Effects of *Danae racemosa* on Spermatogenesis in Rat

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Abstract

Background: ***Danae racemosa* is used medicinally and as a culinary spice. Its constituents are stated to have antithrombotic, antihepatotoxic, antinociceptive and antioxidant.**

Objective: Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus.

Methods: Wistar male rat (n=30) were allocated into three groups, control (n=10) and two test groups (each of ten). Animals in test groups were subdivided into groups of 2 that received fresh extract equivalent to 200 & 400 mg/Kg/Rat/day *Danae racemosa* extract. Fresh *Danae racemosa* extract was administered with gavages for 28 consecutive days. Animals were kept in standard condition. On twenty eighth day, the testes of rats in the all groups were removed and sperm was collected from epididymis and was prepared for analysis.

Results: The percentage of sperm viability and motility in both test groups significantly increased ($p<0.05$), but the sperm concentration significantly increased only in the group that received the high dose of Danae racemosa extracted, ($p<0.05$). It was evident that there was no difference on sperm morphology and testis weight in test groups comparing to control group.

Conclusion: In our study Danae racemosa extract has significantly affected the sperm number and percentage of viability and motility; it seems that using 400 mg/Kg/Rat/day of Danae racemosa extract is effective in sperm health parameters.

Keywords: Danae racemosa, Extract, Sperm, Rat

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Introduction

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 [1, 2, 3, 4]. Researches have reported that using antioxidants and vitamins A, B, C, E in daily diet can protect sperm DNA from free radicals and increase blood testis barrier stability [5]. 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 [6]. Nowadays Danae racemosa is used worldwide as a spice. Both antioxidative [7] and antinociceptive activity [8] of Danae racemosa were reported in animal models. Besides, other researches showed that it has dominative protective effect on cell damage induced by H2O2 and might act as a scavenger of oxygen radical and might be used as an antioxidant [8]. Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men [9, 10]. Therefore, the role of nutritional and biochemical factors in reproduction and sub- fertility treatment is very important. The present study was planned to asses the ability of Danae racemosa to promote sperm parameters in rats [11, 12].

Materials and Methods

Experimental animals

The 30 adult Wistar albino male rats were 8 weeks old and weighing 250 ± 10 g, they were obtained from animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25^0C) with constant humidity (40 - 70%) and 12h/12h light/ dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz medical 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 in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly selected and divided into control (n=10) and experimental (n=20) groups. The experimental groups split into two groups of ten. One of which received 200 mg/Kg/Rat/day and the other group received 400 mg/Kg/Rat/day fresh of *Danae racemosa* extract with gavages for 28 consequence day. The control group just received 1cc distilled water by gavages [12].

Surgical Procedure

In the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether, and liver tissues in control & experimental groups were immediately removed.

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (11). After 5 min incubation at 37°C (with 5% CO2), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method [12].

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Measurement of Serum Total Antioxidant capacity (TAC)

TAC was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L) [13].

Measurement of Serum MDA

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxy propane as the standard [14].

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 were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

Total Antioxidant capacity (TAC) and Malondialdehyde (MDA) concentration

Measurement in Serum

Administration of 200 & 400 mg/Kg/Rat/day fresh Danae racemosa extract daily for 28 consecutive days significantly decreased level of Malondialdehyde (MDA) concentration in experimental groups compared to control group ($p<0.05$). Administration of 400 mg/Kg/Rat/day Danae racemosa extract daily for twenty consecutive days could significantly increased level of TAC. But 200mg/Kg/Rat/day of Danae racemosa extract does not have any significant effect on TAC in experimental groups (Table 1).

Table 1- The effect of the 200 & 400 mg/Kg/Rat/day on sperm parameters &serum TAC and MDA in control and experimental groups in the rats

Groups	control (n=10)	*Danae racemosa* (D1) (200 mg/Kg/Rat/day) (n=10)	*Danae racemosa* (D2) (400 mg/Kg/Rat/day) (n=10)
(TAC) (nmol/ml)	0.70±0.03	0.80±0.03 * (0.006)	0.83±0.04 * (0.003)
(MDA) (nmol/ml)	0.25±0.04	0.24±0.212 * (0.004)	0.22±0.06 * (0.008)
Sperm concentration (total count) (No of sperm/rat $\times 10^6$)	45.68±7.70	50.90±5.16	66.60±2.34*
Motility (%)	31.75±6.88	70±4.35*	77±5.33*
Viability (%)	66.25±4.73	95.80±1.68*	97.80±80*

Data are presented as mean ± SE.
* P-value less than 0.05 were considered significant and are writing in the parentheses, (compared with the control group).

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Results

Administration of 200 & 400 mg/Kg/Rat/day Danae racemosa extract for 28 consecutive days significantly increased sperm motility and viability in both experimental groups as compared to the control group with Dunnett homogeneity test (Table 1). Sperm count was significantly increased in the experimental group that received 400mg/Kg/Rat/day Danae racemosa as compared with the control group (Table 1). Sperm abnormality wasn't significantly different in the experimental group that received 400mg/Kg/Rat/day Danae racemosa with the control group (Table 1).

Discussion

Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Base on ancient Persians traditional books Use of herbal medicine has positive effect on treatment of different diseases especially on diabetes mellitus [1]. Numbers of plants which have effect on sexual stimulation are: barberry, tarragon, sumac, cinnamon, some tea specious and onion. Investigation into chemical compounds of onion and ginger shows these plants contain antioxidant agent [2, 11]. Danae racemosa extract contains vitamins, flavonoids which their antioxidant role has been proved. Make use of Danae racemosa inhibition of acetic acid-induced abdominal constriction in rat models [15, 16, 17]. The importance of many of these factors is not yet clearly understood. A better understanding of underlying mechanisms in (sub) fertility and better study results clarifying the effectiveness of nutritional and biochemical factors are important to improve diagnosis and treatment. Smart choices for better foods might prevent body from many diseases [5]. The main advice for a healthy diet is to eat more fruits and vegetables. But published intervention trials do not support this message yet [2, 18]. Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radicals, is an essential attribute of aerobic life [18]. A disturbance in the pro-oxidant/antioxidant system has been defined as oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion (O─2), nitrogen oxide (NO) and hydroxyl radical (HO─). Even though naturally present in the organism, they are mainly confined to cell compartments and counterbalanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers [19, 20]. Thus, this disease induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function [21, 22, 23]. Besid, these productive effects is reflected by the decrease of malonaldehyde level and increase in total anti oxidants capacity (Table 1). Therefore suggested, increased use of herbal medicine, fruit, vegetables, onion, tea and black burgundy grape which are full of flavonoids and *Danae racemosa* extract can decrease side effects of sperm abnormality and increased sperm parameters in male infertile mans.

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