Original Article

Protective effects of the traditional herbal formulation on oxidative stress, learning and memory in the animal model of type 2 diabetes

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Abstract

Introduction: Diabetes mellitus (DM) is one of the most frequent metabolic diseases that affect various body systems. Cognitive impairment caused by diabetes is gaining more acceptance and attention. In this study, we have investigated the effects of a traditionally herbal formulation (THF) on oxidative stress (OS) and cognitive deficits in type 2 diabetic rats.

Methods: Thirty-six male Wistar rats were divided into six groups: control group, diabetic group, diabetic+100, 200 or 300mg/kg THF, diabetic+glibenclamide (G) 5mg/kg. Streptozotocin-nicotinamide was used to induce type-II diabetes mellitus. Spatial and passive avoidance learning and memory function were evaluated by Morris Water Maze (MWM), novel object recognition test (NORT) and open field test (OFT). The OS biomarkers were also analyzed. The THF was standardized using RP-HPLC according to phenolic and flavonoids compounds.

Results: Indicated that in the diabetic treated (300mg/kg THF and G) vs. diabetic groups, body weight and insulin were significantly increased and the levels of fasting blood glucose significantly reduced. OS was improved in the treated (300mg/kg THF) groups. Furthermore, we noticed that diabetic treated groups (300mg/kg THF) vs. diabetes caused in significant decreases of the travelled distance and escape latency to find the hidden platform, also increased in the time spent and travelled distance in the target quadrant in MWM test, exploration time in NORT and total distance moved in OFT.

Conclusion: These findings suggest that THF ameliorated learning and memory deficits in type 2 diabetic rats via reducing OS. THF can be used with a caution against human DM.

http://dx.doi.org/10.32598/ppj.24.3.30

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease and is major public health problems in the world and studies showed that 592 million worldwide will suffer DM by 2035 (Guariguata et al., 2014). The prevalence of dementia and cognitive dysfunction is frequently observed in DM patients (Kodl and Seaquist, 2008; Zarrinkalam et al., 2017). The mechanisms underlying the development of cognitive dysfunction in DM have not been fully elucidated. Recently recognized that apoptosis, neuron degeneration and oxidative stress are significantly raised in the hippocampus of animal models of DM (Seto et al., 2015). The oxidative stress imposed by reactive oxygen species plays an important role in many chronic and degenerative diseases (Ardjmand et al., 2019; Goli et al., 2019).

Studies suggested that antidiabetic drugs could offer therapeutic benefits to DM-related impairment of cognitive functioning in both patients and animal models (Risner et al., 2006). On the other hand, these drugs frequently exerted side effects, including bone loss, weight gain and increased risk of cardiovascular events. These side effects could become more prevalent due to continuous use. Furthermore, treatment is very costly as well, since type 2 diabetes mellitus (T2DM) is a chronic disease and long-term medications are necessary (Li et al., 2004). Traditional herbal medicine (THM) can be a good alternative to replace or at least supplement to medications. A large number of THM has been used to treat T2DM complications for over thousands of years (Wang et al., 2013). THM treating T2DM can target multiple mechanisms such as raised insulin secretion and sensitivity, or decreased of carbohydrate absorption, anti-inflammatory and exert antioxidant effects (Li et al., 2004; Rahimi et al., 2018). Results from T2DM mouse model studies show that glycemic control using natural products and herbal extracts significantly prevented cognitive impairment via attenuation of cerebral oxidative stress and increase in cerebral brain-derived neurotrophic factor (Seto et al., 2015). THM contains oligomeric proanthocyanidins, flavonoids and polyphenols, which are well-known for their antioxidant, anti-inflammatory and antidiabetic properties (Ravan et al., 2017; Rios et al., 2015). Since the natural products and THM is relatively safe and have fewer side effects compared to other antidiabetic drugs, in this study we uses a combination of various herbs (cinnamon, nettles, *Achillea millefolium* [Yarrow], *Arctium lappa*, *Portulaca oleracea*, *Acroptilon repens*, *Sativus crocus*, Walnut and *Rhus coriaria* [Sumac)]) that are medically important and have great use in traditional medicine. Studies show that the mentioned plants have a high content of phenols and flavonoids such as gallic acid, benzoic acid, naringenin, rutin, quercetin and apigenin (Mansouri et al., 2013; Salehi et al., 2018).

Therefore, the purpose of this article was to examine the effect of the THF on impaired learning and memory function and oxidative stress indices in T2DM rats.

Materials and methods

Chemicals and drugs

Streptozotocin, nicotinamide, thiobarbituric acid, ferric chloride hexahydrate, n-butanol and sodium dodecyl sulfate were purchased from the Sigma Chemical Co. (United States). Nitric acid, perchloric acid and acetic acid (glacial) were purchased from Merck (Germany). All the other chemicals used were of analytical grade.

Plant extraction

The plants were purchased from the market and voucher samples were deposited at the Herbarium of the Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. About 100g of powdered plants were extracted with 500ml of distilled water for 48h using the maceration method and a deep brown aqueous extract was obtained. The extract was dried using a rotary evaporator and freeze drying and finally stored in universal bottles and refrigerated at 4°C prior to using. The dried substance was dissolved in normal saline (pH 7.4). Animals were treated with 100, 200 or 300mg/kg body weight of the THF, by gavage every day for 60 days.

Standardization of herbal formulation by RP-HPLC-PDA

Standardization of herbal formulation was done according to phenolic and flavonoids by RP-HPLC-PDA. The quantitative analysis was performed with external standardization by measurement of the peak areas using LabSolutions (Shimadzu) software. Seven different concentrations of ferulic acid, gallic
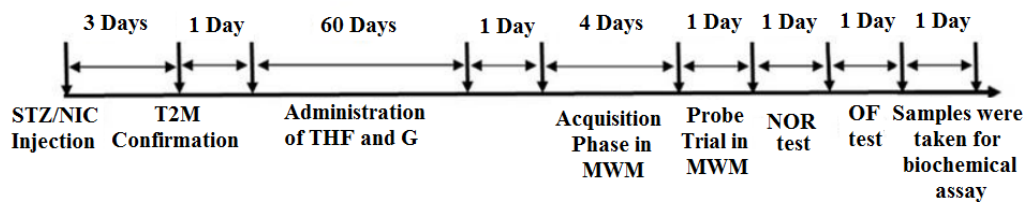
Fig.1. Experimental timeline. Type 2 diabetes mellitus model was induced by intraperitoneal injection of streptozotocin-nicotinamide, and confirmed by a fasting blood sugar level of >126mg/dl 3 days later. Administration of traditional herbal formulation (THF) in 100, 200 and 300 mg/kg, and glibenclamide 5mg/kg began 1 day after confirmation of T2DM, by gavage every day for 60 days. For measurement of learning and memory Morris water maze (MWM), novel object recognition (NOR) and open field (OF) test were used. Also, oxidative stress parameters in the hippocampus, FBS and serum insulin was measured.

acid, caffeic acid, benzoic acid, naringenin, rutin, quercetin and apigenin (Sigma-Aldrich) were used for the calibration curve. The HPLC column was a Spherisorb ODS-2 (5µm) reversed phase 4.6mm × 250mm and the flow rate of mobile phase (MeOH in H_2O/acetic acid (5–100% MeOH) was carried out at a 1ml/min. Different parameters including UV spectra, retention times and comparison with phenolic and flavonoid standards were used for the identification of compounds.

Animals and experimental designs
Thirty-six male Wistar rats (220±20g) were housed under standard conditions (12h dark/light cycle at 22±2°C). Animals were divided into 6 groups (n=6): group C, normal control; group D, diabetic control; diabetic rats treated with THF (100, 200 or 300mg/kg and glibenclamide [G] 5mg/kg respectively for 60 days). All procedures of this study were approved by the Medical Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1396.101).
For induction of T2DM, 15min after the injection of nicotinamide (120mg/kg; ip), streptozotocin (60mg/kg; ip) dissolved in 0.1M of citrate buffer (pH 4.5) was injected in fasted rats. To confirm the T2DM, after 72h fasting blood sugar (FBS) level of rats was measured with a glucometer (Accuchek; Roche, Germany), the animals were considered diabetic when FBS level of >126mg/dl (Shirwaikar et al., 2006). At the end of experiments, under ether anaesthesia blood samples were taken from the inferior vena cava, serum was separated and stored at –20°C for biochemical analysis. Also, the hippocampus tissue was collected from each rat and cleaned with an ice-cold saline solution and frozen in liquid nitrogen immediately after separation and

stored at –70°C until analysis. The experimental timeline is shown in Figure 1.

Assay of serum insulin and hippocampus oxidative stress parameters
Insulin concentration was determined using the rat insulin ELISA Kit (Mercodia, Uppsala, Sweden). The amount of hippocampus lipid peroxidation was predicted as the concentration of thiobarbituric acid reactive output malondialdehyde (MDA) according to Yagi's method (Zanganeh et al., 2018). Thiobarbituric acid reacts with MDA and is formed thiobarbituric acid reactant substances, as biomarkers of oxidative damage to polyunsaturated fatty acids. Total antioxidant capacity (TAC) determined by Benzi and Strain method. Hippocampus TAC was measured using the ferric reducing ability of serum which is a test shows antioxidant capacity and quantifies the ability of serum to reduce ferric ion to ferrous ion (Benzie and Strain, 1996). Hippocampus total thiol molecules (TTM) were determined using Ellman's reagent (DTNB; 5,5'-dithio-bis-[2-nitrobenzoic acid]) according to the Hu method and total oxidant status (TOS) of liver samples was determined by the ferric-xylenol orange 1 reagent, according to the method described by Erel, also the oxidative stress index (OSI) was calculated by dividing TOS/TAC (Kheiripour et al., 2019).

Behavioral tests
Morris Water Maze (MWM)
The MWM was used to measure the ability of spatial learning and memory. In brief, the water maze was a circular tank (180cm in diameter, 60cm in height, black coloured, filled to a depth of 25cm with water at 22±1°C) located in a room containing a variety of

Table 1: The effect of THF on rats' body weight, FBS and insulin in different studied groups

Parameters / Group	C	D	D + 100 mg/kg THF	D + 200 mg/kg THF	D + 300 mg/kg THF	D + G
Final body weight (g)	265.50±12.51	190.67±8.28 [a]*	200.00±9.40	210.33±10.34	257.78±11.03 [b]*	226.50±21.78 [b]*
FBS (mg/dl)	75.50±6.44	292.26±16.19 [a]*	269.50±18.49	253.67±17.50 [b]†	79.33±11.11 [b]*	207.17±17.88 [b]*
Insulin (μU/ml)	12.05±1.54	6.44±0.49 [a]*	7.09±0.66	7.35±0.57	12.51±0.0516 [b]*	8.13±0.71 [b]*

Results were expressed as mean±SD. C: control; D: diabetic; THF: traditional herbal formulation; G: glibenclamide 5mg/kg; FBS: fasting blood sugar. [a]significantly compared to the healthy control group. [b]significantly compared to the diabetic control group. [†]$P<0.05$, [‡]$P<0.01$ and [*]$P<0.001$.

visual cues. Low light was used for illumination and the room was sound insulated. The pool had four quadrants with four starting lines named north (N), east (E), south (S) and west (W) and an invisible Plexiglas platform (10cm in diameter) centrally located 1cm beneath the water in quadrant N. In our studies, 4-day training trials of animals were conducted at nearly the same time and each day had three blocks with four trials (90s). If an animal did not escape within 90s, it was manually guided to the escape platform by the experimenter. There was a 20s gap between three trials on the platform and the rest time was 5min between two consecutive blocks. Twenty-four hours after 4th training test, the probe test was performed in which the platform was removed from the pool and the rat was allowed to swim for 60s while we recorded the ratio of time spent in the target quadrant and the swimming speed (Asadbegi et al., 2017). All trials were processed online by a video track tracking system. The escape latency, the time spent in the target quadrant and the times crossing the platform were used to evaluate the animals spatial learning and memory ability (Zarrinkalam et al., 2017).

Novel object recognition test (NORT)
The NORT is used extensively to investigate the visuospatial memory of animals in a familiar environment. Twenty-four hours before the test, each of the rats was placed in an individual apparatus used for object recognition tasks (70×50×40cm) for 20min for acclimatization so that their exploratory behavior does not interfere with their interaction with objects. The next day, two identical objects (round or square bowls) were placed in the box, following which each rat was placed alone at the midpoint as well as next to the front wall of the box opposite the objects. They

were allowed to explore the objects for 10min and were then taken back to the cages (familiarization phase). One hour later, one of the familiar objects was replaced with a novel object and the rats were placed once more in the apparatus with the same object and novel object for them to explore for 5min (testing phase). A video camera mounted 100cm above the center of the box was used to monitor and record the activity of rats. The discrimination ratio was defined as the time spent with the novel object to the total time spent exploring either object. Object presentation was randomized and counterbalanced across animals and groups. After each trial, the box and the objects were cleaned with a 75% ethanol solution in order to prevent olfactory cues from being perceived by other rats (Dong et al., 2017).

Open field test (OFT)
Motor deficits, locomotor activity and anxiety in animals were measure by OFT. The animals were carried to the test room in their home cages and allowed to acclimate for 30min before the start of the locomotion test. The open field apparatus consisted of a clear Plexiglas box (100×100cm) with 30-cm-high walls and a white floor marked with a grid containing 16 squares. During a 5-min observation period, the animals were placed in one corner of the apparatus facing the wall. All paths taken by the rat were recorded with a computer-controlled tracking system. The number of squares crossed with all four paws was determined from the video recording. The open field box was washed with a 70% ethanol before other animals were placed in it in order to prevent olfactory cues from being perceived by other rats (Adebiyi et al., 2016).

Statistical analysis

Table 2. Standardization of traditional herbal formulation (THF) according phenolic and felavonoids components.

compounds	content (µg/g DW)	Retention time	λmax
Ferulic acid	123.1 ± 0.4	3.31	320
Gallic acid	2447.5 ± 8.4	6.42	270
Caffeic acid	58.6 ± 0.1	22.54	295
Benzoic acid	1301.0 ± 0.5	28.15	250
Naringenin	265.7 ± 0.4	30.30	285
Rutin	349.3 ± 0.2	33.44	360
Quercetin	134.7 ± 0.6	41.33	370
Apigenin	53.3 ± 0.2	52.22	336

Results are mean of three replicates with standard errors (mean±SEM, n=3), $P < 0.05$.

DW: dry weight

The SPSS software version 23.0 (SPSS Inc., Chicago, IL, U.S.A) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) were applied for statistical analysis. All data analyzed by one-way analyses of variance (ANOVA) followed by Tukey's post hoc test. Results were considered significantly different if $P < 0.05$.

Results

The effect of THF on body weight, FBS and insulin

At the end of the experiment, the final body weights and insulin in diabetic groups significantly decreased compared to the healthy control group ($P < 0.001$). Treatments (THF 300mg/kg and G) increased body weight and serum insulin level compared to the diabetic group ($P < 0.001$). FBS level was significantly increased in diabetic rats compared to the control group. In this regard, the results showed that the treatment with THF 200, 300mg/kg ($P < 0.001$) or G ($P < 0.05$) significantly decreased FBS compared to the diabetic group (Table 1).

The effect of THF on hippocampus oxidative toxic stress

The comparison of the hippocampus oxidative stress parameters levels between all groups is summarized in Figure 2. As shown, the streptozotocin/ nicotinamide resulted in a notable decrease in the TAC and TTM, also increase in the MDA, TOS and OSI level ($P < 0.001$). The administration of THF could relieve these oxidative markers by a significant increase in the TAC (THF 300mg/kg vs. D; $P < 0.001$) and TTM (THF 300mg/kg, $P < 0.01$ vs. D), and remarkable decrease in the MDA (THF 200mg/kg,

$P < 0.01$; 300 mg/kg, $P < 0.001$ vs. D), TOS (THF 300mg/kg, $P < 0.001$ vs. D) and OSI (THF 300mg/kg , $P < 0.001$, and G, $P < 0.01$vs. D).

The effect of THF on MWM, NORT and OFT

As shown in Figure 3, diabetes significantly increased of the travelled distance (Fig. 3A) and escape latency (Fig. 3B) to find the hidden platform in the diabetic group at all three blocks compared to the control ($P < 0.05$). THF 300mg/kg significantly decreased the distance and time to find the hidden platform in three blocks compared to diabetic group ($P < 0.05$). Diabetes resulted in significant decreases in the time spent (Fig. 3C) and travelled distance (Fig. 3D) in the target quadrant than those of the control group ($P < 0.001$). Animals of THF 300mg/kg group significantly showed an increase in these parameters compared to the diabetes group ($P < 0.001$).

Effect of THF on NORT illustrated in Figure 4. There was a significant decrease in exploration time in the diabetes group compared to the control group ($P < 0.001$). THF 300mg/kg returned this ration toward normal situation ($P < 0.05$).

Effects of THF on locomotion and anxiety-like behaviors in OFT are shown in Figure 4. There was a significant difference in the total distance moved (Fig. 4A) in the diabetes group compared to the control group ($P < 0.05$). Also these results confirmed that administration of THF 300mg/kg significantly increased in the total distance moved compared to diabetic group ($P < 0.05$). In addition, velocity (Fig. 4B) between all groups did not significantly different.

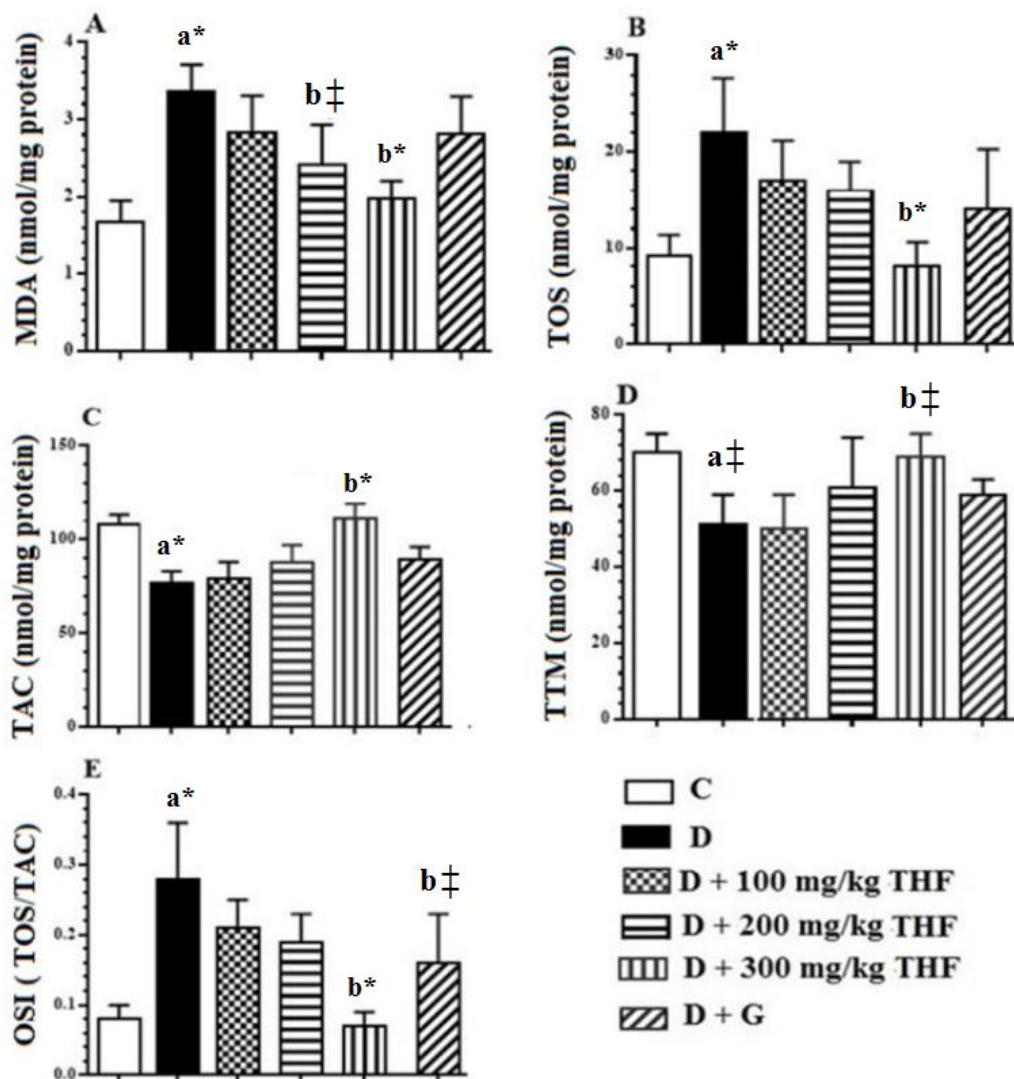### Standardization of herbal formulation by RP-HPLC-PDA

Fig.2. The effect of THF on hippocampus A: MDA, B: TOS, C: TAC, D: TTG, E: OSI. Results were expressed as mean±SD. C: control; D: diabetic; THF: traditional herbal formulation; G: glibenclamide 5mg/kg; MDA: malondialdehyde; TOS: total oxidant status; TAC: total antioxidant capacity; TTM: total thiol molecule; OSI: oxidative stress index. [a]significantly compared to the healthy control group. [b]significantly compared to the diabetic control group. [†]$P<0.05$, [‡]$P<0.01$ and [*]$P<0.001$.

Quantitative HPLC analysis indicated that gallic acid and benzoic acid (2447.5 to 1301.0 µg/g dry weight) were the main phenolic compounds in the herbal formulation. Also, the main flavonoids in herbal formulation were rutin and naringenin (349.3 to 265.7 µg/g dry weight).

Discussion

Millions of people around the world suffer from DM and the prevalence of this disease continues to the rise. DM is an endocrine metabolic disorder defined by chronic hyperglycemia due to the deficiency in insulin secretion and peripheral insulin resistance. DM is one of the main causes of the prevalence of cognitive impairment (Arvanitakis et al., 2004). In this regard, the result of the present study showed learning and memory were impaired in T2DM rats. Also, our investigation showed that natural antioxidants THF decreased FBS, hippocampus oxidative stress and ameliorated behavioral ability in T2DM rats.

Insulin resistance are usually present in patients with classic T2DM. Insulin is the main glucoregulator that promotes the uptake of glucose by tissues and its subsequent use as an energy source (Rodriguez-Araujo et al., 2013). This hormone also performs unique functions within the central nervous system. Studies have demonstrated that the reduces insulin secretion or resistance to its function in the central nervous system creates various complications,
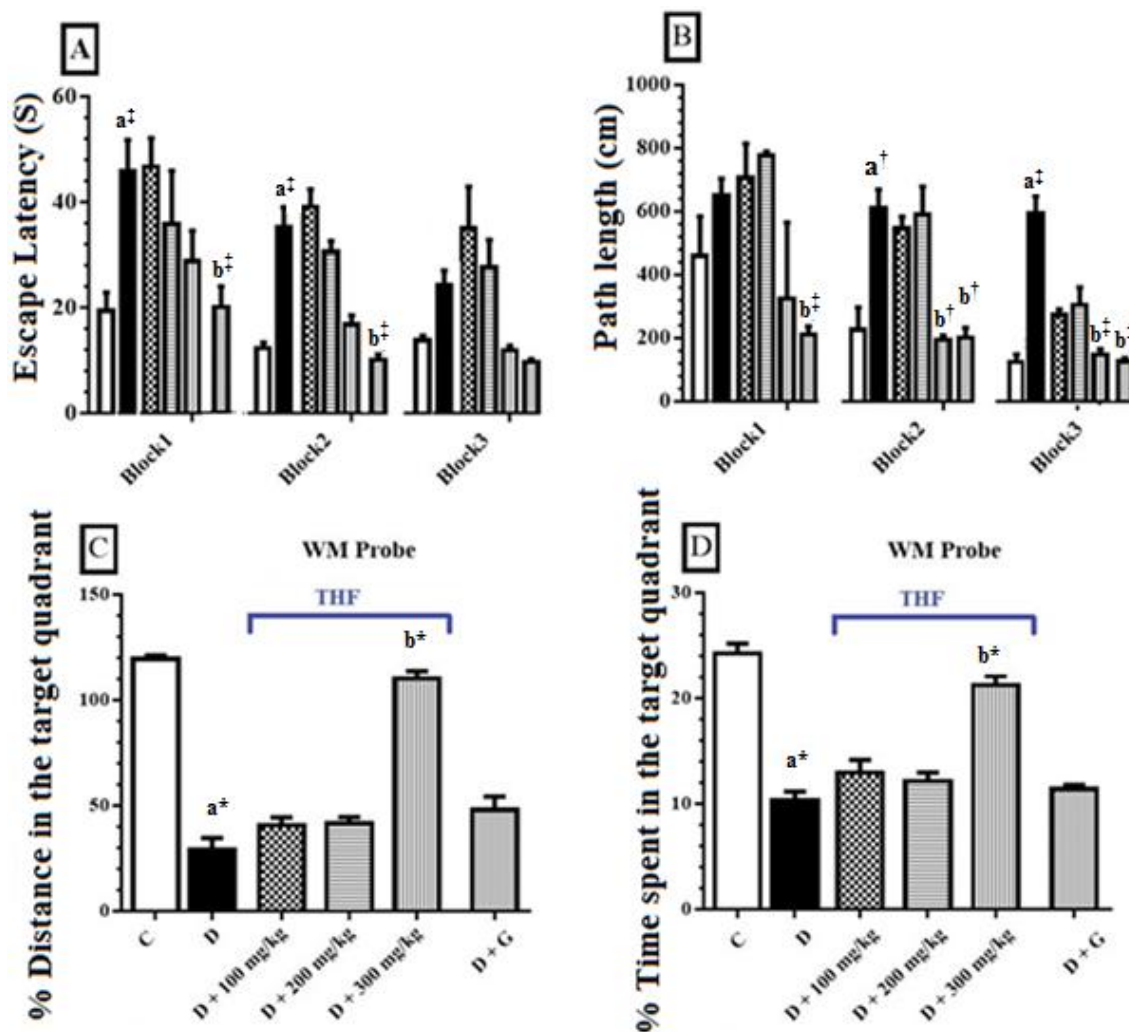
Fig.3. The effect of THF on spatial learning and memory in MWM test. C: control; D: diabetic; THF: traditional herbal formulation; G: glibenclamide 5mg/kg. Travelled distance (A) and escape latency (B) to find the hidden platform in three blocks. Time spent (C) and travelled distance (D) in the target quadrant. Data are shown as mean±SD. [a]significantly compared to the healthy control group .[b]significantly compared to the diabetic control group. [†]$P<0.05$, [‡]$P<0.01$ and [*]$P<0.001$.

ranging from mild forgetfulness to Alzheimer's disease (Ma et al., 2015). In our experiment, serum insulin concentration was decreased in the diabetic group. Whereas, treatments with THF stimulated secretion of insulin, this factor may improve the memory and learning of the studied rats.

Also, our results showed that the FBS of diabetic control rats was significantly increased, but in diabetic treatment with THF (200 and 300 mg/kg) and G, it was significantly decreased. Studies show that hyperglycemic control appears to play an important role in determining the degree of cognitive decrements or dementia detected in patients with T2DM (Watson and Craft, 2003). Although hyperglycemia may not lead to cognitive decrements or dementia directly, it could certainly reduce the threshold for these disorders. Oxidative stress has

been implicated as a contributor to both the onset and the progression of diabetes and its related complications. Hyperglycemia can lead to increase the production of oxidative stress markers, such as lipid peroxidation LPO and protein oxidation, with an accompanying reduction in antioxidant content, which have been suggested to be responsible for the induction of cognitive deficits often observed in diabetic rats (Comin et al., 2010; Fukui et al., 2002). In addition, hyperglycemia can contribute to the increased generation of advanced glycated end products (AGEs) (Wright et al., 2006). The cell surface receptor of AGEs (RAGE) can cause an increase in the production of intracellular oxidative stress (Ahmad et al., 2005). A diabetic animal with showed cognitive deterioration has been found to have over-expression of RAGEs in neurons in the
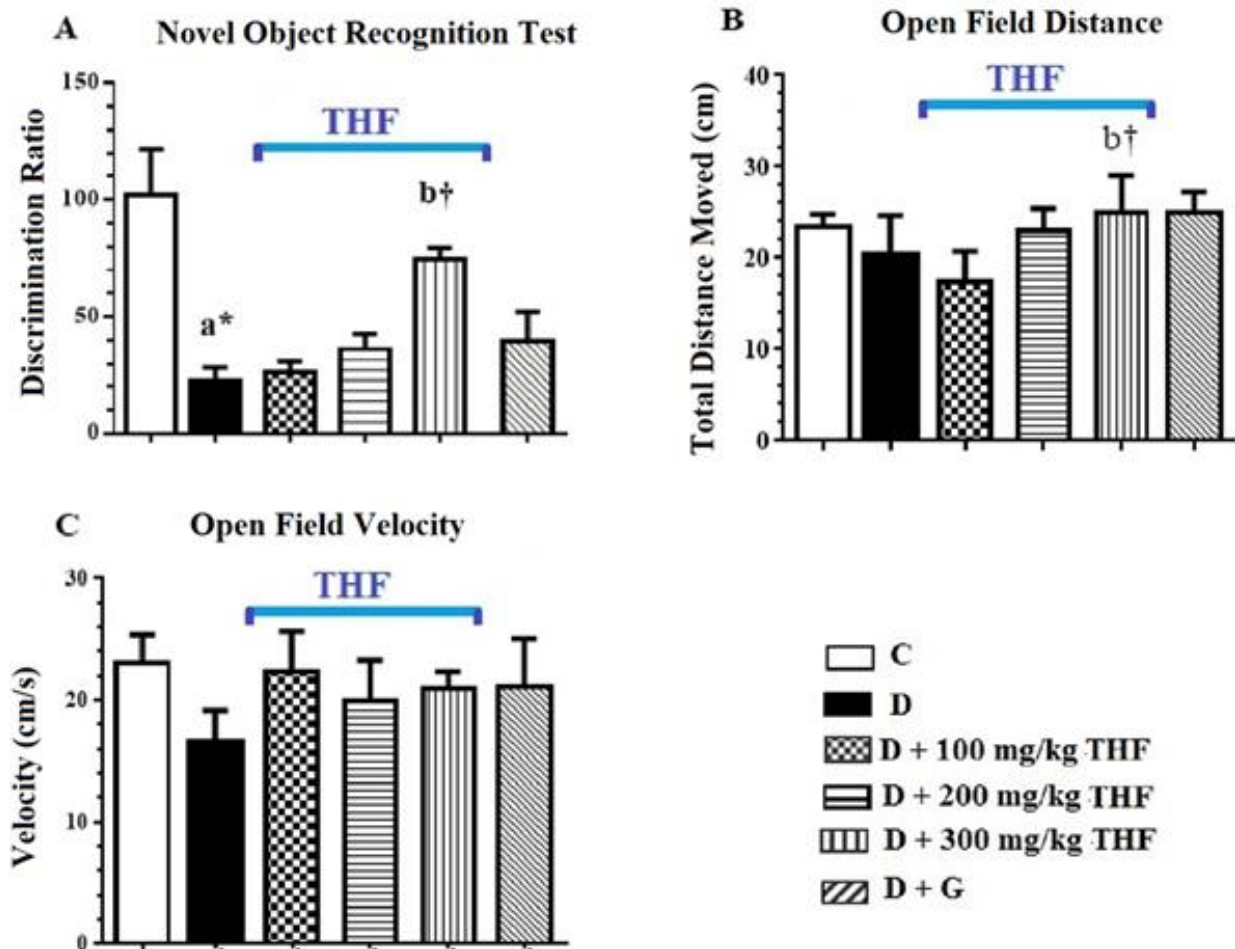
Fig.4. The effect of THF on novel object recognition test, locomotion and anxiety-like behaviors in open field test. C: control; D: diabetic; THF: traditional herbal formulation; G: glibenclamide 5mg/kg. There was a significant decrease in exploration time in the diabetes group compared to the control group (A, novel object recognition test). There was a significant difference in the total distance moved (B) and no significant difference in the velocity (C) between experimental groups. Data are shown as mean±SD. [a]significantly compared to the healthy control group .[b]significantly compared to the diabetic control group. [†]$P < 0.05$.

brain, suggesting the possible role of RAGEs in the progress of cerebral dysfunction (Toth et al., 2006). Therefore, insulin evaluation, FBS, and oxidative stress reduction may have a pivotal role in the improvement of diabetes-behavioral deficits. Results of our experiment demonstrate that serum FBS was more and insulin was decreased in the diabetic group. Whereas, THF treatments decreased serum FBS and increased the level of insulin.

The conditions of hyperglycemia in diabetics inhibits mitochondrial complex III and leads to the production of reactive oxygen spices in the hippocampus and other tissue. Increased oxidative stress in the brain seems to have an important role in the cognitive impairments caused by normal aging and neurodegenerative diseases (Ahmad et al., 2017). In this study, oxidative stress was investigated and the result indicated that the level of MDA, TOS and OSI

in streptozotocin/nicotinamide diabetic rat increased and TTM and TAC level was decreased compared to the healthy group. Treatment with THF can reduce the level of MDA, TOS and OSI and increase the concentration of TTM and TAC compare to the diabetic group.

Numerous studies have demonstrated the beneficial effects of THM on cognitive dysfunction and dementia in diabetic condition (Ganji et al., 2017; Xu et al., 2013). The pharmacological mechanisms of the medicine herbs can be classified as reducing carbohydrate absorption, stimulating insulin secretion, increasing insulin sensitization, increasing peripheral glucose uptake, antioxidant effects and increasing the glycogenesis or inhibiting hepatic glycogenolysis (Wang et al., 2013). As mentioned reduced antioxidative levels and increased oxidative stress production is closely associated with the

pathogenesis of diabetes and its complications such as neurodegenerative diseases. Studies have suggested the use of antioxidant supplementation to reduce oxidative stress and decelerate or prevent the development of disease-associated complications. Also, antioxidant properties of herbal medicines have been demonstrated in numerous studies (Sharifzadeh et al., 2017). Previous studies have investigated the antioxidant, antidiabetic and memory effects of the herbs used in the combination have been studied separately (Rezvani-Kamran et al., 2017; Shahidi and Yeo, 2018). Herbal formulation by RP-HPLC-PDA showed the THF contain ferulic acid, gallic acid, caffeic acid, benzoic acid, naringenin, rutin, quercetin and apigenin. The THF had an about 52% of gallic acid (3, 4, 5-trihydroxybenzoic acid). Pharmacological studies have demonstrated gallic acid is a potential antioxidant and neuroprotective effects from the family of phenolic compounds (Mansouri et al., 2013; Salehi et al., 2018). Gallic acid with three hydroxyl groups showed good antioxidant activity and can efficiently scavenge reactive oxygen specious (Velika and Kron, 2012). Also The THF had an about 23% of benzoic acid. Benzoic acid belongs to a group of phenolic compounds and showed antioxidant properties against different type of reactive oxygen specious and can prevent or decrease overproduction of them (Velika and Kron, 2012). The results of the present study indicate that the combined effects of these herbs are effective and may be a useful therapeutic option for memory disorders caused by diabetes.

In this study administration of a traditional herbal formulation has been shown to improve spatial learning and memory in MWM, NORT and OFT. Likewise, several studies have demonstrated that administration of traditional herbal formulation effect significantly greater than alone treatment on diabetes (Pirbalouti et al., 2012) and DM-associated cognitive impairment (Seto et al., 2015). Several mechanisms have been proposed as underlying the effect of traditional herbal formulation on DM-mediated cognitive dysfunction, such as through reduction of AGEs mediated neuroprotective and anti-inflammation effect, and reducing cerebral amyloid-beta (Wang et al., 2013; Xu et al., 2013). In our study, antioxidant effects, increased insulin level, as well as hypoglycemic effects of traditional herbal formulation, may improve learning and memory in water mazes,

NORT and OFT. Pal et al. (2012) determined a similar modulatory effect about traditional extract protects animals against CCl4 induced renal oxidative impairments and necrotic cell death. These results clearly showed the antioxidative and improving learning and memory of traditional herbal formulation in T2DM induced cognitive dysfunction. Although these findings need to be confirmed in humans, it provides important preclinical data to support the potential benefits of traditional herbal formulation in preventing and slowing the progression and development of DM-associated cognitive dysfunction.

Conclusion

In summary, our results showed that the administration of the THF reduced oxidative stress, enhanced learning and memory and reversed the memory impairment induced by a T2DM. Although, further studies are essential to improve our understanding of the neurobiological mechanisms underlying the effects of the THF administration on learning and memory with the aim of to provide THF as a pharmacological agent to humans.

Acknowledgments

Conflict of interest

No potential conflict of interest was reported by the authors.

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