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Original article

Resistance training and hawthorn extract ameliorate cognitive deficits in streptozotocin-induced diabetic rats

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ABSTRACT

It has been shown that diabetic rats display cognitive impairment. The aim of this study was to investigate the effects of resistance training and natural antioxidants on learning and memory in type 1 diabetic rats. For this purpose, fifty male Wistar rats were randomly divided into five groups: (i) Control (Con, n = 10), (ii) Diabetic (D, n = 10), (iii) Diabetic + Resistance training (DRT, n = 10), (iv) Diabetic + natural antioxidants (DHE, n = 10), and (v) Diabetic + Resistance training + natural antioxidants (DRH, n = 10). Climbing the ladder for a period of 5 days/week for 10 consecutive weeks was considered as the resistance training model in our study. Natural antioxidants (100 mg/kg per day) were administered to natural antioxidant groups for a period of 10 weeks. Moreover, spatial and passive avoidance learning and memory function were evaluated by Morris Water Maze (MWM) and shuttle box tests. The results showed that, mean of total escape latency decreased 25% ($P < 0.0001$) in the DRH group compared with the D group in MWM. The percentage of time spent in the target quadrant identically decreased (34%) in the D and DHE groups compared with the Con group ($p = 0.001$). In this regard, time spent in the dark Compartment (TDC) respectively rose 86% and 95% in the D and DHE groups compared with the Con group ($p < 0.05$), and decreased 88% in the DRT and DRH groups compared with the D group in the shuttle box test ($p < 0.05$). Furthermore, we noticed that total antioxidant capacity increase and lipid peroxidation decrease in response to the treatments in the diabetic rats as well. Therefore, the current study indicated that exercise training and natural antioxidants synergistically ameliorated learning and memory deficits in type 1 diabetic rats via reducing oxidative stress. Hence, it may propose a potential role of resistance training and natural antioxidants as an adjuvant therapy for the prevention and treatment of diabetic complications.

1. Introduction

The prevalence of diabetes mellitus as a common chronic metabolic disease is expected to rise to over 550 million by 2030, with alarming rates in most of the developed countries [1]. Besides its more commonly recognized complications, such as macrovascular disease, retinopathy, and nephropathy, diabetes related cognitive disorders have gained growing attention [2].

In this regard, it has been reported that diabetes accelerates mild cognitive impairment in dementia in old people [3]. Cerebrovascular changes and neurodegeneration are implicated in the development and progression of diabetes-mediated cognitive dysfunction [4]. Overall, a review of studies in this area showed that hippocampal neurogenesis, neuroplasticity, and dendrite remodeling were impaired, and it has been shown that apoptosis, neuron degeneration, and oxidative stress are significantly increased in the hippocampus of diabetic rats [5,6].

To date, many studies have been conducted on diabetes-mediated cognitive dysfunction, but there are no diabetes-specific treatments to prevent or ameliorate learning and memory dysfunction [4]. Nevertheless, abundant data have established the therapeutic effect of exercise training therapies in learning and memory dysfunction [7–9]. The exact molecular mechanism of this process is not yet clear. The effectiveness of endurance training in the amelioration of learning and memory deficits have been demonstrated [10], but the effect of resistance training on cognitive impairment in type 1 diabetic is not yet clear.

On the other hand, natural products and herbal extracts have become well recognized supplements for the prevention and remedy of

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various diseases [11,12]. Hawthorn contains oligomeric proanthocyanidins, flavonoids, and polyphenols, which are well-known for their antioxidant [13] and anti-inflammatory properties [14]. This plant has been traditionally used in various diseases, such as cardiovascular disease, in China, India, and many European countries [15]. Hawthorn fruit as a good source of natural antioxidants scavenges superoxide anions, hydroxyl radicals, and hydrogen peroxides, and inhibits lipid peroxidation [16]. Oxidative stress and inflammation are the main reasons for cognitive impairment in diabetic rats [17]. Hawthorn extract reduces infarct volume and improves neurological score by reducing oxidative stress in the rat brain following middle cerebral artery occlusion. The beneficial effects of exercise training and Hawthorn are remarkable in that they have an almost diametrically opposed effect to the pattern of structural and functional deficits seen following diabetes. In our knowledge, no study has been done on the effect of Hawthorn extract on cognitive deficits in diabetics.

Therefore, the aim of our study was to perform a preliminary study on the synergistic effect of resistance training and natural antioxidants on impaired learning and memory function and stress oxidative indices in type 1 diabetic rats.

2. Material and methods

2.1. Animal care

Fifty male Wistar rats (6–8 week old and 193 ± 19 g) were housed in a room with controlled temperature (22 ± 2 °C), humidity (55 ± 5%), and light (8:00 AM. to 8:00 P.M.). Food and water were available ad libitum. Five rats were housed per cage and all rats were weighed once a week. Animals used in these experiments were treated in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA), and the study protocols were approved by the Institutional Animal Care and Use Committee at Hamadan University of Medical Sciences (Ethics committee approval number: ir.umsha.rec.1394.261).

2.2. Experimental design

Type 1 diabetes model was induced by repeated intraperitoneal injection of freshly prepared 60 mg/kg streptozotocin (twice; second injection occurred 24 h after the first injection, i.p.), and confirmed by a fasting glucose level of ≥ 300 mg/dL 3 days later. Streptozotocin (STZ; Sigma Chemical Company, St. Louis, MO, USA) was dissolved in 0.01 M sodium citrate buffer of pH 4.3 [6]. The buffer was prepared by mixing of 47 mL of 0.1 M citric acid solution with 53 mL of 0.1 M sodium citrate solution [18]. Diabetic rats showed symptoms of diabetes such as polydipsia, polyphagia, and polyuria. This model offers a very effective technique that can be used in most rodents [19]. After that, diabetic rats were randomly distributed into four groups: (i) Diabetic (D, n = 10), (ii) Diabetic + Resistance training (DRT, n = 10), (iii) Diabetic + natural antioxidants (DHE, n = 10), and (iv) Diabetic + Resistance training + natural antioxidants (DRH, n = 10). Furthermore, 10 healthy rats (did not consist of diabetic rats) assigned as a control group. General characteristics of the experimental groups is shown in Table 1 and Experimental timeline is shown in Fig. 1.

2.3. Resistance training

Exercise training began 1 day after confirmation of diabetes. It was stipulated that the rats undergo 10 weeks (5 days per week) of progressive resistance training. The resistance training consisted of climbing a 1-m-high homemade ladder, inclined at 85°, twelve times. Initially, the rats gained familiarity with the ladder by climbing without weight for 3 days during the adaptation period. The initial weight attached to each animal's tail was 50% of its body weight, and was increased progressively up to 130% after 10 weeks (1st-2nd week: 50–60% of body weight; 3rd-5th week: 70–90% of body weight; 6th-8th week: 100–110% of body weight; 9th-10th week: 110–130% of body weight). Each training session consisted of 3 sets of 4 repetitions, with a 3 min rest between sets and 15 s rest between trials [20].

2.4. Plant extraction

Extract preparation was done according to the reported method with slight modifications [16,21]. The berries of Hawthorn were collected from the Zagrous Mountains in West of Iran and identified at the Botanic Institute of this University. A voucher specimen was deposited in the department of pharmacognosy and biotechnology, school of pharmacy, Hamadan University of Medical Sciences. The fresh pulp (3 kg) of the fruits was dried at 40 °C with air circulation and then ground into a powder (100 g) with an electric grinder, and distilled water was added. Water was added to fruits containing bottle to cover them completely. The extraction was done using the maceration procedure for 72 h at 4 °C in the dark room. The extract was then centrifuged and the resulting supernatant was filtered. The filtered extract was then concentrated and dried in a rotary evaporator under reduced pressure at a constant temperature of 40° C. The resulting extract was stored in a refrigerator. The dried substance was dissolved in 0.9% NaCl with pH 7.4 to form an extract of 5 mg/mL. Natural antioxidants rats were treated with 100 mg/kg body weight of the extract by gavage every day for 10 weeks. Previous studies have demonstrated the effectiveness of this dose [16,22].

2.5. Blood sampling and biochemical analyses

Rats were fasted for 12 h and fasting blood glucose levels were monitored and assessed from the tail vein with a glucometer (OneTouch Ultra, LifeScan, Johnson & Johnson Company, Milpitas, CA, USA) one day before (72 h after second STZ injection) and one day after treatments (10 weeks resistance training and natural antioxidants consumption) in accordance with the manufacturer's instructions. Furthermore, serum malondialdehyde (MDA) concentration assayed as a biomarker of lipid peroxidation. MDA was measured at 532 nm by spectrophotometer and MDA content was expressed as μmol/ml according to the method of Niehaus and Samuelsson [23]. Also, the total antioxidant capacity (TAC) of serum samples were assayed by commercially available kits (Randox labs, Grumlin, UK).

For determining the dosage of hawthorn extract is safe, liver enzymes were measured. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are biomarkers for hepatocyte injury [24]. ALT and AST were measured using a Roche kit according to the instructions of the company (Penzberg, Germany).

2.6. Spatial and aversive learning and memory evaluation

For measurement of spatial (acquisition and retention) and aversive (acquisition and retention) learning and memory after the training programs, we used Morris Water Maze (MWM) and shuttle box test, respectively, in type 1 diabetic rats 1 day after treatments.

2.6.1. Morris Water Maze

Learning and spatial memory capability was done using a MWM device [25], including a circular pool (180 cm in diameter, 60 cm in height), black colored, filled to a depth of 25 cm with water at 22 ± 1 °C. Low light was used for illumination and the room was sound insulated. Various visual cues were present. The pool had four quadrants with four starting lines named north (N), east (E), south (S), and west (W), and an invisible Plexiglas platform (10 cm in diameter) centrally located 1 cm beneath the water in quadrant N. Animal training lasted for 4 days at nearly the same time, and each day had two blocks with four trials (90 s). There was a 30 s gap between two trials