



Evaluation of the serum sex hormones levels and alkaline phosphatase activity in rats' testis after administering of berberine in experimental varicocele

Hamed Najaran¹ · Hassan Hassani Bafrani^{1,2} · Hamid Rashtbari¹ · Fatemeh Izadpanah³ · Mohammad Reza Rajabi⁴ · Hamed Haddad Kashani² · Abouzar Mohammadi⁵

Received: 11 August 2017 / Accepted: 25 February 2019
© Institute of Korean Medicine, Kyung Hee University 2019

Abstract

Current study was aimed to investigate the protective effect of berberine (BB) on the serum gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B (INHB), testosterone (T) and alkaline phosphatase (Alk-p) activity in the testis of experimental varicocele-induced animals. For the current objective, 30 mature-male Wistar rats were randomly divided into control (n = 6 rats), control-sham (n = 6 rats) and experimental groups (n = 18 rats). The animals in the experimental groups were undergone experimental varicocele and simple laparotomy was conducted in control-sham group. 60 days after varicocele (VCL) induction the experimental group subdivided into: non-treated VCL-induced and 50 mg/kg and 100 mg/kg BB-treated groups (intra-peritoneally). Following 60 days, the animals were euthanized and serum levels of testosterone and testicular activity of alkaline phosphatase were measured. Non-treated VCL-induced animals indicated a significant ($P < 0.05$) reduction in serum levels of T and INHB and a remarkable ($P < 0.05$) increase in GnRH, FSH, LH and Alk-p activity compared to control and control-sham groups. Insignificant changes were found between control and control-sham groups. Meanwhile, each BB administered group showed a remarkable ($P < 0.05$) increase in serum levels of T and INHB and a significant ($P < 0.05$) decrease in GnRH, FSH, LH and alkaline phosphatase activity in testis tissue. According to the current findings, BB by increasing serum levels of testosterone and INHB increases the testicular endocrine capacity and protects Leydig cell against inflammatory and oxidant injury of varicocele. In addition, BB by inhibiting GnRH, FSH, LH and alkaline phosphatase activity, regulate the levels of serum sex hormones in experimental varicocele and reduces varicocele-induced inflammatory reactions.

Keywords Varicocele · Berberine · Gonadotropin-releasing hormone · Inhibin B · Testosterone · Alkaline phosphatase

✉ Hassan Hassani Bafrani
hassani.bafrani.h@gmail.com

Hamed Najaran
hamednajaran2009@yahoo.com

Hamid Rashtbari
Hamid.Rashtbari123@gmail.com

Fatemeh Izadpanah
fatemehizadpanah123@gmail.com

Mohammad Reza Rajabi
MohammadRezaRajabi123@gmail.com

Hamed Haddad Kashani
hamedir2010@gmail.com

Abouzar Mohammadi
Abouzar.Mohammadi12345@gmail.com

- 1 Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran
- 2 Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran
- 3 Food and Drug Laboratory Research Center and Food and Drug Reference Control Laboratories Center, Food and Drug Administration of Iran, MOH & ME, Tehran, Iran
- 4 Faculty of Medicine, Shahed University of Medical Sciences, Tehran, Iran
- 5 Surgical Technology Department, Nursing and Midwifery College, Kashan University of Medical Sciences, Kashan, Iran

Abbreviations

VCL	Varicocele
BB	Berberine
GnRH	Gonadotropin-releasing hormone
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
INHB	Inhibin B
T	Testosterone
Alk-p	Alkaline phosphatase

Introduction

Varicocele (VCL) was indicated in 15% of population, who are in fertile age (Warner and Frey 2013). Indeed, more than 35% of men with primary infertility and 80% of men with secondary infertility problems were reported as varicoceles (Goldstein and Tanrikut 2006). Back-flowing of toxic metabolites from adrenal and/or kidney, deficiency of the hypothalamic–gonadal axis, hypoxia, hyperthermia (Benoff and Gilbert 2001; Marmar 2001; Miyaoka and Esteves 2012) and heat-dependent apoptosis (Said 2012) were reported as main factors involving in varicocele pathogenesis.

The prosperous and perfect development of male germ cell depends on tri balance between the hypothalamus, pituitary and the testis. Gonadotropin releasing hormone (GnRh) released by the hypothalamus caused the release gonadotropins such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Sokol 2009). FSH binds with the receptors in the sertoli cells and causes the stimulation of spermatogenesis. LH stimulates testosterone production in the Leydig cells, which may in turn affect the Sertoli and peritubular cells of the seminiferous tubules and stimulate spermatogenesis (O'donnell et al. 1994). The failure of FSH and LH secretion from the pituitary lead to testicular dysfunction can lead to infertility. Testosterone, estradiol, and inhibin regulate the secretion of gonadotropins (Weinbauer and Nieschlag 1995). Since keeping the serum level of LH and FSH at normal concentration is very important for initiating and supporting spermatogenesis, changes in the concentration of these hormones may be the exhaustion of Sertoli cells as well as GE cells (Akkoyunlu et al. 2007; Shan et al. 1995).

Varicocele results in a significant reduction in serum testosterone level of men as well as in animal models (Tejada et al. 1984). Several studies were carried out on animal models to portray possible reasons of the significant reduction in serum testosterone levels in varicoceles (Razi 2011; Ricci et al. 2004). The results showed a significant reduction in the synthesis of testosterone and a sharp decline in the distribution of Leydig cells per 1 mm² of the interstitial

connective tissue in animal models (Ricci et al. 2004). The differentiation and growth processes of cells are related to the alkaline phosphatase (Alk-p) activity. Martin and Jacoby (1949) showed that alkaline phosphatase is important in spermatogenesis. It was shown that in varicocele-induced rats, serum testosterone levels are lower and Alk-p levels are higher in the testis versus control animals (Ghosh and York 1994). Regrettably, there is a few information on the impact of varicocele on alkaline phosphatase activity in different reproductive organs. However, a cross link between spermatogenesis and Alk-p activity was previously illustrated (Zhang et al. 2011). In general, varicocele can reduce T level in the left testicular tissue and serum (Rajfer et al. 1987). Simultaneously, the varicocele increases Alk-p (Ghosh and York 1994), GnRH (Hudson et al. 1981), luteinizing hormone (LH) (Guarino et al. 2003), and FSH (Sofikitis et al. 1992) levels and decreases inhibin B (INHB) levels (Plymate et al. 1992) in the serum by its effect on the hypothalamic–pituitary–gonadal (HPG) axis.

Berberine, as an alkaloid salt, is found in many plants, including oregon grape (*Berberis aquifolium*), barberry (*Berberis vulgaris*), turmeric (*Berberis aristata*). Up to now, several studies reported various medical effects of berberine. Antioxidant activity of berberine was widely shown (Jung et al. 2009; Račková et al. 2004; Shirwaikar et al. 2006; Hosseyni et al. 2012; Haddad-Kashani et al. 2012; Moghadasi Mohammad et al. 2012) primordial studies indicated BB-induced antioxidant properties by reporting its neutralizing impact against reactive oxygen species (ROS) and activated species of nitrogen (RNS) (Račková et al. 2004; Hsieh et al. 2007). In continue, it was demonstrated that BB can inhibit lipid peroxidation and demonstrate the protective effect against oxidation of low-density lipoprotein (LDL) (Shirwaikar et al. 2006; Hsieh et al. 2007). Further findings illustrated anti-inflammatory effect of beberin in in vitro and in vivo (Cheng et al. 2013). A study also showed that berberine increases the antioxidant capacity of hippocampal brain tissue, thereby reduce lipid peroxidation and increase superoxide dismutase activity (Plant and Marshall 2001). In another study, berberine was known to reduce oxidative stress induced by mitochondria and increases the antioxidant capacity of liver tissue (Rege et al. 1979). Dong et al. showed that berberine significantly reduced the amount of experimental oxidative stress induced by 3T3-L1 cells (Gorelick and Goldstein 1993). The antioxidant activity of berberine is widely shown. First, it has been reported that berberine can scavenge reactive oxygen species (ROS) and reactive nitrogen (RNA). Secondly, berberine can inhibit lipid peroxidation and protect against low density lipoprotein oxidation (LDL) oxidation (Pierik et al. 2001; Romeo et al. 2007).

Minding progressive oxidation, severe inflammatory reactions and hormonal changes made in varicocele-induced testes, the current study was aimed to uncover possible protective effect of Berberine as an antioxidant and anti-inflammatory and regulating hormones chemical against varicocele-related pathogenesis. For this purpose, the serum level of testosterone and GnRH, FSH, LH, INHB (as marker for endocrine potential) and tissue Alk-p (as marker for inflammatory reactions) were analyzed in current study.

Materials and methods

Animals

Thirty mature male Wistar rats weighting 150–200 g were obtained from the Experimental Animal Center of Kashan University of Medical Sciences. All of the animals were fed ad libitum and kept in an environmental controlled room (temperature: 20–22 °C with 12 h light/12 h dark). In this study all of the experiments were in accordance with the Kashan University of Medical Sciences guidelines for research on laboratory animals.

Experimental varicocele technique

In the experimental groups, left varicocele was induced as previously reported (Rajfer et al. 1987). Briefly, each animal was anesthetized with an intraperitoneal injection of ketamine 5% (Razak, Iran), 40 mg/kg and xylazine 2% (Trittau, Germany) 5 mg/kg. Abdominal wall was prepared for surgery and a midline incision was made. Left renal vein were detected. The renal vein diameter was reduced to 1 mm. A loose ligature was placed around the left renal vein to spermatic vein incorporation. The renal vein diameter was reduced to 1 mm. Then, the anastomotic branch between the left spermatic vein and the left common iliac vein was ligated. Following the procedure, the renal and spermatic veins of each animal dilated. The midline incision was closed. Animals in the control-sham groups were anesthetized and only simple laparotomy was performed on them, and none was blocked veins in their bodies.

Experiment design and administration of compounds

Following 1-week acclimatization, the animals were assigned into five groups (NO=6 rats in each group) as control, control-sham and experimental groups. The animals in control-sham group were undergone simple laparotomy. 60 days after simple laparotomy, 0.1 mL of saline (0.85% w/v) was administered to control-sham group by intraperitoneal injection for 60 days.

60 days after VCL induction, the experimental group subdivided into three groups:

- (a) Non-treated VCL-induced group
- (b) 50 mg/kg berberine (Sigma-Aldrich CAS NO: B3251)-administrated VCL-induced group (Kong et al. 2004; Bhutada et al. 2011). The active ingredient was dissolved in distilled water and made up to volume. The dose of berberine in animals is 50 and 100 mg/kg of body weight.
- (c) 100 mg/kg berberine-administrated VCL-induced group (Kong et al. 2004; Bhutada et al. 2011). All chemicals were intraperitoneally administrated for 60 continuing days.

Evaluating serum level of FSH, LH, GnRH, Inhibin B and T

In order to evaluate the level of testosterone, blood samples were taken from heart. Afterwards, the blood was centrifuged at 5000 r/min for 10 min and the serum was separated and then stored at –80 °C for ELISA. The serum level of these hormones were evaluated using ELISA Kit for Rat FSH (Westang Biotechnology Co., Ltd.), ELISA Kit for Rat LH (Westang Biotechnology Co., Ltd.), ELISA Kit for Rat GnRH (Westang Biotechnology Co., Ltd.), ELISA Kit for Rat Inhibin B (Westang Biotechnology Co., Ltd.), ELISA Kit for Rat T (Westang Biotechnology Co., Ltd.). Procedures were performed according to the manufacturer's instructions.

Assessment of Alk-p activity in testis

In order to evaluate the activity of tissue Alk-p, the testicular tissues were immediately frozen in liquid nitrogen, homogenized, and centrifuged. Alkaline phosphatase level of testis tissue was measured by the commercial available standard kit (ALP, 744, Man Inc. Tehran, Iran) according to manufacturer's instructions.

Western blotting protein GnRH-R, FSH-R, Inhibin BR

Western blotting techniques was used to determine the presence or absence of a particular protein in a tissue (Haddad Kashani et al. 2017; Kashani and Moniri 2015; Hosseini et al. 2016). In this method, protein extraction is carried out by means of a specific antibody against an antigen or a specific antigen derived from the protein (Kashani et al. 2018; Ferdosian et al. 2015; Kashani et al. 2013; Nikzad et al. 2013). The sensitivity of this technique is high and can even show slight protein expression.

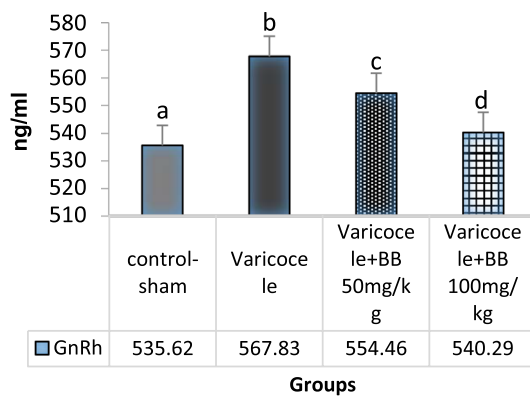


Fig. 1 All data are given as mean ± SD (n=6). a–d present the significant differences ($P < 0.05$) between differently marked data

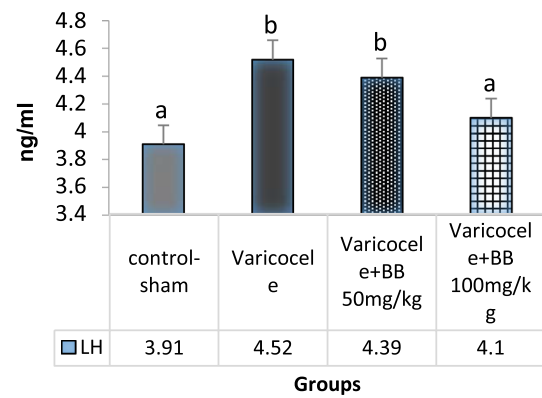


Fig. 3 All data are given as mean ± SD (n=6). a–d: present the significant differences ($P < 0.05$) between differently marked data

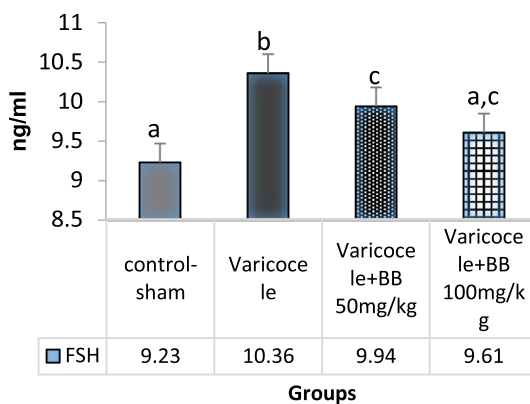


Fig. 2 All data are given as mean ± SD (n=6). a–d: present the significant differences ($P < 0.05$) between differently marked data

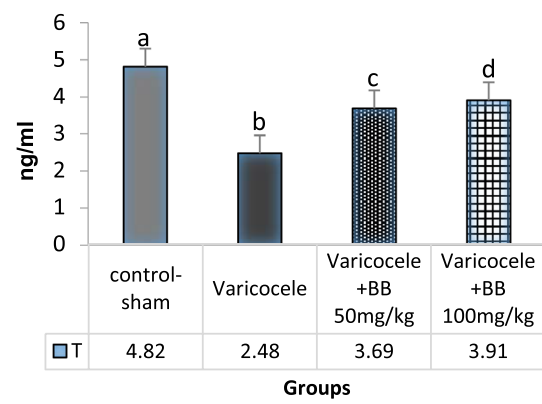


Fig. 4 All data are given as mean ± SD (n=6). a–d: present the significant differences ($P < 0.05$) between differently marked data

Statistical analysis

All data are presented as mean ± standard error (Moghadam et al. 2018; Naeini et al. 2014; Lotfi et al. 2016; Sharif et al. 2016). One-way ANOVA was performed to analyze data obtained (Amini Mahabadi et al. 2013; Sharif et al. 2017). All of the statistical analyses were performed using SPSS software (Version 21.00) and $P < 0.05$ was statistically considered significant.

Results

Changes in serum GnRH, FSH, LH, INHB, T level

The serum levels of GnRH (Fig. 1), FSH (Fig. 2) and LH (Fig. 3) in non-treated VCL-induced group are higher than those in the control-sham group ($P < 0.05$), and those in

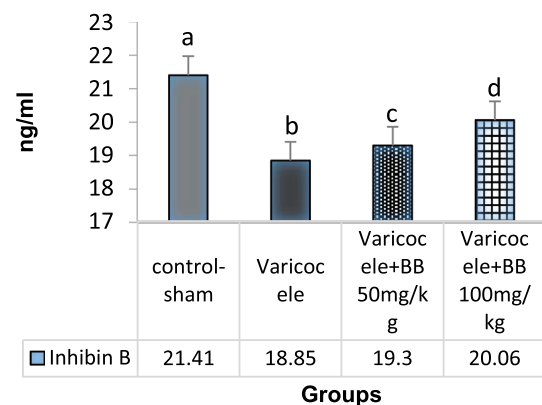


Fig. 5 All data are given as mean ± SD (n=6). a–d: present the significant differences ($P < 0.05$) between differently marked data

each BB administered group are lower than that in the VCL-induced group ($P < 0.05$).

The serum levels of T (Fig. 4) and INHB (Fig. 5) in the non-treated VCL-induced were lower than those in the control-sham group ($P < 0.05$), and those in each BB administered group were higher than those in the VCL-induced group ($P < 0.05$). There was no significant difference between control and control-sham groups.

Changes in Alk-p activity in testis

As shown in Fig. 6, the activity of Alk-p in testis significantly ($P < 0.05$) increased in non-treated VCL-induced groups as compared to control-sham animals. There was no significant difference between control and control-sham groups. Meanwhile, the activity of Alk-p was significantly diminished each BB administered group ($P < 0.05$).

In western blot analysis, the expression of GDNF protein in the varicocele group showed a significant decrease ($P < 0.05$) relative to control and control-sham groups. However, administration of berberine significantly increased the expression of GDNF protein. Comparison between therapeutic groups did not show any significant difference between berberine groups 50 mg/kg and berberine 100 mg/kg. There is no significant difference between control group and control-sham group. The expression level of GDNF protein increased in treatment groups and there was no significant difference compared to control and control-sham groups.

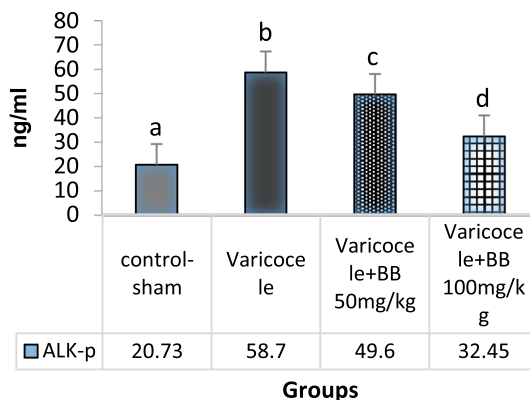
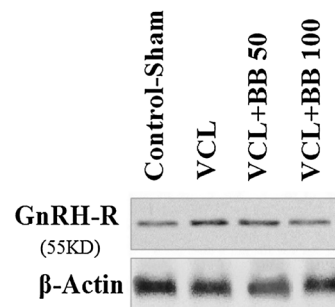
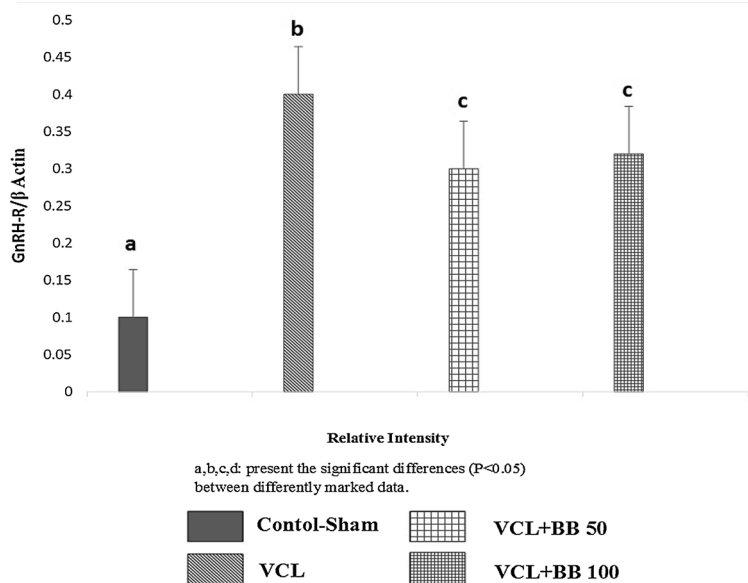
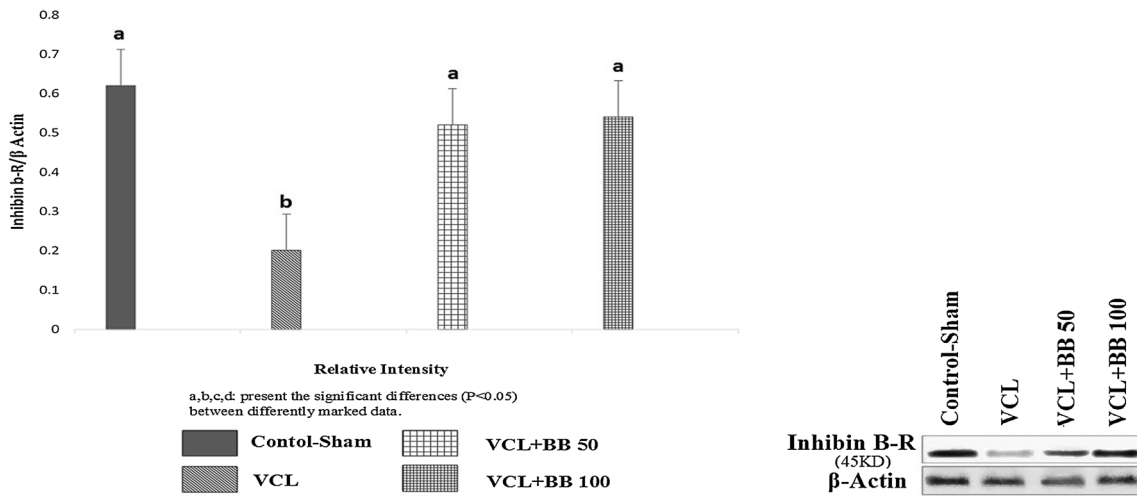


Fig. 6 All data are given as mean \pm SD (n=6). a-d: present the significant differences ($P < 0.05$) between differently marked data

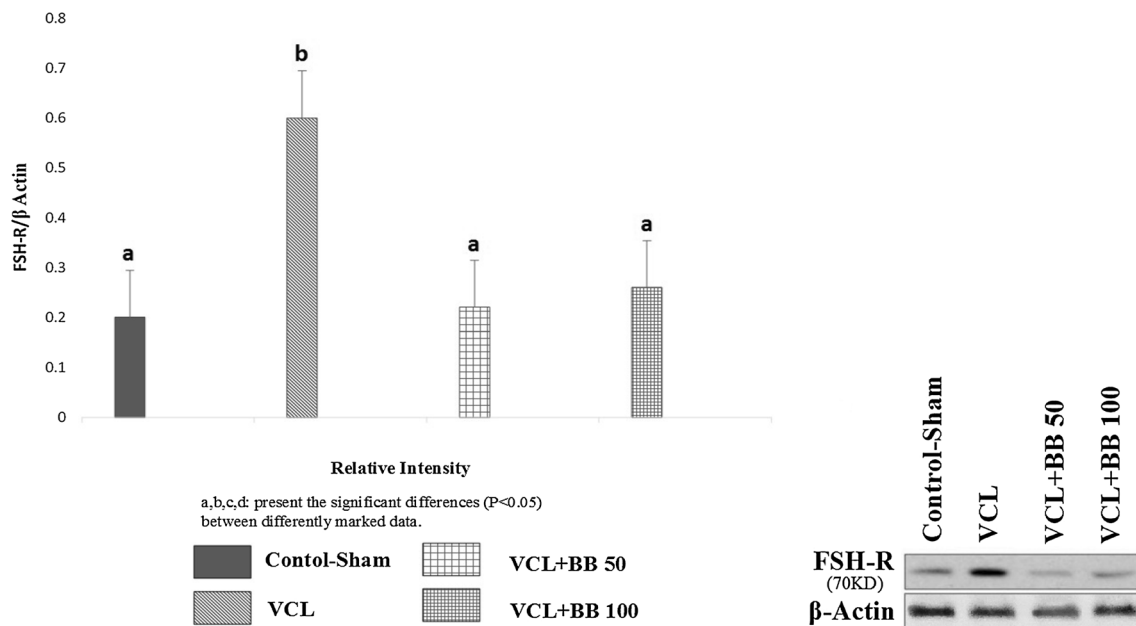
Western blot analysis of GnRH-R in testes tissue. Western blot showing the enhancement of GnRH-R in VCL group in comparison with control-sham group. In treated group with BB 50 and BB 100 expression of the GnRH-R significantly decreased.





Western blot analysis of Inhibin b-R in testes tissue. Western blot showing the reduction of Inhibin b-R in VCL group in comparison with control-sham group. In treated group with BB 50 and BB 100 expression of the Inhibin b-R significantly increased.

Western blot analysis of FSH-R in testes tissue. Western blot showing the enhancement of FSH-R in VCL group in comparison with control-sham group. In treated group with BB 50 and BB 100 expression of the FSH-R significantly decreased.



Discussion

As previously mentioned Varicocele with disrupting hormone levels affect the Leydig cells, Sertoli cells and the hypothalamic–pituitary–gonadal (HPG) axis. In fact, varicocele decreases T and INHB levels, while increases GnRH, FSH, LH levels in the serum and also increases Alk-p in the testes (Guarino et al. 2003; Zhang 2016). Berberine is one of the main components of *Coptis chinensis* used as a Chinese herbal remedy for the treatment of inflammation. Our study for the first time revealed that BB adjusts the levels of serum sex hormones in experimental varicocele.

Accordingly, previous studies reported that varicocele rats represent diminished testosterone levels (Jung et al. 2009; Račková et al. 2004; Shirwaikar et al. 2006) and support our study finding. Reduced tubular diameter, Leydig cell hyperplasia and atrophy with cytoplasmic vacuolization and diminished numbers of testosterone positive Leydig cells were reported for biopsy samples of varicoceles (Zhang 2016). Ghosh and York demonstrated decreased testosterone level and increased Alk-p activity of varicocele-induced ones (Shan et al. 1995). According to the previous studies, there is a positive correlation between germ cells degeneration and Alk-p level (Hsieh et al. 2007). Indeed, the endothelial cells and the tubular basement membrane of the adult rat testis are known as main sources of Alk-p (Kong et al. 2004; Moshtaghion et al. 2013). Also, it was shown that the alkaline phosphatase activity can be influenced by hormonal factors (Moshtaghion et al. 2013). Moreover, serum levels of testosterone were lower and alkaline phosphatase levels were higher in patients with grade 3 varicocele as compared to low grade varicocele (Cheng et al. 2006). Razi et al. showed that Sertoli cells in varicocele-induced rats illustrate up-regulated intracytoplasmic alkaline phosphatase levels, suggesting Sertoli cells directly affected by inflammation (Armagan et al. 2012; Dun 2015). Any inflammatory deleterious effects on Sertoli cells could have an impact on spermatogenesis, especially during the first part of maturity (Luo et al. 2011; Sirvent et al. 1990).

Inhibin B is a glycoprotein regulated by supporting cells and regulated by other factors produced by FSH and germ cells (Hayes et al. 1998). The secretion of inhibin B depends on the interaction between the supporting cells and the germ cells. In particular, sperm cells are known to affect the production of statins, and these cells are susceptible to high temperatures (Blevrakis et al. 2016). Increased temperature of the scrotum associated with varicocele may impair sperm cell function, thereby reducing the production of statin B (Zhang et al. 2018).

In our study, we showed that varicocele increased the levels of GnRH, FSH, LH and Alk-p while BB cancelled this effect, since BB can adjust the abnormal serum sex

hormones levels and return them to the normal levels in varicocele. Secondly, varicocele reduced serum levels of T and INHB. This effect was improved by BB. In summary, our results illustrated that BB could adjust the abnormal serum levels of sex hormones induced by varicocele and return them to the normal levels, thus BB can improve the male reproductive system at the perfect level. Thus, BB may influence the hypothalamic–pituitary–gonadal (HPG) axis and increases the ability of sexual glands.

Conclusions

In summary, BB indeed could increase the serum level of testosterone and INHB and reduce the levels of GnRH, FSH, LH and Alk-p activity in testis in varicocele-induced rats versus control and control-sham groups. Moreover, mechanisms of action for berberine may be attributed to their ameliorative impact on antioxidant status and down-regulating the VCL-induced inflammation. The positive change in serum testosterone, INHB levels and GnRH, FSH, LH and Alk-p activity in testis in varicocele-induced rats was reported in our study. However, additional studies are needed of rats with high grade of varicocele.

Acknowledgements We would like to appreciate Dr. Mazdak Razi, the staffs of Histology laboratory, for their kind technical support. Also the authors wish to thank Kashan University of medical sciences for financial supports.

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.

Funding The research grant provided by Research Deputy of Kashan University of Medical Sciences.

Compliance with ethical standards

Ethical statement 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. The ethical reference number is kaums-94127.

Conflict of interest This manuscript described has not been published before; not under consideration for publication anywhere else; and has been approved by all co-authors.

Availability of data and materials The primary data for this study is available from the authors on direct request.

References

- Akkoyunlu G et al (2007) Immunolocalization of glial cell-derived neurotrophic factor (GDNF) and its receptor GFR- α 1 in varicocele-induced rat testis. *Acta Histochem* 109(2):130–137
- Amini Mahabadi J et al (2013) Effect of diet contains sesame seed on adult Wistar rat testis. *Int J Morphol* 31(1):197–202
- Armagan A et al (2012) The effect of micronized purified flavonoid fraction on the prevention of testicular pathologies in adolescent rats with experimentally induced varicocele. *J Urol* 188(5):2007–2013
- Benoff S, Gilbert BR (2001) Varicocele and male infertility: part I. *Hum Reprod Update* 7(1):47–54
- Bhutada P et al (2011) Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. *Behav Brain Res* 220(1):30–41
- Blevrakis E et al (2016) Impact of varicocele on biological markers of gonadal function. *Hernia* 20(3):435–439
- Cheng D et al (2006) Effects of epidermal growth factor on sperm content and motility of rats with surgically induced varicoceles. *Asian J Androl* 8(6):713–717
- Cheng F et al (2013) Berberine improves endothelial function by reducing endothelial microparticles-mediated oxidative stress in humans. *Int J Cardiol* 167(3):936–942
- Dun R et al (2015) Traditional Chinese herb combined with surgery versus surgery for varicocele infertility: a systematic review and meta-analysis. *Evid-Based Complement Altern Med*. <https://doi.org/10.1155/2015/689056>
- Ferdosian M et al (2015) Identification of immunotopes against *Mycobacterium leprae* as immune targets using PhDTm-12mer phage display peptide library. *Trop J Pharm Res* 14(7):1153–1159
- Ghosh PK, York JP (1994) Changes in testicular testosterone and acid and alkaline phosphatase activity in testis and accessory sex organs after induction of varicocele in Noble rats. *J Surg Res* 56(3):271–276
- Goldstein M, Tanrikut C (2006) Microsurgical management of male infertility. *Nat Rev Urol* 3(7):381
- Gorelick JI, Goldstein M (1993) Loss of fertility in men with varicocele. *Fertil Steril* 59(3):613–616
- Guarino N, Tadini B, Bianchi M (2003) The adolescent varicocele: the crucial role of hormonal tests in selecting patients with testicular dysfunction. *J Pediatr Surg* 38(1):120–123
- Haddad-Kashani H et al (2012) Pharmacological properties of medicinal herbs by focus on secondary metabolites. *Life Sci J* 9(1):509–520
- Haddad Kashani H et al (2017) A novel chimeric endolysin with antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *Front Cell Infect Microbiol* 7:290
- Hayes FJ et al (1998) Differential control of gonadotropin secretion in the human: endocrine role of inhibin. *J Clin Endocrinol Metab* 83(6):1835–1841
- Hosseini ES et al (2016) Purification of antibacterial CHAP K protein using a self-cleaving fusion tag and its activity against methicillin-resistant *Staphylococcus aureus*. *Probiotics Antimicrob Proteins* 8(4):202–210
- Hosseyini ES et al (2012) Mode of action of medicinal plants on diabetic disorders. *Life Sci J* 4(9):2776–2783
- Hsieh Y-S et al (2007) Protective effects of berberine against low-density lipoprotein (LDL) oxidation and oxidized LDL-induced cytotoxicity on endothelial cells. *J Agric Food Chem* 55(25):10437–10445
- Hudson RW, Crawford VA, McKay DE (1981) The gonadotropin response of men with varicoceles to a four-hour infusion of gonadotropin-releasing hormone. *Fertil Steril* 36(5):633–637
- Jung HA et al (2009) Anti-Alzheimer and antioxidant activities of Coptidis Rhizoma alkaloids. *Biol Pharm Bull* 32(8):1433–1438
- Kashani HH, Moniri R (2015) Expression of recombinant pET22b-LysK-cysteine/histidine-dependent amidohydrolase/peptidase bacteriophage therapeutic protein in *Escherichia coli* BL21 (DE3). *Osong Public Health Res Perspect* 6(4):256–260
- Kashani HH et al (2013) Expression of galectin-3 as a testis inflammatory marker in vasectomized mice. *Cell J (Yakhteh)* 15(1):11
- Kashani HH et al (2018) Recombinant endolysins as potential therapeutics against antibiotic-resistant *Staphylococcus aureus*: current status of research and novel delivery strategies. *Clin Microbiol Rev* 31(1):e00071-17
- Kong W et al (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 10(12):1344
- Lotfi A et al (2016) Comparing the effects of two feeding methods on metabolic bone disease in newborns with very low birth weights. *Glob J Health Sci* 8(1):249
- Luo D-Y et al (2011) Effects of varicocele on testosterone, apoptosis and expression of StAR mRNA in rat Leydig cells. *Asian J Androl* 13(2):287
- Marmar JL (2001) Varicocele and male infertility: part II: the pathophysiology of varicoceles in the light of current molecular and genetic information. *Hum Reprod Update* 7(5):461–472
- Martin B, Jacoby F (1949) Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J Anat* 83(Pt 4):351
- Miyaoka R, Esteves SC (2012) A critical appraisal on the role of varicocele in male infertility. *Adv Urol* 2012:597495
- Moghadam SE et al (2018) Neuroprotective effects of oxytocin hormone after an experimental stroke model and the possible role of calpain-1. *J Stroke Cerebrovasc Dis* 27(3):724–732
- Moghaddasi Mohammad S, Kashani HH, Azarbad Z (2012) *Caparis spinosa* L. propagation and medicinal uses. *Life Sci J* 9(4):684–686
- Moshtaghian S-M et al (2013) Silymarin protects from varicocele-induced damages in testis and improves sperm quality: evidence for E2f1 involvement. *Syst Biol Reprod Med* 59(5):270–280
- Naeini ZK, Bafrani HH, Nikzad H (2014) Evaluation of ebselen supplementation on cryopreservation medium in human semen. *Iran J Reprod Med* 12(4):249
- Nikzad H et al (2013) Expression of galectin-8 on human endometrium: molecular and cellular aspects. *Iran J Reprod Med* 11(1):65
- O'donnell L et al (1994) Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle. *Endocrinology* 135(6):2608–2614
- Pierik FH et al (2001) Increased serum inhibin B levels after varicocele treatment. *Clin Endocrinol* 54(6):775–780
- Plant TM, Marshall GR (2001) The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocr Rev* 22(6):764–786
- Plymate SR, Paulsen CA, McLachlan R (1992) Relationship of serum inhibin levels to serum follicle stimulating hormone and sperm production in normal men and men with varicoceles. *J Clin Endocrinol Metab* 74(4):859–864
- Račková L et al (2004) Antiradical and antioxidant activities of alkaloids isolated from *Mahonia aquifolium*. Structural aspects. *Bioorg Med Chem* 12(17):4709–4715
- Rajfer J et al (1987) Inhibition of testicular testosterone biosynthesis following experimental varicocele in rats. *Biol Reprod* 36(4):933–937
- Razi M et al (2011) Histological impact of long term varicocele-induction on right and left testes in rat (evidence for the reduction of sperm quality and mating abilities). In: Veterinary research forum. Faculty of Veterinary Medicine, Urmia University
- Rege N et al (1979) Serum gonadotropins and testosterone in infertile patients with varicocele. *Fertil Steril* 31(4):413–416

- Ricci J-E et al (2004) Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. *Cell* 117(6):773–786
- Romeo C et al (2007) Altered serum inhibin b levels in adolescents with varicocele. *J Pediatr Surg* 42(2):390–394
- Said TM et al (2012) Oxidative stress, DNA damage, and apoptosis in male infertility. In: Agarwal A, Aitken RJ, Alvarez JG (eds) *Studies on men's health and fertility*. Humana Press Inc., NJ, pp 433–448
- Shan L et al (1995) Effects of luteinizing hormone (LH) and androgen on steady state levels of messenger ribonucleic acid for LH receptors, androgen receptors, and steroidogenic enzymes in rat Leydig cell progenitors in vivo. *Endocrinology* 136(4):1686–1693
- Sharif MR et al (2016) The effect of a yeast probiotic on acute diarrhea in children. *Probiotics Antimicrob Proteins* 8(4):211–214
- Sharif A et al (2017) The role of probiotics in the treatment of dysentery: a randomized double-blind clinical trial. *Probiotics Antimicrob Proteins* 9(4):380–385
- Shirwaikar A et al (2006) In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. *Biol Pharm Bull* 29(9):1906–1910
- Sirvent J et al (1990) Leydig cell in idiopathic varicocele. *Eur Urol* 17:257–261
- Sofikitis N et al (1992) Surgical repair versus medical treatment of varicocele in the rat: pharmacological manipulation of the varicoelized testicle. *Eur Urol* 22:44–52
- Sokol RZ (2009) Endocrinology of male infertility: evaluation and treatment. *Semin Reprod Med* 27(2):149–158
- Tejada RI et al (1984) A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. *Fertil Steril* 42(1):87–91
- Warner JN, Frey KA (2013) The well-man visit: addressing a man's health to optimize pregnancy outcomes. *J Am Board Fam Med* 26(2):196–202
- Weinbauer GF, Nieschlag E (1995) Gonadotropin control of testicular germ cell development. *Adv Exp Med Biol* 317:55–65
- Zhang J et al (2011) Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. *Agric Sci China* 10(5):786–796
- Zhang L et al (2016) Effects of *Morinda officinalis* polysaccharide on experimental varicocele rats. *Evid-Based Complement Altern Med*. <https://doi.org/10.1155/2016/5365291>
- Zhang J et al (2018) Roles of Fas/FasL-mediated apoptosis and inhibin B in the testicular dysfunction of rats with left-side varicocele. *Andrologia* 50(2):e12850

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.