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Original Article

The effect of *Cinnamomum zeylanicum* bark water extract on memory performance in alloxan-induced diabetic mice

Azadeh Mesripour[1,*], Fatemeh Moghimi[2], and Mahmoud Rafieian-Kopaie[2]

[1]Department of Pharmacology and Toxicology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[2]Medical plant research center, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran.

Abstract

Cinnamomum zeylanicum (cinnamon) has a wide range of beneficial effects including mild glucose lowering activity. The aim of the present study was to investigate whether cinnamon bark extract has the potential to improve memory performance and glucose profiles in diabetic mice. Memory was assessed by the novel object recognition task in male Balb/c mice. In this method, the difference between exploration time of a familiar object and a novel object was considered as an index of memory performance (recognition index, RI). The water extract was prepared by boiling cinnamon bark for 15 min. Alloxan induced diabetes in animals (serum glucose levels were 322 ± 7.5 mg/dL), and also impaired memory performance (RI= -3.3% ± 3.3) which differed significantly from control animals (RI = 32% ± 6.5). Although treatment with cinnamon only reduced fasting blood glucose level moderately but it improved memory performance remarkably (RI = 25.5% ± 5.6). Oxidative stress following administration of cinnamon extract was lower in diabetic mice. It was concluded that cinnamon water extract could be a useful alternative medicine in diabetic patients' daily regimen which not only reduces blood glucose levels but also improves memory performance and lipid peroxidation level.

Keywords: Memory; Cinnamon; Diabetes mellitus; Cognitive; Alloxan; Oxidative stress

INTRODUCTION

Long-term diabetes is associated with end-organ damage in the eyes, kidneys, peripheral nerves and also in the brain. Gradually progressive alterations in cerebral performance and structure that occur in association with diabetes are referred to as "diabetic encephalopathy" (1). The number of studies that show clinically relevant changes in cerebral functioning in diabetic patients is growing. Manifestations of diabetic encephalopathy in type 1 diabetes mellitus patients include changes in cognitive functioning, which are considered by a mild to moderate slowing of mental speed and a diminished mental flexibility, without evident impairments of learning and memory (2,3), and in type 2 diabetes mellitus patients include moderate impairments of verbal memory and mental speed (4,5). In elderly patients with type 2 diabetes mellitus the cognitive impairments may be more obvious, that can hamper their daily functioning (6). In addition, the prevalence of dementia appears to be doubled in the elderly with diabetes (7).

Plant-derived spices are added to food preparations to improve their taste and aroma. Their medicinal properties are well known and they are documented to possess antioxidant, antidiabetic, antiallergic, antiatherosclerotic effects in animals (8). *Cinnamomum zeylanicum* (cinnamon), an evergreen tree, belongs to the family Lauraceae which has been traditionally harvested in Asian countries. It is one of the oldest herbal medicines that have been mentioned in Chinese texts as early as 4,000 years ago (9). The bark of cinnamon possesses significant antiallergic, anti-ulcerogenic, antipyretic and antioxidant properties (9,10). We were interested in studying the bark of cinnamon used as culinary herb in oriental countries. The possible role of cinnamon on insulin action has

*Corresponding Author: A. Mesripour
Tel: 0098 31 36688064, Fax: 0098 31 36680011
Email: a_mesripour@yahoo.com

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been presented in several *in vitro* studies (11-13). Water soluble cinnamon compounds in the diet could reduce risk factors associated with diabetes and cardiovascular disease because of its antioxidant effects (14).

Owing to the beneficial effects of cinnamon on glucose profile and its antioxidant activity, this research was designed to firstly investigate the impact of diabetes on cognition in mice and secondly to evaluate the effect of cinnamon extract on memory performance in a mouse model.

MATERIALS AND METHODS

Animals

Male Balb/C mice weighing 25-30 g were housed in cages of six animals each at 21 ± 2 ºC in a 12 h light-dark cycle with the lights on at day time 6 AM - 6 PM. Tap water and standard food pellets were available *ad libitum*. Tests were performed only after the mice had acclimated to the above environment for at least 1 day. In order to minimize circadian rhythm influence, all experiments were conducted between 08:00 and 13:00 h, in a special noise-free room with controlled illumination. Minimum of six mice were used for each treatment group. Experiment was comprised of 5 groups: control group (received normal saline), cinnamon group (10 mL/kg cinnamon extract), diabetic group (received water), treatment 1 (diabetic mice received 10 mL/kg cinnamon extract), and treatment 2 (diabetic mice received 10 mL/kg one-fold diluted cinnamon extract). All animal procedures were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Novel object recognition task

The novel object recognition task was performed as described previously (15,16). Briefly, square wooden open-field (35 × 35 × 40 cm) with the inside painted in dark black and a white floor was used. It was placed in a dark room with a uniform dim light toward the apparatus. At the first day, animals were submitted to a habituation session in the open field and allowed to freely explore the arena in the presence of two objects for at least 15 min. On experimental day, animals were submitted to two trials spaced by an interval of 20 min. During the first trial (acquisition trial, T1), animals were individually placed in the arena containing two identical objects for an amount of time necessary to explore the objects for 20 s. Any mouse not exploring the objects for 20 s within the 12 min period was excluded from experiments. Exploration is defined as the animal directing the nose within 2 cm of the object while looking at, sniffing, or touching it. At the second trial (test trial, T2), one of the objects presented in the first trial was replaced by a new object, animals were placed back in the arena for 5 min and total time spent to explore each of the objects were determined.

The following parameters were measured: time required to achieve 20 s of object exploration on T1 (duration of T1), time spent for active exploration of the familiar (F) or novel (N) object on T2. Recognition memory was evaluated using a recognition index (RI) calculated for each animal using the formula: (N−F/N+F) × 100 corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects (15). Positive values indicate a good discrimination performance, while negative values or those around zero indicate very poor discrimination capacity. Animals' behaviors were recorded by EasyCap mounted on the top of the apparatus and analyzed later.

Induction of diabetes

Alloxan monohydrate (Sigma Aldrich, St. Louis, MO, USA; stored at 4 °C) was dissolved in normal saline at room temperature and was injected intraperitoenally at 120 mg/kg in overnight fasted animals once daily for 3 consecutive days. Four days later (96 h) blood glucose levels of the animals were measured by glucometer (On-Call Plus, USA) and animals with glucose levels greater than 250 mg/dL were considered diabetic.

Cinnamon water extract

Cinnamon bark was prepared from local market in city of Isfahan (Iran) and approved by the Medical Plant Research Center, Shahrekord University of Medical Sciences,

Shahrekord (Iran). In order to prepare cinnamon water extract, 1 g cinnamon bark powder was boiled in 100 mL water for 15 min. It was then filtered and the water extract was used (17). The final solution was injected at 10 mL/kg to the animals. Additionally, the solution was diluted by normal saline (1/1 v/v and 1/2 v/v) and injected in the same volume. Since the solution prepared by two-fold dilution did not lower the blood fasting glucose level in animals it was excluded from further investigations.

Sample collection

Mice were lightly anesthetized with diethyl ether inhalation, rapidly decapitated and trunk blood samples were collected. Following centrifugation of blood samples, serum was transferred to small-capped vials and stored frozen at -20 ºC until analyzed.

Lipid peroxidation assay

The analysis of lipid peroxidation was carried out as described by Buege *et al.* with a minor modification. The reaction mixture was prepared by adding 250 mL homogenate into 2 mL reaction solution (15% trichloroacetic acid/0.375% thiobarbituric acid/0.25N HCl, 1:1:1, w/v) and heated at 1008 ºC for 15 min. The mixture was cooled to room temperature, centrifuged at 10000 g for 10 min and the absorbance of the supernatant was recorded at 532 nm. Malonodialdehyde (MDA) results were expressed as nmol/mg protein in the homogenate (18).

Data processing and statistical analysis

Results were expressed as group mean ± SEM. All results were analyzed by a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests, p values less than 0.05 were considered significant. GraphPad Prizm 5 software was used for data analyzing and constructing the graphs.

RESULTS

Fig. 1 represents serum glucose levels of different groups of animals. Diabetic group showed very high fasting serum glucose levels (322 ± 7.5; $p < 0.001$) compared to control animals.

Treatment of diabetic animals with cinnamon extracts significantly reduced glucose levels though did not reach to the normal values.

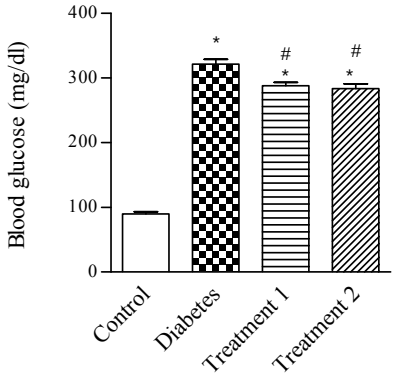

Fig. 1. Blood fasting glucose levels (mg/dL) measured on the day of memory experiments. Diabetes was induced by aloxan (120 mg/kg). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's multiple comparison tests, n = 6. * P <0.05 compared with control group, # $P < 0.05$ compared with diabetic group. Treatment 1; diabetic mice received 10 mL/kg cinnamon extract, treatment 2; diabetic mice received 10 mL/kg one-fold diluted cinnamon extract.

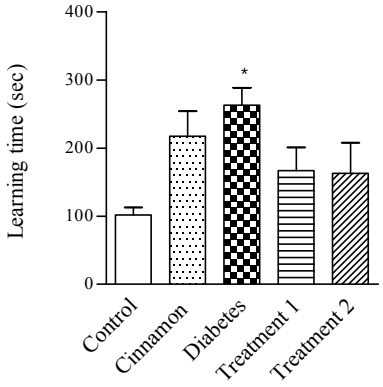

Fig. 2. Effect of different treatments on duration of T1 (time required to achieve 20 second of object exploration in the first trial) in mice, n = 6. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's multiple comparison tests, n = 6. * $P < 0.05$ compared with control group, Treatment 1; diabetic mice received 10 mL/kg cinnamon extract, treatment 2; diabetic mice received 10 mL/kg one-fold diluted cinnamon extract.

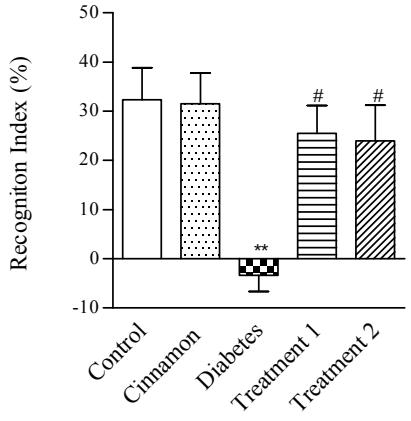

Fig. 3. The effect of different treatments on memory performance. Memory was assessed by recognition index (RI), in the novel object recognition task. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's multiple comparison tests, n = 6. ** p <0.01 compared with control (blank bar), $^{\#}p < 0.05$ compared with diabetic group. Treatment 1; diabetic mice received 10 mL/kg cinnamon extract, treatment 2; diabetic mice received 10 mL/kg one-fold diluted cinnamon extract.

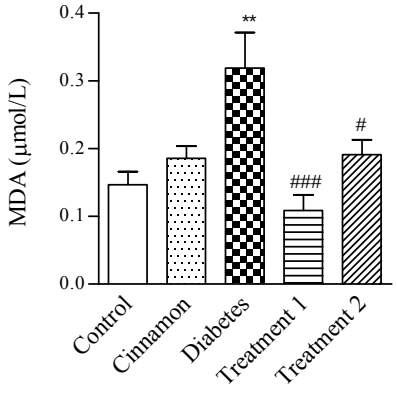

Fig. 4. Serum malonodialdehyde level µmol/L. Malonodialdehyde was assessed by spectrophotometer absorbance of the supernatant that was recorded at 532 nm. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's multiple comparison tests, n = 6. $^{**}p < 0.001$ compared with control (blank bar), $^{\#}p < 0.05$ compared with diabetic group, $^{\#\#\#}p < 0.001$ compared with diabetic group. Treatment 1; diabetic mice received 10 mL/kg cinnamon extract, treatment 2; diabetic mice received 10 mL/kg one-fold diluted cinnamon extract.

The effects of different treatments with cinnamon extract on learning behavior are illustrated in Fig. 2. The time required for learning in the first trial (T1) was significantly longer in diabetic animals ($p < 0.05$) compared to control group. Cinnamon treatment could restore the learning time roughly to its normal values.

Memory performance was assessed by RI, as presented in Fig. 3. Diabetic animals did not differentiate the familiar object with the new one and had recognition index near naught. Treatments with cinnamon significantly improved memory performance (treatment 1, RI = 26% ± 5.7, and treatment 2, RI = 24% ± 7.2).

Lipid peroxidation assay showed that diabetic animals had higher levels of MDA (0.32 ± 0.05 µmol/L) representing higher levels of oxidative stress (Fig. 4). Nevertheless, treatments with cinnamon reduced oxidative marker significantly. MDA was reduced to 0.11 ± 0.02 µmol/L and 0.19 ± 0.02 µmol/L following treatment 1 and treatment 2, respectively.

DISCUSSION

The present study proved recognition memory impairment following alloxan-induced diabetes in mice. Serum peroxide levels were considerably higher in diabetic animals compared to the healthy group. Although cinnamon water extract did not reduce glucose levels significantly it could have beneficial effects not only on lipid peroxidation but also on memory performance in diabetic animals.

In order to evaluate the validity of this memory paradigm, we examined the effect of scopolamine on mice memory performance in the object recognition task (data not shown). In agreement with the results of other studies on the object recognition task (15,19) a single injection of 0.3 mg/kg scopolamine administered after learning trial caused amnesia in mice.

It has been shown that using cinnamon water extracts achieved the desired blood glucose effects while avoiding the non-polar constituents in whole cinnamon or the cinnamon flavor components that have been linked to deleterious effects, oral lesions and mutagenicity (20). The non-polar constituents contain minimal quantity of potentially toxic cinnamaldehyde and coumarin, while greater amount of the active ingredients of polyphenolic compounds for controlling blood glucose level are present. Recently, it has been

found that cinnamon bark water extract elevates glucose uptake through the promotion of insulin sensitivity and inhibits angiogenesis through blocking vascular endothelial growth factor 2 signaling (13,14). These results indicate that the observed pharmacological activities may have originated from the polyphenolic compounds of the bark water extract.

The blood glucose lowering potential and pharmacological properties of cinnamon has been demonstrated previously *in vitro* and *in vivo* in animals (12,21). Cinnamon polyphenols display insulin like properties and stimulate glucose uptake in skeletal muscle and adipose tissue. In our study, following administration of cinnamon bark water extract glucose levels were reduced but they did not reach to the normal values. This could be because of the short course of therapy, compared to previous studies, or inefficiency of using cinnamon alone.

Diabetic animals required more time in order to identify the object in the first trial. This is consistent with previous reports that, the brain is not protected from the adverse effects of diabetes, with both cognitive deficits and brain structural abnormalities reported in the literature (22). The hippocampus is especially vulnerable to atrophy and decrements of hippocampal-dependent cognitive functions, such as delayed verbal recall and apparent early in disease development (23). Previously extension in P300 latency of the diabetic patients showed that the speed of information classifying is lower than in normal persons. Substantial reduction in P300 amplitude of the patients with type 1 diabetes mellitus proved that working memory in these patients cannot recognize new events and update its situation in harmony with them (24). However cinnamon bark water improved learning time; this rapid effect of cinnamon seems to be distinct from its effects on the plasma glucose level.

After diabetes was induced in animals memory performance was impaired in the novel object recognition task. Recognition index was near zero in diabetic animals indicating that they could not discriminate between the new and the old objects which is different from normal animals. Interestingly the RI values were significantly improved by treating diabetic animals with cinnamon bark water extract. Recently a similar study using lyophilized aqueous extract of cinnamon showed its beneficial effect on memory of streptozotocin-induced diabetic rodents which was attributed to the anti acethylcholinesterase activity of cinnamon (25). In our study the water extract of cinnamon was used which can be easily prepared and conveniently consumed by patients which results in more patients' compliance. In the present study alloxan-induced diabetic animals were used to assess the effect of diabetes on performance of memory. It has been shown that the development and chronic state of diabetes in mice following streptozotocin and alloxan are not identical (26).

Previously it was shown that meal ingestion induces acute memory deficits in patients with diabetes mellitus 2 (27,28). Chui and Greenwood in their article suggested that postprandial oxidative stress is a potential contributor because deficits in memory performance after test meal consumption could be minimized by co consumption of the test meal with high doses of antioxidant vitamins (22). The oxidative stress induced by diabetes on cardiac function was prevented by the extract of *Allium eriophyllum* leaves (29). Our study also determined the lower oxidative stress following cinnamon administration in diabetic animals. As far as the following experiments show oxidative stress is the main cause of cognition failure and by overcoming this risk factor memory performance could be improved. Further experiments are suggested in this regard.

CONCLUSION

To sum up, cinnamon water extract could be useful as an adjuvant remedy in diabetic patients' daily regimen. Cinnamon extract could potentiate the glucose lowering property of insulin or oral diabetic drugs thus, lower doses could be administered. Additionally our results proved that this extract is not only useful on memory performance but also on lipid peroxidation level, which are troublesome in diabetes.

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