The Effect of Co-Administration of *Portulaca Oleracea* and *Plantago Psyllium* Plus Submaximal Swimming Training on Memory Deficit in Streptozotocin/Nicotinamide-Induced Type 2 Diabetic Rats

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Research Article

Keywords: Swimming training, Portulaca Oleracea, Plantago Psyllium, Type 2 diabetes, learning and memory, Metabolic disorders, Rat

Posted Date: May 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-539677/v1

Abstract

Many studies have assessed the effect of exercise training and the use of various herbs on the cognitive deficit in type-2 diabetic patients. The aim of the current study was to assess the effect of a combination of two traditional plants, *Portulaca oleracea* and *Plantago psyllium,* and swimming training on cognitive decline in type 2 diabetic rats. Fifty male Wistar rats (weight: 275±25g) were selected. Type-2 diabetes was induced by a single IP injection of streptozotocin and nicotinamide. Then, the subjects were randomly assigned to the following groups: control-healthy (Con), control-diabetic (D), diabetic-training (D+Tr), diabetic-*P.oleracea* plus *P. psyllium* (D+PO+PP), and diabetic- *P.oleracea* plus *P. psyllium* plus training groups (D+PO+PP+Tr). Training groups were subjected to submaximal swimming training for 12 weeks (5 days per week). Learning abilities and memory retention were evaluated using shuttle box, elevated plus maze, open field, and novel recognition object tests. Step-through latency period in retention phase in the shuttle box test and discrimination index in the novel recognition object test increased in response to the simultaneous use of two herbal medicines. Swimming training had no effect on learning and memory indices in diabetic rats, but co-administration of *P. oleracea* and *P. psyllium* with swimming training for 12 weeks ameliorated passive avoidance memory, general locomotor activity, and exploratory behavior in diabetic rats. These results indicated that co-administration of *P. oleracea* and *P. psyllium* with submaximal swimming training for 12 weeks can reverse the cognitive impairment present in type-2 diabetic rats.

Introduction

Diabetes mellitus is a serious health problem in the world that causes complications in the peripheral and central nerves. It is associated with structural and functional harmful changes in the peripheral and central nervous systems (Zochodne, 2007). Diabetes reduces the density of neurons in the dentate gyrus area, which is involved in learning and memory processes (Beauquis et al., 2006). It causes mild cognitive impairment in a short period and dementia in a long period (Pal et al., 2018; Albai et al., 2019). Although the mechanism of these disorders in the diabetic community is not well understood, it has been found that the cerebral cortex and hippocampus, which are the main areas associated with learning and memory, are greatly affected by diabetes. The proposed mechanisms in this regard are: 1) stress oxidative and inflammation extension, 2) dentate gyrus neural density reduction, 3) neuronal nitric oxide synthase depression, which plays an important role in synaptic plasticity, and 4) a decrease in gene expression of proteins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) that have a great role in learning and memory processes (Burdo et al., 2009; Takeda et al., 2010).

Several factors, such as physical activity and eating medicinal herbs have a positive effect on learning and memory in diabetic patients (Ganji et al., 2017; Heidarianpour et al., 2021). Exercise training is one of the best strategies to prevent and treat diabetics. Exercise training increases the generation of new neurons in the hippocampus, increases the size of the hippocampus, and improves specific types of learning and memory (Erickson et al., 2011). It also increases the mitochondrial content of skeletal muscle and oxidative enzymes, thereby dramatically improving glucose and fatty acids oxidation (Phielix

et al., 2010). Resistance and aerobic training decrease hyperglycemia and promote insulin sensitivity in diabetic patients (Hall et al., 2013). On the other hand, exercise training reduces the risk of Alzheimer's disease (Moore et al., 2016). Exercise training in young rats increases memory and learning through neurotrophic factors and plasticity changes in the brain (Baek, 2016). The hippocampus, the most important center for spatial memory and learning, changes after exercise training.

The biological and medical properties of medicinal herbs against diabetes have been recently widely considered (Samarghandian et al., 2017). *Portulaca oleracea*, which is referred to as Purslane, is an herb from the Portulacaceae family with anti-diabetic properties (Dehghan et al., 2016). *P. oleracea* decrease the total cholesterol (TC), triglyceride (TG), and fasting blood sugar (FBS) levels in type 2 diabetes because of its polyunsaturated fatty acids, flavonoids, and polysaccharides (El-Sayed, 2011). *Portulaca oleracea*lt exerts anti-hyperglycemia and anti-hyperlipidemia effects by a reduction in oxidative stress and inflammation depression (Bai et al., 2016; Samarghandian et al., 2017). In this regard, Yu et al. concluded that the anti-diabetic effect of *P. oleracea* can be due to its antioxidant and anti-inflammatory activities (Bai et al., 2016). The *P. oleracea* protective effects against cognitive deficit in type 2 diabetes remain uncertain. One of the properties of this plant is the development of the brain and nervous system. In this regard, Zhang et al. demonstrated the neuroprotective effects of purslane aqueous extract against D-galactose-related neurotoxicity through a p21(waf1)-dependent and p53-independent pathway (Hongxing et al., 2007). On the other hand, *Plantago psyllium* is another herb with anti-diabetic effects. It is a bulk-forming laxative with high levels of fiber and mucilage. In this respect, it caused a decrease in serum glucose and glycosylated hemoglobin significantly in diabetic outpatients (Ziai et al., 2005).

Hence, we investigated the effect of co-administration of *P. oleracea* and *P. psyllium* plus submaximal swimming training on memory deficit in streptozotocin (STZ)/nicotinamide-induced type 2 diabetic rats.

Material And Methods

Subjects

Fifty male Wistar rats aged 9–10 weeks with a weight range of 250 to 300 g were purchased from the animal laboratory of Hamadan University of Medical Sciences. The rats were kept in a standard condition (12 hours of light and 12 hours of darkness and a temperature of 22±1 ° C, relative humidity: 55% to 60%). Rats were fed with laboratory chow. The study protocol was approved by the Institutional Animal Ethics Committee.

Induction of Type 2 Diabetes Mellitus

The rats were deprived of food 12 h before the induction of type 2 diabetes. Induction of type 2 diabetes was done by nicotinamide (120 mg/Kg, soluble in normal saline, Sigma-Aldrich, St. Louis, USA) that was administrated by intraperitoneal (IP) injection following the fasting period. The IP injection of STZ (65 mg/kg, soluble in citrate buffer (0.05M, pH=4.5), Sigma-Aldrich, St. Louis, USA) was injected 15 min after nicotinamide injection (Nasri et al., 2020). The control group rats received only subcutaneous injections

of citrate buffer. Seventy-two hours after injections, the FBS level in blood samples collected from the tail vein was measured and animals with the FBS of above 250 mg/dl were regarded as diabetic and used in the study (Fig. 1).

Experimental design

After a short-term acclimation of rats to the laboratory environment, healthy and diabetic rats were divided into the following groups:

Group 1: Control (Con)

Group 2: Untreated type 2 diabetic rats (D(

Group 3: Type 2 diabetic rats + *P. oleracea* and *P. psyllium* (D+PO+PP)

Group 4: Type 2 diabetic rats + exercise training (D+Tr)

Group 5: Type 2 diabetic rats + *P. oleracea* and *P. psyllium* + exercise training (D+PO+PP+Tr)

Exercise training

To alleviate stress without promoting adaptation to exercise, rats in the training groups were familiarized with water and swimming in the pool (70 × 80 × 100 cm, water temperature: 30-32° C) filled with water to a depth of 60 cm for 3 days (2 sessions per day, each session 10 min). The training protocol consisted of 12 weeks of progressive submaximal swimming exercise (5 days per week) (Chen et al., 2018, Lin et al., 2020). After the habituation, the rats swam for 15 min in the first two weeks, and then, gradually the swimming time increased to 50 minutes in the overload phase from the third to the tenth weeks, and in the last two weeks, the duration of swimming increased to 60 min. All rats swam while wearing a weight of 2% of their body weight attached to the tail. The body weight of all groups was monitored and recorded weekly.

Co-administration of *P. oleracea* and *P. psyllium*

P. oleracea and *P. psyllium* were collected from a local herb store of Hamedan and approved by the Department of Pharmacy of the Hamedan University of Medical Science. To prepare the food with *P. oleracea* and *P. psyllium*, 5 mg of *P. psyllium,* and 5 mg of *P. oleracea* (3 mg of *P. oleracea* seeds powder plus 2 mg of *P. oleracea* dried plant) were mixed with one liter of water to obtain a homogeneous solution. The solution was then mixed with 90% of normal food. *P. psyllium* and *P. oleracea* were mixed with standard pelleted food at a weight ratio of 10% and were received by the rats in the D+PO+PP and D+PO+PP+Tr groups for 12 weeks.

Shuttle box test

Passive avoidance memory was evaluated by the shuttle box test. The method of working with the device and process were fully mentioned in our previous papers (Zarrinkalam et al., 2016, Zarrinkalam et al., 2018, Karimi et al., 2020, Ahmadi et al., 2021). The device had two light and dark sections (20× 20 ×30 cm), with a grid stainless-steel rod floor attached to a shock generator and a guillotine door separated two compartments. At first, for acclimatization, the animals were placed in a lighted section and then, the guillotine door was opened and after 30 s of the entrance to the dark section, it was transferred to its home cage. Thirty minutes later, this test was repeated again. When the rat had its whole body in the dark section, the entrance latency to the dark chamber (step-through latency, STLa) was measured. The guillotine door between two sections was closed and then an electrical shock (0.8 mA) was applied to the rat for 2 s. Thirty seconds after an electrical shock, the rat was transferred to its home cage. The test was conducted again after 2 min. Each time the rat re-entered the dark section, it received an electric shock. When an animal stayed in the dark section for 120 s, the test was terminated and the number of trials was recorded (Zarrinkalam et al., 2016, Zarrinkalam et al., 2018, Ghaderi et al., 2020).

The retention test was executed 24 h after the PAL acquisition trial. In this phase, the rat was placed in the light section and the guillotine door was raised to the rat for 5 s and then, the step-through latency (STLr) and the time spent in the dark section (TDC) were measured for 600 (Zarrinkalam et al., 2016, Shiri et al., 2017).

Open field (OF) test

To determine the general locomotor activity and exploratory behavior of the subjects, the open field (OF) test was used. As described by our laboratory (Etaee et al., 2019), briefly, we carried out the test in a 100 × 100 × 40 cm hypethral box with the bottom divided into four identical squares on the floor of the arena. The rat was placed in the central square and had 10 min to explore. The total distance moved (locomotor activity) was recorded using a video camera and the data were analyzed through video track software (Etaee et al., 2019).

Novel object recognition (NOR) test

We used the novel object recognition (NOR) test to assess non-spatial memory in type 2 diabetic rats. As previously described (Lueptow, 2017, Kassab et al., 2019) with some minor modifications, the NOR test is a simple test that can be done over 3 days. During training, the rat could explore two similar objects. On the test day, one of the objects was replaced with a new one having a different shape and color. The rats prefer novelty, thus, when they recognize the familiar object, they prefer to spend most of their time with the new one (Lueptow, 2017, Kassab et al., 2019). The time spent exploring each object (sniffing or touching the object not standing, sitting on, or leaning against the object) was noted. The discrimination index (DI= (TNO – TFO)/(TNO + TFO) was also determined (TNO: the exploration time of the new object and TFO: the exploration time of the familiar object (Kassab et al., 2019, Shekarian et al., 2020). It should be noted that all sessions were video recorded and analyzed blindly.

Elevated plus-maze test (EPM)

The elevated plus-maze (EPM) was employed to evaluate anxiolytic activity. As previously described (Cavalcanti et al., 2020), the EPM apparatus consists of two opposing closed arms (10 × 50 cm) and two opposing open arms (50 × 10 × 50 cm) connected through a central square (10 × 10 cm), and the maze is 80 cm above the floor. Each rat was placed in the center of the device in front of one of the closed arms and could explore the maze for 10 min. The time spent in closed arms was video recorded and analyzed. The light intensity was 130 lux in the closed arms and 220 lux in open arms. The maze was cleaned using 10% ethanol after each test to get rid of any remaining odors.

Statistical analyses

Data were analyzed by SPSS version 20.0 (IBM SPSS Statistics). The Shapiro-Wilk test was used to assess the normal distribution of the data. The statistical difference between groups was estimated using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Values were expressed as mean ± SD. Values with a p-value of ≤ 0.05 were considered significant.

Results

Shuttle box

We assessed the effect of co-administration of *P. oleracea* and *P. psyllium* and swimming training for 12 weeks on passive avoidance memory in rats with type 2 diabetes. As shown in Figure 2, STLa in the D, D+PO+PP, and D+Tr groups was less than the control group. This result showed that diabetes reduced STLa. On the other hand, co-administration of *P. oleracea* and *P. psyllium* with swimming training significantly increased STLa compared with the D group.

According to Figure 3, no significant difference was detected in the number of trials to acquisition between the experimental groups (P>0.05).

Regarding STLr, the experimental groups showed significant differences (Figure 4). This parameter was lower in the diabetic groups than in the healthy control group (P<0.01). The STLr of the D+PO+PP and D+PO+PP+Tr groups were significantly higher than the D group (P<0.01).

Diabetic groups showed an increase in TDC than the healthy control group (p<0.05). Also, TDC was significantly lower in the D+PO+PP+Tr group than in the D group (Figure. 5).

Open field test

General locomotor activity and exploratory behavior of the experimental rats were evaluated by the open field test. The results showed that the total distance traveled was different between groups (Figure. 6). The distance traveled in the D group reduced significantly compared with the healthy control group, and the general locomotor activity and exploratory behavior in the D+PO+PP+Tr group showed a significant increase than the D group (P<0.05).

Novel object recognition test

Statistical analyses showed a meaningful reduction in discrimination index in the D group when compared to the healthy control group. Co-administration of *P. oleracea* and *P. psyllium* treatment for 12 weeks significantly elevated DI compared to the D group (Figure. 7).

Elevated Plus Maze

The effect of co-administration of *P. oleracea* and *P. psyllium* with submaximal swimming training on anxiolytic activity was assessed by EPM. The results showed that the time spent in close arms was similar between the experimental groups (Figure 8).

Discussion

We assessed the effect of co-administration of *P. oleracea* and *P. psyllium* with submaximal swimming training for 12 weeks on the cognitive deficit in type 2 diabetic rats.

Cognitive decline in diabetes

The results of this research showed that diabetes induction significantly reduced passive avoidance memory (↓36% STLa, ↓57%STLr, and ↑94%TDC), locomotor activity and curiosity (↓32% distance traveled), and non-spatial cognitive memory (↓34 % DI) compared with the healthy control rats. These findings are consistent with studies, in which it was shown brain abilities reduced in STZ-induced diabetic rats (Popoviç et al., 2001, Hasanein and Shahidi, 2010). Memory deficit in diabetic patients is associated with enhanced blood-brain barrier permeability, inflammation and oxidative stress, deregulated expression of 54 genes related to angiogenesis, inflammation, vasoconstriction/vasodilation, and an increase of least 2-fold in platelet activation pathways (including eNOS, TNFα, TGFβ1, VCAM-1, E-selectin, several chemokines, and MMP9), attenuated coverage of pericytes (Rom et al., 2019), elevated cortical atrophy, microstructural abnormalities in white matter tracts, neuronal function impairment, neural plasticity depression, and neurotransmitter reduction (McCrimmon et al., 2012, Tumminia et al., 2018).

Effect of *P. oleracea* and *P. psyllium* on cognitive deficit

Another main finding in this paper was that STLr and DI rose by 42% and 86%, respectively after co-administration of *P. oleracea* and *P. psyllium* for 12 weeks. This result showed that co-administration of *P. oleracea* and *P. psyllium* ameliorates spatial and non-spatial memory in type 2 diabetic rats. To date, no study has assessed the simultaneous effects of both herbs on memory impairment in diabetic rats, however, it was shown that *P. oleracea* possesses remarkable anxiolytic activity and can improve spatial cognitive performance, locomotor deficit, and stress in diabetic ovariectomized female rats (Tabatabaei et al., 2016). According to our knowledge, so far, no study has found the supportive effect of *P. psyllium* on the cognitive deficit in diabetic rats, however, it has been demonstrated that the diet supplemented with psyllium fiber facilitated the treatment of cases suffering from both diabetes and hepatic encephalopathy (Uribe et al., 1985).

Most diabetes complications and cognitive impairment in diabetic patients are caused by hyperglycemia. Since previous studies have shown that both *P. oleracea* and *P. psyllium* reduce hyperglycemia, co-administration of *P. oleracea* and *P. psyllium* could attenuate hyperglycemia.

On the other hand, *P. oleracea* and *P. psyllium* have antioxidant effects. Enhancement of inflammation and stress oxidative in the hippocampus and cerebral cortex of mammals, which play a pivotal role in a diverse set of cognitive functions, leads to a significant motor and memory deficit in behavioral functions of diabetic animals.

The positive effect of co-administration of *P. oleracea* and *P. psyllium* may be attributed to the antioxidant and anti-diabetic properties and neuroprotective effects.

Effect of exercise training on cognitive deficit

The result of this research showed that brain deficits were not affected after 12 weeks of submaximal swimming training in type 2 diabetic rats. Swimming training independently did not affect cognitive deficit in type 2 diabetic rats. This finding is not in agreement with previous studies indicating the amelioration of memory deficit by exercise training in diabetic rats (de Senna et al., 2017, Zarrinkalam et al., 2018). Most relevant studies have assessed the effect of running training (Mehta et al., 2019) and resistance training (Zarrinkalam et al., 2018, Cho et al., 2020) and have shown the positive effects of these training modes on reducing memory impairment in diabetic rats. To our knowledge, no study has yet examined the long-term effects of swimming training on memory and learning deficit in type 2 diabetic rats. Therefore, one of the possible causes of conflict with the results of previous research is the difference in the type of exercise. On the other hand, it is possible that low-intensity swimming is also a factor in the ineffectiveness of swimming training.

In general, studies have shown that exercise training modes stimulate neurogenesis in the hippocampus by reducing oxidative stress and inflammation, thereby improving memory cognitive in diabetic rats.

The effect co-administration of *P. oleracea* and *P. psyllium* plus Submaximal swimming training on memory deficit

A notable finding of this study was that co-administration of *P. oleracea* and *P. psyllium* plus exercise training ameliorated passive avoidance memory (↑64% STLa, ↑76%STLr, and ↓36%TDC) and locomotor activity and curiosity (↑36% distance traveled) compared with the diabetic control rats.

This finding suggests that the coexistence of several stimuli has a greater effect on reducing memory impairment in diabetic rats. According to our data, the molecular mechanism of such changes is still unclear. In the future, it is better to measure the changes in inflammatory and neurogenic indices to address the possible role of inflammation and neurogenesis in improving memory in diabetic rats.

Conclusion

Our research demonstrated that co-administration of *P. oleracea* and *P. psyllium* plus submaximal swimming training reversed the cognitive impairment in type 2 diabetic rats.

Declarations

Authors' contributions

All authors have assumed responsibility for data integrity and accuracy of the data analysis. Study concept and design: HP, FG, AK. Data acquisition: FG, KR. Data analysis and interpretation: HP, AK. Drafting of the manuscript: AK, KR. Critical revision of the manuscript for important intellectual content: AK and HP. Study supervision: AK. HP. All authors read and approved the final manuscript.

Data availability statements

The authors declare that the data supporting the findings of this study are available within the article [and its supplementary information files].

Acknowledgments

The authors would like to express their gratitude to the staff of the Neurophysiology Research Center for helping us to carry out this project. This study was supported by a grant (Grant number: 2663764) of the Hamadan University of Medical Sciences, Hamadan, Iran.

Compliance with ethical standards

Conflict of interest statement

We confirm that the authors do not have any conflict of interest with this publication.

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Figures

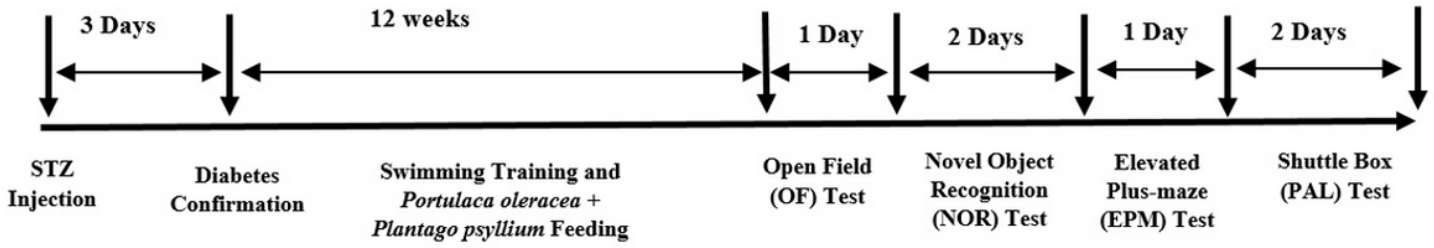

Figure 1

The experimental timeline. Type 2 diabetes was induced by a single IP injection of streptozotocin (65 mg/kg) and nicotinamide (120 mg/kg), and approved by a fasting glucose level of ≥250 mg/dL three days later. Swimming training was started one day after confirmation of diabetes. The rats underwent 12 weeks of progressive swimming training. During swimming training, the treated groups received Portulaca oleracea+Plantago psyllium mixed with standard pelleted food at a weight ratio of 5% for 12 weeks. To assess cognitive memory, the novel object recognition (NOR) and elevated plus maze (EPM) tests were used shuttle box test was used to measure aversive (acquisition and retention) learning and memory after the training programs, and the open field test was employed to measure locomotor activity.

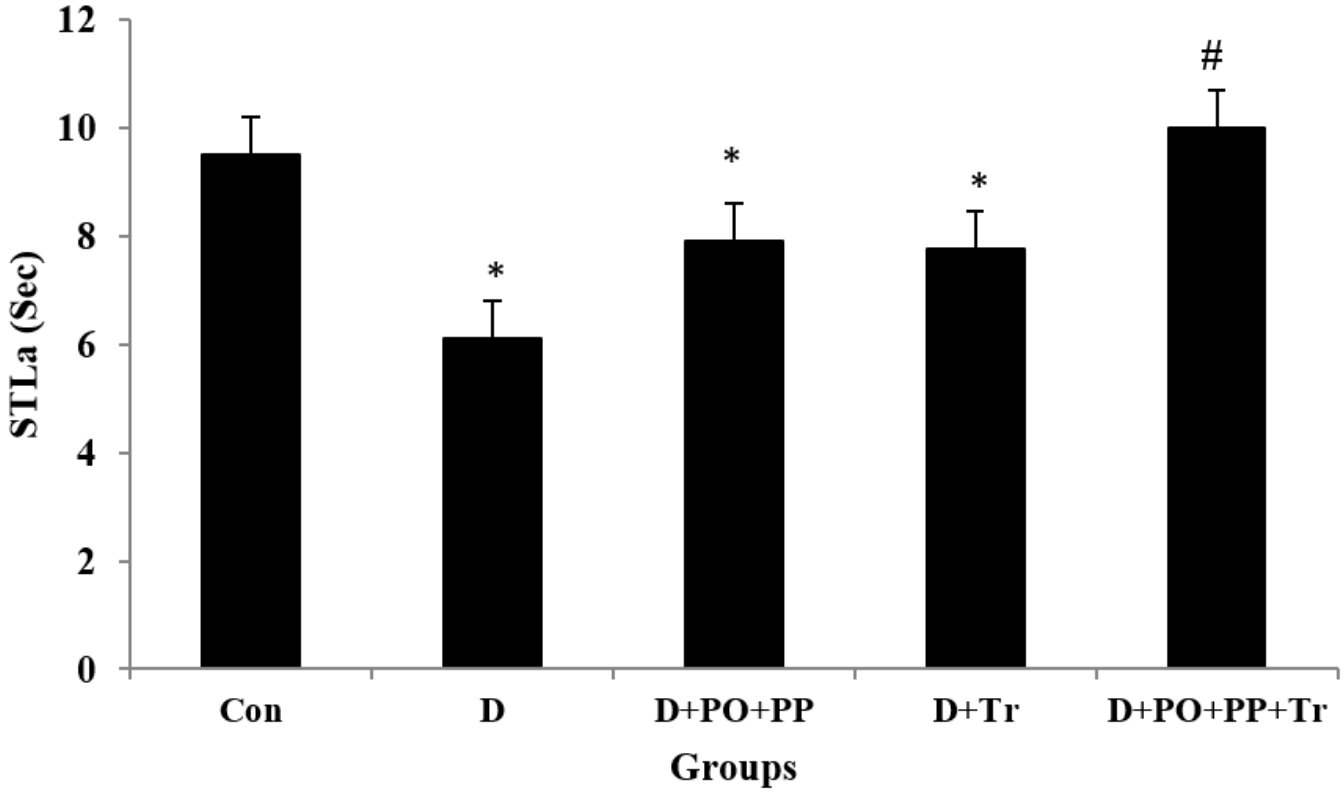

Figure 2

Comparison of the step-through latency (STLa) in the shuttle box test between groups. Values are presented as mean ± SEM. # Significant differences with the diabetic group ($p \leq 0.05$) and * significant differences compared with the control group ($p \leq 0.05$).

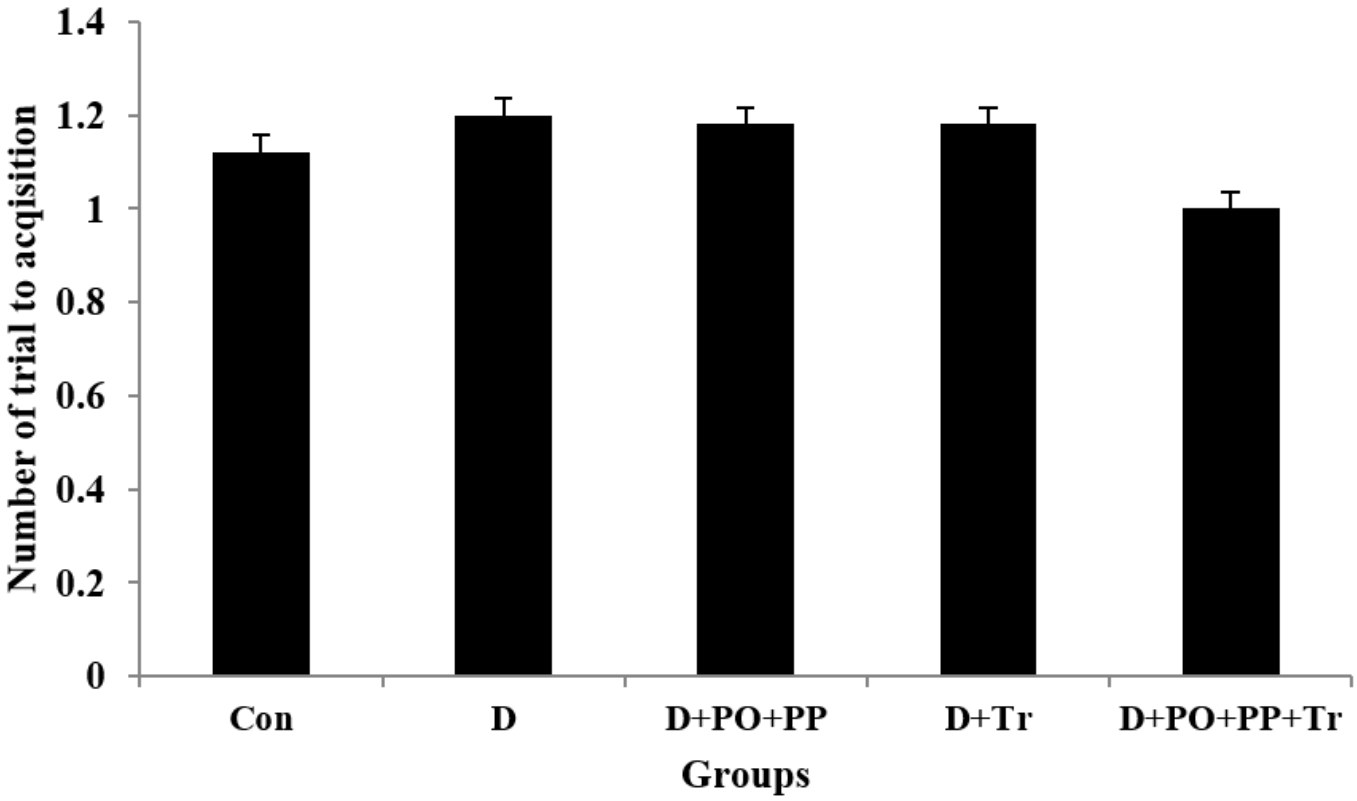

Figure 3

The number of trials to acquisition between experimental groups. Values are presented as mean ± SEM.

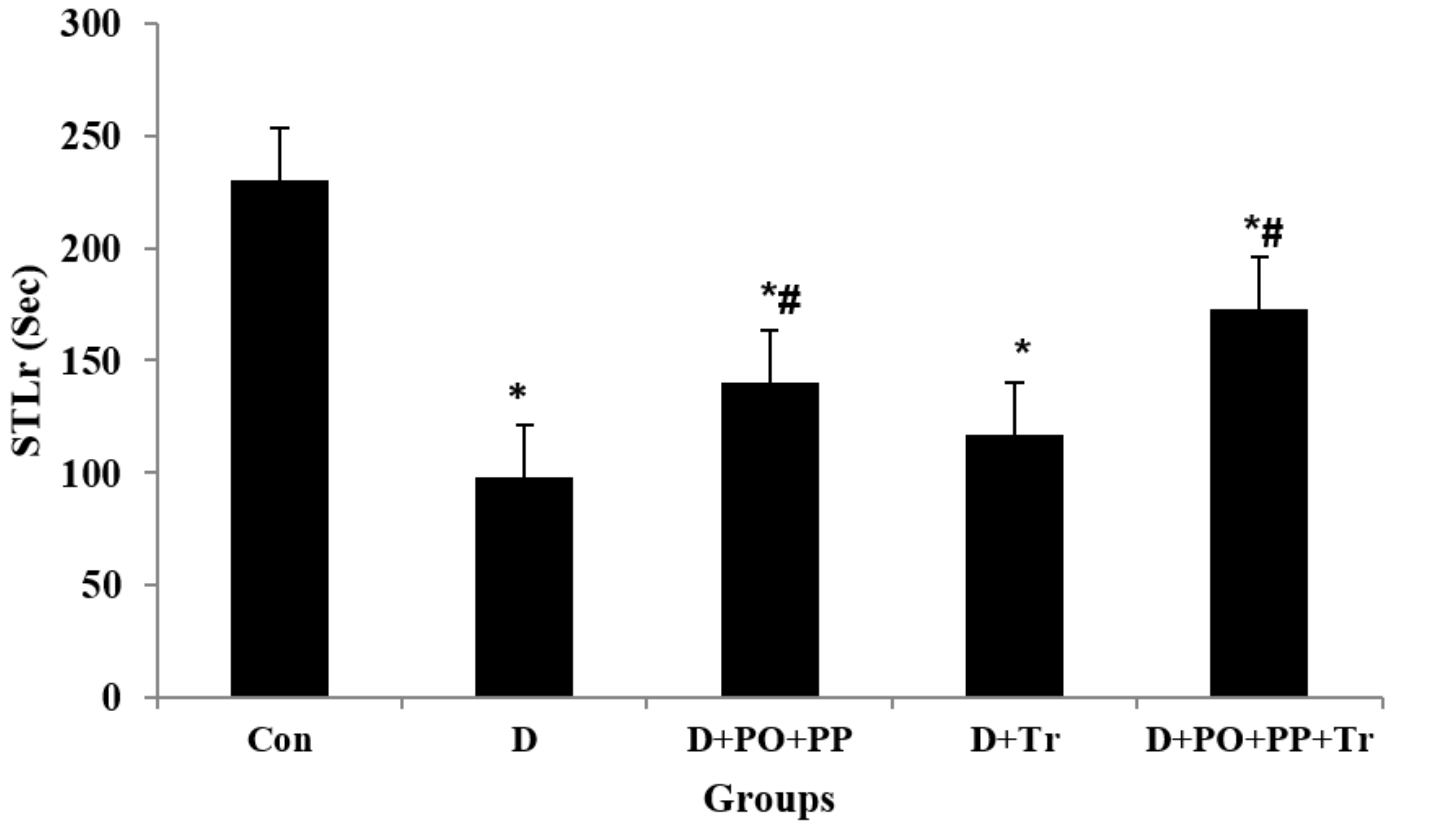

Figure 4

Step-through latency in retention phase (STLr) obtained in a passive avoidance test. Values are presented as mean ± SEM. # Significant difference compared with diabetic rats group ($p \leq 0.05$) and * significant difference compared with the control group ($p \leq 0.05$).

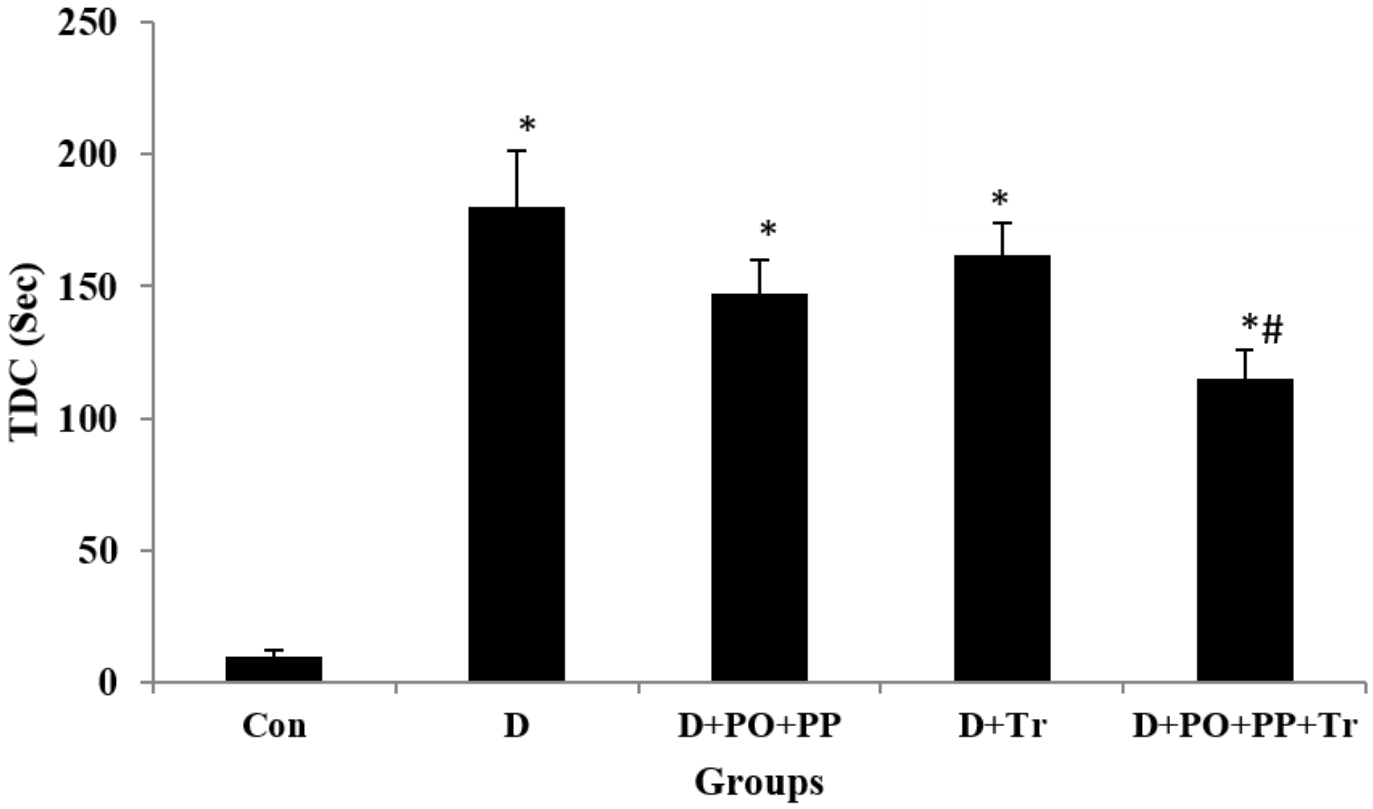

Figure 5

Comparison of the time spent in the dark compartment (TDC) between groups. Values are presented as mean ± SEM. # Significant difference compared with the diabetic group ($p \leq 0.05$) and * significant difference compared with the control group ($p \leq 0.05$).

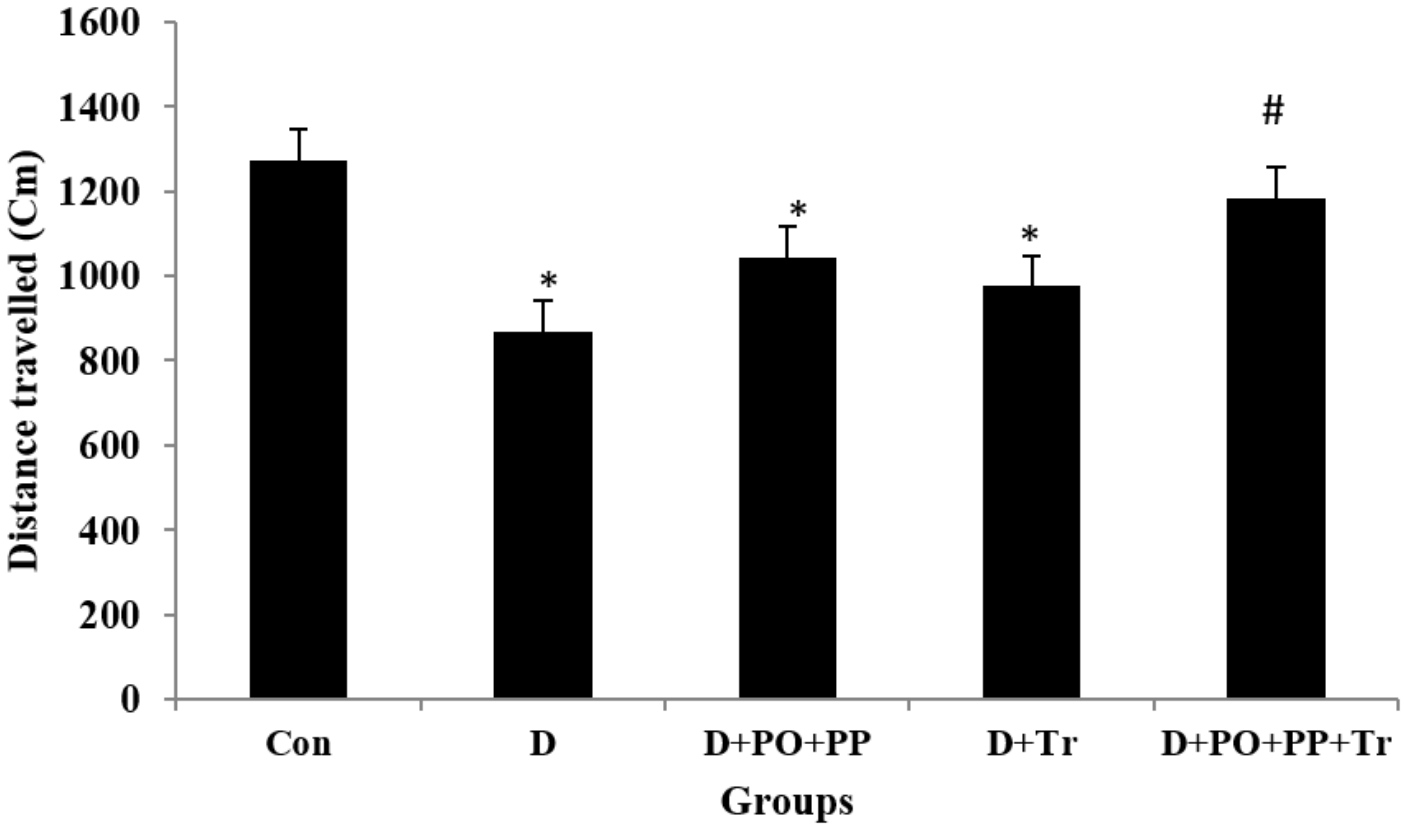

Figure 6

General locomotor activity and exploratory behavior were different between the experimental groups. Values are presented as mean ± SEM. # Significant difference compared with the diabetic group ($p \leq 0.05$) * significant difference compared with the control group ($p \leq 0.05$).

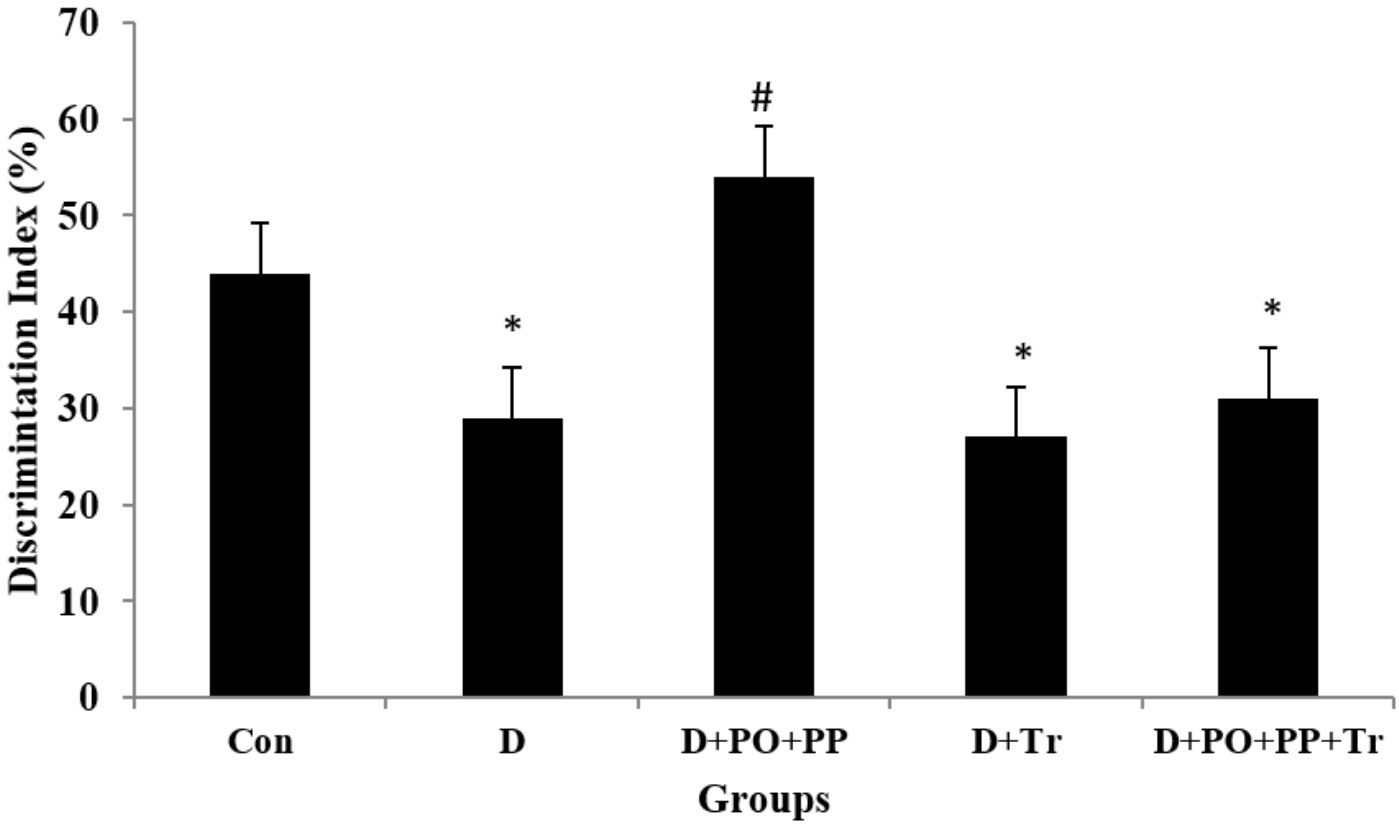

Figure 7

Comparison of the discrimination index in the novel object recognition test between groups. Values are presented as mean ± SEM. # Significant difference compared with the diabetic group ($p \leq 0.05$) and * significant difference compared with the control group ($p \leq 0.05$).

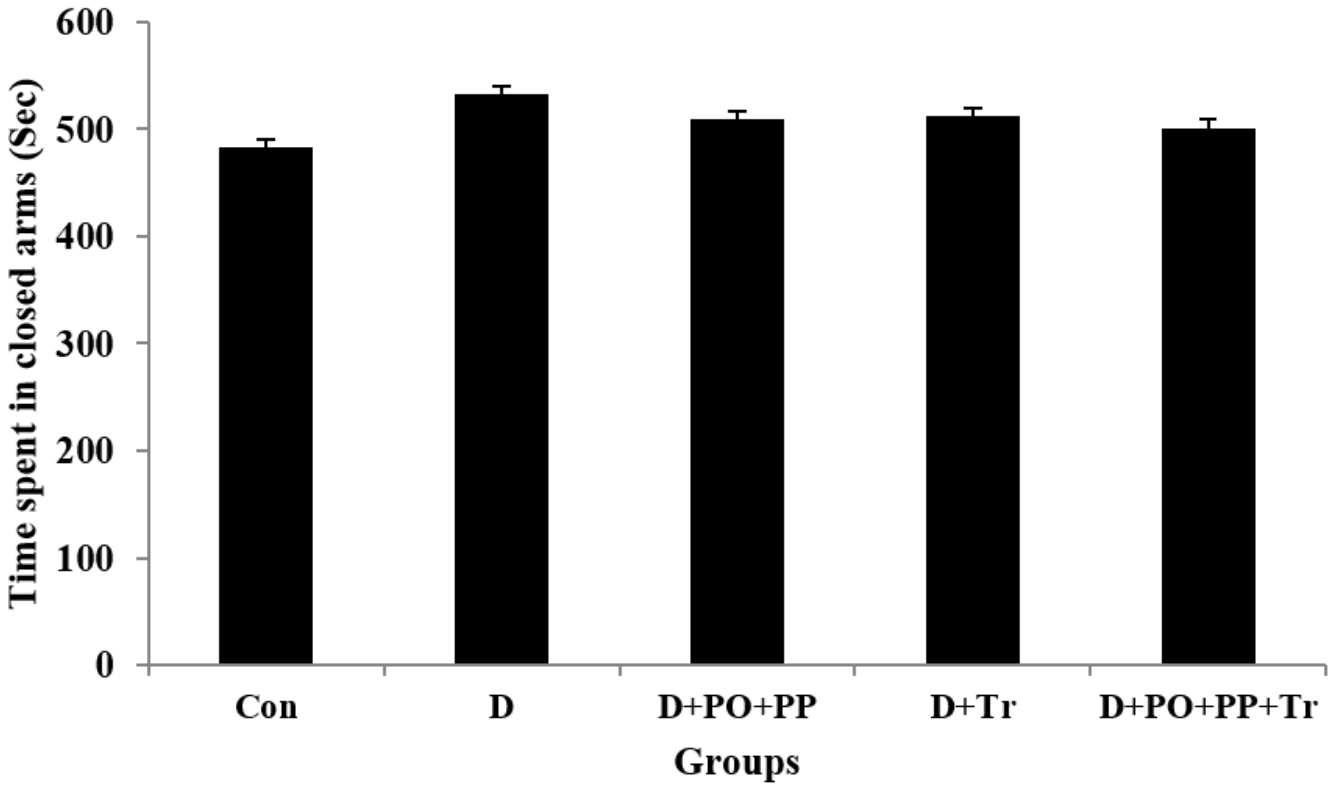

Figure 8

Anxiolytic activity was not different between groups. Values are presented as mean ± SEM.