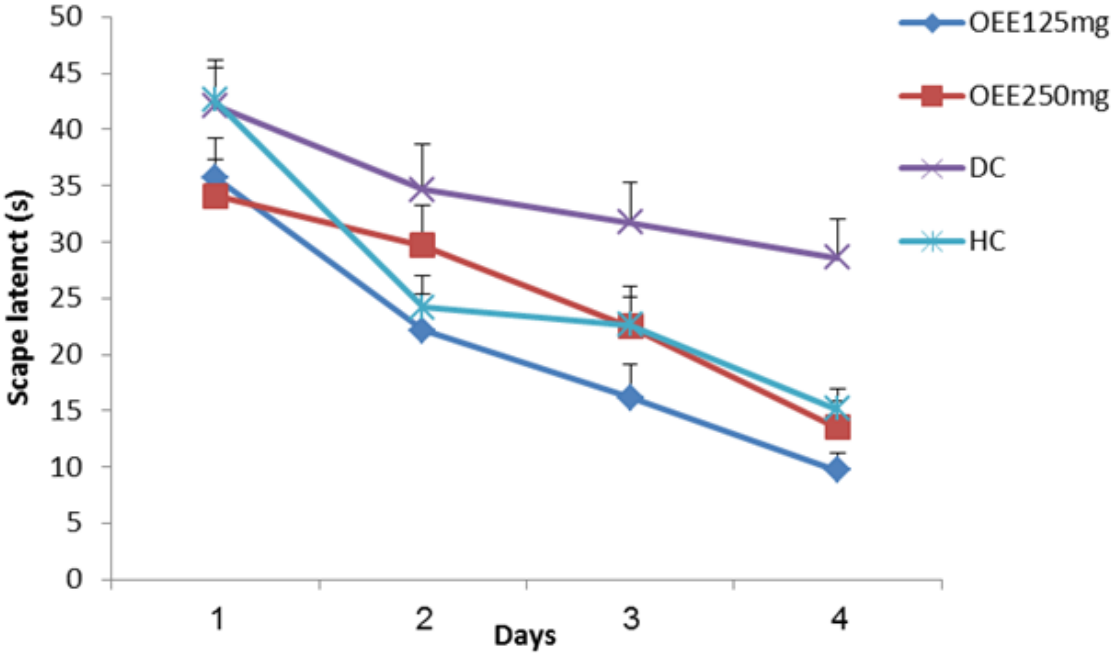


**Figure1.** Effects of ethanolic extract of red onion on latency to escape in Morris water-maze. Data are expressed as Mean± SEM. Significance was determined by two-Way ANOVA followed by Bonferroni post hoc test (n = 8). **OEE:** Onion ethanolic extract, **DC:** Diabetic group, **HC:** Health group

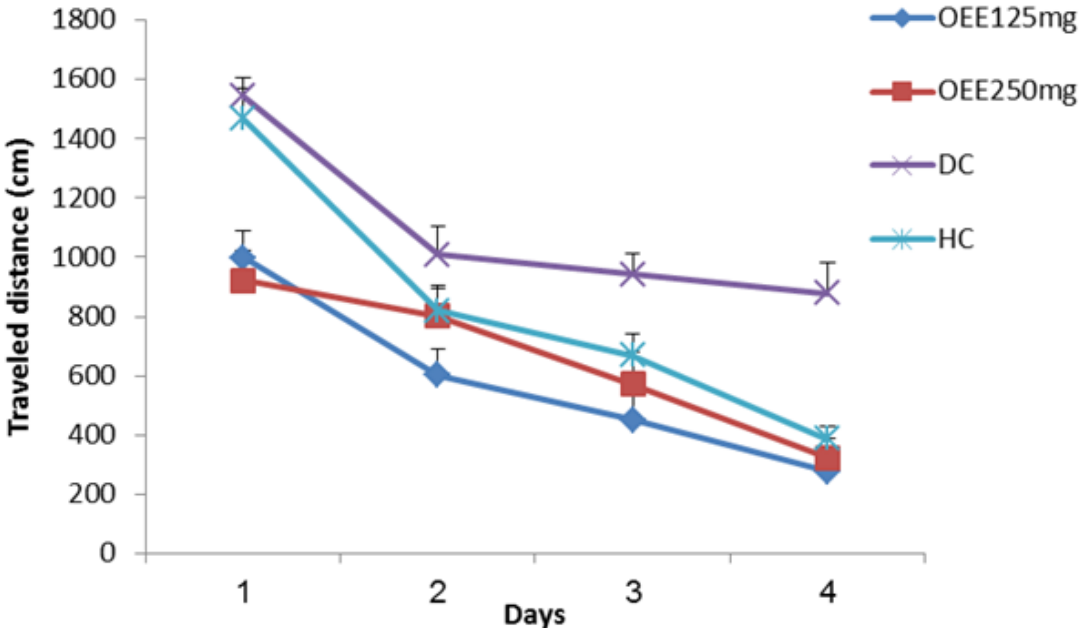


**Figure 2.** Effects of ethanolic extract of red onion on traveled distance in Morris water-maze. Data are expressed as Mean± SEM. Significance was determined by two-Way ANOVA followed by Bonferroni post hoc test (n = 8). **OEE:** Onion ethanolic extract, **DC:** Diabetic group, **HC:** Health group

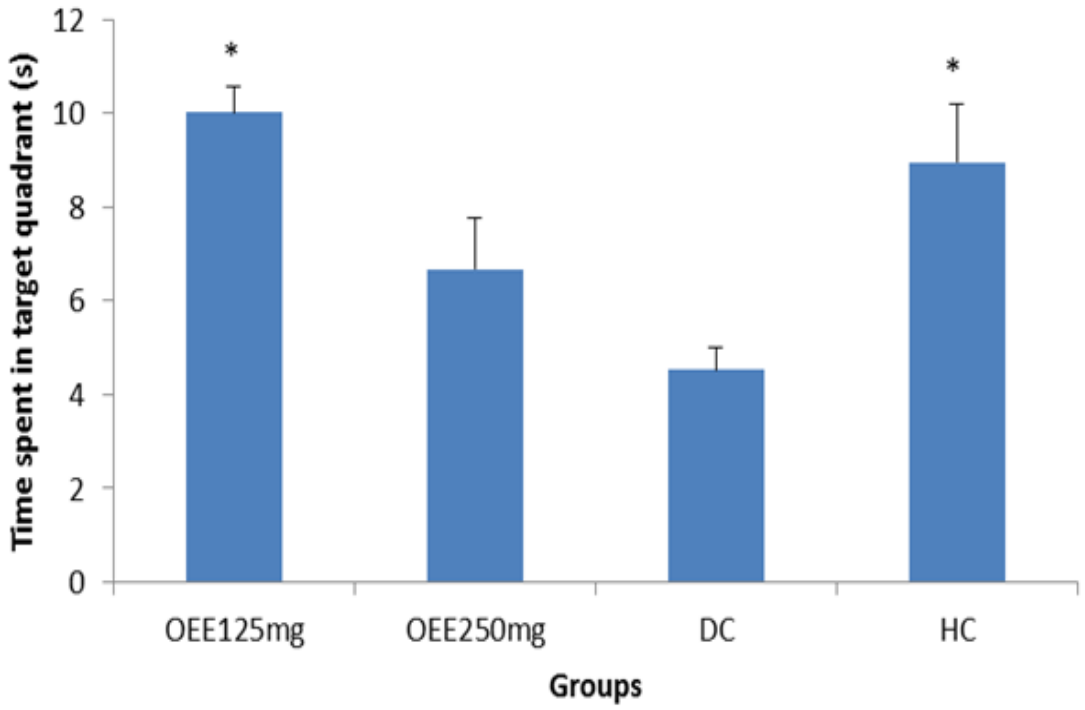


**Figure 3.** The time was elapsed by the rats in correct quadrant in the probe trial test. Data are expressed as Mean± SEM. Significance was determined by two-Way ANOVA followed by Bonferroni post hoc test: * Difference between DC and other groups; P<0.05 (n = 8).
**OEE:** Onion ethanolic extract, **DC:** Diabetic group, **HC:** Health group

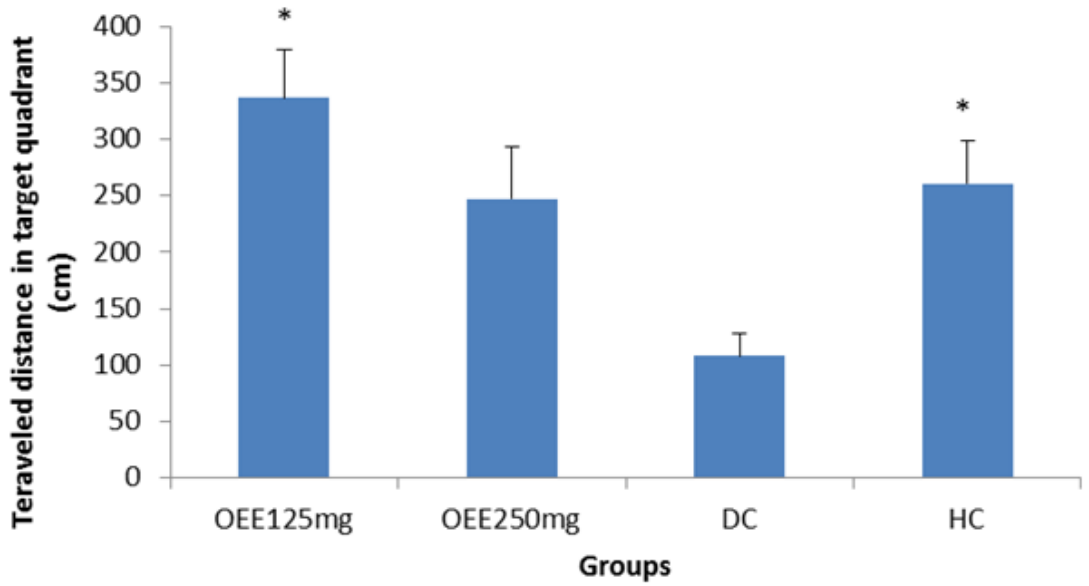


**Figure 4.** The distance was traveled by the rats in correct quadrant in the probe trial test. Data are expressed as Mean±SEM. Significance was determined by two-Way ANOVA followed by Bonferroni post hoc test: * Difference between groups with DC; P<0.05 (n = 8).
**OEE:** Onion ethanolic extract, **DC:** Diabetic group, **HC:** Health group

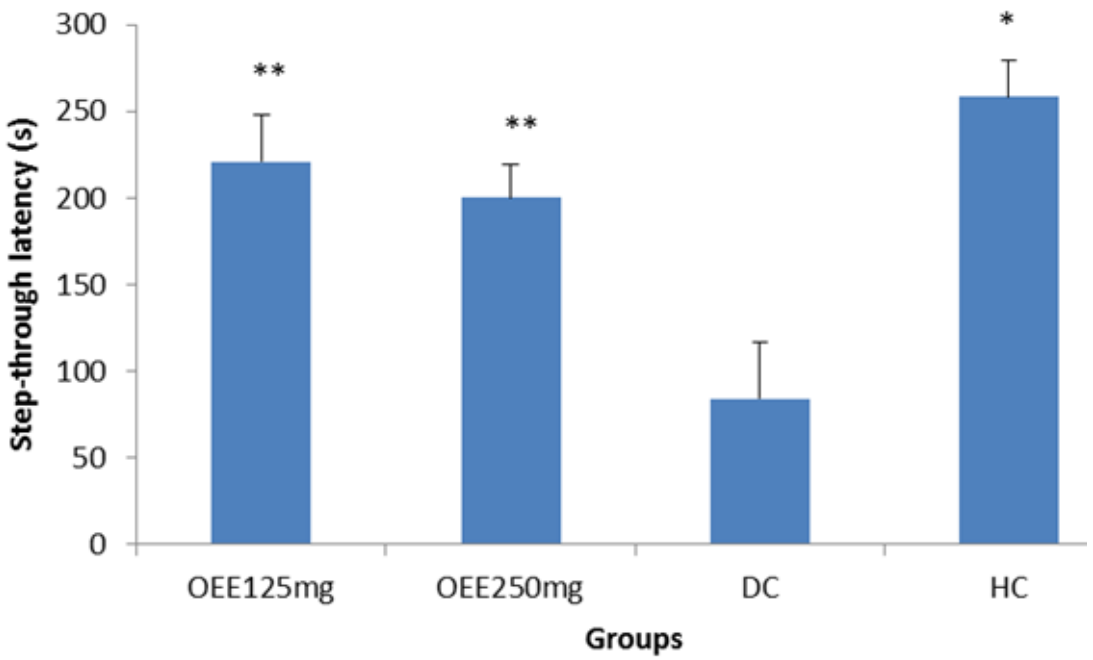


**Figure 5.** Effect of OEE on the step-through latency in the studied groups. Data are expressed as Mean±SEM. Significance was determined by two-Way ANOVA followed by Bonferroni post hoc test: * Difference between DC with HC groups; P<0.0001, ** Difference between DC and groups received OEE; P<0.05 (n = 8).
**OEE:** Onion ethanolic extract, **DC:** Diabetic group, **HC:** Health group

memory in diabetic patients and animal models of diabetes [13, 27]. We were measured fasting blood glucose by routine laboratory kit. In our study, administration of onion at 125 and 250 mg/kg had significant effects in improvement of hyperglycemia. Previously studies by some researchers also showed that administration of onion leads to improvement of diabetes. For instance, Demerdash *et al*. described the benefit effect of onion juice in attenuation of blood glucose in diabetic rats [28]. The consumption of diet containing 7% onion for 5 weeks alleviated serum levels of lipid and blood glucose and inhibited free radicals in animal model of diabetes [29]. Therefore, impairment of memory ability in diabetic rats may be due to the elevation of hyperglycemia-induced oxidative stress. Administration of onion extract as an antioxidant and anti-diabetic plant may improves memory ability by inhibition of hyperglycemia-induced oxidative stress. The anti-diabetic and antioxidant activities of onion extract can be due to flavonoid and polyphenol compounds. Our phytochemical analysis showed that the ethanolic extract of red onion contains large amounts of flavonoid compounds. Our results agree with previous studies that showed this plant contains flavonoid compounds such as quercetin and rutin [16]. These compounds have significant anti-diabetic and antioxidant properties in diabetes [30]. Our study had some limitations. We did not evaluate biochemical and histological study in brain of rats. The evaluating administration of onion on cognitive dysfunction in nervous system dysfunction is interesting. More studies are suggested for evaluating effects of onion on cognitive performance and mechanisms involved in it.

## Conclusions

Oral administration of the ethanolic extract of red onion improves learning and memory dysfunction in streptozotocin-diabetic rats.

## Acknowledgement

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## Conflict of Interest

The authors declare no conflict of interest.

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