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THE ANTI-OXIDANT EFFECTS OF GINGER AND CINNAMON ON SPERMATOGENESIS DYS-FUNCTION OF DIABETES RATS.

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Abstract

Background: Diabetes rats have been linked to reproductive dysfunction 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;Group2: rats received ginger (100mg/kg/rat) daily; Group 3: rats received cinnamon (75mg/kg) daily; Group 4: rats received ginger and cinnamon, (100mg/kg/rat 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.

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. In fact there has been an established relationship between the reduction in glucose load and reduction in insulin resistance and oxidative stress in diabetic people (Wang et al., 2013). Poor glucose control over the time (hyperglycemia) causing tissues to damage and produce life-threatening health complications such as micro vascular or macro vascular disease and infertility. Infertility is a health declining issue for both women and men in reproductive age. Male factor is considered a predominant factor at almost half of the infertility cases (Kovac et al., 2013). A number of studies both in human and animals have shown the correlation between increased blood sugar and low sperm quality (count) and subsequently infertility (Khaki et al., 2010; Mallidis et al., 2009). Various studies showed that even experimentally induced diabetes with streptozotocin (STZ) had a destructive effect on testis tissue structure (Khaki et al., 2010; Altay et al., 2003).

Diabetes has been also shown to alter steroid hormonal (Testosterone, luteinizing hormone, LH and follicle stimulating hormone, FSH) levels as well and subsequently to diminish spermatogenesis (Arikawe et al., 2012; La Vignera et al., 2012). Among various hypothesis for mechanism behind spermatogenesis impairment, oxidative stress has been shown to be the keen concern for researchers and considered accountable for most of disintegrating effects of both diabetes and infertility (Birben et al., 2012**;**Karunakaran and Park, 2013). It has been speculated that semen of diabetic males is susceptible for DNA damage and low sperm quality due to oxidative harm. It has been understood that increased cell dead signaling through mitochondrial membrane destruction is responsible for most of these changes and subsequent infertility (Agarwal and Sekhon, 2010;Suresh et al., 2013). Oxidative stress causes protein damage and plays a major role in the development of diabetes (Birben et al., 2012). At the same time there is this predominant view that binding of glucose to proteins or lipids has a critical role in oxidative stress and DNA damage in reproductive system of diabetic males (Suresh et al., 2013; Mallidis et al., 2011).

Highly reactive molecules or free radicals are produced constantly inside cells and oxidative stress occurs. Moreover membrane of spermatozoa is rich in polyunsaturated fatty acids and very capable of reacting with free radicals so prone to per oxidative damage (Sanocka D, Kurpisz, 2004; Henkel, 2005). On the other hand, LH and FSH hormones are the main regulatory hormones used for stimulation of steroid hormone production including testosterone and gametogenesis in both men and women (Arikave et al., 2012). When natural anti-oxidant response can't manage oxidative stress and free radicals' overload, oxidative damage occurs which is correlated to etiology of many diseases such as diabetes (Birben et al., 2012). Common synthetic drugs used for diabetes treatment and its complication, infertility, could have serious side effects such as hypoglycemia, increase in weight and toxicity of liver. Use of alternative source of medicine, and herbal medicine has aroused researchers interest these days given its little or no side effects and have been in use for the treatment of diabetes and its complications since ancient times (Levetan , 2007;Plutzky, 2011). Herbal medicine has a long history of being used for diabetes treatment in China, India and Iran (60, 82). In fact beneficial effect of natural anti-oxidants existed in various herbs and plants and their application in curing male diabetic infertile subjects has been evaluated by some researches

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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 (*Cinnamon zeylanicum*), both known for their anti-oxidant (Shagauo and Davidson, 2006; Shing et al., 2007; Krim et al., 2013; Fathiazad etal., 2013;Škrovánková 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).
Group2: 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 56^{th} 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 spermatozoa 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 consequences 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

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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 Troloxequivalents (in nmol/mL) (Quintanilhaet 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). (Quintanilhaet 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 H_2O_2 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 H_20 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 H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 cm^{-1} was used for calculation. The specific activity of catalase was expressed as moles of H_2 reduced per minute per mg protein. At 240nm of 40.0M^{-1} cm^{-1} was used for calculation. The specific activity of catalase was expressed as moles of H_2O_2 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).

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Results

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 was 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 valuesare shown in parentheses

	Control(n =10)	Ginger 100mg/kg-per day (n=10)	cinnamon 75mg/kg-per day (n=10)	ginger + cinnamon 50mg/kg-per day+ 100mg/kg-per day (n=10)	Streptozotocin 55 mg/kg (IP) (n =10)	STZ + ginger 55mg/kg (IP) +100mg/kg-per day (n=10)	(STZ+cinnamon) 55mg/kg (IP)+ 75mg/kg-per day (n=10)	(STZ+ginger+cinnamon) 55mg/kg (IP) Streptozotocin+75mg/kg-per day+ 100mg/kg-per day (n=10)
Body weight (gr)	251 ±0.365	250±0.005 (0.0001)	250±0.005 (0.0001)	251 ±0.005 (0.0001)	190.1±0.731* (0.0001)	200±0.005* (0.0001)	201±0.005 * (0.0001)	239±0.005* (0.0001)
Testis weight(gr)	1.40 ±0.821	1.41±0.579 (0512)	1.40 ±0.611 (0.5)	1.39±0.82 1 (.489)	1 ±0.05* (.079)	1.10 ±0.821 (0.212)	1.11±0.82 1 (0.22)	1.21±0.821 (0.306)
Sperm concentration (total count) (No of sperm/rat ´10^6)	38.40±1.2 9	61.60±2.34 1	50.20±2.35 1	70.40±1.2 9 1	20.1±0.731* (0.0001)	39.60±2.34* (0.001)	41.50±2.3 4* (0.001)	60.60±0.34 1
Motility (%)	33±3	81±5.33 1	63.3±0. 30 1	83±5.11*	11.05 ±5.77* (0.0001)	25±5.33* (0.0001)	22±5.33* (0.0001)	75±5.33* (0.0001)
Viability (%)	58±2.55	98.80±80 0.929	77±0.40 1	90±0.40 1	44.20±1.33* (0.0001)	52.70±80* (0.0001)	50±0.40* (0.0001)	65±0.40* (0.0001)
Testosterone(ng /ml)	4.01 ±0.50	3.71±0.387 (0.076)	3.65±0.22* (0.030)	3.88±0.22 (0.233)	1.50 ±0.05* (0.0001)	2.07±0.22* (0.0001)	2.87±0.22 * (0.0001)	3.07±0.22* (0.0001)
Blood glucose (mg/dl)	135.3 ±0.943	100.3 ±0.845* (0.0001)	104.3 ±0.842* (0.0001)	90.1±0.87 3* (0.0001)	382.6 ±0.702* (0.0001)	200.3 ±0.943* (0.0001)	233.3 ±0.943* (0.0001)	170.3 ±0.943* (0.0001)
insulin(μU/ml)	24.7 ±0.411	22.7 ±0.411* (0.0001)	21.7 ±0.411* (0.0001)	20.7 ±0.411* (0.0001)	12.1 ±0.547* (0.0001)	15.7 ±0.421* (0.0001)	14.7 ±0.401* (0.0001)	19.7 ±0.401* (0.0001)
Serum LH(ng/ml)	1.51±0.13 8	2.23±0.323* 0.997	2.00±0.413 1	2.02±0.15 3* (0.0001)	1.00±0.253* (0.0001)	1.22±0.421* (0.030)	1.23±0.42 1* (0.030)	1.33±0.453* (0.968)
Serum FSH(ng/ml)	20.37±1.7 88	21.68±2.11* 0.924	22.35±1.748 * (0.989)	21.33±1.7 48* (0.879)	15.27±1.555* (0.0001)	16.57±1.798 * (0.0001)	16.07±1.7 88* (0.0001)	16.17±1.788* (0.0001)
Total antioxidant capacity(TAC) (nmol/ml)	0.70 ±0.03	0.84±0.341* (0.886)	1.05±0.03 1	1.70 ±0.03 1	0.32 ±0.04* (0.0001)	0.51±0.05* (0.0001)	0.60±0.03 * (0.0001)	0.68±0.03* (0.0001)
Malondialdehyd e (MDA) (nmol/ml)	4.05±0.55	0.91±0.192* (0.0001)	2.65±0.55* (0.0001)	1.81±0.19 2* (0.0001)	4.1 ±0.06* ((0.0001)	2.1±0.08* (0.005)	1.99±0.08 * (0.0001)	1.1±0.08* (0.0001)
Super oxide dismutase(SOD) ,(u/g Hb)	1000±0.5 5	1500±0.55 1	1247±0.83 1	1600±0.55 1 1	765±0.55* (0.0001)	900±0.55* (0.0001)	976±0.55* (0.0001)	1000±0.55* (0.0001)
GPX),(u/mg Hb)	125±2.7	165±2.7 1	138.4±2.7 1	165±2.7 1	100±2.7* (0.0001)	111±2.7* (0.0001)	99±2.7* (0.0001)	101±2.7* (0.0001)
Catalase),(u/mg Hb)	306.1±4.0 5	320.1±4.05 1	336.1±3.05 1	350.1±4.0 5 1	200.1±4.05* (0.0001)	231.4±4.05* (0.0001)	221.4±4.0 5* (0.0001)	265.4±4.05* (0.0001)

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Data are presented as mean ± SE.
*Significant different at P< 0.05 level, (compared with healthy control and STZ control group).
*Significant different at P< 0.05 level, (compared with healthy control and STZ groups).

STZ induced diabetics showed decreased in sperm count, motility and viability significantly compare 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 compare to ginger and cinnamon alone (Table 1).

Serum glucose increased and serum insulin decreased fallowing STZ induced diabetes. After treatment with ginger, cinnamon and combined ginger and cinnamon glucose decreased and insulin increased significantly in almost all groups (P<0.05), (Table 1). In diabetic rats fed with combined ginger and cinnamon showed marked decrease in glucose and intense increase in insulin compared with ginger and cinnamon alone.

STZ induced diabetes caused decrease in serum Testosterone level, when comparing STZ group treatments(ginger, cinnamon and combined ginger and cinnamon) against healthy control it was significant increase in the total serum testosterone level after all 3 treatments with combined ginger and cinnamon having more increase (P<0.05), (Table 1).

Serum LH and FSH level went down in STZ induced diabetic control group significantly (P<0.05). Also treatment of STZ groups with ginger, cinnamon and combined ginger and cinnamon showed significant increase in serum LH and FSH level, with combined ginger and cinnamon having more intense increase (P<0.05), (Table 1).

TAC decreased significantly in STZ groups but increased significantly after treatment in all 3 ginger, cinnamon and combined ginger and cinnamon group with combined ginger and cinnamon having more intense increase when compared against healthy controls (P<0.05), (Table 1).

The MDA level showed a significant increase in STZ group. Significant decrease observed in all 3 STZ treatments with ginger, cinnamon and combined ginger and cinnamon with combined ginger and cinnamon having more intense increase when comparing against control. When compared with treatments against STZ control, there were also significant (P<0.05), (Table 1).

SOD decreased significantly in STZ induced diabetic but increased significantly after the treatment in all 3 STZ treatment (ginger, cinnamon and combined ginger and cinnamon) with combined ginger and cinnamon having more intense increase (P<0.05), (Table 1).

GPX decreased significantly in STZ induced diabetics, It has increased significantly after treatment in all 3 STZ treatment (ginger, cinnamon and combined ginger and cinnamon) with combined ginger and cinnamon having more intense increase. When compared combined ginger and cinnamon against STZ control it was also significant (P<0.05), (Table 1).

Catalase decreased in STZ induced diabetics but increased significantly after treatment in all 3 STZ treatment (ginger, cinnamon and combined ginger and cinnamon) with combined ginger and cinnamon having more intense increase (P<0.05), (Table 1).

Discussion

Diabetes has defecting 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).

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 specially 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 synergetic 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 (Roessner 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).

Serum antioxidants level decrease indicated (TAC,SOD,GPX and CAT)at hyper glycemic state, known to affect cellular antioxidants level and as a result cell injury or cell death would occur; this will in turn impair spermatogenesis process and consequently will decrease sperm count (moslemi et al., 2011;Arikawe et al.,2012; Hesham et al.,2008).Moreover, polyunsaturated fatty acids of spermatozoa plasma membrane are very vulnerable to oxidative damage and could easily get destroyed by overload of free radicals'. Loss of sperm motility is believed to be related to the same mechanism in the membrane of spermatozoa (Sanocka and Kurpisz, 2004; Henkel, 2005).Decrease in anti-oxidant capacity is manifested by an increase in MDA level. MDA is a byproduct of lipid per-oxidation, and has a reverse relation with serum anti-oxidants' capacity. Our results are consistent with the results of other investigators indicating that semen of infertile men are depleted in antioxidants' level of TAC,SOD, GPX and catalase accompanied by increase in seminal MDA level (Yüce et al., 2012). Increased lipid per-oxidation is considered as responsible factor for these changes in infertile men (**A**garwal et al., 2003; Hesham et al., 2008).

The sperm cells of human are indicated to possess all required antioxidants for their defense to scavenger free radicals overload. Among them GPX, plays a determining role in antioxidants defense system and spermatozoa protection. Any interruption in the cellular natural antioxidant system by free radical overload (in our case caused by diabetes) would result in antioxidant function impairment. Understanding the free radicals, being responsible for most cases of infertility and finding a proper way to the cure than medications (Levetan 2007), is an important health inclining

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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;Yüce et al., 2012; Tempest et al., 208; 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;HemayatkhahJahromi et al., 2012;Riaz et al., 2011;Onwuka et al., 2011; Modaresi et al., 2009; Hafez 2010;Mashhadi 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., 2003) 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; Hafez et al.,2010) . In addition to testosterone, we examined the LH and FSH hormones and serum anti-oxidants. Significant decrease in testosterone, LH and FSH level foundin 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 (Steger and Rabe, 1996) and plays an important role in testosterone production (Parivzi and Ellendorff, 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 synergetic recovery effects on treatment groups in terms of serum antioxidant and all other indices such as sperm parameters and hormonal levels. The role of the anti-oxidant potency of polyphenol content of ginger and cinnamon which resembles the reduced effect of naturally existed 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 ginger and cinnamon are more effective at higher dosage (above 50 mg/kg).

This study is the first to show the synergetic 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.

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