Introduction

Diabetes mellitus has always been linked to reproductive dysfunction in research interest nowadays. Diabetes is among a number of disorders caused by oxidative stress and plant medicine has been shown to be effective in its treatment. Anti-oxidants have distinctive effects on spermatogenesis, sperm biology and oxidative stress, and changes in anti-oxidant capacity are considered to be involved in the pathogenesis of chronic diabetes mellitus. Ginger and cinnamon are strong anti-oxidants and have been shown to reduce oxidative stress in the long-term treatment of streptozotocin (STZ)-induced diabetes in animal models. The present study examined the influence of combined ginger and cinnamon on spermatogenesis in STZ-induced diabetes in male Wistar rats.

Materials and Methods:

Animals (n = 80) were allocated randomly into eight groups, 10 each: Group 1: Control rats given only 5cc Normal saline (0.9% NaCl) daily; Group 2: rats received ginger (100mg/kg/rat) daily; Group 3: rats received cinnamon (75mg/kg) daily; Group 4: rats received ginger and cinnamon, (100mg/kg/day ginger and 75mg/kg cinnamon) daily; Group 5: Diabetic control rats received only normal saline; Group 6: Diabetic rats received 100mg/kg/day ginger; Group 7: Diabetic rats received 75mg/kg day cinnamon; Group 8: Diabetic rats received ginger and cinnamon (100mg/kg/day and 75mg/kg /day). Diabetes was induced with 55 mg/kg, single intra-peritoneal injection of STZ in all groups. At the end of the experiment (56th day), blood samples were taken for determination of testosterone, LH, FSH, total anti-oxidant capacity, and levels of malondialdehyde, SOD, Catalase and GPX. All rats were euthanized, testes were dissected out and spermatozoa were collected from the epididymis for analysis.

Results:

Sperm numbers, percentages of sperm viability and motility, and total serum testosterone increased in ginger and cinnamon and combined ginger and cinnamon treated diabetic rats compared with control groups. Serum testosterone, LH and FSH were higher compared to control group and also serum anti-oxidants (TAC, SOD, GPX and catalase) all were increased at the end of treatment. Combined ginger and cinnamon showed more intense increase in all parameters compare to ginger and cinnamon alone. Most of the results were significant (P<0.05).

Conclusion: We concluded that combined ginger and cinnamon have significant beneficial effects on the sperm viability, motility, and serum total testosterone, LH, FSH and serum anti-oxidants’ level and could be effective for maintaining healthy sperm parameters and male reproductive function in diabetics.

Key words: Ginger; Cinnamon; Streptozotocin; Spermatogenesis; rat.

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(Agarwal and Sekhon, 2010; Khaki et al., 2010; Kota et al., 2012). Various traditional herbs and spices were indicated to have blood sugar lowering activities and this made it a choice medicine for the treatment of Type 2 diabetes. Aloe Vera, Bitter Melon, Cinnamon, Allium cepa, and ginger are among the plant-based therapies shown to be effective in the treatment of diabetes (Khaki et al., 2010). Polyphenol and anti-oxidant content of herbal medicine plays a critical role in increasing anti-oxidant defense, consequent reduction in oxidative state and, genotoxic effects and improvement of fertility similar to other natural anti-oxidants such as vitamin A,C and E, (Rajeev et al., 2006; Yang et al., 2006; Bahmanpour et al., 2012).

There has been evidence that herbal plants treatment could have protective effect on reproductive hormones level disturbances such as LH, testosterone (T) and FSH (102). Ginger (Zingiber officinale R.), and cinnamon (Cinnamom zeylanicum), both known for their anti-oxidant (Shagau and Davidson, 2006; Shing et al., 2007; Krim et al., 2013; Fathiazad et al., 2013;Škrlovánková et al., 2012), anti-inflammatory (Saenghong et al., 2012) and curing effects for different diseases (Kamath et al., 2003; Anderson et al., 2004; Alinkina et al., 2012; Wattanathorn et al., 2011), are among mostly used herbs for treatment of diabetes since antiquity (khan et al., 2003). They are both considered safe with little or no side effects compared to synthetic drugs (Yiming et al., 2012). The methanolic extracts of ginger and ethanolic extracts of cinnamon have been shown to be effective in treating fertility issues (Shalaby and Hamowieh, 2010; Shah et al., 1998). No prior studies have hitherto been focused on evaluating the combination effect of ginger and cinnamon on spermatogenesis out come in diabetics.

The aim of this study was to examine the synergetic anti-oxidant effects of dietary ginger in combination with cinnamon on fertility and spermatogenesis improvement in diabetic male rats.

Material and Methods

Animals

Eighty adult Wistar albino male rats, of 8 weeks old and weighing 250±10g, were obtained from the 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.

STZ induced diabetes

Diabetes was induced by a single intra peritoneal (i. p.) injection of streptozotocin (STZ, Sigma-Aldrich, StLouis, 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 regularly. Therefore, the Wistar male rats were divided into eight groups comprising ten animals in each group as follows:

Group 1: Control rats given only 5cc Normal saline (0.9% NaCl).
Group 2: Control rats given ginger (100mg/kg/rat) daily.
Group 3: Control rats given cinnamon (75mg/kg) daily.
Group 4: Control rats given ginger and cinnamon, (100mg/kg/rat ginger and 75mg/kg cinnamon) daily.
Group 5: Diabetic control (55 mg/kg, single intra peritoneal injection of STZ).
Group 6: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) received 100mg/kg/day ginger.
Group 7: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) received 75mg/kg/day cinnamon.
Group 8: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) received ginger and cinnamon (100mg/kg/day and 75mg/kg /day) (Fathiazad et al., 2013).

All feedings were by gavage method, daily for 8 weeks, respectively; however, the control group just received an equal volume of distilled water daily.

At the end of the experiment on the 56th day, blood was collected into heparinized tubes, and serum were separated by centrifugation and used for further analysis. All rats were euthanized, testes were dissected out and spermatooza were collected from the epididymis.

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, and Basel, Switzerland). Samples were then taken 24 hrs 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. The experimental protocol was started 48 hrs after the induction of experimental diabetes.

Serum insulin level

Serum insulin concentrations were determined by using radioimmunoassay kit (Boehringer Mannheim, Germany). The insulin level in serum was expressed in μU/ml.

Cinnamon preparation

Cinnamon zeylanicum were bought in Istanbul province, Istanbul city of Turkey. By mixer 100 grams of Cinnamon zeylanicum were condensed and powdered. Daily 75mg/kg of it was dissolved in 2cc distilled water and each rat was received it daily for 56 consequencies days.

Ginger preparation

Ginger roots were purchased from Tabriz traditional market, Tabriz city of Iran. Dried and powdered. Daily 100 mg/kg of it was dissolved
in 2cc distilled water and each rat was received it daily for 56 consequences days. It was dissolved in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. The solution was freshly prepared each week.

Surgical procedure

On the 56th day (at the end of the treatment period) the rats were sacrificed, after sodium pentobarbital solution (40 mg/kg) was administered intra-peritoneal as an anesthetic, and the peritoneal cavity was opened with a lower transverse abdominal incision. Both testes were then immediately removed from the control and experimental groups. The weight of the testes for each group member was recorded. Animals were then decapitated between 10:00 and 12:00 hrs. At the end of 4 weeks of treatment, testis was dissected from each rat, 24 hrs after the last administration.

Sperm analysis (count, viability and motility)

Spermatozoa from the cauda epididymidis were released by cutting the organ into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin. After 5 min incubation at 37°C (under 5% CO2 in air), the epididymal sperm reserves were determined using the standard hemocytometric method [WHO] and sperm motility was analyzed microscopically (Olympus IX70) [X40 magnification] in 10 fields according to the World Health Organization (WHO, 1992) recommended method. Sperm abnormalities were evaluated according to Khaki et al. (2008). Briefly, sperm smears were made on clean glass slides and stained with periodic acid-Schiff’s reaction plus hematoxylin. The stained smears were observed under a light microscope using a 40 X objective. Sperm were classified as normal or abnormal. The total sperm abnormality was expressed as percentage incidence. Sperm viability was performed by the eosin nigrosin staining. One drop of semen was mixed with two drops of 1% eosin Y. After 30 s, three drops of 10% nigrosin were added and mixed well. A smear was made by placing a drop of mixture on a clean glass slide and allowed to air dry. The prepared slide was examined using a phase contrast microscope. Pink-stained dead sperm were differentiated from unstained live sperm, and there numbers were recorded.

Measurement of serum total anti-oxidant capacity (TAC)

TAC was measured in serum using a commercial kit (Randox Laboratories, Crumlin, UK). The assay is based on the incubation of 2, 2′-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) with a peroxidase (methmyoglobin) and H2O2 to produce the radical cation ABTS+, which has a relatively stable blue-green color measured spectrophotometrically at 600 nm. The suppression of the color is compared with that of Trolox, which is widely used as a standard for TAC measurements and the assay results are expressed as Trolox equivalents (in nmol/mL) (Quintanilha et al., 1982).

Measurement of serum malondialdehyde (MDA)

Serum MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with a spectrophotometer. A calibration curve was prepared using 1,1′,3,3′-tetramethoxypropane as the standard (Randox Laboratories Crumlin, UK). (Quintanilha et al., 1982).

Measurement of serum LH, FSH and testosterone hormone

Serum concentration of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA). Rat FSH / LH kits obtained from Biocode Company-Belgium, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2ng/ml and 0.14ng/ml for FSH and LH respectively. Total serum concentration of testosterone was measured using a double-antibody RIA kit (ImmunoTech Beckman Coulter Co., USA). The assay sensitivity per tube was 0.025 ng/ml (Huang et al., 1995).

Measurement of serum super oxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD) was measured by following the method of Beyer and Fridovich (106).

Measurement of serum glutathione peroxidase (GPX) activity

GPX activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H2O2 in the presence of reduced glutathione (10 mM), NADPH, (4 mM), and 1 U enzymatic activity of GR (106).

Measurement of serum catalase (CAT) activity

Serum catalase activity was determined by measuring the decrease in absorbance at 240nm due to the decomposition of H2O2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50mM, pH 7.0) and 2.9ml of 30mM H2O2 in phosphate buffer pH 7.0. An extinction coefficient for H2O2 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2 reduced per minute per mg protein. At 240nm of 40.0M-1cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2O2 reduced per minute per mg protein.

Statistical analysis

Statistical analysis was done using the ANOVA and T-test for comparison of data in the control group with the experimental group. 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. The data were analyzed by SPSS software (version 17).
The results showed that there was a significant decrease in the mean body weight in the STZ induced diabetes group. After STZs fed with ginger, cinnamon and combined ginger and cinnamon, the increase in body weight was significant (P < 0.05) (Table 1). There were no significant changes in testis weight in all treatment groups compared to control group aside being a slight decrease in testis weight of STZ induced diabetes. Feeding with these herbs showed increase in all STZ treatments but was not significant (Table 1).

Table 1: The effect of streptozotocin with and without 56 days of treatment with ginger, cinnamon and combined ginger and cinnamon on sperm parameters, serum total testosterone, LH, FSH, total anti-oxidant capacity, malondialdehyde, SOD, catalase, GPX levels, blood glucose, insulin and testis weights. P values are shown in parentheses

<table>
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<th>Table 1: The effect of streptozotocin with and without 56 days of treatment with ginger, cinnamon and combined ginger and cinnamon on sperm parameters, serum total testosterone, LH, FSH, total anti-oxidant capacity, malondialdehyde, SOD, catalase, GPX levels, blood glucose, insulin and testis weights. P values are shown in parentheses</th>
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<td><strong>Control (n=10)</strong></td>
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<td><strong>Body weight (gr)</strong></td>
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<td><strong>Testis weight (gr)</strong></td>
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<td><strong>Sperm concentration (total count) (No of sperm/rat per day)</strong></td>
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<td><strong>Motility (%)</strong></td>
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<td><strong>Serum LH (ng/ml)</strong></td>
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<td><strong>Serum FSH (ng/ml)</strong></td>
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<td><strong>Total antioxidant capacity(TAC) (nmol/ml)</strong></td>
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<td><strong>Malondialdehyde (MDA) (mmol/ml)</strong></td>
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<td><strong>Catalase (u/mg Hb)</strong></td>
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STZ induced diabetics showed decreased in sperm count, motility and viability significantly compared to the control group. When STZs fed with ginger, cinnamon and combined ginger and cinnamon, increase observed in all 3 parameters and in all 3 treatment groups. Comparing all 3 against healthy controls was significant (P<0.05). The last group, combined ginger and cinnamon showed intense increase compared to ginger and cinnamon alone (Table 1).

The present study revealed that diabetes had significant harmful effects on sperm parameters (count, motility and viability) and serum levels of sex hormones (testosterone, LH and FSH). Our study also revealed a significant decrease in serum antioxidants levels(TAC, SOD, CAT and GPX) with significant increase in MDA associated with impaired spermatogenesis evidenced by decrease in sperm parameters (P<0.05). Nevertheless, diabetic rats fed with ginger, cinnamon and specialty ginger together with cinnamon showed significant ameliorating effects on blood glucose, insulin level, damaged sperm parameters, increase in levels of LH and FSH and also increase in serum antioxidants levels with decrease in MDA level and subsequent positive fertility outcome (p<0.05). All together the present study indicated that ginger plus cinnamon might have synergistic protective effects on testis. This study is the first to show the beneficial synergetic effects of ginger plus cinnamon on spermatogenesis in diabetic rats. This is consistent with the results of previous investigators Khaki et al., 2010 and also others (khaki et al.,2009;Yüce et al.,2013;Hesham et al.,2008) who showed the same destructive results in diabetic subjects. Although there are some debates over mechanisms involved in these changes but, oxidative stress is known to be the key factor responsible for most alterations (Ashrafi et al., 2013).Oxidative stress causes sperm metabolism impairment and decreases sperm quality and quantity (Gomez et al., 1996). It has shown that diabetic male patients’ sperms are more susceptible to DNA damage and these patients to have low sperm quality due to oxidative harm (Roesnser et al., 2012).On the other hand, glucose is a fuel for testicular cells when its metabolism gets disturbed in diabetes; testicular cell function gets altered and as a result spermatogenesis process destroys. In addition, reactive oxygen species considered toxic to spermatozoa and its plasma and antioxidants, the first line of defense, are affected by free radicals’ disturbance (Sikka et al., 1996; Sanocka and Kurpisz, 2004;Henkel, 2005).

Discussion

Diabetes has detecting consequences on male reproductive system including testicular function, sperm maturation and sexual hormone alteration (Arikawe et al., 2012; Steger and Rabe, 1997;Rato et al., 2013;Alves et al.,2013;La Vigneraet al., 2012; Trindade et al. 2013). Furthermore, some researchers concluded that increase glucose level could alter natural anti-oxidant enzyme level and glycolytic activities in Sertoli cells (Tabak et al.,2011) resulting in damaged sperm DNA and subsequently infertility (Suresh et al., 2012;Roessner et al, 2012; Mallidis et al.,2011).

Data are presented as mean ± SE.

*Significant different at P< 0.05 level, (compared with healthy control and STZ groups).

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issue for men in reproductive age. Plant medicine and natural antioxidants with less or no side effects have been considered for treatment of diabetes and different diseases since ancient time (Plutzky, 2011; Karunakaran and Park, 2013; Bahmanpour et al., 2012). Inevitably herbs such as ginger, cinnamon and allium cepa owe their therapeutic effects to their polyphenols content and antioxidant properties which have been shown to be effective in treatment of different ailments such as STZ-induced hyperglycemia and infertility (Khaki et al., 2010; Yuce et al., 2012; Tempest et al., 2008; Mallidis et al., 2009). Moreover, ginger and cinnamon both have been indicated to improve testicular function, sperm quality and quantity, sex hormones levels (Testosterone, LH and FSH), and serum antioxidants level (Shalaby et al., 2010; Hemayatkhah Jahromi et al., 2012; Riaz et al., 2011; Onwuka et al., 2011; Modaresi et al., 2009; Hajef 2010; Mashhadì et al., 2013). Our study showed that serum levels of anti-oxidants including TAC, SOD, catalase and specially GPX as well as sperm parameters could be enhanced following treatment with ginger, cinnamon and particularly ginger plus cinnamon. GPX and MDA showed significant changes in all our treatment groups. These findings were consistent with those of previous studies (Yuce et al., 2013; Khaki et al., 2009; Priya et al., 2011). Ginger and cinnamon contribution to the recovery of sperms, and their uptake on free radicals, are related to their very high antioxidant virtue (Moselhy et al., 2012; Khan et al., 2005) and increasing antioxidant capacity of male diabetic sperms manifested by recovered sperm parameters and decreased MDA level.

There are earlier studies similar to our study however they have investigated the effect of ginger and cinnamon (at the dose of 250mg/kg and 500mg/kg) separately on fertility in male diabetics for 65 days (Shalaby et al., 2010; Hajef et al., 2010). In addition to testosterone, we examined the LH and FSH hormones and serum antioxidants. Significant decrease in testosterone, LH and FSH level found in our diabetic rats. This finding is parallel to that of the previous investigations (Hemayatkhah Jahromi et al., 2011; Khaki et al., 2009). It is understood that high blood glucose induces changes in leydig cells, including decrease in androgen synthesis (Foglia et al., 1996) and changes in the pituitary–testicular axis with subsequent decrease in LH level. LH itself is responsible for normal Leydig cell function (Steiger and Rabe, 1996) and plays an important role in testosterone production (Parivzi and Endellendorf, 1982). The significant improvement in the hormone levels of diabetic rats following ginger and cinnamon treatment in our study is most probably due to decrease in glucose level and treatment of diabetes. In the present study ginger plus cinnamon extracts rather than ginger and cinnamon alone showed synergistic recovery effects on treatment groups in terms of serum antioxidant and all other indices such as sperm parameters and hormonal levels. The effect of the anti-oxidant potency of polyphenol content of ginger and cinnamon which resembles the reduced effect of naturally existing anti-oxidants within the cells and disturbs the oxidative stress accumulation when they powered up together (Kelen and Tepe, 2007). However there has been opposing findings showing that probably using these herbs at low dosage does not have enough influence on anti-oxidant/free radical balance within the cells (Buch et al., 1988). Never the less this particular finding, has not been supported by other researchers. To illustrate more, our two previous similar investigations (Khaki et al., 2009) and the present study are in a tide agreement with each other in terms of improvements in serum antioxidants level and subsequently spermatogenesis. In conclusion the present showed that the application of ginger plus cinnamon compared with ginger and cinnamon alone in diabetic rats significantly improved the damaging effects of oxidative stress on spermatogenesis and fertility parameters. It seems that the anti-oxidant content of herbs could be increased dramatically when used in combination. Polyphenols in cinnamon and ginger are more effective at higher dosage (above 50 mg/kg).

This study is the first to show the synergistic effect of ginger and cinnamon together on spermatogenesis in diabetic rats. We suggest the habitual use of ginger and cinnamon along with other polyphenol containing herbs such as onion to lower the biomarkers of oxidative stress to improve the antioxidant defense.

Acknowledgments

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References


