

Effects of Ginger on Spermatogenesis in Streptozotocin-induced Diabetic Rat

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Abstract

Background: Ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae) is used medicinally and as a culinary spice. The medicinal use of ginger dates back to ancient China and India.

Objective: Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory, stimulant, cholagogue and antioxidant. 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=40) were allocated into three groups, control group (n=10) and ginger (G) group that received 100mg/kg-perday (oral), (n=10), and Diabetic group that received 55 mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55mg/kg (IP) STZ plus ginger 100mg/kg-perday (G), daily for, 4 weeks, respectively; however, the control group just received an equal volume of distilled water daily(IP). Diabetes was induced by a single (IP) injection of streptozotocin (55 mg/kg). Animals were kept in standard condition. In 28day after inducing diabetic 5cc blood were collected for TAC, MDA and sperm parameters and testis tissues of Rat in whole groups were removed then prepared for analyzes.

Results: TAC, MDA and sperm parameters significantly decreased in diabetic group. treatment group that has received Ginger 100 mg/kg (oral) show increasing in TAC and sperm parameters (p<0.05) in comparison to experimental groups (p<0.05).

Conclusion: Since in our study 100 mg/kg (oral) ginger have significantly Preventive effect on sperm parameters, so it seems that using it can be effective for treatment in Diabetic Rat.

Keywords: Diabetic, Ginger rhizome, Streptozotocin, Sperm, Rat



Introduction

Diabetes is a chronic disease that due to hyperglycemic. Hyperglycemic in long time have side effect in other tissues especially in liver. Liver dysfunctional has seen in diabetic patients especially in patients with uncontrolled blood sugars level. Liver has regulative effects on blood sugar level with glyconeogenesis and glycogen's ways, Disorders in these pathways yield in disorders i.e. carbohydrate metabolism dysfunction. Recently due attention has been paid to the search of effective drugs in the field of traditional Chinese medicine (TCM). 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 [1,2,3]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus [4,5]. Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well understood, hyperglycemia lead patients to increased oxidative stress because the production of several reducing sugars (through glycolysis and the polyol pathway) is enhanced [3,4]. These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation reaction), increasing the production of reactive oxygen species (ROS) [6]. Diabetes is the most common endocrine disease that leads to metabolic abnormalities involving regulation of carbohydrate metabolism. In addition to imbalanced carbohydrate metabolism, yet another major concern in diabetes is increased oxidative stress. Increased production of free radicals or ROS formation may induce oxidized LDL (Ox-LDL), which is key step in the sequence of events leading to atherosclerosis

Sustained hyperglycemia and increased oxidative stress, are the major players in the development of secondary complications in diabetes. 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 [4, 5]. Nowadays ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae), is used worldwide as a spice. Both antioxidative [6] and androgenic activity of *Z. officinale* were reported in animal models. All major active ingredients of *Z. officinale*, such as Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols, have antioxidant activity [7]. Besides, other researches showed that ginger oil has dominative protective effect on DNA 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 ginger to promote sperm parameters in diabetic rats [11, 12].

Material and Methods

Animals

Forty adult Wistar albino male rats were 8 weeks old and weighing 250±10g, they were obtained from animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to use in 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 Ginger group that received 100 mg/kg/day (G); (IP). (n=10), and Diabetic group that received 55mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus 100 mg/kg/day (G); (IP). The control group just received an equal volume of 1cc distilled water daily (IP). Diabetes was induced by a single intra peritoneal (I.P) injection of streptozotocin (STZ, Sigma- U.S.A.) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight [12].

Induction of experimental type 1, Diabetes

Experimental type 1 diabetes was induced in rats by intra peritoneal (I.P) injection of 55 mg/kg streptozotocin (STZ) in distilled water. Control rats were received distilled water, only.

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). Sample collections were then made 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, protocol was started.

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 [12,13].

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) [14].

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'-tetramethoxypropane as the standard [15].



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 $\pm$ S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

Results

Results of total blood anti oxidant capacity

Amount of total blood anti oxidant capacity in control group was (0.70 ± 0.03 mmol/ml) and in experimental groups was 0.75 ± 0.03 , 0.32 ± 0.04 and 0.61 ± 0.05 mmol/ml respectively. Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group ($p < 0.05$) (Table 1).

Results of MDA (malondi aldehyde) level in blood

MDA level in control group was 0.25 ± 0.04

mmol/L and in experimental groups was 0.27 ± 0.212 , 4.1 ± 0.06 , 1.1 ± 0.08 mmol/L respectively.

Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group ($p < 0.05$) (Table 1).

Results of sperm motility, viability and count

Streptozotocin-induced diabetic model by 55 mg/kg significantly decreased sperm count, motility and viability in diabetic group as compared with those observed in the control and other experimental groups. The sperm concentrations, motility and vitality in STZ group and the corresponding values in QR group and the corresponding values in treatment (STZ+G) group were shows significant differences between experimental groups in comparison to control group ($p < 0.05$). (Table 1). There weren't any significant change in sperm abnormality in experimental groups as compared with control group, (Table 1).

Table 1- The effect of the 100 mg/Kg/Rat/day ginger on sperm parameters & serum TAC and MDA in control and experimental groups in the rats

Groups	control (n=10)	ginger (100mg/kg- perday) (n=10)	STZ (55mg/kg (IP)) (n=10)	ginger +Stz 55mg/kg (IP) streptozotocin plus 100mg/kg-perday ginger (n=10)
(TAC) (nmol/ml)	0.70 ± 0.03	$0.75 \pm 0.03^*$ (0.006)	$0.32 \pm 0.04^*$ (0.003)	$0.61 \pm 0.05^*$ (0.004)
(MDA) (nmol/ml)	0.25 ± 0.04	$0.27 \pm 0.212^*$ (0.004)	$4.1 \pm 0.06^*$ (0.008)	$1.1 \pm 0.08^*$ (0.005)
Sperm concentration (total count) (No of sperm/rat $\times 10^6$)	45.68 ± 7.70	54.90 ± 5.36	$31.60 \pm 2.34^*$	$39.60 \pm 2.34^*$
Motility (%)	31.75 ± 6.88	$72 \pm 4.35^*$	$22 \pm 5.33^*$	$25 \pm 5.33^*$
Viability (%)	66.25 ± 4.73	$95.80 \pm 1.68^*$	$45.80 \pm 80^*$	$51.80 \pm 80^*$

Data are presented as mean $\pm$ SE.

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

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]. Onion contains A, B, C vitamins, flavonoids and selenium which their antioxidant role has been proved. Make use of onion and Quercetin in diabetic patient treatment has been experimented [16, 17]. The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects [17]. 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 ($HO\cdot$). 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 [18, 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]. Diabetic testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction (ED) is a well-recognized complication of diabetes mellitus (DM). The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research [24]. However, an altered testicular axis was noted in experimental studies Seethalakshmi et al. [25] found that testicular weight, sperm count and motility significantly decreased in diabetic rats. Moreover, Cameron et al [26]. In the present study, administration of 50mg/kg/rar and 100mg/kg/rat ginger for twenty consecutive days significantly increased sperm motility and viability in both experimental groups as compared with the control group (Table 1). These results are supported by the finding of Aitken et al., (1995) who reported that the conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen [25]. This increase in sperm motility of experimental groups in comparison to control group could be due to the protective effect of ginger rhizoma administration. 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 ginger can decrease side effects of



diabetes mellitus on sperm parameters in diabetic patient.

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