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Ameliorating effect of propolis extract against equigan induced testicular toxicity and oxidative stress in rat testes

Ahmed Massoud[a], Ehab Tousson[a], Nahla Zedan[b] and Ali E. Abd elwahab[a]

[a] *Department of Home Economics, Faculty of Specific Education, KafrElShaikh University, Egypt*
[b] *Department of Nutrition and Food Science, Faculty of Home Economics, University of Tabuk, Tabuk 71491, Saudi Arabia*

(Corresponding author e.mail. toussonehab@yahoo.com)

Abstract

Equigan is an androgenic steroid that improves the growth and food conversion in meat producing animals. The present study was performed to determine the ameliorating effect of propolis in the toxicity of the rat testes induced with Equigan. Fourty male albino rat were divided into four groups (10 animals each); the control group includes animals that injected intramuscularly with olive oil. The second group includes rats received propolis. The third group is the experimental group included animals that received intramuscular injections of Equigan. The last group was co-administrated group where rats received Equigan along with propolis. The results suggest that misuse of growth promoter Equigan may contribute to continuous damage of the testicular function and structure that shown a significant increase in nitric oxide (NO), malondialdehyde (MDA), testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in Equigan group when compared to the control group, these results decrease in co-administrated Equigan with propolis. On the other hand a significant decrease in superoxide dismutase (SOD), catalase, Glutathione (GSH) and total thiol in Equigan group when compared with control group, and a significant increase in co-administrated Equigan with propolis. It was concluded that; propolis has ameliorating role on the biochemical alterations in Equigan induced testicular toxicity in male rat.

Keywords: Steroid hormones*;* Equigan*;* Propolis*;* Testes*;* Oxidative stress; Antioxidants; Rats.

1 Introduction

Boldenone were developed mainly for veterinary use, mostly for horse treatment and well known under the trade names Equigan, Equipoise, Ganabol, and Ultragan. Equigan is used to enhance strength and endurance in canine, equine and human athletes through increasing muscle protein production, it is a synthetic substance related to the primary male sex hormone, testosterone (Kicman, 2008; Guan et al., 2010; Tousson et al., 2012). Equigan (Boldenone, 1, 4-androstadiene-17β-ol-3-one) is an androgenic steroid that improves the growth and food conversion in meat producing animals (Alm-Eldeen and Tousson, 2012).

In most countries worldwide, this anabolic steroid is forbidden for meat production (Cannizzo et al., 2007; Soma et al., 2007; Noppe et al., 2008). It has been demonstrated that precursors of 17α-Equigan can be detected in the faeces of rats fed with phytosterols (Song et al., 2000; Le Bizec et al., 2006). Equigan has a very long half-life that show up on a steroid test for up to 1.5 years and this is because of the long undecylenate ester attached to the parent steroid. Trace amounts of the drug can be easily detected for months after discontinued use (Brookhouse, 2007; El-Moghazy et al., 2012; Tousson et al., 2012). Recently, it is used by bodybuilders in both off-season and pre-contest periods, where it is well known for increasing vascularity while preparing for a bodybuilding contest (Tousson, 2013).

Propolis is a resinous natural product collected from cracks in the bark of trees and leaf buds which are enriched with the salivary enzymes of honeybees (Talas et al., 2014). The antioxidant activities of propolis are

related to its ability to scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite (Türkez et al., 2010). The primary mechanism of the effect of propolis may involve the scavenging of free radicals that cause lipid peroxidation. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated (Kanbura et al., 2009).

Propolis is a honeybee product with a broad spectrum of biological properties (Mello and Hubinger, 2012). The exact composition of which is dependent upon the source plant. Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids (El Sayed and Ahmad, 2012). In addition, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Banskota et al., 2001; Lotfy, 2006). Propolis also contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids (ElMazoudy et al., 2011). It has gained false popularity and was extensively used in drinks and foods to improve well-being and prevent diseases such as inflammation, heart disease, diabetes and even cancer. Propolis possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibiotic and antifungal activities (Marquele et al., 2005; Yousef et al., 2010). Therefore, this study was designed to investigate the possible effect of using growth promoter Equigan on the rat testes structure and functions and the possible ameliorating role of propolis extract as a protective agent against Equigan -induced testes toxicity.

2. Materials and Methods

The experiments in this study adhered to the guidelines of the ethical committee of the national research center (Dokki, Giza, Egypt).

Animals

The experiments were performed on 40 male albino rats weighing 170 ± 10 g and of 10-12 weeks of age. The rats were kept in the laboratory for 1 week before the experimental work and maintained on a standard diet and water available ad libitum, and at ambient temperature (23 ± 2°C) with a relative humidity of 55 ± 5%. The experiment continued for 12 weeks on which constant amount of diet was given for each rat.

Forty male albino rat were divided into four groups (10 animals each). Control group (G1) includes animals that injected intramuscularly with olive oil. Propolis group (G2) includes animals that received intragastrically a dose of 200 mg/kg body weight (0.5 ml) of propolis extract 3 times a week, respectively. Equigan group (G3) rats that received intramuscular injections of 5 mg/Kg body weight Equigan at beginning of the experiment and after 2, 4, 6, 8 and 10 weeks respectively (Gabr et al., 2009). Co-administrated group (G4) rats that received intramuscular injections of 5 mg/Kg body weight Equigan at beginning of the experiment and after 2, 4, 6, 8 and 10 weeks respectively, in addition to, rats were received intragastrically a dose of 200 mg/kg body weight propolis extract (0.5 ml) 3 times a week.

At the end of the experiment, the rats were fasted for 10 hr and then anesthetized with Thiopental and subjected to a complete necropsy.

2.1 Clinical chemistry

Total protein were measured using diagnostic kits according to the method described by Henry (1974) and Fawcett (1960), total lipids in serum by using the sulfophospho-vanillin colorimetric according to Frings (1972) and Chapman (1998), Hormone profiles, testosterone, follicle-stimulating hormone (FSH) and plasma luteinizing hormone (LH) levels were assayed using the same radioimmunoassay kit according to Tietz (1994).

2.2 Homogenate

Testes homogenate 10 % (W/V) was prepared in ice-cold 0.067 M phosphate buffer (PH=7) then the homogenate was centrifuged at 3000 rpm for 15 minutes. The resulting supernatant was used to determine the total protein by comassie blue according to Bradford (1976); Nitric oxide (NO) according to Vodovotz (1996); Super oxide dismutase activity (SOD) according to Oyanagui (1984); glutathione (GSH) according to Beutler et al. (1963); Malondialdhyde (MDA) according to Lahouel et al.(2004); Total thiol content were performed according to Sedlak and Lindsay (1986) and catalase activity was detected according to the method of Aebi (1984) and Fossati et al. (1984).

2.3 Statistical Analysis

Data were expressed as mean value ± SE and statistical analysis was performed using one-way analysis of variance to assess significant differences among administrated groups. The criterion for statistical significance was set at $p<0.05$ for the biochemical data. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) statistical version 16 software package (SPSS1 Inc., Chicago, Illinois, USA).

3. Results

Figure 1 shows that, the changes in the relative body weight rate in different group under study. The relative body weight rates were significantly increased after the administration of Equigan when compared to control group.

Figure 2&3 shows that significant elevation in NO and MDA in Equigan group when compared to control and propolis groups. On the other hand, NO and MDA shows decreased in co-administrated propolis group when compared to Equigan group.

Total thiol, SOD, catalase, and GSH levels were

significantly decreased in Equigan group when compared to control and propolis groups (Figures 4-7).

In contrast, total thiol, SOD, catalase, and GSH levels showed increased levels in co-administrated propolis group when compared to Equigan group (Figures 4-7). Figures 8&9 shows significant increase in total lipid and total protein in Equigan group when compared to control and propolis groups, while total lipid and total protein showed decreased levels in co administrated propolis group when compared to Equigan group.

Figures 10&11&12 shows a significant increase in testosterone, LH , and FSH hormones in boldenone group, these elevations decreased in co-administrated group with propolis.

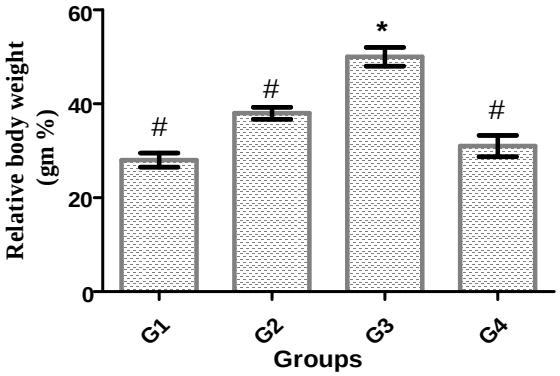

Figure 1: Changes in relative body weight in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

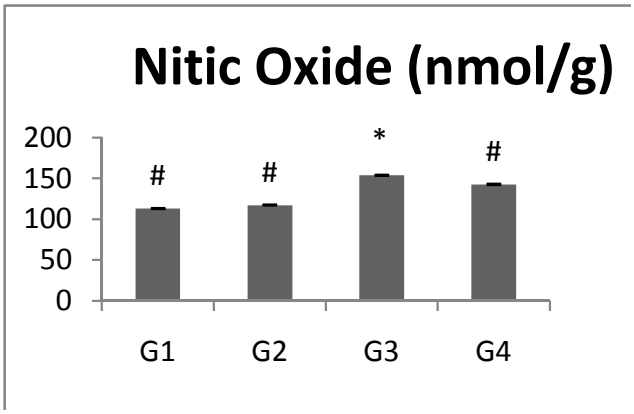

Figure 2: Changes in testes nitric oxide (nmol/g tissue) levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

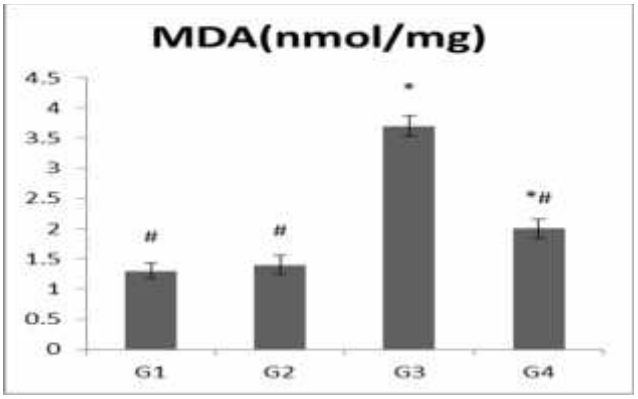

Figure 3: Changes in testes MDA levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

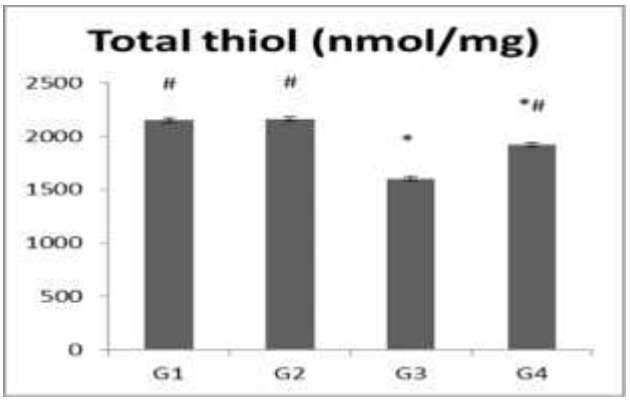

Figure 4: Changes in testes total thiol levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

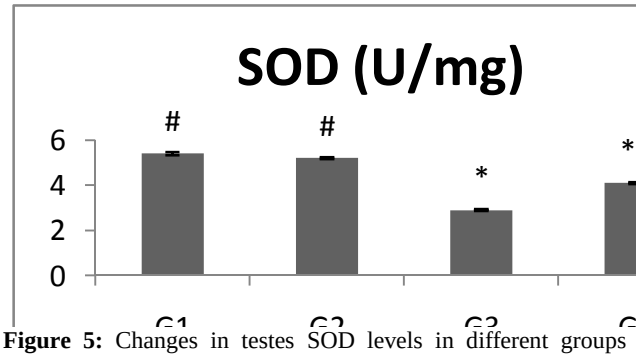

Figure 5: Changes in testes SOD levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

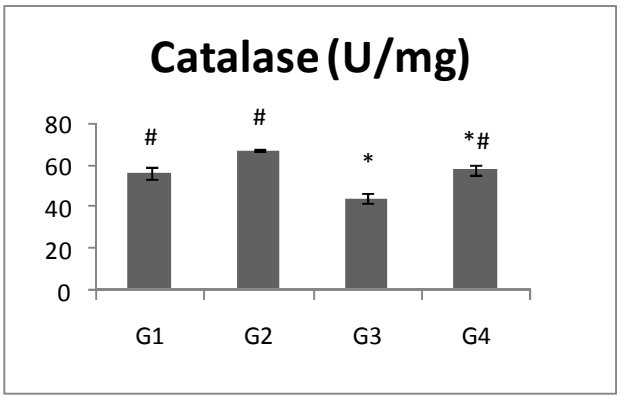

Figure 6: Changes in testes catalase (U/mg tissue) levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

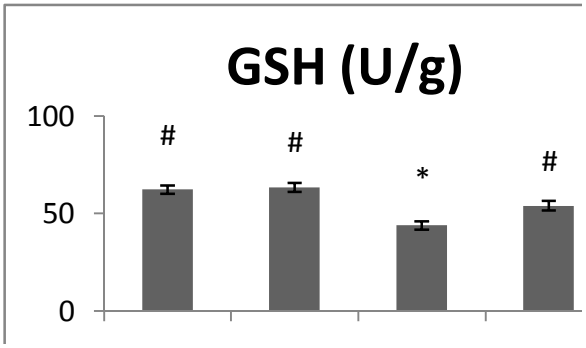

Figure 7: Changes in testes GSH (U/g tissue) levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group

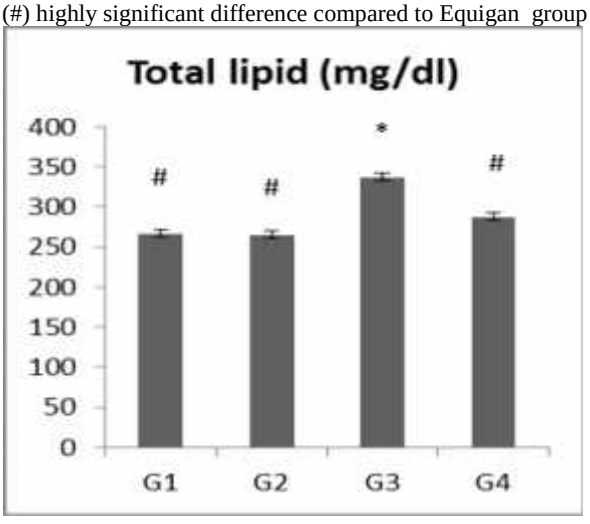

Figure 8: Serum total lipid (mg/dl) levels in in different groups under study, where G1, control group; G2, propolis group; G3, boldenone group; G4, co-treated boldenone group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to boldenone group.

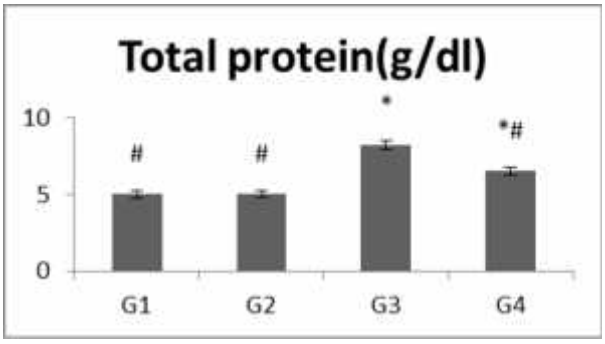

Figure 9: Changes in testes total protein (g/dl) levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

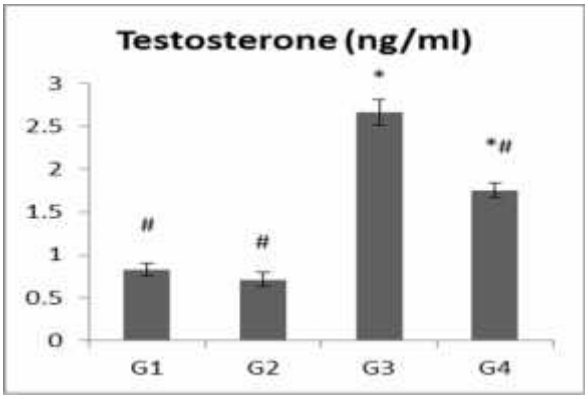

Figure 10: Serum Testosterone (ng/ml) levels in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

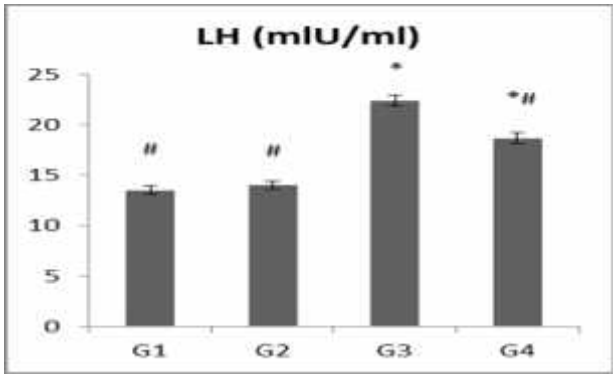

Figure 11: serum LH (mlU/ml) levels in in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-administrated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group.

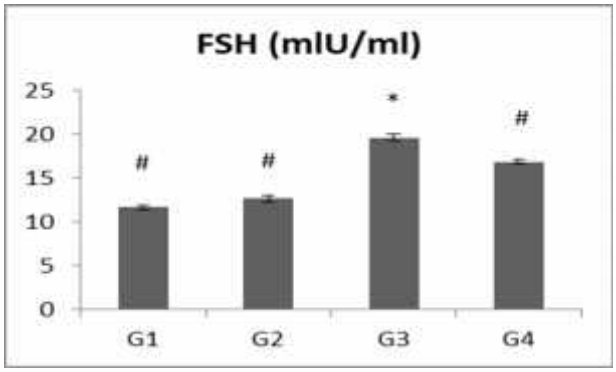

Figure 12: Serum FSH (mlU/ml) levels in in different groups under study, where G1, control group; G2, propolis group; G3, Equigan group; G4, co-treated Equigan group with propolis. (*) significant difference compared to control group. (#) highly significant difference compared to Equigan group

4. Discussion

Antioxidants are molecules that are capable of slowing or preventing the oxidation of other molecules, thereby protecting cells from damages caused by exposure to free radicals, including reactive oxygen species, which are produced during oxidation reactions in biological cells. Antioxidants can be either phytochemicals or vitamins and other nutrients; they range from micro molecules such as glutathione, vitamins, to macromolecules such as catalase, glutathione and peroxidase (Khan et al., 2009; Grigorov, 2012). In the developing countries with the rapid growth of population, like Egypt, there is a more demand for edible protein than supply. Equigan is used heavily in Egypt, not only in the field of veterinary practice, but also by athletes and bodybuilders. So, Equigan has dual effects on humans, both directly and indirectly; directly through injection to build muscles and indirectly by consuming meat of Equigan administrated animals (Toffolatti et al., 2006; Tousson et al., 2012). Equigan increases muscle size owing to promotion of positive nitrogen balance by stimulating protein production and reducing protein destruction, as well as causing retention of body water, nitrogen, sodium, and potassium and calcium ions (De Brabander et al., 2004; Oda and El-Ashmawy, 2012). The abuse of Equigan can lead to serious and irreversible organ damage (Maravelias et al., 2005). Among the most common adverse effects of anabolic androgenic steroid that have been described are reduced fertility, cardiovascular disorders, hepatic neoplasms and carcinoma, tendon damage, psychiatric and behavioral disorders in both sexes (Velazquez and Alter, 2004). The purpose of the present study was to investigate the ameliorating role of propolis extract as a protective agent against Equigan - induced testes toxicity.

The results in this study are in agreement with Zahran et al. (2015) who reported that the body weight rate was increasing after treatment with Equigan undecylenate when compared with control group. Anabolic androgenic steroids increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles. Anabolic steroids also have androgenic and virilizing properties including the development and maintenance of masculine characteristics such as the growth of the vocal cords, testicular, and body hair (secondary sexual characteristics). Tousson et al. (2012) said that there is strong indication that the duration, dosage, and chemical structure of the anabolic steroids are important for the serum concentration gonadotropins.

A moderate decrease of gonadotropin secretion causes atrophy of the testes, as well as a decrease of sperm cell production, oligo, azoospermia and an increased number of abnormal sperm cells have been reported in athletes using anabolic steroids use, the gonadal functions will restore within some months. The results are also agreed with Mottram and George, (2000) who reported that anabolic androgenic steroids administration would disturb the regular endogenous production of testosterone and gonadotrophins that may persist for months after drug withdrawal. Many other adverse effects associated with anabolic androgenic steroids misuse include disturbance of endocrine and immune function, alterations of sebaceous system and skin, changes of haemostatic system and urogenital tract. Tousson et al., (2012) reported that intramuscular injection with Equigan adversely affects spermatogenesis.

The administrated animal tests also showed marked histological changes in the seminiferous tubules such as thickened in basement membrane together with focal areas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, fibrosis, degeneration of germinal epithelium with abnormal distribution of spermatozoa and many syncytial cells were found in the seminiferous tubule lumen. The results showed significant increase in NO and MDA in Equigan group when compared to control and propolis groups. On the other hand, NO and MDA decreased in co-administrated propolis group when compared to Equigan group and this indicated that propolis has a prophylactic role on MDA and nitric oxide production as a result of Equigan injection.

The preset results indicated also that propolis administration improved antioxidant activity which spermatozoa, like any other aerobic cell, are constantly facing the “oxygen-paradox”, The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and contaminating leukocytes has been defined as one of the few etiologies for male infertility (Maneesh and Jayalekshmi, 2006). ROS also cause injury to the basic cell structures such as proteins, lipids, carbohydrates, and nucleic acids. Oxidative stress is considered to play a main role in the progress of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders and other conditions (Grigorov, 2012). The results in the present study showed significant decrease in catalase and SOD levels in Equigan group when compared to control and propolis groups. On the other hand, catalase and SOD levels showed increased levels in co-administrated group when compared to Equigan

group. These results are in agreement with Dobrowolski et al, (1991) who reported that extracts of propolis are receiving renewed attention worldwide because of their beneficial effects and their antioxidant activity.

CONCLUSION

Propolis extract ameliorate the Testicular toxicity and oxidative stress on rat testes induces by Equigan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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