Introduction

Diabetes is a group of metabolic syndromes associated with increased serum glucose level and memory impairment. In diabetic patients, hyperglycemia can increase reactive oxygen species (ROS) generation in liver, kidney, and brain. Free radicals and ROS may be involved in the pathogenesis of diabetes. These compounds can be quickly removed by glutathione peroxidase, superoxide dismutase, and catalase. Nowadays, there is an increasing trend toward the use of herbal supplements in the treatment of diabetes. Herbal drugs can be effective by lowering oxidative damage and reducing the production of free radicals. Natural antioxidants also have the potential to reduce hyperglycemia-induced brain lipid peroxidation and provide long-term remission for diabetes-induced neurological damages. Herbal drugs are much safer in comparison with chemical ones. Nevertheless, most of the clinicians are still prescribing synthetic drugs for diabetic patients without regarding their side effects. Hundreds of traditional plants have been reported to contain hypoglycemic and antioxidant activities, but only a small number of them have received academic evaluations. Spistan (Cordia myxa) is a widely used drug in central Asian and Middle Eastern Arab countries. Fenugreek or Trigonella foenum-graecum is a plant
from the Fabaceae family, which is cultivated in dry regions of central Asia and southern Europe. Fenugreek seeds contain numerous compounds that are biologically active such as Quercetin flavonoids and glycosides with strong antioxidant activity. In Iran's traditional medicine, Fenugreek seeds are used as astringent and appetizer. This plant is also used for headaches, muscles aches, and arthritis. Previous reports have pointed analgesic, anticoagulant, and antioxidant properties of fenugreek. Cordia myxa is a plant of Boraginaceae family, widely cultivated in central Asia. This is a naturally growing plant in Sistan and Baluchistan Province in Iran. In Baluchistan's traditional medicine, C. myxa is used as an antitussive, purgative and antipyretic. C. myxa has a wide range of antioxidant compounds as well as trace elements including magnesium, copper, iron, zinc, and selenium. Being a tropical country, Iran is abundant in medicinal plants used in ethnomedicine. Cordia myxa, along with other plants in Sistan and Baluchestan province, such as Prosopis farcta and Momordica charantia is a new candidate for novel research in the treatment of diabetes and obesity. In this regard, two medicinal plants known for their hypoglycemic effects were selected for this study in order to compare their effects against diabetes-induced lipid peroxidation and memory impairment. We selected the aqueous plant extracts to mimic the conventional drugs.

Methods

Animals

Locally bred adult male rats (270-300 g) were used for this experimental study. Animals were maintained in rooms with appropriate ventilation at a constant temperature of 20–23°C and 12 (h) light/dark cycles with free access to normal rodent food (Javaneh-Khorasan, Iran) and tap water.

Diabetes Induction

The alloxan monohydrate (120 mg/kg BW) (Sigma Ltd., USA) was utilized for diabetes induction in rats. A single intraperitoneal injection was administered to the rats. Diabetes induction was verified 3 days post-injection if blood glucose level was higher than 250 mg/dL.

Plant Extracts Preparation

The herbs were purchased from a shop in Zabol, Iran and were identified at Department of Biology, Faculty of Science, Zabol University by a plant taxonomist. The plants were powdered using an electric blender (Pars Khazar, Tehran, Iran). T. foenum-graecum L. seeds were extracted according to Xue et al. The standard method employed in a study conducted by Pirnia et al was used for the extraction of C. myxa fruits.

Treatments

Frothy-eight adult male rats were allocated to 4 groups. The first group was the healthy control that was orally treated with tap water. The second group was untreated diabetic rats (administrated with sterile tap water. The third group was diabetic rats treated with T. foenum-graecum seeds extract (TE) at a dose of 870 mg/kg. The fourth group was diabetic rats treated with C. myxa fruit extract (CE) at a daily dose of 500 mg/kg.

Serum and Brain Tissue Preparation

Blood samples were obtained by conventional methods. The obtained samples were quickly sent to the laboratory to separate the serum. Blood samples were centrifuged at 3000 rpm for 10 minutes. The collected serum samples were kept in a freezer at −20°C until analysis. After taking the blood samples, animals were euthanized by cervical dislocation and brain tissues were isolated. The fresh brain tissues were immediately washed with 0.9% NaCl and stored at −80°C for further measurement of malondialdehyde (MDA).

Measurement of Blood Glucose and Lipid Peroxidation

Fasting serum glucose level was determined enzymatically using ELISA commercial kit (Pars Azemoon, Tehran, Iran), according to the instructions of the manufacturer. As an advantageous marker of tissue lipid peroxidation, the level of brain malondialdehyde was determined by the standard method described previously by Buege and Aust. The Buege method is based on the chemical reaction of 2-thiobarbituric acid (TBA) with tissue MDA. The final product of MDA-TBA has an absorbance peak at 535 nm which can be read by a spectrophotometer.

Passive Avoidance Learning Test

The protocol was done as described previously. The apparatus which determines step-through passive avoidance consisted of a light chamber, and a dark chamber. The chambers consisted of light and dark plastic walls, respectively. The floor of the dark chamber was electrified by an electric shock generator. The shock was transferred to the animal's feet using stainless steel bar-shaped tools (with a diameter of 3 mm) located beneath both chambers. For each rat, 2 trials were conducted to habituate the animal to the testing procedure. They were all placed in a lighted chamber of the apparatus and 5 seconds later the door was opened. All the rats had an intrinsic inclination for the dark chamber. As a rat entered the dark chamber, the guillotine was closed and after 30 seconds the animal was taken out of the dark.

At the end of the trial, rats were moved to their cages. The mentioned protocol was performed again after 30 minutes. The latency to enter the dark chamber (step-through latency, STLa) was recorded when the rat completely entered the dark chamber. After it had spontaneously placed all 4 paws into the dark chamber, the door was closed and an electrical shock (0.5 mA) was applied for 3 seconds. A half- minute later, the rat was taken from

the dark chamber to its cage. The previous procedure was repeated two minutes later for the third time. When an animal remained in the light chamber for 2 minutes, the experiment was considered terminated. For each rat, the number of entries into the dark compartment was carefully recorded.

Retention Test
In retention test, avoidance learning was tested without the electric shock. The subject rat was placed in the illuminated chamber and the door was opened after 5 seconds. The time (seconds) each rat spent in the dark chamber and the STLr or STL were documented for about 6 minutes. In cases in which the animal did not enter the chamber within 6 minutes, the test was stopped, as the maximum score of six minutes was assigned for the test.20

Statistical Analysis
The data was analyzed using the one-way analysis of variance (ANOVA) and SPSS software (version 18.0). The results were expressed as mean ± standard deviation (SD). The Kolmogorov-Smirnov test served to verify the normality of the data distribution. Multiple comparisons between groups were performed using Tukey post hoc test. Statistical significance was determined at \( P \leq 0.05 \).

Results
According to Figure 1, diabetic rats showed severe hyperglycemia compared with rats in control group \( (P<0.001) \). The oral gavage of TE (870 mg/kg) notably reduced the elevated level of serum glucose, compared to control rats \( (P<0.001) \). Similarly, CE administration (500 mg/kg) decreased the elevated level of serum glucose in treated diabetic rats compared to the control diabetic animals \( (P<0.001) \). No significant difference was detected in serum glucose levels between CE and TE treated groups.

Oral supplementation of CE significantly decreased the brain MDA levels compared to the untreated diabetic rats. Oral supplementation of TE significantly reduced the brain level of MDA in diabetic rats compared to untreated diabetic animals \( (P<0.05) \) (Figure 2). No significant difference was found between CE and TE treated groups regarding brain MDA level. These results indicate that aqueous extracts of both plants have similar effects against diabetes-induced lipid peroxidation.

Compared to untreated diabetic rats, chronic oral feeding of TE significantly reduced TDC (the time spent in the dark chamber), number of trials, while it increased STL. On the other hand, the oral administration of CE did not have any significant effects on diabetes-induced cognitive impairment \( (P>0.05) \) (Figure 3). Results of Tukey’s post hoc test revealed that STL in TE group was significantly higher than CE group \( (P<0.001) \). Furthermore, treatment with TE significantly reduced the time spent in the dark compartment compared to the CE treated animals \( (P<0.01) \).

Discussion
Our aim was to examine the effects of 2 conventional antidiabetic herbs on diabetes-induced memory loss and brain lipid peroxidation in diabetic rats. As demonstrated by the results, the oral administration of aqueous extract of \( C. myxa \) fruits could markedly reduce serum glucose and brain lipid peroxidation levels. In addition, TE supplementation had the potential to significantly decrease brain MDA level.

Diabetes mellitus is the main cause of memory problems, as well as liver injury, and kidney failure. High glucose levels could result in lipid peroxidation and increased brain MDA contents, leading to long-term memory problems.21 Brain lipid peroxidation was linked to the severity of brain degeneration in animal models of diabetes mellitus.23 Herbal antioxidants have been noted to decline brain lipid peroxidation and oxidative damages.22 In the current study, higher serum glucose levels in diabetic rats were significantly reduced after being treated with \( C. myxa \) fruit extract, which was in accordance with previous observations.24 \( T. foenum-graecum \) seed extract also reduced the high levels of

![Figure 1. The Effects of CE and TE on Serum Glucose Level in Diabetic Rats (n = 12).](image1)

![Figure 2. The Effects of CE and TE on Cerebral MDA in Diabetic Rats (n = 12).](image2)
Concerning the antidiabetic properties of these plants, the hypoglycemic effects of both extracts verified their ethnobotanical applications.25,26 Aqueous extract of fenugreek seeds was capable of abolishing the elevated levels of serum malondialdehyde in a model of chronic ethanol toxicity.27 Furthermore, the polyphenol compounds of *T. foenum graecum* seeds significantly decreased serum lipid peroxidation.28 In insulin-resistant mice; fenugreek seeds extract improved high glucose-induced insulin resistance.29

**Conclusion**
Administration of TE prevented cognitive impairment and prevented lipid peroxidation significantly. In addition, the treatment with CE notably decreased the serum glucose and brain MDA but did not affect diabetes-induced cognitive impairment. Both herbal extracts had antidiabetic and anti-lipid peroxidative effects and can be considered as useful therapeutic agents against diabetes-induced memory impairment in rats.

**Competing Interests**
There are no conflicts of interest.

**Ethical Approval**
Experimental animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health [NIH] publication 86–23; revised 1985).

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**References**


