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ORIGINAL ARTICLE

The protective effect of coenzyme Q10 and berberine on sperm parameters, with and without varicocelectomy in rats with surgically induced varicoceles

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Abstract

The current study aimed to investigate the protective effects of coenzyme Q10 (Co Q10) and berberine (BB) with and without varicocelectomy on sperm parameters in postoperative varicocele rats. For the current purpose, a total of 60 mature male Wistar rats were randomly divided into control ($n = 6$ rats), control-sham ($n = 6$ rats), and experimental ($n = 6$ rats) groups. The animals in the experimental groups were undergone experimental varicocele, and simple laparotomy was performed in control-sham group. The experimental group was subdivided into the following groups 60 days after varicocele (VCL) induction: non-treated VCL-induced rats ($n = 6$ rats), VCL-induced rats administered 100 mg (kg per day) BB ($n = 6$ rats), VCL-induced rats administered Co Q10 75 mg (kg per day) ($n = 6$ rats), VCL-induced rats administered 100 mg (kg per day) BB + Co Q10 75 mg (kg per day) ($n = 6$ rats), varicocelectomy rats ($n = 6$ rats), varicocelectomy rats administered 100 mg (kg per day) BB ($n = 6$ rats), varicocelectomy rats administered Co Q10 75 mg (kg per day) ($n = 6$ rats), varicocelectomy rats administered 100 mg (kg per day) BB + Co Q10 75 mg (kg per day) ($n = 6$ rats). Following 60 days, the animals were euthanized and sperm parameters were evaluated. Non-treated VCL-induced animals indicated a significant ($P < 0.05$) decrease in sperm parameters and a significant ($P < 0.05$) increase in sperm DNA damage compared to control and control-sham groups. Insignificant changes were found between control and control-sham groups. Meanwhile, each treatment group showed a remarkable ($P < 0.05$) increase in sperm parameters as well as a significant ($P < 0.05$) decrease in sperm DNA damage. Based on current results, BB and Co Q10 alone and/or together could improve sperm parameters and reduce sperm DNA damage in varicocele-induced rats compared to control and control-sham groups. Varicocelectomy alone will improve sperm parameters, but this recovery will be greater when combined with Co Q10 and BB.

Keywords Varicocele · Berberine · Co Q10 · Sperm parameters · DNA damage · Varicocelectomy

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Introduction

Varicocele is an abnormal vasodilation of the plexus of the testis (Benoff and Gilbert 2001). Varicocele is the most obvious common cause of male infertility (Haddad et al. 2014). Clinical varicocele found that in 15% of the general male population, 35% had primary infertility and up to 81% had secondary infertility (Meacham et al. 1994). Many patients with varicocele have altered spermatogenesis which may be due to various factors, including reflux of toxic kidney and adrenal metabolites, abnormal hormone levels, spermatic venous hypertension, oxidative stress, testicular abnormal temperature, and severe venous stasis hypoxia (Sakamoto et al. 2008).

Varicocele can cause abnormalities in sperm parameters, especially sperm count, motility, and morphology (Naftulin et al. 1991). The number of sperm with fragmented DNA in the ejaculate of patients with varicocele is higher compared to that of healthy individuals (Mostafa et al. 2009). This may be associated with increased reactive oxygen species (ROS) leading to oxidative stress (OS), causing sperm plasma membrane peroxidation and nuclear DNA damage (Enciso et al. 2006). To prevent sperm abnormality and infertility, early diagnosis of varicocele seems to be required (Skoog et al. 1997). Varicoceles are mostly modified through surgery in order to correct male infertility (Abdel-Maguid and Othman 2010). Varicocelectomy can improve semen parameters and testosterone levels in patients with varicocele (Li et al. 2012). Moreover, it can reduce the concentration of reactive oxygen species and enhance antioxidant capacity in these patients (Mostafa et al. 2001). However, there is some evidence that there are still some cases of varicocelectomy in which sperm parameters and fertility are not improved; in fact, there is no difference in the pregnancy rate between surgical varicocelectomy and non-surgical treatment (Baazeem et al. 2011). In addition, varicocelectomy is associated with complications, including hydrocele genesis, surgical infection, chronic orchia, and/or recurrence of varicocele (Fretz and Sandlow 2002). Studies have shown that the application of antioxidants can reduce the number of DNA damage and improve semen parameters (Oliva et al. 2009; Ahmadi et al. 2016; Fallah et al. 2017). Therefore, the use of antioxidants appears to be useful in the treatment of infertile patients with varicocele (Ahmadi et al. 2016; Cocuzza et al. 2007; Mohammadi et al. 2018). Coenzyme Q10 (Co Q10) is also referred to as ubiquinone; it is a stronger antioxidant than vitamin E, so it neutralizes free radicals (Nagaoka et al. 2000).

Coenzyme Q10 is an integral part of the electron transport chain that participates in aerobic cell respiration and ultimately produces energy. Studies have shown that sperm concentration, motility, and semen parameters are related to CoQ10 concentration, because CoQ10 can reduce stress oxidation, increase antioxidant enzyme activity, and improve overall antioxidant capacity (Balercia et al. 2009; Lafuente et al. 2013).

Berberine (BB) is present in many plants as alkaloid salts, including Oregon grape (*Berberis aquifolium*), barberry (*Berberis vulgaris*), and turmeric (*Berberis aristata*). Berberine has been found to have broad antioxidant activity (Shirwaikar et al. 2006). In addition, the anti-inflammatory effect of berberine is demonstrated in vitro and in vivo (Cheng et al. 2013).

Due to the oxidative stress and the inflammatory conditions in varicocele as well as the complications and failure of varicocelectomy in some cases, the current study aim to evaluate the protective effects of Co Q10 and berberine as an antioxidant and anti-inflammatory chemical and the effect of varicocelectomy as a surgical procedure to varicocele correction and also the effect of these factors together against varicocele pathogenesis.

To this end, sperm parameters, including sperm count, motility, and morphology, and sperm DNA fragmentation were studied in the current study.

Materials and methods

Animals

A total of 60 mature male Wistar rats, deliberation 150–200 g, were purchased from the Experimental Animal Center of Kashan University of Medical Sciences. The rats were kept under controlled environmental conditions in a 12-h light and dark cycle. Rats had free get entry to food and water. All experiments have been in accordance with worldwide standards for the care and use of laboratory animals.

Following 1-week acclimatization, rats were randomly divided into three groups: group I, control group ($n = 6$ rats); group II, sham operation group ($n = 6$ rats); group III, varicocele-induced rats ($n = 48$ rats). The animals in experimental groups were subjected to an experimental varicocele, and a simple laparotomy was performed in a sham operation group. The experimental group was divided into the following groups 60 days after varicocele (VCL) induction:

a) Non-treated VCL-induced rats ($n = 6$ rats)
b) VCL-induced rats administered 100 mg (kg per day) BB intraperitoneally ($n = 6$ rats)
c) VCL-induced rats administered Co Q10 75 mg (kg per day) by gavage ($n = 6$ rats)
d) VCL-induced rats administered 100 mg (kg per day) BB intraperitoneally + Co Q10 75 mg (kg per day) by gavage ($n = 6$ rats)
e) Varicocelectomy rats ($n = 6$ rats)
f) Varicocelectomy rats administered 100 mg (kg per day) BB intraperitoneally ($n = 6$ rats)
g) Varicocelectomy rats administered Co Q10 75 mg (kg per day) by gavage ($n = 6$ rats)

h) Varicocelectomy rats administered 100 mg (kg per day) BB intraperitoneally + Co Q10 75 mg (kg per day) by gavage ($n = 6$ rats)

All chemicals were administrated for 60 continuing days.

Surgical procedure

In varicocele-induced rats, a left varicocele was induced. Briefly, rats were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine (1 mg/kg body weight) (Cam et al. 2004; Kashani et al. 2013). We made a midline abdominal incision. Then, we carefully dissected the left renal vein. After the semen vein is connected, the loose suture is tied around the renal vein. The diameter of the renal vein is reduced to 1 mm.

The midline incision was sewn into two layers of 3-point silk suture. In the sham surgery group, only a simple laparotomy was performed and the renal vein was dissected, but it was not bound (Herbenick et al. 2008).

In varicocelectomy groups (e, f, g, h) 60 days after varicocele induction, after anesthesia and midline incision, the suture tied around the renal vein was cut and exited, and then, the midline incision was closed.

Epididymal sperm count, viability, motility, and sperm DNA damage

Male rats were killed for sperm collection and the epididymis was carefully separated from the testis using a stereo zoom microscope at × 20 magnification (Model TL2, Olympus Co., Tokyo, Japan). The caudal parts of the left 1were grinded in 5-mL Hanks' medium.

The epididymal tissue was separated from the released spermatozoa. The released spermatozoa (10 μL) were transferred to a hemocytometer, and the sperm was counted under an optical microscope at × 40 magnification (million/mL) (Cheng et al. 2006).

Sperm motility was determined with a microscope (Olympus IX70) [× 40 magnification] in ten fields according to the World Health Organization recommended method.

Smear was prepared by eosin-nigrosin to assess death, abnormalities, and morphologically immature sperm (MIS). Staining red sperm is considered to be infeasible; those with cytoplasmic residues are considered morphologically immature (Fig. 1). For this purpose, unstained live sperms were analyzed in ten fields (Khaki et al. 2010).

To evaluate sperm DNA damage (denaturation), an acridine orange staining kit (Sigma Co., St. Louis, MO, USA) was used. Briefly, a drop of 10 μL of sample was placed in the slide and a smear of samples was prepared. After drying smear in the air, Lam using fixative Carnoy's solution fixed away from light and in a humid environment at a temperature of 4 °C for 2 h. About 80 mL of acridine orange color bleeds on the slides and we put them in a dark place for 90 min. The stained shoots were washed with PBS. The sample was analyzed by an epifluorescence microscope (Model GS7, Nikon Co., Japan). Sperm showing green fluorescence has normal DNA, while sperm showing yellow-orange to red fluorescence has damaged DNA (Fig. 1) (Chohan et al. 2004; Tejada et al. 1984).

Statistical analyses

All values are expressed as mean ± standard deviation (SD) (Ferdosian et al. 2015; Jalali et al. 2016; Dehghani et al. 2016). One-way analysis of variance (ANOVA) was performed using SPSS software version 13.OSS to determine differences in staining characteristics for all groups. $P < 0.05$ was considered statistically significant.

Data availability The primary data for this study is available from the authors on direct request.

Results

Changes in sperm parameters

The results of the comparison of sperm parameters in ten groups including control, sham, and varicocele-induced rats are shown in Table 1. The sperm count, vitality, motility, and morphology of the left epididymis of varicocele group were significantly decreased in comparison with those of control and sham groups ($P < 0.05$). Sperm parameters of the left epididymis in the treatment group increased significantly in comparison with those in the varicocele group ($P < 0.05$). However, there was no significant difference between control and control-sham groups. Among the treatment groups, the sperm parameters of the varicocelectomy + BB + Co Q10 group are very close to those of the control group, and with it, there is no significant relationship (except morphological immature sperm parameter).

Changes in *sperm* DNA damage

The results of the comparison of sperm DNA damage in ten groups are shown in Table 1. There was no significant difference between control and control-sham groups, but the varicocele group showed significant differences in comparison to the control group ($P < 0.05$). Sperm DNA damage significantly improved in the treatment groups compared to that in the varicocele groups in AO test ($P < 0.05$). Among the treatment groups, the sperm DNA damage of the varicocelectomy + BB + Co Q10 group are very close to that of the control group, and with it, there is no significant relationship.

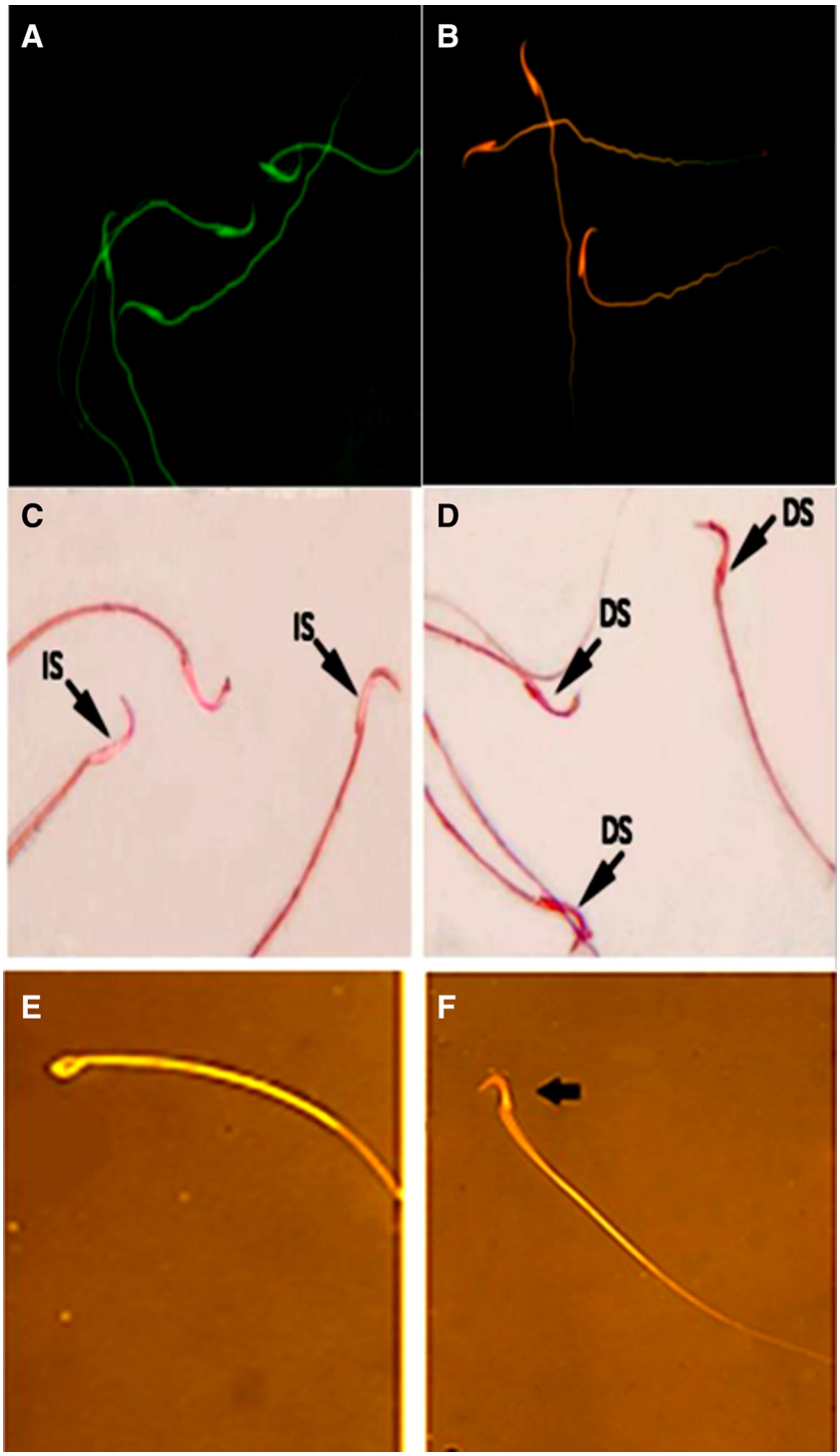

Fig. 1 **a** AO^{-} (sperm with normal DNA), × 100. **b** AO^{+} (sperm with damaged DNA), × 100. **c**, **d** Eosin-nigrosin staining for the examination of live sperm, × 400; IS (intact sperm), live and healthy sperm that has a colorless cytoplasm; DS (dead sperm), dead sperm with pink cytoplasm. **e**, **f** Eosin-nigrosin staining for the examination of morphological immature sperm (MIS) × 100

Discussion

Varicocele is a common disease in men (de la Calle et al. 2001) and is commonly diagnosed as infertility (Hauser et al. 2001). Factors of varicocele include hyperthermia (Miyaoka and Esteves 2012), androgen disease (Comhaire 1991), exposure to toxic substances (Benoff et al. 2004), ovarian hypoxia (Li et al. 1999), increased oxidative stress (Benoff and Gilbert 2001), adrenal retrograde blood, and increased apoptosis of spermatogenic cells (Wang et al. 2010). Resection of varicocele is considered to be an effective method of treatment in patients with varicocele (Fretz and Sandlow 2002; Ding et al. 2012).

However, some patients did not improve their sperm parameters after surgery (Unal et al. 2001). In addition, varicocelectomy surgery has complications that are

Table 1 Comparison of sperm parameters in ten groups including control, sham, and varicocele-induced rats

Sperm parameters/groups	Sperm count	Sperm motility	Morphological immature sperm	Sperm viability	Sperm DNA damage
Control	70.5 ± 7.77[a]	87.75 ± 4.78[a]	15.4 ± 4.42[a]	91.4 ± 4.48[a]	13.48 ± 4.08[a]
Sham	69.8 ± 4.82[a]	87.01 ± 3.96[a]	15.3 ± 4.15[a]	90.98 ± 4.01[a]	13.89 ± 4.13[a]
Varicocele	37.4 ± 3.90[b]	44.96 ± 8.61[b]	49.94 ± 1.64[b]	54.72 ± 7.9[b]	55.13 ± 6.1[b]
Varicocele + BB	49.65 ± 5.68[c]	69.53 ± 3.43[c]	26.43 ± 2.48[c]	76.35 ± 3.41[c]	21.64 ± 2.85[c]
Varicocele + Co Q10	50.01 ± 5.23[c]	71.46 ± 3.94[c]	23.05 ± 2.68[d]	77.28 ± 4.85[c]	24.32 ± 4.22[c]
Varicocele + BB + Co Q10	51.84 ± 5.06[c]	73.01 ± 4.76[c]	22.08 ± 2.88[d]	82.69 ± 2.87[d]	17.98 ± 2.58[d]
Varicocelectomy	48.93 ± 5.84[c]	67.34 ± 4.98[c]	26.28 ± 2.12[c]	70.96 ± 5.72[c]	30.08 ± 4.69[e]
Varicocelectomy + BB	50.53 ± 4.98[c]	70.98 ± 4.65[c]	23.06 ± 2.8[d]	77.74 ± 3.68[c]	17.75 ± 2.23[d]
Varicocelectomy + Q10	54.84 ± 4.73[c]	73.34 ± 4.94[c]	22.64 ± 2.69[d]	82.1. ± 2.01[d]	20.8 ± 1.67[c]
Varicocelectomy + BB + Co Q10	63.11 ± 3.34[a]	85.48 ± 2.94[a]	19.28 ± 3.74[d]	89.2 ± 1.85[a]	13.96 ± 4.42[a]

All data are given as mean ± SD ($n = 6$). a, b, c, d, and e present the significant differences ($P < 0.05$) between differently marked data

sometimes irreparable (Fretz and Sandlow 2002). On the other hand, this type of surgery requires the surgeon's extensive experience and skills (Raman and Goldstein 2004).

However, some patients did not improve their sperm parameters after surgery (Unal et al. 2001). In addition, complications of varicocele surgery sometimes cannot be repaired (Sautter et al. 2002). On the other hand, this type of surgery requires the surgeon's extensive experience and skills (Raman and Goldstein 2004).

There is evidence that the success rate of varicocelectomy in patients with severely degenerated testicular cells after long-term varicocele is very low (Tapanainen et al. 1993). In addition, varicocele causes an increase in ROS production, sperm plasma membrane peroxidation, nuclear DNA damage, and a decrease in total antioxidant capacity (TAC) that may impair sperm (Agarwal et al. 2006).

As mentioned earlier, varicocele can lead to abnormalities of sperm parameters and sperm DNA (Naftulin et al. 1991). Based on these results, our study examined the effects of BB (as an antioxidant and anti-inflammatory chemical) and Co Q10 (as an antioxidant) with and without varicocelectomy (as the most common method of varicocele repair) in varicocele-induced rats.

In the current study, it was demonstrated that varicocele-induced rats showed a significant decrease in sperm count, motility, morphology, and vitality and a significant enhancement in sperm DNA damage. There were significant differences between varicocele and the control group. Previous studies have confirmed the results of our research (Köksal et al. 2003; Bahmanzadeh et al. 2008). Increased ROS and decreased testosterone levels may be due to changes in sperm parameters (Tanrikut et al. 2011; Ozbek et al. 2000). It was indicated that sperm parameters and sperm DNA damage in treatment groups without varicocelectomy (VCL + BB, VCL + Co Q10, VCL + BB + Co Q10) and treatment groups with varicocelectomy (varicocelectomy, varicocelectomy + BB, varicocelectomy + Co Q10, varicocelectomy + BB + Co Q10) significantly improved compared to those in varicocele groups.

However, this improvement was more pronounced in the treatment group of varicocelectomy combined with varicocelectomy and anti-oxidation therapy (varicocelectomy + BB, varicocelectomy + Co Q10, varicocelectomy + BB + Co Q10).

Administration of BB and Co Q10 in this study reduced excess ROS production in varicose model. On the other hand, varicocelectomy can improve drainage of testicles.

Conclusions

In summary, BB and Co Q10 alone and/or together could improve sperm parameters and reduce sperm DNA damage in varicocele-induced rats compared to those in control and control-sham groups. Varicocelectomy alone will enhance sperm parameters; however, this recuperation will be more prominent when joined with Co Q10 and BB. BB and Co Q10 could be suggested as natural sources of antioxidants. Therefore, it may be useful for the treatment of varicocele and possibly other clinical conditions involving excessive free radical production. The positive change in sperm parameters and DNA damage to sperm in varicocele-induced rats was reported in our study. However, additional studies are required in rats with a high degree of varicocele.

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Authors' contributions HN and HHB developed the concept and designed the study. HN was involved in subject recruitment and laboratory analysis. All other three authors were involved in data analysis and helped draft the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving animal participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Competing interests The authors declare that they have no conflict of interest.

Consent for publication Not applicable

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