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Effects of cinnamon (*Cinnamomum zeylanicum*) bark oil on testicular antioxidant values, apoptotic germ cell and sperm quality

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Summary

Cinnamon and its contents have multifactorial properties such as antioxidant, anti-inflammatory and antidiabetic. Male infertility is one of the major health problems in life. The aim of this study was to investigate the effects of long-term cinnamon bark oil (CBO) ingestion on testicular antioxidant values, apoptotic germ cell and sperm quality of adult rats. Twelve male healthy Wistar rats were divided into two groups, each group containing six rats. While olive oil was given to control group, 100 mg kg^{-1} CBO was administered to the other group by gavage daily for 10 weeks. Body and reproductive organ weights, sperm characteristics, testicular lipid peroxidation and antioxidant enzyme activities, and testicular apoptosis via terminal deoxynucleotidyl transferase–mediated dUTP nick end labelling (TUNEL) method were examined. A significant decrease in malondialdehyde level and marked increases in reduced glutathione level, glutathione peroxidase and catalase activities were observed in rats treated with CBO compared with the control group. CBO consumption provided a significant increase in weights of testes and epididymides, epididymal sperm concentration, sperm motility and diameter of seminiferous tubules when compared with the control group. However, CBO consumption tended to decrease the abnormal sperm rate and apoptotic germ cell count, but it did not reach statistical significance. It is concluded that CBO has improvement effect on testicular oxidant–antioxidant balance and sperm quality, and its consumption may be useful for asthenozoospermic men.

Introduction

Reactive oxygen species (ROS) are highly reactive oxidising agents belonging to the class of free radicals. The production of ROS in various organs including the testis is a normal physiological event; however, the alterations in their synthesis stimulate the oxidation and DNA damage of cells (Sikka, 1996). The plasma membrane of spermatozoa contains a high amount of polyunsaturated fatty acids (PUFAs). Therefore, it is particularly susceptible to peroxidative damage. The lipid peroxidation (LPO) destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the defects of membrane integrity (Sanocka & Kurpisz, 2004; Henkel, 2005). Antioxidants, in general, are compounds that scavenge and suppress the formation of ROS and LPO. Among the well-known biological antioxidants, glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase have a significant role as a suppressor or scavenger of free radical. Hence, the application of ROS scavengers is likely to improve sperm function (Sikka, 1996; Vernet *et al.*, 2004).

Cinnamon has been used as a spice and as traditional herbal medicine for centuries. The genus cinnamomum comprises of about 250 species. The most important volatile oils derived from cinnamon are *Cinnamomum zeylanicum* bark and leaf oils, *C. cassia* (cassia oil) and

C. camphora. However, a number of other cinnamomum species are distilled on a smaller scale, and the oils are used either locally or exported to regional markets (Jayaprakasha & Rao, 2011). Eugenol is the main chemical compound of oil extracted from *C. zeylanicum* leaf. However, the principal bioactive compound of oil extracted from *C. zeylanicum* bark is the cinnamaldehyde (Gruenwald *et al.*, 2010; Jayaprakasha & Rao, 2011). Recent studies have demonstrated that cinnamon has anti-inflammatory (Kim *et al.*, 2007), antibacterial (Lopez *et al.*, 2007), antifungal (Velluti *et al.*, 2004), antiviral (Premanathan *et al.*, 2000), antineoplastic (Ka *et al.*, 2003; Schoene *et al.*, 2005), antihyperglycaemic and antihyperlipidaemic (Kim & Choung, 2010) properties. Additionally, many investigators (Jayaprakasha *et al.*, 2006; Prasad *et al.*, 2009; Ciftci *et al.*, 2010; Azab *et al.*, 2011) have reported that extracts from different cinnamomum species have free radical scavenger and potent antioxidant activity.

Some studies revealed that ethanolic extract of *C. zeylanicum* bark had an improvement effect on reproductive organ weights (Shah *et al.*, 1998), sperm quality parameters (Shah *et al.*, 1998; Hafez, 2010; Shalaby & Mouneir, 2010) and LH, FSH, testosterone concentrations (Modaresi *et al.*, 2009; Hemayatkhah Jahromi *et al.*, 2011) in laboratory animals. However, there is no evidence about the effect of long-term ingestion of cinnamon bark oil (CBO) on testicular apoptosis and the changes in testicular tissue oxidant–antioxidant balance. Therefore, to see the effects of CBO, a potent antioxidant, on these parameters, we examined apoptotic germ cells, testicular tissue LPO and antioxidant enzyme activities in conjunction with the epididymal sperm characteristics of rats in this study.

Materials and methods

CBO and chemicals

Cinnamon bark oil was purchased from a local store (Altınterim Co., Elazığ, Turkey). According to the manufacturer's procedure, *C. zeylanicum* barks were transported in polypropylene bags and were dried to constant weight in room temperature. CBO was obtained by hydro-distillation method. The plant materials (about 100 g) were then ground into small pieces and were placed in a flask (2 l) together with double distilled water (1.5 l). The mixture was boiled for 4 h. The extract was condensed in cooling vapour to collect the essential oil. The extracted oil was dried over anhydrous sodium sulphate. CBO was kept at 4 °C until used. The other chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and experimental design

The experimental protocols were approved by the local Animal Use Committees of Firat University (Elazig, Turkey): animal care and experimental protocols complied with the NIH Guide for the Care and Use of Laboratory Animals. Twelve healthy adult male Wistar albino rats, aged 5 months, were obtained and maintained from Firat University Experimental Research Centre (Elazig, Turkey). The animals were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of 24 ± 3 °C, humidity of 45–65%. During the whole experimental period, animals were fed with a balanced commercial diet (Elazig Food Company, Elazig, Turkey) *ad libitum* and fresh distilled drinking water was given *ad libitum*.

Cinnamon bark oil was administered by gavage at the dose of 100 mg kg^{-1} day^{-1} for 10 weeks. To equalise the total amount (1 ml) that each rat will receive in each application, CBO was further suspended in olive oil and the CBO ratio in 1 ml of olive oil was adjusted according to the weight of each rat. As the toxicity studies suggest that dose of 100 mg kg^{-1} day^{-1} has no toxic effect (Shah *et al.*, 1998), therefore, this dose was used in this study. Because the spermatogenic cycle, including spermatocytogenesis, meiosis and spermiogenesis, is 48–52 days (Bennett & Vickery, 1970) and epididymal transit of spermatozoa is approximately 1 week (Kempinas *et al.*, 1998) in rats, the treatment period in this study was set at 10 weeks for the maximum effect. Each rat was weighed weekly, and dosing suspensions were adjusted for changes in body weights during experimental period. The rats were randomly divided into two groups, each group containing six rats. Only 1 ml of pure olive oil was administered by gavage to rats in the first group, and they served as *control*. One millilitre of olive oil containing 100 mg kg^{-1} CBO was given by gavage to rats in the second group and named as *CBO*.

Sample collection and homogenate preparation

The rats were sacrificed using ether anaesthesia at the end of 10th week. Testes, epididymides, seminal vesicles and ventral prostate were removed, cleared of adhering connective tissue and weighed. One of the testis samples was fixed in Bouin's solution for histological examination. The other testis samples were stored at −20 °C for biochemical analyses. Testes were taken from a −20 °C freezer and immediately transferred to the cold glass tubes. Then, the testes were diluted with a 9-fold volume of PBS (pH 7.4). For the enzymatic analyses, testes were minced in a glass and homogenised by a Teflon-glass

homogenizator for 3 min in cold physiological saline on ice (Türk *et al.*, 2010).

Testicular tissue lipid peroxidation (LPO) level and antioxidant enzymes

Lipid peroxidation level was measured according to the concentration of thiobarbituric acid reactive substances, and the amount of produced malondialdehyde (MDA) was used as an index of LPO. One volume of the test sample and two volumes of stock reagent (15%, *w/v* trichloracetic acid in 0.25 N HCl and 0.375%, *w/v* thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated in boiling water for 15 min. After cooling, the precipitate was removed by centrifugation at 1500 ***g*** for 10 min, and then, absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). The MDA level was expressed as mmol g protein^{-1} (Placer *et al.*, 1966).

Reduced glutathione (rGSH) level was measured using the method of Sedlak & Lindsay (1968). The samples were precipitated with 50% trichloracetic acid and then centrifuged at 1000 ***g*** for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris–EDTA buffer (0.2 M; pH 8.9) and 0.1 ml of 0.01 M 5,5′-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer. The level of rGSH was expressed as nmol g protein^{-1}.

Glutathione peroxidase (EC 1.11.1.9) activity was determined according to the method of Lawrence & Burk (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 IU/ml oxidised glutathione (GSSG)-reductase, 1 mM GSH and 0.25 mM H_2O_2. Enzyme source (0.1 ml) was added to 0.8 ml of the above mixture and incubated at 25 °C for 5 min before initiation of the reaction with the addition of 0.1 ml of peroxide solution. The absorbance at 340 nm was recorded for 5 min on the spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidised per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. The GSH-Px activity was expressed as IU g protein$^{-1.}$

Catalase (EC 1.11.1.6) activity was spectrophotometrically determined by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm and was expressed as kg protein^{-1}, where k is the first-order rate constant (Aebi, 1983). Protein concentration was determined using the method of Lowry *et al.* (1951).

Determination of apoptotic germ cells in the testis

The apoptotic germ cells were defined by terminal deoxynucleotidyl transferase–mediated dUTP nick end labelling (TUNEL) assay with the ApopTag Peroxidase *In Situ* Apoptosis Detection kit (Chemicon, Temecula, CA, USA). Briefly, the fixed testicular tissue in Bouin's solution was embedded in paraffin and sectioned at 4 μm thickness. The paraffin sections were deparaffinised in xylene, dehydrated through graded alcohol and washed in PBS. The sections were treated with 20 mg ml^{-1} proteinase K for 5 min, which was followed by treatment with 3% H_2O_2 for 5 min to inhibit endogenous peroxidase. After re-washing with PBS, sections were then incubated with the TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT) enzyme and digoxigenin-11-dUTP at 37 °C for 1 h in humidified chamber, and then, stop-wash buffer was applied for 30 min at 37 °C. Sections were visualised with 3-amino-9-etilcarbazole (AEC) substrate. Negative controls were performed using distilled water in the place of the TdT enzyme. Finally, sections were counterstained with Mayer's haematoxylin, rinsed in top water and mounted with glycerol. TUNEL-positive apoptotic germ cells (from spermatogonia to spermatids) in 25 seminiferous tubules (ST) per animal were counted, and mean apoptotic cell in both groups was presented.

Sperm analyses

The sperm concentration in the right cauda epididymal tissue was determined with a haemocytometer (Türk *et al.*, 2007). Freshly isolated left cauda epididymal tissue was used for the analysis of sperm motility. The percentage of sperm motility was evaluated using a light microscope with a heated stage (Sönmez *et al.*, 2005). To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin, and 0.1 M of sodium citrate) were prepared. The slides were then viewed under a light microscope at 400× magnification. A total of 300 spermatozoa were examined on each slide (1800 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as percentage (Türk *et al.*, 2007).

Histological examination

Testis tissues were fixed in Bouin's solution for 48 h, they were dehydrated through graded concentrations of ethanol, embedded in paraffin wax, sectioned at 5 μm thicknesses and stained with Mayer's haematoxylin and eosin. Twenty-five seminiferous tubules (ST) were randomly

examined per section, and their diameters and germinal cell layer thickness (GCLT; from the basal membrane towards the lumen of the tubule) were measured using an ocular micrometre in a light microscope, and the mean size of ST and GCLT was calculated. Johnsen's testicular scoring (Johnsen, 1970) was performed for control and CBO groups. Twenty-five ST from each section were evaluated, and a score between 1 (very poor) and 10 (excellent) was given to each tubule according to Johnsen's criteria.

Data analysis

Data are presented as mean ± SEM. The degree of significance was set at $P < 0.05$. Nonparametric Mann–Whitney-*U* test was used to determine the differences between the groups with respect to all parameters. All the analyses were carried out using the SPSS/PC (version 15.0; SPSS, Chicago, IL, USA) package program.

Results

Body and reproductive organ weights

Although CBO administration significantly increased the weights of testes ($P < 0.01$), whole epididymis ($P < 0.05$) and right cauda epididymis ($P < 0.05$), it did not affect the weights of final body, seminal vesicle and ventral prostate in comparison with the control group (Table 1).

Testicular tissue LPO level and antioxidant enzyme activities

The MDA and rGSH levels, GSH-Px and CAT activities of all the groups are given in Table 2. While CBO administration caused significant decreases in MDA ($P < 0.01$) level, it provided significant increases in GSH ($P < 0.01$) level, GSH-Px ($P < 0.05$) and CAT ($P < 0.05$) activities when compared with the control group.

Table 1 Effect of cinnamon bark oil (CBO) on final body weight and reproductive organ weights (mean ± SEM)

Weights (g)	Control ($n = 6$)	CBO ($n = 6$)	*P*-value
Final body	322.400 ± 11.316	330.333 ± 14.242	1.000
Testis	1.410 ± 0.039	1.635 ± 0.030*	0.004
Whole epididymis	0.494 ± 0.016	0.535 ± 0.013#	0.029
Right cauda epididymis	0.162 ± 0.005	0.196 ± 0.009#	0.020
Seminal vesicle	0.858 ± 0.099	0.753 ± 0.112	0.628
Ventral prostate	0.432 ± 0.017	0.398 ± 0.045	0.842

*Significantly different from control ($P < 0.01$).
#Significantly different from control ($P < 0.05$).

Epididymal sperm parameters

The effects of CBO on epididymal sperm concentration, sperm motility and abnormal sperm rate are presented in Table 3. The sperm concentration ($P < 0.01$) and sperm motility ($P < 0.01$) were found significantly to be higher than control group after CBO administration. Head, tail and total abnormal sperm rates of rats treated with CBO were numerically lower than control group rats, but these decreases did not reach the statistical significance.

Histological and apoptotic findings

The histological structures of control and CBO-treated rats were normal (Fig. 1a,b). No prominent difference was observed in testicular histological view in terms of germ cell order, Sertoli and Leydig cell populations, GCLT and Johnsen's testicular scoring between control and CBO-treated groups. However, CBO treatment provided significant ($P < 0.001$) increases in diameters of ST when compared with the control group (Table 3). Although the presence and distribution of apoptotic cells in both groups showed similarity to each other (Fig. 1c,d), CBO consumption tended to decrease the apoptotic germ cell count in comparison with the control group (Table 3).

Discussion

Essential oils, also known as volatile oils, are concentrated natural plant products that contain volatile aroma compounds. These mixtures of volatile compounds exert different biological actions on humans and animals (Adorjan & Buchbauer, 2010). The volatile oils obtained from the bark, leaf and root of *C. zeylanicum* vary significantly in chemical composition, which suggests that they vary in their pharmacological effects as well (Shen *et al.*, 2002). These oils of three different parts of the plant possess the same array of monoterpene hydrocarbons in different proportions. However, each oil has a different

Table 2 Effect of cinnamon bark oil (CBO) on testicular tissue malondialdehyde (MDA) and reduced glutathione (rGSH) levels and glutathione-peroxidase (GSH-Px) and catalase (CAT) activities (mean ± SEM)

Variables	Control ($n = 6$)	CBO ($n = 6$)	*P*-value
MDA (mM g protein^{-1})	7.59 ± 0.46	5.26 ± 0.14*	0.004
rGSH (nmol g protein^{-1})	5.49 ± 0.31	8.41 ± 0.20*	0.004
GSH-Px (IU g protein^{-1})	1.36 ± 0.42	2.76 ± 0.29#	0.032
CAT (k g protein^{-1})	34.42 ± 9.24	104.70 ± 26.43#	0.017

*Significantly different from control ($P < 0.01$).
#Significantly different from control ($P < 0.05$).

Table 3 Effect of cinnamon bark oil (CBO) on sperm quality parameters, diameters of seminiferous tubules (ST), germinal cell layer thickness (GCLT), Johnsen testicular score and apoptotic cell count (mean ± SEM).

Variables	Control (n = 6)	CBO (n = 6)	P-value
Sperm concentration (million/right cauda epididymis)	85.00 ± 3.11	122.83 ± 5.83#	0.004
Sperm motility (%)	77.32 ± 3.71	90.22 ± 0.22#	0.003
Abnormal sperm rate (%)			
Head	4.00 ± 0.83	3.00 ± 0.44	0.429
Tail	7.00 ± 1.22	4.50 ± 0.84	0.126
Total	11.00 ± 1.87	7.50 ± 0.92	0.126
Diameter of ST (μm)	254.14 ± 1.98	266.96 ± 1.64*	0.000
GCLT (μm)	104.14 ± 1.15	104.10 ± 0.90	0.653
Johnsen testicular score (1–10)	9.60 ± 0.24	9.83 ± 0.16	0.524
Apoptotic cell count	2.32 ± 0.19	1.97 ± 0.19	0.286

*Significantly different from control ($P < 0.001$).
#Significantly different from control ($P < 0.01$).

primary constituent: cinnamaldehyde (in the bark oil), eugenol (in the leaf oil) or camphor (in the root oil, Gruenwald *et al.*, 2010). In addition, water extract of *C. zeylanicum* has five phenolic compounds: protocatechuic acid, cinnamtannin B-1, urolignoside, rutin and quercetin (Jayaprakasha *et al.*, 2006). With this study, we demonstrated that long-term ingestion of the volatile oil of *C. zeylanicum* bark by adult rats provided significant improvements in sperm concentration, motility and testicular oxidant–antioxidant balance.

In this study, CBO administration significantly increased the weights of testes and epididymides. This finding is similar to the results reported by some authors who demonstrated that *C. zeylanicum* causes an increase in reproductive organ weights of normal (Shah *et al.*, 1998; Hemayatkhah Jahromi *et al.*, 2011) and diabetic animals (Hafez, 2010; Shalaby & Mouneir, 2010). However, it has been reported that *C. zeylanicum* leads to significant increases in LH, FSH and testosterone concentration without affecting the testicular weight (Modaresi *et al.*, 2009). The significant increase in the absolute weights of the testis and epididymis observed in the present study could possibly be due to the CBO-stimulated increased testosterone concentration (Modaresi *et al.*, 2009; Hemayatkhah Jahromi *et al.*, 2011), which is the necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands, and its level is positively correlated with the weight of male reproductive organs. In addition to testosterone effect on reproductive organ weights, it is plausible that the increased weight of the epididymal tissue reflects a dual effect of increased testosterone levels and also increased sperm count in this organ as evidenced by a significant increase in the epididymal sperm concentration in this study.

Many compounds, metabolised by cells, cause increase in the levels of electrophilic radicals that can react with oxygen giving rise to ROS, one of the main sources of free radicals like H_2O_2, singlet-oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$) or peroxynitrite. ROS are normally synthesised in several essential metabolic processes for living cells including the spermatozoa; however, excessive generation of ROS produced by spermatozoa, especially abnormal spermatozoon, themselves (de Lamirande *et al.*, 1997) induces the formation of toxic lipid peroxides. When ROS begin to accumulate, cells exhibit a defensive

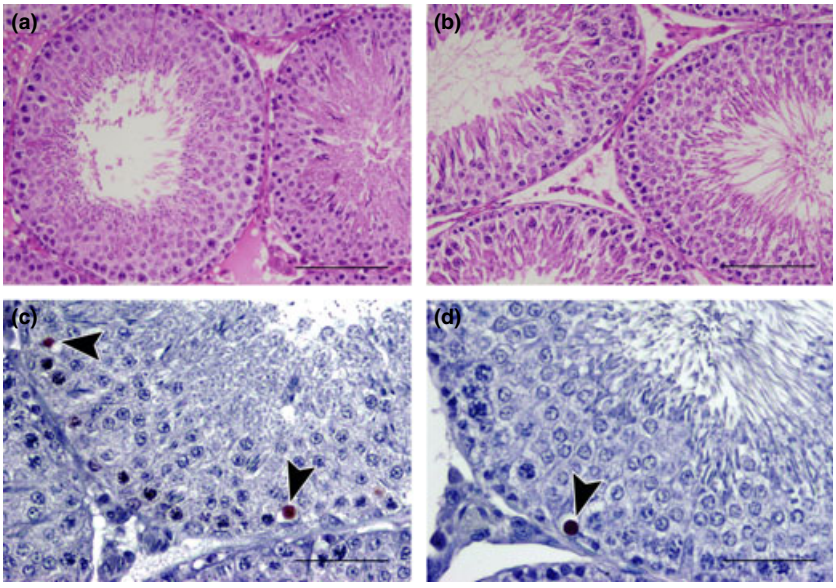

Fig. 1 Representative photomicrographs of normal histologic- and terminal deoxynucleotidyl transferase–mediated dUTP nick end labelling (TUNEL)-staining in the testes of control and cinnamon bark oil (CBO)-treated rats. (a) Hematoxylin & eosin-staining of control group. Calibration bar = 80 μm. (b) Hematoxylin & eosin-staining of CBO-treated group. Calibration bar = 80 μm. (c) TUNEL-staining of control group. Calibration bar = 40 μm. (d) TUNEL-staining of CBO-treated group. Calibration bar = 40 μm. Arrows indicate apoptotic cells.

mechanism using various antioxidant enzymes. The main detoxifying systems for peroxides are CAT and GSH. CAT is an antioxidant enzyme, which destroys H_2O_2 that can form a highly reactive $^{\bullet}OH$ in presence of iron as a catalyst. By participating in the glutathione-redox cycle, GSH together with GSH-Px converts H_2O_2 and lipid peroxides to nontoxic products (Sikka, 1996; Sanocka & Kurpisz, 2004). The antioxidant, metal chelating and free radical scavenging activity of phenolic (Jayaprakasha *et al.*, 2006; Cao *et al.*, 2008; Prasad *et al.*, 2009) and oil (Ciftci *et al.*, 2010; El-Baroty *et al.*, 2010) compounds derived from *C. zeylanicum* have been reported. A significant decrease in MDA level, byproduct of LPO, and marked increases in rGSH level, GSH-Px and CAT activities were observed in testicular tissue of rats consumed CBO in the present study. These findings demonstrate that CBO has a potent antioxidative effect.

Reactive oxygen species are highly reactive and can react with many intracellular molecules, mainly PUFAs (phospholipids, glycolipids, glycerides and sterols) and trans-membrane proteins with oxidisable amino acids. The oxidation of these molecules causes increase in the cellular membrane permeability. ROS can attack to the unsaturated bonds of the membrane lipids in an autocatalytic process, with the genesis of peroxides, alcohol and lipidic aldehydes as byproduct of the reaction. Thus, the increase in free radicals in cells can induce the LPO by oxidative breakdown of PUFAs in membranes of cells (de Lamirande *et al.*, 1997; Henkel, 2005). Spermatozoa are especially susceptible to peroxidative damage because of high concentration of PUFAs, which are involved in regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion, and low antioxidant capacity. Obviously, peroxidation of sperm lipids destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability and increased midpiece morphological defects, and even it completely inhibits spermatogenesis in extreme cases (Sikka, 1996; Sanocka & Kurpisz, 2004; Vernet *et al.*, 2004). It has been reported that *C. zeylanicum* consumption causes significant increases in sperm motility and concentration in normal (Shah *et al.*, 1998) and diabetic (Hafez, 2010; Shalaby & Mouneir, 2010) laboratory animals. However, Buch *et al.* (1988) alleged that cinnamon oil was spermicidal when it was incubated with human semen samples for a long period. In the present study, it was observed that CBO treatment provided significant increases in the epididymal sperm concentration and sperm motility when compared with the control. These findings are in agreement with the results of earlier studies (Shah *et al.*, 1998; Hafez, 2010; Shalaby & Mouneir, 2010). The contradictory between our findings and results of Buch *et al.* (1988) is due to the *in vitro* addition of CBO to semen. Improvements observed in sperm quality of CBO-treated rats may be attributed to reduction of LPO and increments of antioxidant enzymes as evidenced by a significant decrease in MDA level and increase in rGSH level, GSH-Px and CAT activities.

In this study, CBO consumption for a long period tended to decrease the apoptotic germ cells. No evidence was found with regard to direct effect of cinnamon bark or its oil and other extracts on testicular apoptosis. However, phenolic compounds like ellagic acid have anti-apoptotic effect against LPO-induced testicular apoptotic cell count (Türk *et al.*, 2010, 2011). Besides, it has been reported that an increase in free radicals results in increased testicular apoptotic germ cell (Maheshwari *et al.*, 2009). The tendency to decrease in apoptotic germ cell count after CBO ingestion may possibly related to the decreased LPO level as evidenced by a significant decrease in MDA level.

In the present study, CBO administration had no prominent effect on testicular histological architecture of rats except diameters of ST in which significant increase was observed when compared with the control group. Although Hemayatkhah Jahromi *et al.* (2011) reported that the use of cinnamon hydroethanolic extract provided increases in germ cell count in ST, Modaresi *et al.* (2009) alleged that it had no any effect on testicular histological findings. Besides, Türk *et al.* (2008) demonstrated that daily consumption of pomegranate juice, which is rich from phenolic compound, by rats enlarged the diameter of ST and increased spermatogenic cell density associated with the decreased LPO level and increased antioxidant enzymes. Our findings are partially supported by earlier studies mentioned earlier with respect to testicular histological view. Not being a marked change in testicular view and germ cell density, though, sperm concentration and diameters of ST increased after CBO consumption may be explained by the tendency to decrease in apoptotic cell count in the present study.

In conclusion, the findings of the present study clearly suggest that long-term CBO ingestion results in improved sperm quality and a tendency to decrease in apoptotic germ cells associated with the decreased testicular LPO and increased antioxidant enzyme activities in rats. It was concluded that daily ingestion of CBO at least for 10 weeks may be useful for asthenoazoospermic men.

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