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Dietary Propolis Supplement Improves Semen Characteristics in Egyptian Buffaloes

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A B S T R A C T

Buffalo bulls frequently present with infertility, and reproductive improvement in such animals is of great importance. The present study aimed to investigate the effects of dietary propolis supplementation on semen characteristics, including volume, sperm motility, viability, physiological abnormalities, sperm count, seminal antioxidant level, seminal α-glucosidase activity, and serum testosterone level. This study was carried out on nine buffalo bulls divided into three groups supplemented daily with 150 mg/kg DM/head propolis, 100 mg/kg DM/head propolis, or fed a basal control. After 2 months, semen samples were collected for biochemical analysis of various parameters and serum samples were collected for measuring total testosterone levels. Results showed that propolis supplementation increased semen volume, sperm motility, and live sperm percentage, with a concomitant reduction in the percentage of abnormal sperm. Antioxidant activity of glutathione peroxidase and superoxide dismutase increased in groups that received propolis, with a concurrent decline in malondialdehyde concentration. Also, α-glucosidase activity in seminal plasma and serum testosterone levels showed a marked increase after propolis supplementation. In conclusion, these results suggest that dietary propolis may enhance semen characteristics in Egyptian buffaloes.

Keywords: Antioxidants, α-glucosidase, Buffaloes, Propolis, Testosterone

1. Introduction

Buffaloes (Bubalus bubalis) are a major source of meat, milk, and leather in many developing countries (Sarwar et al., 2009). Fertility of buffalo bulls is a very important factor for the success of any breeding program, as well as for improving the milk yield of a herd. Reduced fertility can result in massive economic losses to livestock owners (Blaschek et al., 2011). Fertility is a multifactorial issue influenced by herd population genetics, environmental conditions, nutrition, and herd management procedures (Rekwot et al., 1988).

Propolis is a resinous material produced by honeybees that can be obtained from a variety of plant sources. It contains a multitude of pharmacologically active compounds, such as polyphenols, terpenoids, steroids, and amino acids, and has been used for decades in traditional medicine (Burdock, 1998). A wide range of biological activities have been reported for propolis, including antibacterial, antiviral, antiinflammatory, and potent antioxidant properties (Medić-Šarić et al., 2009). Propolis also has a strong cytoprotective effect against a wide range of toxic stimuli (Rizk et al., 2014).

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It has been reported that propolis showed protective effects against aluminum chloride-induced reproductive toxicity in males which causes testicular dysfunction, reduced testosterone levels, and low-quality semen (Yousef and Salama, 2009, Ogretmen et al., 2014). Propolis may specifically enhance synthesis of proteins and metabolites that regulate male fertility (Fernandez-Gago et al., 2013). Metabolic products in seminal plasma serve multiple purposes related to sperm function, including ATP assembly, sperm motility enhancement, various functions in cellular immunity, control of biochemical reactions, and regulation of metabolic rates (Bieniek et al., 2016).

Tiny amounts of free radicals are present in seminal plasma, including hydroxyl radicals, superoxide anions, and hydrogen peroxide, which are produced continually by sperm in response to environmental and endogenous stimuli (de Lamirande and Gagnon, 1993). Low levels of free radicals are necessary for critical processes such as intracellular signaling and sperm maturation (Suarez, 2008). Alternatively, excessive output of reactive oxygen species (ROS) results in oxidative stress, leading to diminished sperm motility, viability, and aberrant spermatogenesis that contributes to infertility or impaired acrosome reactions (Aitken, 1997). Sperm are rich in polyunsaturated fatty acids, and are therefore vulnerable to ROS interference (Shen and Ong, 2000). To combat the drawbacks of ROS, seminal plasma contains a wide variety of both enzymatic and metabolic antioxidants (Agarwal et al., 2014). Furthermore, propolis has been reported to reduce production of ROS and free radicals, including superoxide anions, hydrogen peroxide, and hydroxyl radicals (Olczyk et al., 2017).

Neutral α-glucosidase (NAG) breaks down α-glycosidic bonds of maltooligosaccharides and maltodextrins resulting from glycogen metabolism by α-amylase enzymes (Karpiak et al., 1977, Martiniuk and Hirschhorn, 1981). α-glucosidase hydrolyzes α-1,2-, α-1,3-, and α-1,6-glycosidic bonds (Chiba, 1997), though at high substrate concentrations, the enzyme also exhibits transferase activity and can synthesize oligosaccharides such as maltodextrins. Distinct from lysosomal α-glucosidase (acidic type), neutral α-glucosidase is a cytosolic enzyme that is active at pH 6–7. This enzyme is broadly conserved among living organisms, and is ubiquitously expressed in diverse body tissues and organs, including in blood sera (Richter, 1990). Recently, several studies have focused on the activity of neutral α-glucosidase in human semen and its value in determining semen quality (Levrant et al., 2009, Vivas-Acevedo et al., 2014).

This study explores the effect of dietary propolis supplementation on characteristics of buffalo semen, including volume, sperm motility, count, viability, and abnormalities of sperm, in addition to activity of both seminal antioxidants and α-glucosidase and serum testosterone level.

2. Material and methods

All experiments were performed in accordance with the Guidelines for the Care and Use of Animals approved by the Animal Production Research Institute, Ministry of Agriculture, Egypt.

Nine Egyptian buffalo bulls (average 450 kg, 24-30 months old) were used in this study. This study was conducted at the Animal Production Experimental Station, Mehallet Moussa village, Kaferelsheikh Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Animals were divided into three

groups (three bulls each); group I was supplemented daily with propolis (Sigma Pharmaceutical Industries for International Business Establishment Co., Egypt, 400 mg capsules) at a dose of 150 mg/kg dry matter (DM)/head; group II was supplemented daily with propolis at a dose of 100 mg/kg dry matter/head; group III (control) was fed only a basal diet without propolis supplementation. All bulls were fed the recommended ration according to the instructions of NRC (NRC, 1984).

1.Experimental procedures.

1.1.Semen collection and evaluation.

Semen samples were collected after two months of dietary propolis supplementation using an artificial vagina. Bulls were allowed two false mounts on teaser bulls prior to ejaculation. Semen was evaluated using the following parameters: ejaculate volume, percentage of sperm motility, percentage of live versus dead sperm, percentage of sperm bearing physiological abnormalities, and sperm cell concentration (Salisbury et al., 1978). Ejaculate volume (mL) was determined using a graduated cylinder. Sperm motility was scored using a drop of undiluted fresh semen on a warm glass slide that was viewed under a heated-stage microscope at 37°C. For determination of live/dead sperm and abnormalities (double-headed sperm, elongated head, bent tail, proximal protoplasmic droplet, etc.), a drop of fresh semen was stained by eosin-negrosin mixture (5%) and then dried at room temperature. Counting of live, dead, and abnormal sperm (200 per slide) was performed manually. Concentration of sperm cells (×109/mL) was measured using spectrophotometer (JENWAY 6305, Dunmow, United Kingdom) at 550 nm wavelength using a mixture of 4 mL of sodium chloride solution (0.9%) and 20 µL of semen.

1.2. Measurement of seminal antioxidants.

Total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, as well as malondialdehyde (MDA) concentrations, were determined in seminal plasma using a commercially available kit (Biodiagnostic, Cairo, Egypt). Sperm were homogenized in phosphate-buffered saline using an ultrasonic disintegrator, centrifuged at 1000 rpm for 10 min at 4°C, and supernatant was collected. T-SOD activity was normalized to the amount of sample protein required to inhibit the reduction of nitro blue tetrazolium by 50% of maximum inhibition. GSH-Px activity was normalized to the amount of enzyme necessary to oxidize 1 µmol NADPH/min at pH 7.0 and 25°C. Malondialdehyde (MDA) concentration was determined using the thiobarbituric acid method, based on the colorimetric reaction of MDA with thiobarbituric acid (Fan et al., 2011), and expressed as nmol/mg protein.

1.3. α-glucosidase activity in seminal plasma.

α-glucosidase activity was determined via the glucose oxidase method (Mahmoud et al., 1998) using a commercially available EpiScreen Plus-α-glucosidase assay (FertiPro, Beernem, Belgium) according to the manufacturer's instructions. Optical density was measured at 505 nm, and enzymatic activity was normalized to the volume of sample that produced 1 µmol of D-glucose/min at pH 6.8 and 37°C.

1.4.Blood sampling.

Blood samples were taken from the jugular vein of each bull. Samples were incubated at 37°C until blood clotted, then centrifuged to separate the sera, which was used for measuring total testosterone levels. Total testosterone hormone levels were measured via enzyme-linked immunosorbent assay (BioVendor) in accordance with previous reports (Snowdon and Ziegler, 2007). Data was measured using an ELISA reader (Spectra).

1.5.Statistical analysis.

Data were analyzed by one-way analysis of variance (ANOVA) and Turkey's post hoc test for multiple comparisons using SPSS (version 22) software (Richmond, VA, USA). Biochemical results were statistically analyzed by one-way ANOVA using GraphPad Prism 5 (San Diego, USA). Significance was determined by p values <0.05.

3. Results:

3.1. Evaluation of semen characteristics

Dietary supplementation with propolis resulted in significant increase ($p < 0.05$) in semen volume compared to the control group (Table 1). In addition, sperm motility increased significantly ($p < 0.05$) in groups I and II as compared with control group, with group I displaying the highest motility. Also, the percentage of live sperm increased significantly ($p < 0.05$) in group I as compared with group II and the control group; conversely, the percentage of dead sperms was significantly higher ($p < 0.05$) in the groups II and III, with the lowest percentage of dead sperm found in group I. Similarly, there was significant increase ($p < 0.05$) in percentage of abnormal sperm in both the control group and group II as compared with group I. Total sperm count was not significantly different among these groups.

3.2. Seminal antioxidants status

Propolis dietary supplementation in bulls caused a significant increase of GSH-Px activity in seminal plasma (Fig. 1). The largest increase of GSH-Px activity occurred in group I, followed by group II. Furthermore, T-SOD activity in seminal plasma was significantly increased in both propolis-treated groups as compared to the control group. MDA concentration in seminal plasma was highest in the control group; propolis supplementation caused a significant decline in MDA concentration in group I compared to the control group.

3.3. α-glucosidase activity

A significant increase in α-glucosidase activity was observed in seminal plasma in group I as compared to control group. A-glucosidase activity was also increased in group II, although to a lesser degree than in group I (Fig. 2).

3.4. Testosterone levels

A significant increase of serum testosterone levels were observed in group I as compared to the control group (Fig. 3). Group II displayed increased serum testosterone as well, although to a less degree than group I.

4. Discussion

Reproductive efficiency of buffalo bulls can be affected by inherited defects of the genital tract, hormonal imbalance, contagious diseases of the reproductive system or other systems, imbalanced rations, psychological defects, and harsh climate conditions (Perumal, 2017).

Propolis is a resinous substance collected from trees and leaf buds that are rich in enzymes secreted in the saliva of honeybees. It contains more than one hundred active ingredients, including phenolic acids and flavonoids (Kocot et al., 2018, Gunduz et al., 2005). Propolis has recently gained popularity due to its antioxidant properties (Borges et al., 2011). Also, it is commonly used as a feed additive for prevention against disease conditions such as diabetes, cancer, inflammations and heart diseases (Meurer et al., 2009).

Our results demonstrate that administration of propolis may cause a significant increase in semen volume, sperm motility, and the percentage of live sperm, with a reduction in percentage of abnormal sperm. Sperm counts were unaffected by propolis treatment. The positive effects of propolis on semen parameters measured were more apparent in group I, which received 150 mg propolis/kg DM/head, as compared with group II, which received 100 mg/kg DM/head. The results of this study are in agreement with a previous study, which reported that propolis induced a decline in the number of abnormal and dead sperm (Elmazoudy et al., 2011). Propolis treatment was also shown to lead to increased weight of reproductive organs, total body weight, percentage of motile sperm, levels of seminal plasma enzymes, and number of normal-shaped sperm (Yousef et al., 2010). In addition, administration of propolis to rats reduced both testicular injury and effects of toxic compounds on reproductive function (Ball et al., 2001).

Sperm cell membranes contain abundant unsaturated fatty acids, which predisposes them to lipid peroxidation. This higher rate of lipid peroxidation contributes to membrane damage as well as decreased sperm motility (Sharma and Agarwal, 1996). Our data demonstrate that dietary supplementation with propolis may improve antioxidation in seminal plasma via the elevated activities of both glutathione peroxidase and super oxide dismutase, concurrent with a decline in lipid peroxidation.

ROS, particularly hydrogen peroxide and superoxide, are major causes of defective sperm in infertile males (Goyal et al., 2007). Oxidative stress can stimulate lipid peroxidation, which is especially relevant as unsaturated fatty acid content is higher in sperm. Lipid peroxidation can result in less fluid volume in sperm or dysfunctions of the plasma membrane, which can contribute to male infertility (Tavares et al., 2007). Decreased oxidative stress and increased seminal quality induced by propolis may be a result of phenolic compounds, such as caffeoylquinic acid and prenylated cinnamic acid, which may have the ability to neutralize free radicals produced by sperm (Moura et al., 2011).

Neutral α-1,4-glucosidase has two forms, an acidic isoform originating from the prostate and a neutral isoform originating from the epididymis.

The neutral isoform secreted mainly in the epididymis plays a role in the maturation of sperm (Loko et al., 1997). Although L-carnitine and glycerophosphorylcholine have been used as biomarkers of epididymis function, NAG is considered the most sensitive and specific epididymis marker (Camargo et al., 2018).

The epididymis can produce antioxidants necessary to counteract harmful products of excessive ROS production. Decreased NAG activity in semen has been shown to contribute to DNA fragmentation, decreased integrity of the sperm membrane, and reduced binding to hyaluronic acid (Depuydt et al., 1996). To our knowledge, this study is the first to portray the impact of propolis on NAG in buffalo semen. Our data revealed a marked increase in NAG activity in both propolis-treated groups as compared to the control group.

Low levels of seminal α-glucosidase activity are possibly linked to epididymitis and inflammation of the genital tract (ALI et al., 1994, Mahmoud et al., 1998), and have also been associated with defective maturation of sperm in the epididymis. Higher enzymatic activity correlated with strong binding efficiency of sperm to the oocyte zona pellucida (Henkel et al., 2006). It has been demonstrated that activity of α-glucosidase in seminal plasma strongly correlated with sperm concentration, ejaculate volume, and the acrosome reaction (Lemmens et al., 2016). In the same way, α-glucosidase activity may possibly be used to determine probability of in vitro fertilization success (Elzanaty, 2007).

Dietary propolis significantly increased the level of testosterone; this finding is in agreement with a previous study which reported that propolis treatment resulted in a decline in abnormal and dead sperm and increased testosterone levels in male rats (Zahmatkesh et al., 2014). In addition, it was shown that increased steroid reductase enzymatic activity and total testosterone concentration in groups that received propolis correlated with improved sperm proliferation and increased fertility in rats (Yousef and Salama, 2009).

5. Conclusion

In conclusion, these data reveal that oxidative stress may play a negative role in reproduction in sexually mature bulls due to adverse effects on sperm characteristics and testosterone production in these animals. Propolis, due to beneficial biological properties of its components, exhibits antioxidant capabilities, and was shown to boost semen characteristics in Egyptian buffaloes, along with improvement of seminal antioxidant status. Furthermore, propolis treatment increased the activity of seminal α-glucosidase and serum testosterone levels.

Conflict of interest.

The authors declare no conflict of interest.

6. References

Agarwal, A., Virk, G., Ong, C. and Du Plessis, S. S., 2014. Effect of oxidative stress on male reproduction. The world journal of men's health, 32, 1-17.

Aitken, R. J., 1997. Molecular mechanisms regulating human sperm function. Mol hum reprod, 3, 169-73.

Ali, H. B., Guerin, J. F., Pinatel, M. C., Mathieu, C., Boulieu, D. and Tritar, B., 1994. Relationship between semen characteristics, a-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida. International journal of andrology, 17, 121-126.

Ball, B. A., Medina, V., Gravance, C. G. and Baumbe, J,. 2001. Effect of antioxidants on preservation of motility,viability and acrosomal integrity of equine spermatozoa during storage at 5 degrees c. Theriogenology, 56, 577-89.

Bieniek, J. M., Drabovich, A. P. and Lo, K. C., 2016. Seminal biomarkers for the evaluation of male infertility. Asian j androl, 18, 426-33.

Blaschek, M., Kaya, A., Zwald, N., Memili, E. and Kirkpatrick, B. W. 2011. A whole-genome association analysis of noncompensatory fertility in holstein bulls. J dairy sci, 94, 4695-9.

Borges, K. S., Brassesco, M. S., Scrideli, C. A., Soares, A. E. E. and Tone, L. G., 2011. Antiproliferative effects of tubi-bee propolis in glioblastoma cell lines. Genetics and molecular biology, 34, 310-314.

Burdock, G. A., 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food chem toxicol, 36, 347-63.

Camargo, M., Intasqui, P. & Bertolla, R. P., 2018. Understanding the seminal plasma proteome and its role in male fertility. Basic and clinical andrology, 28, 6.

Chiba, S., 1997. Molecular mechanism in α-glucosidase and glucoamylase. Bioscience, biotechnology, and biochemistry, 61, 1233-1239.

De Lamirande, E. & Gagnon, C., 1993. Human sperm hyperactivation in whole semen and its association with low superoxide scavenging capacity in seminal plasma. Fertil steril, 59, 1291-5.

Depuydt, C. E., Bosmans, E., Zalata, A., Schoonjans, F. and Comhaire, F. H., 1996. The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. J androl, 17, 699-707.

Elmazoudy, R., S, A. and El-Shenawy, N., 2011. Evaluation of protective effect of propolis against testicular oxidative damage, lipid peroxidation and infertility induced by chlorpyrifos in albino rats. Egyp. J. Exp. Biol. (zool).

Elzanaty, S., 2007. Association between age and epididymal and accessory sex gland function and their relation to sperm motility. Archives of andrology, 53, 149-156.

Fan, Y. Y., Ran, Q., Toyokuni, S., Okazaki, Y., Callaway, E. S., Lupton, J. R. and Chapkin, R. S., 2011. Dietary fish oil promotes colonic apoptosis and mitochondrial proton leak in oxidatively stressed mice. Cancer prev res (phila), 4, 1267-74.

Fernandez-Gago, R., Dominguez, J. C. and Martinez-Pastor, F., 2013. Seminal plasma applied post-thawing affects boar sperm physiology: a flow cytometry study. Theriogenology, 80, 400-10.

Goyal, A., Chopra, M., Lwaleed, B. A., Birch, B. & Cooper, A. J., 2007. The effects of dietary lycopene supplementation on human seminal plasma. Bju int, 99, 1456-60.

Gunduz, C., Biray, C., Kosova, B., Yilmaz, B., Eroglu, Z., Sahin, F., Omay, S. B. and Cogulu, O., 2005. Evaluation of manisa propolis effect on leukemia cell line by telomerase activity. Leuk res, 29, 1343-6.

Henkel, R., Maass, G., Schuppe, H. C., Jung, A., Schubert, J. and Schill, W. B., 2006. Seasonal changes of neutral alpha-glucosidase activity in human semen. J androl, 27, 34-9.

Karpiak, S., Ziarko, D., Zoltowska, K. and Gwara, K., 1977. Relations between glycogen phosphorylase activity and activities of amylolytic enzymes in rabbit skeletal muscle. Arch immunol ther exp (warsz), 25, 199-212.

Kocot, J., Kiełczykowska, M., Luchowska-Kocot, D., Kurzepa, J. and Musik, I., 2018. Antioxidant Potential of propolis, bee pollen, and royal jelly: possible medical application. Oxidative medicine and cellular longevity, 2018, 7074209-7074209.

Lemmens, L., Kos, S., Beijer, C., Brinkman, J. W., Van Der Horst, F. A., Van Den Hoven, L., Kieslinger, D. C., Van Trooyen-Van Vrouwerff, N. J., Wolthuis, A., Hendriks, J. C. and Wetzels, A. M., 2016. Predictive value of sperm morphology and progressively motile sperm count for pregnancy outcomes in intrauterine insemination. Fertil steril, 105, 1462-8.

Levrant, S., Watanabe, M., Land, S., Sauer, R. and Jeyendran, R. S., 2009. The relevance of neutral alpha-glucosidase activity in andrology. Syst biol reprod med, 55, 116-9.

Loko, F., Alihonou, E., Goufodji, B., Fagla, B. and Hounton, M., 1997. [neutral alpha-glucosidase, a specific marker for epididymal secretion in seminal pathology]. Contraception, fertilite, sexualite (1992), 25, 939-941.

Mahmoud, A. M., Geslevich, J., Kint, J., Depuydt, C., Huysse, L., Zalata, A. and Comhaire, F. H., 1998. Seminal plasma alpha-glucosidase activity and male infertility. Hum reprod, 13, 591-5.

Martiniuk, F. and Hirschhorn, R., 1981. Characterization of neutral isozymes and human α-glucosidase differences in substrate specificity, molecular weight and electrophoretic mobility. Biochimica et biophysica acta (bba) - enzymology, 658, 248-261.

Medić-Šarić, M., Rastija, V., Bojić, M. and Maleš, Ž., 2009. From functional food to medicinal product: systematic approach in analysis of polyphenolics from propolis and wine. Nutrition journal, 8, 33.

Meurer, F., Matiuzzi Da Costa, M., De Barros, D. A. D., Leal De Oliveira, S. T. and Da Paixão, P. S., 2009. Brown propolis extract in feed as a growth promoter of nile tilapia (oreochromis niloticus, linnaeus 1758) fingerlings. Aquaculture research, 40, 603-608.

Moura, S. A. L. D., Negri, G., Salatino, A., Lima, L. D. D. C., Dourado, L. P. A., Mendes, J. B., Andrade, S. P., Ferreira, M. A. N. D. and Cara, D. C., 2011. Aqueous extract of brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. Evidence-based complementary and alternative medicine, 2011, 8.

NRC., 1984. Nutrient requirements of beef cattle, washington, dc, the national academies press.

Ogretmen, F., Inanan, B. E. and Ozturk, M., 2014. Protective effects of propolis on cryopreservation of common carp (cyprinus carpio) sperm. Cryobiology, 68, 107-12.

Olczyk, P., Komosinska-Vassev, K., Ramos, P., Mencner, L., Olczyk, K. and Pilawa, B., 2017. Free radical scavenging activity of drops and spray containing propolis-an epr examination. Molecules (basel, switzerland), 22, 128.

Perumal, P., 2017. Biochemical attributes of mithun semen. International journal of bio-resource and stress management, 8, 796-801.

Rekwot, P. I., Oyedipe, E. O., Akerejola, O. O. and Kumi-Diaka, J., 1988. The effect of protein intake on body weight, scrotal circumference and semen production of bunaji bulls and their friesian crosses in nigeria. Animal reproduction science, 16, 1-9.

Richter, G., 1990. Handbook of amylases and related enzymes. Edited by the amylase research society of japan. Pergamon press, oxford 1988. 274 pp., with 57 figures and 102 tables. Isbn 0-08-036141-2. Hardcover dm 152. Starch - stärke, 42, 325-325.

Rizk, S. M., Zaki, H. F. and Mina, M. A., 2014. Propolis attenuates doxorubicin-induced testicular toxicity in rats. Food chem toxicol, 67, 176-86.

Salisbury, G. W., Vandemark, N. L. and Lodge, J. R., 1978. Physiology of reproduction and artificial insemination of cattle, san francisco, w.h. freeman and company.

Sarwar, M., Khan, M. A., Nisa, M., Bhatti, S. A. and Shahzad, M. A., 2009. Nutritional management for buffalo production. Asian-australas j anim sci, 22, 1060-1068.

Sharma, R. K. and Agarwal, A., 1996. Role of reactive oxygen species in male infertility. Urology, 48, 835-50.

Shen, H. and Ong, C., 2000. Detection of oxidative dna damage in human sperm and its association with sperm function and male infertility. Free radic biol med, 28, 529-36.

Snowdon, C. T. and Ziegler, T. E., 2007. Reproductive hormones. Handbook of psychophysiology, 3rd ed. New york, ny, us: cambridge university press.

Suarez, S. S., 2008. Control of hyperactivation in sperm. Hum reprod update, 14, 647-57.

Tavares, D. C., Lira, W. M., Santini, C. B., Takahashi, C. S. and Bastos, J. K., 2007. Effects of propolis crude hydroalcoholic extract on chromosomal aberrations induced by doxorubicin in rats. Planta med, 73, 1531-6.

Vivas-Acevedo, G., Lozano-Hernández, R. and Camejo, M. I., 2014. Varicocele decreases epididymal neutral α-glucosidase and is associated with alteration of nuclear dna and plasma membrane in spermatozoa. Bju international, 113, 642-649.

Yousef, M. I., Kamel, K. I., Hassan, M. S. & El-Morsy, A. M., 2010. Protective role of propolis against reproductive toxicity of triphenyltin in male rabbits. Food chem toxicol, 48, 1846-52.

Yousef, M. I. and Salama, A. F., 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food chem toxicol, 47, 1168-75.

Zahmatkesh, E., Najafi, G., Nejati, V. and Heidari, R., 2014. Protective effect of royal jelly on the sperm parameters and testosterone level and lipid peroxidation in adult mice treated with oxymetholone. Avicenna journal of phytomedicine, 4, 43-52.

Table 1. Effect of propolis supplementation on volume of semen (ml), mass motility (%), live (%), dead (%) spermatozoa, abnormalities (%) and concentration of spermatozoa (x 109/ml).

	Parameter (Mean ± SE)					
Group	Volume (ml)	Mass motility	Live %	Dead %	Abnormalities %	Concentration (millions/ml)
Control	2.8±0.1	61.7±1.9	73.3±1.8	26.7±2.1	18.7±1.4	2 ±0.2
Propolis i	3.6±0.1*	71.1±3*	82.2±1.8*	17.8±1.8*	11±1.1**	2.1±0.2
Propolis ii	3.4±0.2*	63.9±3.3	76.2±2.3	23.8±2.3	15.7±2*	2.1±0.2

Means (± SE) in the same column within the same category carrying different superscripts are significantly different when (P<0.05).

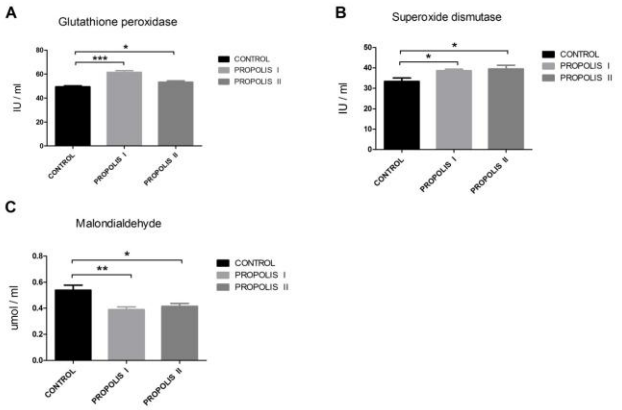

Fig.1. Effect of propolis in comparison to control on seminal plasma oxidative markers; A) GPx (glutathione peroxidase) activity, B) SOD (superoxide dismutase) activity and C) MDA (malondialdehyde) levels.

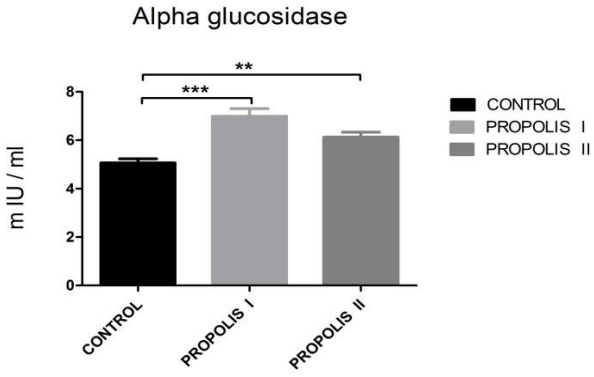

Fig . 2. Effect of propolis in comparison to control on seminal plasma alpha glucosidase activity

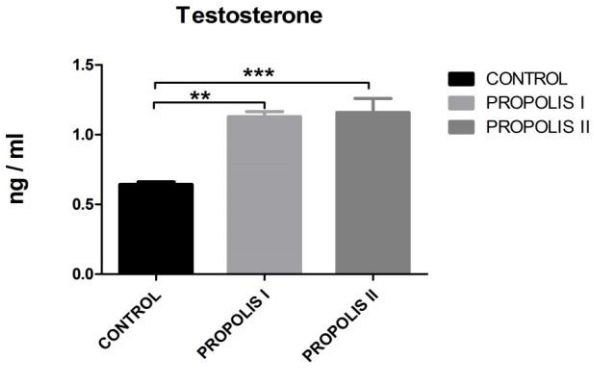

Fig . 3. Effect of propolis in comparison to control on plasma testosterone levels