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STUDY THE EFFECT OF *PANAX GINSENG* ON TESTICULAR MORPHOLOGY, SOME SPERM PROPERTIES AND TESTICULAR HISTOLOGY IN MALE JAPANESE QUAIL

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ABSTRACT : This study was designed to evaluate the levels affected levels of adding *Panax ginseng* powder admisteration on sperm motility, testicular weight and histological evaluation. This study was conducted at the laboratories of Theriogenology Department, College of Veterinary Medicine, Basrah University, during the period extended from 15/ 1/2019 to the end of 15/ 3/2019. The study includes 40 intact straw male birds, aged between (14–15) weeks, weight range between 200-220gm. The birds were divided randomly into four equals groups (10 birds in each group). The 1st group was served as the control group (n = 10), 2nd group, 3rd group and 4th group were treated by ginseng for 50 days. Testis with epididymis were harvested directly after slaughtering birds, the sperm was collected by sliced caudal epididymis for evaluation sperm availability, dead sperm and sperm abnormality and the testis sample was collected in formalin 10% for histological examination. The results illustrated that the rate mass motility, individual motility and forward spermmotility increased significantly value (P< 0.01) in groups A, B and C as compared with the control group. On the other hand, the rate of the dead sperm and sperm deformity decreased significantly value (P< 0.01) in groups A, B and C as compared with the control group. The results also showed highest significant (P<0.01) values of rate mass motility, individual motility, forward sperm motility in Group C as compared with Group B and A. Moreover, the results indicated the total average testicular weight (Right and Left) increased significantly value (P<0.01) in groups A, B and C as compared with the control group. As well as the results showed the total rate of right testis increased significantly value (P<0.01) in groups A, B and C as compared with the control group. On the other hand, the results illustrated that the total rate of left testis increased significantly value (P<0.01) in groups A, B and C as compared with control group. While the results appeared the total average testicular weight right and left testis increased significantly value (P< 0.01) in group C as compared with groups B and A. Ginseng is one of the most popular herbs and antioxidant property, which effects on sexual performance, general tonic, increases the motility and morphology of epididymal sperm and increased male fertility.

Key words : Japanese quails, *Panax ginseng*, testis, epididymis, sperm.

INTRODUCTION

Japanese quails are small, chunky, fast-moving, and able to fly at a low level (Shanaway, 1994). Japanese quial is resistant to common diseases of poultry (Mohammed and Ejiofor, 2015). It does not require intensive vaccination programs, but only Newcastle vaccine, it is considered as one of the best birds in poultry experiments and research because of the great flexibility it brings to obstacles (Priti and Satish, 2014). Japanese quailhas highly sexual activity in production of spermit is approximately 92.5×10^6 (sperm / cloud / testicular tissue / day) (Clulow and Jones, 1988). With the increased activity of the male reproductive system, which leads to an increased chance of developing free radicals (Bartosikova *et al*, 2003). The organs and tissues of high capacity (testis) are exposed to free radicals at a higher rate (Loven and Oberley, 1985). To treat tissues from injuries, antioxidants prevent or slow down the generation of free radicals that are generated by various vital activities in the body, so they act as a defensive line against the destructive activity of free radicals in terms of their generation or their chain of interactions (Bartosikova *et al*, 2003). Recent studied have tended to used medicinal herbs as food additives which help to increase growth and protect them from many diseases (Hassan, 2011). These additives include palmpollen and ginseng extracts that contribute to improve male and female fertility (Yesilbag *et al*, 2013). These herbs containestrogen and estrone as well as other nutrients, proteins, essential and non-essential amino acids, carbohydrates, vitamins and minerals (Hassan, 2011). The major active ingredient of ginseng is the ginseng saponin, which is composed of various ginsenosides (Pak *et al*, 2005). Currently; approximately 30 ginsenosides have been identified (Kim

et al, 2003; Bae *et al*, 2004). There are at least 9 known species of ginseng, including the Asian (*Panax ginseng*), American (*Panaxquin quefolium*) and Japanese (*Panax japonicus*) ginseng. These varieties have shown effects such as antioxidant effects, enhancement of diverse physiologic effects including immunostimulatory effects (Lin *et al*, 2008; Bak *et al*, 2012) effects on the neuronal system (Nah *et al*, 2009; Ye *et al*, 2011) control of the metabolic state (De Souza *et al*, 2015; Zhang *et al*, 2016). In addition to the general properties of ginseng which consider as a general tonic and antioxidant. Therefore, the present aims of study aims the effects of ginseng root powder ontesticular morphology and weight as well as the physiological characteristics of sperm in quail.

MATERIALS AND METHODS

Experimental study

This study was conducted on 40 intact straw male birds, aged between (14–15) weeks weight range between (200-220)g, the study was investigated at the laboratories of Theriogenology, Department of Surgery and Obstetrics, College of Veterinary Medicine, Basrah University, during the period extended from 15/ 1/ 2019 to the end of 15/ 3/ 2019. The male birds were kept in special cages and their food was dried pellets. The bird divided into four equals groups (10 birds in each group). The 1st group was served as the control group (n=10), 2nd group, 3rd group and 4th group were treated by ginseng for 50 days according to the following:

Group A : 10 birds / treated without supplemented(control group).

Group B : 10 birds / treated with supplemented ginseng (1gm/L) dissolving in water.

Group C : 10 birds / treated with supplemented ginseng (2gm/L) dissolving in water.

Group D : 10 birds / treated with supplemented ginseng (3gm/L) dissolving in water.The roots of pure red ginseng were imported from Malaysian market. The ginseng roots are grinds into powders and stored in a clean dray bag.

Studied qualities

After 50 days of experiment, all groups were weighed by electronic balance,for the calculating the average weight of birds. Five birds of each treated group were slaughtered to study the relative weight of testicles (Fig. 1), histological structure and some physiological parameter of sperms (Salhie, 2016).

Collection of sperms

All samples of birds (testis with epididymis) were collected directly after slaughtering and transported to the laboratory of unit center research in the College of Veterinary Medicine, Basrah University. The samples testis with epididymis (Fig. 2), washed by distill water firstly, then with normal saline containing antibiotics and anti-fungal. The epididymis was separated from testis and placed in glass petri dishes, with 3 ml of the media TCM-199 containing 100 IU/ml penicillin streptomycin and 100 IU/mL of Nystatin (Fig. 3) and sliced by sterile blade for small pieces (Habeeb *et al*, 2019). The other step aspiration one drop of media containing Spermatozoa for physiological evaluation of sperm determination (Fig. 4). Total motility, Dead Sperms Percentage, Percentage of Sperms deformity. Progressive motility. All these parameters for sperm evaluation were examined using sperm analyzer (Habeeb *et al*, 2019). On the other hand, the testes were placed in special containers containing 10% formalin for histological examination.

Fig. 1 : Slaughtered of birds.

Fig. 2 : Testis with epididymis.

Fig. 3 : Testis with media.

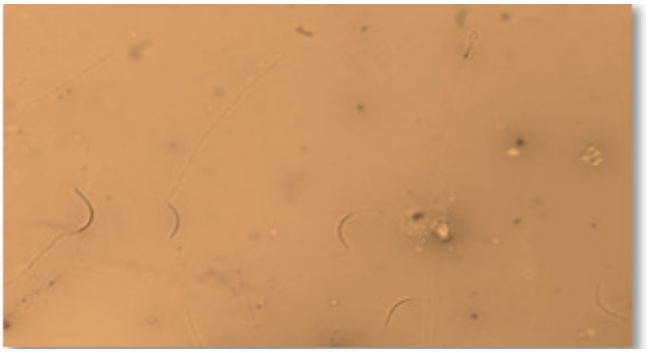

Fig. 4 : Physiological evaluation of sperm.

Statistical analysis

ANOVA one way test was using SPSS for comparison sperm properties, testis weight between different groups. A significance level was used (1%), V.17.

RESULTS

Effect ginseng on some sperm properties

The results illustrated that the rate mass motility, individual motility, forward sperm motility increased significantly value (P< 0.01) in groups A, B and C as compared with the Control group. On the other hand, the rate ofdead sperm and sperm deformity decreased significantly value(P< 0.01) in groups A, B and C as compared with the control group. The results also showed highest significant (P<0.01)values of rate mass motility, individual motility, forward sperm motility in group C as compared with groups B and A as shown in Table 1.

Effect ginseng on testis weight

The results indicated that totalaverage testicular weight (Right and Left) increased significantly value (P< 0.01) in groups A, B and C as compared with Control groups. As well as the results showed the total rate of right testis increased significantly value (P< 0.01) in groups A,B and C as comparedwith the control group. On the other hand, the results illustrated that the total rate of left testis increased significantly value (P<0.01) in groups A, B and C as compared with the control group. While the results appeared the total average testicular weight (Right and Left), right andleft testis increased significantly value (P< 0.01) in group C as compared with group B and A.

Histological evaluation

The histological observations of the control testicle and examined showed that the somniferous tubules lined by a stratified epithelial cells and tall sertoli cells which are few in number and act as supported cells which seated at the basement membrane of the tubules and extended into the lumen of the tubules, the interstitial thin tissue between the tubules interpose by blood vessels and lydig cells, the outer connective tissue capsule are surrounding the organ (Fig. 5 a, b). The epithelial tissue of the seminiferous tubules that form of different stages of spermatogenic cells, the primary and secondary spermatocytes, with the spermatids that found in a cluster at the lumen of the tubules (Fig. 5 c). The treated group A distinguished by a significant augment of primary, secondary spermatocytes and spermatids as compared with the control group, as well as an invaded of blood vessels (Fig. 5 d). B and C showed that they are filled with a high number of sperm cells filled the seminiferous tubules lumen, withan increasein the size of interstitial cells (Fig. 5 e, f).

Table 1 : Effect additiondifferent concentration of ginseng on some sperm parameters (mean ± SE).

Treated	Mass motility	Individual motility	Dead sperm	Forward sperm motility	Deformity sperm
Control group	54.8 ± 1.6a	55.8 ± 2.29A	45.2 ± 1. 6a	52.8 ± 2. 6a	19.2 ± 1.8a
Group A	64.2 ± 0.86b	65.0 ± 1.1B	35.8 ± 0.86b	67.8 ± 1.1b	15.0 ± 1.0b
Group B	77.2 ± 0.73c	74.4 ± 1.29c	22.8 ± 0.73c	70.8 ± 3.1c	8.8 ± 1.16c
Group C	89.8 ± 1. 6d	84.6 ± 1.29d	10.2 ± 1.65d	89.6 ± 1. 6d	4.0 ± 0.07d

Different small letters vertically denotes significant (P<0.01) between groups

Table 2 : Effect addition different concentration of ginseng on testicular weight parameters (mean±SE).

Treated	Testicular weight (Right and Left) /gm.	Right Testis /gm.	Left Testis /gm.
Control group	5.14 ± 0.1a	2.44 ± 0.12a	2.6 ± 0.1a
Group A	6.7 ± 0.2b	3.32 ± 0.1b	3.5 ± 0.22b
Group B	8.34 ± 0.12c	4.26 ± 0.9c	4.08 ± 0.1c
Group C	10.38 ± 0.08d	5.34 ± 0.9d	5.8 ± 0.37d

Different small letters vertically denotes significant (P<0.01) between groups

DISCUSSION

The present study revealed a significant difference between control group and treated groups in epididymis sperm characterized. Various studies have showed that treated by ginseng enhancement of quality sperm motile in animal models (Choi *et al*, 2003). The relationship

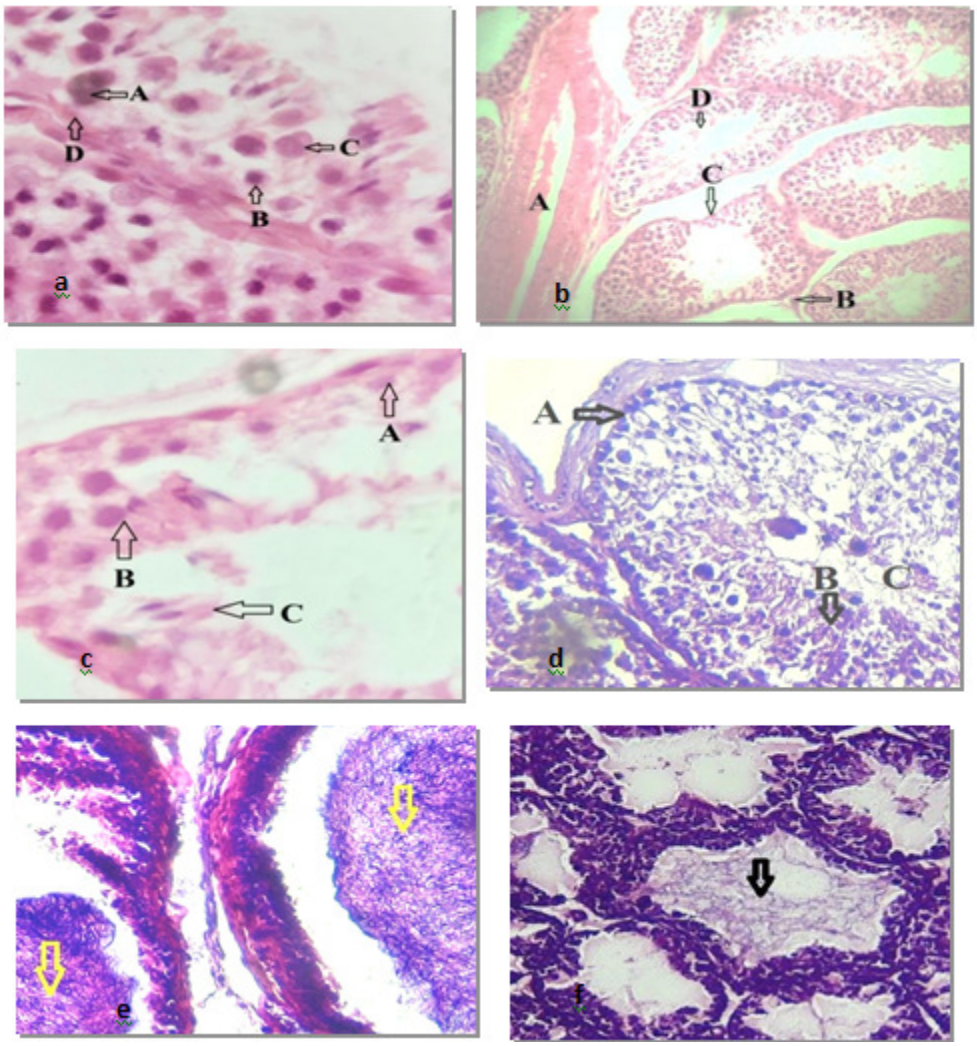

Fig. 5 : (a) Normal seminiferous tubules of Control group, A-Sertoli cell, B.Primary spermatocyte, C. Secondary spermatocyte, d. basement Membrane. H and E, X 1000. **(b)** Cross section of control group testes: A. Blood Vessels, B. lydig cell, C. Seminiferous tubules, D. Spermatids. H and E X 200. **(c)** Seminiferous tubules of control group : A.B and C Primary Spermatogonia H and E, X200. **(d)** group A showed :A. Primary spermatocytes, B. secondary spermatocytes, C Spermatids, H and E, X200. **(e)** Group B showed the seminiferous tubules fill with spermatids. H and E, X 400, **(f)** Group C, the seminiferous tubules fill with spermatids. H and E, X 200.

between reproduction male andginseng has been evaluated for more 20 years ago because ginseng was consideredaseffective factor on sexual performance, general tonic, enhancement libido and improvement copulatory behavior after treated with it (Salvati *et al*, 1996). Choi *et al* (2003) indicated that root ginseng root will increased the motility and morphology of epididymal sperm but it will not change the sperm concentration in male rat. Shin *et al* (1990) pointed that ginseng root is characterized by a high content of active substances such as saponins, phenolic compounds, alkaloids, polyacetylene and polysaccharides that played an important role in reproductive effectiveness (Solakidi *et al*, 2005) mentioned that ginseng root contains substances that promote sexual activity such as substances ginsenoside, which has a structure similar to steroid hormones. Ginsenoside will increase LH secretion in the anterior pituitary gland of male rats (Tsai *et al*, 2003). Liu *et al* (2003) ginseng combines two types of important characteristics for the functional of male reproductive system, they provide protection and support for testicular tissue and cells from harmful substances that may be accidentally produced during the course of biological processes, including active oxygen varieties and free radicals. Therefor, they acted a defensive line against the destructive activity of free radicals in terms of their generation or their chain of interactions (Bartosikova *et al*, 2003). Linjawi (2015) showed that treating by ginseng root improved the fertility of mice which effected on the hypothalamus gland of the testicles where observed a significant increase serum testosterone, luteinizing hormone (LH) and follicular hormone (FSH) levels,

further noted that ginseng root has the ability to inhibit damage in DNA and stimulate gene expression of the gene (CYP19, LH and FSH) responsible for hormonal production FSH and LH hormones. On the other hand, the results showed that the ginseng root has increased the relative weight of the testicles as compared with the control treatment this rise may be attributed to the increase of testosterone production which lead to high effected role on spermatogenesis production within seminiferous tubule of testis (Sturkie, 1986). Saponins from the cultured root of wild *P. ginseng* showed an increase in sperm production in male rats (Park *et al*, 2006). Histological in previous studies, ginseng-treated rats have shown increased thickness of basement membrane seminiferous tubule, germinal layer and increased primary, secondary spermatogenesis this significant due to increase androgen receptor of seminiferous tubule in testis and higher protein production in testicular cells (Hong *et al*, 2002). Massa *et al* (1980) suggested that the relationship between thickness of germinal layer in seminiferous tubule of testicular tissue and testosterone level in male Japanese quail.

CONCLUSION

Ginseng is one of the most popular herbs and antioxidant property, which affect the sexual performance, general tonic, increase the motility and morphology of epididymal sperm and increase male fertility.

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