Effects of *Danae racemosa* on Testosterone Hormone in Experimental Diabetic Rats

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Abstract

Background: ***Danae racemosa*** **is a strong antioxidant and antioxidants have significant effects on spermatogenesis, sperm biology and oxidative stress, and changes in antioxidant capacity are considered to be involved in the pathogenesis of chronic diabetes mellitus.**

Objective: Because STZ causes testicular dysfunction and degeneration under situations of experimentally induced diabetes in animal models, we aimed in this study *Danae racemosa'*s effects in decreasing the harmful effects of STZ on testicular and sperm functions b.

Methods: Male Wistar rats (n = 40) were allocated into four groups: Group 1a: Control rats given 0.5 ml of 20% glycerol in 0.9% normal saline. Group 1b: Control rats given 0.5ml of 0.5 ml citrate buffer (pH4.0).Group 2: streptozotocin (STZ) treated rats. Group 3: rats given *danae racemosa* 400 mg/kg (gavage). Group 4: STZ treated rats given *danae racemosa* 400 mg/kg (gavage). Animals were kept in standard conditions. At 28 days after inducing diabetics, 5 mL blood was collected for measuring testosterone.

Results: Total serum testosterone increased significantly in the group treated with *danae racemosa* ($p < 0.05$) compared with control groups. Testis weights in the diabetic groups decreased significantly in comparison with controls ($p < 0.05$).

Conclusion: *Danae racemosa* had a significant protective effect on the diabetes-induced deteriorations in serum total testosterone, by reducing the levels of reactive oxygen species in serum. Therefore, it could be effective for maintaining healthy in diabetic rats.

Keywords: ***Danae racemosa*****, Diabet, Streptozotocin, Rat**

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Introduction

Diabetes is associated with reproductive impairment in both men and women. About 90% of diabetic patients have disturbances in sexual function, including decreases in libido, impotence and fertility [1, 2]. In this context, attention has been paid to the search for effective antidiabetic drugs in the field of traditional Chinese medicine. Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins, and an increased risk of complications from vascular diseases [3]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus [4]. Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well understood, hyperglycemia causes increased oxidative stress because the production of several reducing sugars is enhanced via glycolysis and the polyol pathway [5]. These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation), increasing the production of reactive oxygen species (ROS) [5]. Diabetes is the most common endocrine disease that leads to metabolic abnormalities involving the dysregulation of carbohydrate metabolism. Another major concern in diabetes is increased oxidative stress. Thus, increased production of free radicals or reactive oxygen species (ROS) may induce oxidized low-density lipoproteins (Ox-LDL), which are key factors in the sequence of events leading to atherosclerosis. Thus, sustained hyperglycemia and increased oxidative stress are the major players in the development of secondary complications in diabetes. These abnormalities produce a variety of pathologies including vasculopathies, neuropathies, ophthalmopathies and nephropathies, among many other medical derangements [6]. Maintaining a balance between ROS and antioxidants is a major mechanism in preventing damage from oxidative stress. Therefore, dietary supplementation with antioxidants such as vitamins, and flavonoids has been used in attempts to prevent the occurrence of many chronic diseases [7]. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution and vitamins insufficient intake may have harmful effects on spermatogenesis and sperm normal production [8 - 10]. *Danae racemosa* (DR) is used medicinally and as a culinary spice. Its constituents are stated to have antithrombotic, antihepatotoxic, antinociceptive and antioxidant [11]. Because STZ causes testicular dysfunction and degeneration under situations of experimentally induced diabetes in animal models [12]. we hypothesized that *Danae racemosa* might decrease the harmful effects of STZ on testicular and sperm functions by reducing ROS production.

Materials and Methods

Animals

Forty male 8-week-old Wistar albino rats weighing 250 ± 10 g were obtained from the animal facility of the Pasture Institute of Iran. Rats were housed in temperature controlled rooms (25 °C) with constant humidity (40 – 70%) and a 12/12 h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz Medical

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University. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments to determine the amount of water needed per experimental animal. Diabetes was induced by a single intraperitoneal (i. p.) injection of streptozotocin (STZ, Sigma-Aldrich, St Louis, MO, USA) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight (Mahesh and Menon, 2004). Blood glucose concentration and changes in body weight was monitored regulary. Rats were allocated into four groups randomly: controls (n = 10); a danae racemosa treatment group receiving 400 mg/kg (gavage) (n = 10), a diabetic treatment group receiving a single injection of STZ (n = 10) plus 400 mg/kg (gavage) danae racemosa, daily for 4 weeks (Coskon et al, 2005), and an STZ control group (n = 10) that received STZ plus an equal volume of distilled water daily (i. p.). after a week rats with diabetes having hyperglycemia with blood glucose concentration 300 mg/dL.

Blood Glucose Determination

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL, Bayer HealthCare, Basel, Switzerland). Samples were then taken 48 h after STZ injection and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dL were declared diabetic and were used in the experimental group. One week after the induction of experimental diabetes, the experimental protocol was started.

Surgical Procedure

At the end of the treatment period, the rats were killed with diethyl ether and the testes in control and experimental groups were removed immediately. The weights of testis were recorded.

Serum Total Testosterone

Serum concentrations of total testosterone were measured by using a double antibody radioimmunoassay (RIA) kit (Immunotech Beckman Coulter Co., Los Angeles, CA, USA). The assay sensitivity per tube was 0.025 ng/mL [13].

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results are expressed as the mean ± standard error of mean (SEM) and $p < 0.05$ was considered significant.

Results

Total Serum Testosterone

STZ treatment caused a significant decrease in the total serum testosterone level in the diabetic group (STZ) compared with the control, *Danae racemosa* and STZ + *Danae racemosa* groups. The values were 1.60 ± 0.05, 3.01 ± 0.40, 5.50 ± 0.15 and 3 ± 0.21, ng/mL, respectively (Table 1).

Discussion

Male reproductive function is clearly impaired in diabetes. Diabetes-induced alterations of Leydig cell functions include a decrease in androgen synthesis and in the total number of these cells [14]. Together, these

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Table 1- The effect of streptozotocin with and without 30 days of treatment with *danae racemosa* on serum total testosterone, and body & testis weights. *P* values are shown in parentheses in italics

Groups (n=10)	Control	*Danae racemosa* 400 mg/kg (gavage)	Streptozotocin 55 mg/kg i. p.	Treatment Streptozotocin 55 mg/kg (i. p) plus *danae racemosa* 400 mg/kg (gavage)
Testis weight (g)	1.40 ± 0.821	1.36 ± 0.821	1 ± 0.05*	1.22 ± 0.20
Body Weight (g)	251 ± 0.365	250.3 ± 0.761	189.3 ± 0.831*	221.7 ± 1.1*
Serum Testosterone levels (ng/ml)	4.01 ± 0.50	5.50 ± 0.15	1.60 ± 0.05*	3 ± 0.21*

effects impair male libido [15]. The diabetes-induced alterations of Leydig cells are related to concomitant alterations in the control mechanisms that modulate the proliferation, differentiation and overall function of these cells [15]. Furthermore, diabetes-related alterations in Leydig cells are also related to changes in the pituitary–testicular axis [16]. Thus, this disease induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function [16, 17]. Diabetes-induced testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction is a well-recognized complication of diabetes mellitus. Infertility among men with diabetes is a less well-examined problem and their gonadal status is not clearly established. The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research [18]. However, an altered testicular axis was noted in experimental studies. Seethalakshmi et al. Oxidative stress also plays a role in the development of diabetic complications [6]. Oxidative damage in rats with STZ-induced diabetes was ascertained in the present study by measuring the MDA levels, ROS generation, alterations in antioxidant defense and the serum level of ox-LDL. There was expansion of the interstitial space with vacuolization and the Leydig cells had an abnormal fibroblast-like appearance. Fibroblastic degeneration appeared in the seminiferous tubules and was increased in the STZ group compared with those observed in the control and other experimental groups. These results are clearly in agreement with other studies [12, 19] Serum total testosterone levels showed marked decreases in the STZ-induced diabetic group compared with those seen in the control and other experimental groups and these results were in agreement with Tang et al [20, 21]. The testicular injury and apoptosis induced by diabetes are partially attributed to augmented oxidative stress in testicular tissue. Flavonoids are antioxidant agents widely distributed in dietary plants frequently consumed by humans such as fruits, vegetables, teas and wine [20, 22]. The dietary intake of flavonoids in humans has been estimated to be 16 – 1000 mg/day. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution and vitamins insufficient intake may have harmful effects on spermatogenesis and sperm normal production [8 - 10]. Researches have reported that using antioxidants and vitamins A, B, C and E in daily diet can protect sperm DNA from free radicals and increase blood

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testis barrier stability [23]. Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood testis barrier stability [24]. Nowadays *Danae racemosa* is used worldwide as a spice. Both antioxidative [11] and antinociceptive activity [25] of Danae racemosa were reported in animal models. As this antioxidant flavonoid is known to decrease the risk of degenerative diseases, we suggest that using dietary plants, fruits, vegetables, onion, teas and red wine rich in flavonoids and *Danae racemosa* could have beneficial effects on subjects with diabetes and possible decrease the risk of infertility in men.

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