INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

EPIYDIDMAL SPERM QUALITY OF VASECTOMIZED ADULT MICE TREATED WITH PHITOFERT®

Muna HussenYousef, Saad S. AL-Dujaly & Jawad K. AL-Arrak
Department of Physiology and Pharmacology Veterinary Medicine –University of Baghdad, Iraq
Chairman of Clinical Reproductive Physiology Department, Institution of Infertility Treatment and Embryo Research, Al-Nahrain University Baghdad- Iraq

ABSTRACT
This study was designed to evaluate the role of Phitofert® in the improvement of sperm function in vasectomized and healthy adult mice. Twenty four adult male mice were randomly and equally divided into four groups (G1-control, G2, G3 and G4). Twelve mice (G3 and G4) were vasectomized and others 12 mice were healthy and treated for 35 days as follows: G1 (control group), mice in G2 were given Phitofert® daily with a dose of 0.035mg/Kg B.W. Mice in G3 were vasectomized without treatment while the mice in G4 were vasectomized and given Phitofert®. At the end day of the experiment (35 days), fasting blood samples were collected by cardiac puncture for measuring testosterone hormone concentrations and all mice were weighted and scarified for evaluation of the sperm function. The sections were taken to conduct histo-pathological test and to measure of the number of leydig cells and diameter of semniferous tubules. The results revealed that serum testosterone hormone concentration was significantly decreased in healthy treated mice (G2) compared with control group, while vasectomized mice were showed significant increase in testosterone hormone concentration compared with vasectomized non treated group (G3). Also treatment of animals with the Phitofert® showed a significant decrease of dead and abnormal percentage of sperms compared with control and other groups. The vasectomy of mice showed a significant increase of the above two parameters compared with all other groups. The results of present study demostrated that Phitofert® caused significant increase in the relative of the testiculer weight (weight of testicle to body weight ratio) in G2 while this ratio decreased significantly in vasectomized mice (G3) compared with other groups. Histological finding revealed harmful effect in vasectomized group specially spermatogensis, while the treatment of mice with Phitofert® revealed improvement of spermatogensis and the development of testicular tissue in both G2 and G4 groups.

KEYWORDS: mice Phitofert®, sperm quality, cardiac puncture.

INTRODUCTION

The World Health Organization (WHO) is defined infertility as lack of ability to get pregnancy within twelve months of regular contact for couples in conception. One of the growing problems in the developed countries all around the world is male infertility. The male factor of couple infertility was estimated between 25 and 50% (Walczak-Jedrzejowska *et al*., 2013) with a mean value up to 30% (Karavolos *et al.*, 2013). The reasons behind infertility classified into three groups; pre-testicular, testicular and post–testicular (Karavolos *et al*., 2013; Kupis *et al*., 2015). Phitofert® is one of the food supplement contains a Maca extract and considered as synthetic drug used for the adaptogenic and tonic action due to its content of components of Maca root extract. Maca was used to treat numerous illness conditions including exhaustion, anemia and infertility because of its claimed anabolic and aphrodisiac effects. The active ingredient of Phitofert® is often referred to as "Peruvian ginseng (Valentová, 2003; Ganzera, 2002 and Zhao, 2005). Some athletes have used *Lepidium meynii* as a substitute to anabolic steroids even though its value is not proven (Cicero, 2001). The ethnobotanical study mentioned that the use of maca against cancer, sexual and menstrual disorders (Bogani, 2006). Other studies referred to its role in the secretion of hormones, immune stimulation and memory perfection (Ganzera, 2002). Vasectomy is a common method for birth control in men that causes several implications as well. However, new surgical techniques could decrease these implications significantly (Lavers *et al*., 2006). The epididymis has been shown to be responsible for the sustenance, conservation, transport, maturation, and storage of spermatozoa (Cooper, 1998). Maturation of sperm within the epididymis needs the interaction of sperm with epithelium and luminal fluid of the epididymis (Klinefelter and Hess, 1998). Although Phitofert® was used for man with selenium and for woman with folic acid to improve the function of reproductive system and fertility (Gonzales *et al*., 2001) but there is no researches about its role in animals, so that we designed the current study on mice as a model of mammals.

MATERIALS & METHODS

Animal's preparation and study design

Twenty four healthy adult male's mice, weighed 25-35g were used. Mice were held in plastic cages and sited in a room for two weeks for acclimation. Room temperature was maintained at 22 ±25˚C, air of the room was altered continuously using ventilation vacuum and with light/dark

cycle of 12:12 hrs per day. The litter of the cages was changed weekly. Animals were allowed freely reached to water and pellets along the experimental period. The study was carried out in the laboratories of Biotechnology Research Center at AL-Nahrain University.

Study protocol

Twenty four male mice were divided randomly into 4 groups, six animals per group and handled as follows:

Control group (G1 group): Healthy animals of this group (6 male mice) received only distilled water.

Healthy treated group (G2 group): Healthy animals of this group (6 male mice) received oral administration (0.035 mg/ mice) of Phitofert® once daily.

Vasectomized group (G3group): Animals of this group (6 male mice) received only distilled water.

Treated vasectomized group (G4 group): Animals of this group (6 male mice) received oral administration (0.035mg/ mice) of Phitofert® once daily.

Blood samples collection

Blood samples were collected via cardiac puncture technique from each anesthetized animal using disposable insulin needles. Blood samples were collected at 35 days to study the some parameters.

Hormonal estimation

Serum testosterone concentration

The testosterone hormone was determined using kits provide by Monobind Inc.USA according to Microplate Enzyme immunoassay (ELIZA) (Tietz, 1995).

Testicular weight to body weight ratio

After the end of treatment period (35 days), mice were weighed, anesthesized by intramuscular injection of Ketamine 90mg/Kg B.W and Xylazine 40mg/Kg B.W), and testes were excised and weighted by sensitive balance after being cleaned from the accessory connective and adipose tissues. Testicular weight to body weight ratio was calculated as in the following equation:

$$\text{Relative weight} = \frac{\text{Testicular weight (gm)}}{\text{Body weight (gm)}} \times 100$$

Preparation of Epididymal tail suspension

After cervical dislocation of animals, the tail of the epididymus of both sides were taken and embedded in one drop of phosphate buffer solution at 37ºC in a watch glass, and then the tail was cut into at least 200 sections by microsurgical scissors, to perform the following microscopical examination on sperm characters.

Sperm function test

Abnormal sperm morphology

The method of Siegmund (1979) was applied to evaluate the abnormal sperm morphology. Abnormal sperm morphology was calculated according to the following equation:

$$\text{Percentage of morphologically abnormal sperms} = \frac{\text{No.of morphologically abnormal sperms}}{\text{Total sperms no.}} \times 100$$

The morphologically abnormal sperms were estimated depending on sperms abnormality which had tapered head, tailless head, coiled tail, bifurcated tail, and broken tail.

Sperms viability

The assessment of live and dead sperms was carried out by method of Chemineau *et al.* (1991).

Sperms viability was calculated as in the following equation:

$$\text{Percentage of dead sperms} = \frac{\text{No.of dead sperms}}{\text{Total sperm No.}} \times 100$$

Sperms concentration

Sperm count was done according to Sakamoto and Hashimoto (1986) by using Hemocytometer (Neubauer Type). Estimation of sperm was made according to the following formula:

$$\text{Sperm concentration} = \text{Number of sperms} \times 10^6$$

Histological study of testes

Histological aspect of the study of testes was done as follow :Paraffin section of a 5 µm thickness were deparaffinized with xylene, then hydrated with descending series of ethyl alcohol solutions, and stained with Hematoxilin and Eosin (H & E) (Luna, 1968). After that the histological sections examined by light microscope to study the histological changes suspected to occur in the four groups.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System-version 9.1). One-way, two ways ANOVA and Least significant differences (LSD) post hoc test were used to assess the significant differences among means. $P<0.05$ was considered statistically significant (SAS, 2010).

RESULTS

Results of relative weight of testicles (testicular weight to body weight ratio)

The testes weight to body weight ratio was illustrated in table (1) The results showed that the mean of ratio in G2 group that treated with Phitofert® was increased significantly ($P<0.05$) compared to control group (G1) and other vasectomized groups (G3, G4 groups). Also vasectomized mice treated with Phitofert® showed significantly increase ($P<0.05$) of mean ratio as compared to vasectomized mice without treatment (G4 group).The results revealed that treatment with Phitofert® in healthy mice showed a high increase in testicular ratio while vasectomy of mice without treatment showed a significant decrease in this ratio compared with other groups.

TABLE 1: Effect of Phitofert® and vasectomy on testicular weight to body weight ratio, in adult male mice

Groups	G1	G2	G3	G4
Relative weight of testicles	0.43 ±0.02 B	1.05 ±0.15 A	0.34 ±0.02 B	0.43 ±0.01 B

LSD = 0.23. Values are presented as Means ±SE (n=6 mice/group)
Different capital letters denote significant differences between groups (P<0.05).
GI: Control group, G2: Healthy mice with Phitofert® group, G3: Vasectomized mice without Phitofert® group, G4: Vasectomized mice with Phitofert® group

Sperm concentration (spermx10^6/ml)

The results of sperm concentration in epididymal suspension of healthy and vasectomized mice are shown in table 2 which showed that sperm concentration in G2 group which treated with Phitofert® was significantly increased (P<0.05) compared to control group and vasectomized mice. Also sperm concentration was significantly decreased (P<0.05) in vasectomized mice (G3 group) compared to control group (healthy mice). On the other hand results showed that sperm concentration in vasectomized mice (G4 group) treated with Phitofert® was significantly increased (P<0.05) compared to mice without treatment (G3 group) which significantly decreased (P<0.05) compared to healthy animals (control group).

Sperms viability (%)

Treatment of mice with Phitofert® caused significant (P<0.05) decrease in dead sperm percentage compared to G3 and G4 groups, while vasectomized mice (G3 group) showed significant (P<0.05) increase of this percentage compared to healthy and healthy treated mice with Phitofert®. The highest increase of dead sperm appeared in vasectomized non treated animals, but the highest decrease observed in healthy treated group compared to other groups (Table 2).

Sperms abnormal morphology (%)

The results of abnormal sperms morphology are shown in table (2). After 35 days, the results showed that sperm abnormality % increased significantly (P<0.05) in vasectomized animals (G3 group) compared to healthy animals (control group and other groups), whereas vasectomized animals treated with Phitofert® (G4 group) revealed improvement of this ratio significantly (P<0.05) compared to vasectomized mice without treatment (G3 group). Also treatment of healthy animals with Phitofert® showed a non- significant decrease (P>0.05) of sperm abnormality compared with control group (G1 group).

TABLE 2: Effect of Phitofert® and vasectomy on sperms concentration, viability and abnormal morphology (%) in adult male mice

Sperm function parameters / Groups	Sperm conc. (sperm X10^6/ml)	Sperm viability (%)	Sperm abnormal morphology (%)
G1	45.5± 0.23 a	13.33 ±0.80 c	13.33 ±0.80 c
G2	11.48 ±1.32 b	15.33 ±1.78 c	15.33 ±2.02 c
G3	4.05 ±0.42 c	45.50 ±2.52 a	45.00 ±2.23 a
G4	8.51 ±0.37 b	29.66 ±1.11 b	29.66 ±1.20 b
L.S.D.	2.15	5.02	4.93

Values are presented as Means ±SE (n=6 mice/group)
Different capital letters denote significant differences between groups (P<0.05).
GI: Control group, G2: Healthy mice with Phitofert® group, G3: Vasectomized mice without Phitofert® group, G4: Vasectomized mice with Phitofert® group
Different small letters denote significant differences between groups (P<0.05).

Testosterone hormone concentration (ng/ml)

In this study, the results revealed the effect of Phitofert® on testosterone concentration (Table 3). The results of healthy animals treated with Phitofert® showed non-significant (P>0.05) decrease in serum testosterone concentration compared the control (G1 group), while vasectomy of mice cause a significant (P<0.05) decrease in the serum testosterone hormone concentration compared the all other groups, on other hand treatment of mice with Phitofert® (G4 group) lead to significant (P<0.05) increase of hormone concentration compared to (G3 group).

TABLE 3: Effect of Phitofert® and vasectomy on Testosterone hormone concentration (ng/ml) in adult male mice

Hormone concen. / Groups	Testosterone (ng/ml)
G1	4.06± 0.11 a
G2	3.30± 0.09 b
G3	1.79± 0.15 c
G4	2.21± 0.10 b
L.S.D.	0.36

Values are presented as Means ±SE (n=6 mice/group).
Different capital letters denote significant differences between groups (P<0.05).

GI: Control group, G2: Healthy mice with Phitofert® group,
G3: Vasectomized mice without Phitofert® group,
G4: Vasectomized mice with Phitofert® group.
Different capital letters denote significant differences between groups (P<0.05).

Histopathological changes

Histological study of testes of mice treated with Phitofert® (G2 group) (Fig 1) indicated a hyper spermatogensis and the tubules impacted with numerous spermatids and hyper chromatic spermatogonia. On other hand the testicular tissue of vasectomized mice showed severs hypospermatogensis and sever necrosis (Fig 2). While vasectomized treated mice showed mild hypospermatogensis and decreased number of spermatids with an increased number of spermatids with increased collagen proliferation and congested blood vessels (Fig 3). Concerning control group (Fig 4) results illustrated that the testicular tissue showed normal architecture; each somniferous tubules filled with serial layers of spermatogenesis.

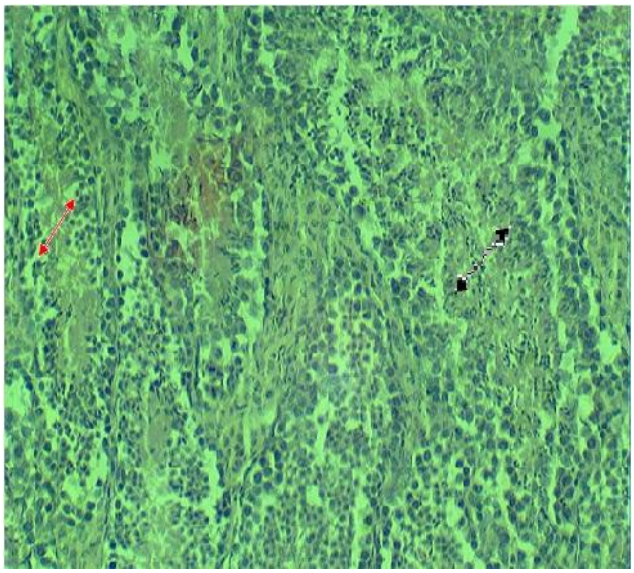

FIGURE 1: Histopathological section of testis of mice in G2 group (healthy with Phitofert® treated group): showed hyper spermatogenesis ◄► each tubule impacted with many numbers of spermatids and more eosinophilic material in their cavities, also there is hyper chromatic spermatogonia . (H&E stain, X40).

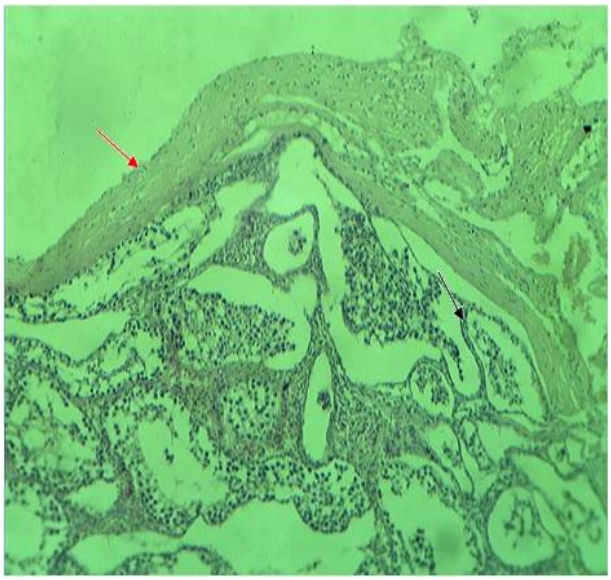

FIGURE 2: Histopathological section of testis of mice in G3 group (vasectomized without treatment group): Showed recognizable hypo spermatogenesis, ⟶ many tubules appeared severely necrotized and great thickened capsule ⟶ (H & E stain, X20).

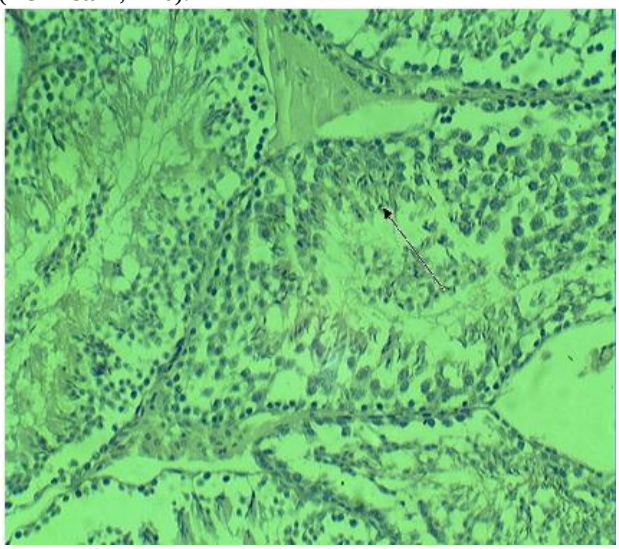

FIGURE 3: Histopathological section of testis of mice in G4 group (vasectomized with Phitofert® treatment group): Showed one seminferous tubule with complete spermato gensis ◄► (H & E stain, X40).

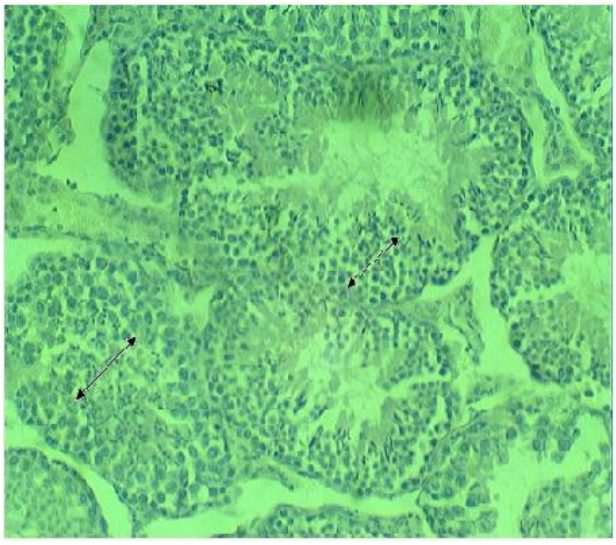

FIGURE 4: Histopathological section of testis of mice in G1 group(control group): the testicular tissue showed normal architecture; each somniferous tubules filled with serial layers of spermatogenesis ◄► (H and E stian, X20).

DISCUSSION

Relative weight of testicles (testicular weight to body weight ratio)

The weight of the testes is one of the indicators of changes in androgen status, the oxidative stress induced release of free radicals leading to testicular degeneration (Partra *et al*., 2011). The finding of the present study showed that testicular weight to body weight ratio was significantly increased in G2 group treated to Phitofert® compared with vasectomized mice, this result may be attributed to the effect of gonadotropins hormones (LH and FSH) in the development of semniferous tubules and Proliferating of cells (Guyton *et al*., 2016) thus the data revealed an increase of the diameters and number of leydig cells under the effect of treatment, also the increased of sperm production may play a role in increscent of this ratio. Phitofert® contains antioxidant substances (Flavonoids, alkaloids) may decrease the reactive oxygen species (ROS) level which caused by vasectomy (G3 group) and improve testes function (Večeřa *et al*., 2007), therefore, it seems that oxidative stress caused by vasectomy alleviated by Phitofert® component may be due to its production activity against oxidative damage by increasing antioxidant defense and improving the testicular functions and increase testosterone level in (G4 group). Furthermore testosterone has important role in increasing the weight of the reproductive organs including the testes and seminal vesicles (Chowdhury and Steinberger, 1976). Testosterone hormone is essential for spermatogensis process of seminiferous tubules and regarded a useful indicator of testicular function (Holdcrraft and Braun, 2004). The antioxidant effect of Phitofert® due to its component may be regulate digestion and metabolism which act as a digestion stimulant by increase the secretion of some digestive enzymes like trypsin, chemotrypsin and lipase (Platel and Srinivasan, 2000) also the high content of essential nutrients in Phitofert® such as amino acid may causes an increase of weight of organs such as testes. The oxidative stress causes decrement of testicular weight to body weight ratio, this result may be resulted from passive effect of free radicals on development and growth of tissues organs (He *et al*., 2010). Also the decrease of testosterone hormone concentration produced by vasectomy may lead to decrease of testicular weight, on the other hand the oxidative stress might be destroyed the germ cells due to membrane damage or macromolecular degeneration by reactive oxygen species (ROS) and lead to decrease of testicular weight (Kini *et al*., 2009). Also the decrease of testicular weight to body weight ratio may be due to decrease in the level of serum testosterone (Simanainen *et al*., 2008), therefore, a reduction in this ratio may be attributed to decreased steroidogenic biosynthesis and number of germ cells with inhibition of spermatogensis (Ponnusamy and Pari, 2011). Exposure of mice to vasectomy operation induced oxidative stress and oxidative damage to testicular tissues caused inhibition of serum hormonal level FSH,LH which induced the signals for testosterone synthesis (Asadi *et al*., 2014). Also, the reduction in testosterone level in vasectomized mice which under oxidative stress may be due to reduction of utilization of cholesterol by leydig cells, thus leydig cells function are impaired by the high level of cholesterol (Zakaria and Al-Busadah, 2015). On the other hand oxidative stress reactive oxygen species (ROS) can promote lipid peroxidation and causes loss in the ability of plasma membrane as a barrier leading to loss of catalytic enzymes and substrates from intracellular stores (Ikediobi *et al*., 2004) and these may be lead to decrease in testosterone concentration level. In group G4 which treated with Phitofert® showed increase in testes weight to body weight ratio as compared with G3 group may be attributed to improvement in testosterone level or FSH level also the anabolic effect of testosterone promote protein synthesis and improvement of body weight (Sinha-Hikim *et al*., 2002) also the increase of testes weight may be due to component of Phitofert® especially vitamins and minerals or due to increase the activity of hypothalamus-pituitary-testes axis and stimulation the activity of steroidogenic enzymes under the effect of antioxidant effect of Phitofert® (He *et al*., 2010).

Sperms concentration

The data of current study revealed significant increase of sperm concentration in mice treated with Phitofert® and this result may be due to the effect of Phitofert®. Semen enhancing properties (Gonzales *et al*., 2013; Lavana *et al*., 2013 and Clément *et al*., 2010b). Gonzales *et al* 2013 reported that the active ingredient component in Phitofert® increased stage of sperm nation and mitosis of germ cells during the spermatogenic cycle in male rats, these findings suggest that Phitofert® improve sperm formation. Also other researches revealed beneficial effect of Phitofert® to increase sperm production and motility, thus Phitofert® and its extract has been shown improve sperm concentration due to its role to enhanced the response to FSH and LH hormones (Gonzales *et al*., 2001). Composition of Phitofert® from alkaloids (Zhao *et al*., 2005; Muhammad *et a*l., 2002) that component was responsible for producing the effect on fertility effect on both ovaries and testes of the animals and it caused higher sperm production in male (Ohta *et al*., 2017). Also the Phitofert® treatment was increased both semen volume and sperm motility because the volume of semen is resulted from the contributions of the accessory glands (seminal vesicles 60%, prostate 30% and epididymus 10%) (Gonzales, 1988a) which were androgenic depended (Gonzales, 1988b) that the sperms concentration may be increased because the semen volume increase. On other hand sperm concentration in vasectomized mice was significantly decrease and such finding may be caused as a result of oxidative stress and free radical release. Thus reactive oxygen species (ROS) has a passive effect on testes function and spermatogensis (Guyton and Hall, 2016). Also, reactive oxygen species (ROS) affected the ultrastructure of sperm and increase of DNA fragmentation so that the percentage sperm viability will decrease. The present study showed that significant increase in sperm concentration, the increment may be attributed to the effect of Phitofert® which activated spermatogensis or may be due to its stimulation of pituitary gland to increase synthesis and release of LH hormone and thereby increased sperm concentration (Hussein, 2013). On the other hand the increment of FSH and LH by Phitofert® (Table 4-4) may can support mitosis and meiosis and leading to stimulate spermatogensis and

this may induced increase sperm concentration (Salem and Moustafa, 2001).

Also, the increasing of the testicular weight of mice confirms the correlates between sperm concentration and testicular weight. The decrement of sperm concentration in vasectomized mice (G3 group) compared with other groups may be due to early failure of spermatogensis and reduced sperm motility under the effect of vasectomy (Ren *et al*., 2011), also vasectomy may be lead to epididymis function (Maturation of sperm) by providing a specific fluid environment secreted from epididymis as a site for sperm maturation (Soudamani *et al*., 2005; Aman *et al* ., 1993)

Sperms viability

The results of this study showed an increase of dead sperms percentage in vasectomized mice which were decreased in the semen sample of Phitofert® treated animals. This improvement in the viability of sperms may be due to the antioxidant properties of Phitofert® due to its alkaloid components and Flavonoids (Bai *et al*., 2015). Also the vasectomy of mice causes oxidant stress that lead to increase of DNA fragmentation and abnormalities of sperms which predisposed to increase the percentage of dead sperms (Desai *et al*., 2010). On other hand, treatment of mice with Phitofert® can caused significant decrease in abnormality percentage (table 4-2) compared to vasectomized mice, these results may be due to component of Phitofert® which increased the activity and intact sperms beside the effect of Phitofert® to improve DNA normality (Tamburrino *et al*., 2012;Schulte *et al*., 2010), while vasectomy causes free radicals generation may be lead to increase abnormality of sperms due to the lipid peroxidation and increase of fragility of sperm membranes (Geilazyn *et al*., 2002).

Although the increase of testosterone in this stage was non significant, but this hormone important in regulated epididymal section and had essential role in sperm maturation, survival and production of fructose so may caused an increase in sperm viability (Ahmed *et al*., 2002). Oxidative stress in mice exposed to vasectomy operation and increase of reactive oxygen species (ROS) may be causes passive effect on motility, viability and lipid peroxidation of sperm and decrease viability in this group, high reactive oxygen species(ROS) levels associated with inhibition of sperm function and viability due to peroxidation of membrane polyunsaturated fatty acid (Yeni *et al*., 2010). Also the decreasing of testosterone hormone may be caused decrement of fructose in the epididymus section and decrease of viability (Hussein *et al*., 2015) in G4 group. The increase of sperm viability compared with G3 group may be due to the role of Phitofert® components which lead to increase vitamins and protect sperm membrane against reactive oxygen species and lipid peroxidation and the improve sperm viability (Yousef *et al*., 2003).

Sperms abnormal morphology

Furthermore the increase of abnormal sperm morphology combined with reactive oxygen species (ROS) production as indicator of damage to sperm DNA induced by vasectomy (Valko *et al.,* 2006) also increase of nitric oxide due to oxidative stress has a negative correlation with sperm morphology and DNA fragmentation (Huang *et al*., 2006). The finding of this study demonstrated a significant decrease in sperm morphology in G4 group compared with G2 group, this result may be due to the effect of Phitofert® as antioxidant and remove the excess reactive oxygen species (ROS) and prevent oxidative stress, thus Phitofert® contains antioxidant compounds which increase vitamins such as A, C led to remove the reactive nitrogen species (RNS) and sperm morphology return to normal (Dini *et al*., 1994).

On the other hand, the increase of gonadotropins and testosterone hormones in G2 group treated with Phitofert® improved male fertility and decrease abnormal sperm morphology (Muhammad *et al*., 2002). On the other hand, after vasectomy (Légaré *et al.,* 2004) found that sperm quality and changes in the function of the epididymal epithelium may occur with time after vasectomy, also the epididymal secretes proteins and other components that create the specific microenvironment required for maturation of sperm (Lavers *et al*., 2006) vasectomy effects may be causes alterations in morphology of epididymal sperms due to change in epididymus pH and impairment of acid-base balance (Caflisch and DuBose, 1990).

Testosterone concentration

Current study showed that semen testosterone concentration doesn´t affected by Phitofert® treatment and this result agreed with who failed to find any increase in serum testosterone levels which may suggested that either bio available testosterone or testosterone receptors binding might be augmented ,or Phitofert® may act without participation of androgen mechanism, this seems to be supported by the fact that the weight of seminal vesicle, target for androgen action was not influenced by Phitofert® in adult male mice (Gonzales *et al*., 2001). On other hand, the decrease of testosterone concentration may be due to inhibition of enzymatic pathways of its synthesis in the testes or the adrenal gland or may be interfering with LH release and all of these leading to decrease testicular testosterone synthesis and release (Pineda and Dooly, 2003). Furthermore, the free radicals may act directly on hypothalamic-pituitary-testicular axis, as a result serum testosterone level will decreased. In vasectomized mice (G4) the decrease of testosterone levels also may be attributed to decrease of gonadotropin hormones which are responsible for sertoli cells response to testosterone production (Hunter *et al*., 1982). Free radicals and oxidative stress in vasectomized mice may be another possible mechanism for decrease of serum testosterone concentration (Behrman and Aten, 1991).

Testicular Histolopathological changes

The histological section of testes of mice treated with Phitofert (G2 group) showed hypospermarogenesis and spermatogonia cells with significant increase of leydig cells, these histological changes may be due to the increase of testosterone and gonadotropin hormones, testosterone andgonadotropine hormones responsible for development and deformation of seminferous tubules and leydig cells, the increase of number of leydig cells (Table 4) caused increase in testosterone hormone and improvement of testes function and hyperspermatogensis.

On the other hand, the increase of gonadotropin levels and antioxidant components of Phitofert® like alkaloid and

flavanoids may be supported the function of testes (Sandoval *et al*., 2002) and activate pituitary to increase synthesis and release of gonadotropins. Histopathological section in testes of vasectomized mice (G3 group) showed hypospermato gensis with sever necrosis. These histopathological changes may be due to effect of vasectomy of mice which led to significant decrease in gonadotropin hormones as a result to oxidative stress so that the gonadotropin and testosterone inhibition caused significant decrease in spermatogensis, and passive effect on testicular somniferous tubules and leydig cells. Also the treatment of vasectomized mice with Phitofert® (G4 group) led to improvement of development of testicular tissues and increase of testosterone, LH and FSH hormones resulted to decrease the harmful effect of oxidative stress due to vasectomy operation (Al-Dujaily, 1996).

REFERANCES

Ahmed, M., Ahmed, R.N., Aladakatti, R.H. and Ghosesawar, M.G. (2002) Reversible anti- fertility effect of benzene extract of *Ocimum sanctum* leaves on sperm parameters and fructose content in rats. J. Basic Clin Physiol. Pharmacol., 13: 51-59.

Al-Dujaily, S.S. (1996) *In vitro* sperm activation and intra-bursalinsemination. Ph.D. Thesis. College of Veterinary Medicine- Baghdad-University.

Aman, R.P., Hammerstedt, R.H. and Veeramachaneni, D.N. (1993) The epididymis and sperm maturation: A perspective. Reprod. Fertil. Dev., 5(4):361-81.

Asadi, M.H., Zafari, F., Sarveazad, A., Abbasi, M., Safa, M., Koruji, M., Yari, A. and Miran, R. (2014) Saffron improves epididymal sperm parameters in rats exposed to cadmium. Nephro Urol MON., 6(1): e12021.

Bai, N., He, K., Roller, M., Lai, C-S, Bai, L. and Pan, M-H. (2015) Flavonolignans and other constituents from *Lepidium meyenii* with activities in anti-inflammation and human cancer cell lines. J Agric Food Chem., (63): 2458-2463.

Behrman, H.R. and Aten, R.F. (1991) Evidence that hydrogen peroxide blocks hormone-sensitive cholesterol transport into mitochondria of rat luteal cells. *Endocrinology*, **128**: 2958–2966.

Bogani, P., Simonini, F., Iriti, M. (2006) *Lepidium meyenii* (maca) does not exert direct androgenic activities. J. Ethnopharmacol., 104(3):415-417.

Caflisch, C.R. and DuBose, T.D.Jr. (1990) Effect of vasectomy on *insitu* pH in rat testis and epididymis. Contraception, 42(5): 589-95.

Chemineau, F., Cogine, Y., Guerin, Y., Orgeure, P. and Valtet, J.C. (1991) Training manual on artificial insemination in sheep andgoats.FAO, Animal Production Health: 83.

Chowdhury, M. and Steinberger, E. (1976) Differences of the effects of testosterone propionate on the production of LH and FSH. Acta Endocrinologica, 82:688-690.

Cicero, A.F., Bandieri, E. & Arletti, R. (2001) *Lepidium meyenii* Walpimproves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. J. Ethnopharmacol., 75(2-3):225-229.

Clément, C., Kneubühler, J., Urwyler, A., Witschi, U. and Kreuzer, M. (2010b) Effect of maca supplementation on bovine sperm quantity and quality followed over two spermatogenic cycles. Theriogenology, 74(2):173–183.

Cooper, T. Epididymis. In: Neill, J.D. and Knobil, E. (1998) (Eds.), Encyclopedia of Reproduction.Vol.2.New York, Academic Press: 1- 17.

Desai, N.R., Mahfouz, R., Sharma, R., Gupta, S. and A. agarwal, A. (2010) Reactive oxygen species levels are independent of sperm concentration, 4 motility, and abstinence in a normal, healthy, proven fertile man: a longitudinal study". Fertility and Sterility, 94(4): 1541–1543.

Dini, A., Migliuolo, G., Rastrelli, L. and Saturnino, P. (1994) Chemical composition of *Lepidium meyenii*. Food Chem. 49(4): 9-347.

Ganzera, M., Zhao, J., Muhammed, I. and Khan, I.A. (2002) Chemical profiling and standardization of Lepidiummeyenii (Maca) by reversed phase high performance liquid chromato-graphy. Chem. Pharm. Bull., 50(7): 988-91.

Geilazyn, M.L., Ringwood, A.H. and Piegorsch, W.W. (2002) Detection of oxidative DNA damage in isolated marine bivalve hemocytes using the comrt assay and for mamidopyrimidine glycosylase (FPG), Mutation research/ genetic toxicology and environmental mutagenesis, S C.Elsevier,Columbia.542:15-12.

Gonzales, G.F., Cordova, A., Gonzales, C., Chung, A., Vega, K. and Villena, A. (2001) *Lepidiummeyenii* (Maca) improved semen parameters in adult men. Asian Journal of Andrology, 3(4):301–303.

Gonzales, G.F., Gonzales-Castañeda, C. and Gasco, M. (2013) A mixture of extracts from Peruvian plants (black maca and yacon) improves sperm count and reduced glycemia in mice with streptozotocin-induced diabetes. Toxicology Mechanisms and Methods, 23(7):509–518.

Gonzales, G.F. (1988a) Functional structure and ultra-structure of seminal vesicles. Arch Androl., 22: 1-13.

Gonzales,G.F. (1988b). A test for bioandrogenicity in men attending an infertility service. Arch Androl, 21: 135-42.

Guyton, A.C. and Hall, J.E. (2016) Textbook of Medical Physiology. (13th.Edition). Philadelphia, PA: Elsevier.

He, Z., Feng, Y. and Xu, L.F. (2010) "In vitro antioxidant activity of ethanol extract of maca (*Lepidium meyenii* walpers) cultivated in Yunnan. Food Science, 31(15): 39–43.

Holdcraft, R. and Braun, R. (2004) Hormonal regulation of spermatogenesis. Int. J Androl., 27: 335-342.

Huang, I., Jones, J. and Khorram, O. (2006) Human seminal plasma nitric oxide: Correlation with sperm morphology and testosterone. Med. Sci. Monit., 12: 103-106.

Hunter, M.G., Sullivan, M.H.F., Dix, C.J., Aldred, L.F. and Cooke, B.A. (1982) Stimulation and inhibition by LHRH

analogues of cultured rat Leydig cell function and lack of effect on mouse Leydig cells. Molec.eel! Endocr., 27: 31-14.

Hussein, Y.M., Hussein, R.M., Amin, Al., Mohamed, A.S. and Hussein, H.S. (2015) Evaluation of Mesenchymal Stem Cells and Vitamin E in Treatment of Infertile Male Albino Rats. Int. J. Multi discplinary and Current research,3: 931-951.

Hussein, Z.F. (2013) Study the effect of *Eruca Sativa* leaves Extract on Male Fertility In Albino Mice.J. Al-Nahrain University, 16(1): 143-146.

Ikediobi, C.O., Badisa, V.L., Ayuk-Takem, L.T., Latinwo, L.M. and West, J. (2004) Response of antioxidant enzymes and redox metabolites to cadmium- induced oxidative stress in CRL-1439 normal rat liver cells. Int J. Mol. Med., 14(1): 87-92.

Karavolos, S., Stewart, J., Evbuomwan, I., McEleny, K. and Aird, I. (2013) Assessment of the infertile male. The Obstetrician & Gynaecologist, 15: 1-9.

Kini, R.D., Tripathi, Y., Raghuveer, C.V., Pai, S.R., Ramswamy, C., Nayanatara, A.K., Vinodhini, N. a. and Ranade, A. (2009) Protective Role of Vitamin E Against Cadmium Chloride Induced Testicular Damage in Rats.JPBS., 22(2): 12-16.

Klinefelter, G. and Hess, R.A. (1988) Toxicology of the male excurrent ductsand accessory sex glands. In: Korach, S. K. (Ed.). Reproductive and Developmental Toxicology. New York, Marcel Decker: 553-91.

Kupis, Ł., Dobroński, P.A. & Radziszewski, P. (2015) Varicocele as a source of male infertility – current treatment techniques. Cent European J. Urol., 68: 365-370.

Lavana, A., Vazquez, R., Palma-Irizarry, M. and Orihuela, A. (2013) Effect of supplementation with maca (*Lepidium meyenii*) in libido and semen characteristics in hair sheep rams (*Ovisaries*) *BoletínLatinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 12(3):238–242.

Lavers, A.E., Swanlund, D.J., Hunter, B.A., Tran, M.L., Pryor, J.L. and Roberts, K.P. (2006) Acute effect of vasectomy on the function of the rat epididymal epithelium and vas deferens. J. Androl., 27(6): 826-36.

Légaré, C., Verville, N. and Sullivan, R. (2004) Vasectomy influences expression of HE1 but not HE2 and HE5 genes in human epididymis. J. Androl., 25(1):30-43.

Luna, L.G. (1968) Manual of histology staining. Methods of armed forces. Institute of Pathology.3rd edition. McGraw-Hill Book Company, New York and London.

Muhammed, I., Zao, J., Dumbar, D.C. and Khan, I. A. (2002) Constituents of Lepidiummeyenii 'maca'. Phytochemistry, 59(1):105–110.

Ohta, Y., Kawate, N., Inaba, T., Morii, H. and Takahashi, K. (2017) Feeding hydroalcoholic extract powder of *Lepidium meyenii* (maca) enhances testicular gene expression of 3β-hydroxysteroid dehydrogenase in rats. Andrologia, 49(10): E 12792.

Partra, R.C., Rautray, A.K. and Swarup, D. (2011) The total antioxidant power of semen and its correlation with the fertility potential of human male subjects. J ClinDiagn Res., 7:991-995.

Pineda, M.H. & Dooley, M.P. (2003) McDonald's Veterinary Endocrinology and Reproduction .5thed.Iowa State Press. A Blackwell Publishing Company, 17-32,239-256.

Platel, K. and Srinivasan, K. (2000) Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats.Nahrung.44: 42-46.

Ponnusamy, M. & Pari, L. (2011) Protective role of dialytetrasulfide on cadmium induced testicular damage in adult rats: A biochemical and histological study. Toxicol Indus Health, 5: 407-416.

Ren, L., Weng, Q., Kishimoto, M., Watanabe, G., Jaroenporn, S. and Taya, K. (2011) Effect of short period vasectomy on FSH, LH, inhibin and testosterone secretions, and sperm motility in adult male rats. Exp. anim., 60(1):47-56.

Sandoval, M., Okuhama, N.N. and Angeles, F.M. (2002) Antioxidant activity of the cruciferous vegetable Maca (*Lepidium meyenii*). Food Chemistry, 79(2):207–213.

Sakamato, J. and Hashimoto, K. (1986) Reproductive toxicity of acrylamide and related compounds in mice. Effect on fertility and sperm morphology. Arch. Toxicol., 95: 201-205.

Salem, M.R. and Moustafa, N. (2001) Histological and quantitative study of the effect of *Eruca sativa* seed oil on the testis of albino rat. Egyptian J. Hosp. Med., 2: 148-162.

Schulte, R.T, Ohl, D.A, Sigman, M. and Smith, G.D. (2010) Sperm DNA damage in male infertility: etiologies, assays, and outcomes. J Assist Reprod Genet., 27:3–12.

SAS (2010) SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C.,USA.

Siegmund, O.H. (1979) Reproductive and urinary systems. In: The Merck Veterinary Manual. Siegmund, O.H. and Fraser, C.M. (eds), Published by Merck and Co. Inc. Rahway, N.J. USA: 794-892.

Simanainen, U., McNamara, K., Davey, R.A., Zajac, J.D. and Handelsman, D.J. (2008) severe subfertility in mice with androgen receptor inactivation in sex accessory organs but not in testis. Endocrino., 149: 3330-8.

Sinha-Hikim, I., Artaza, J., Woodhouse, L., Gonzales-Cadavid, N., Singh, AB. and Lee, MI. (2002) Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am. J. Physiol., 283: 154-164.

Soudamani, S., Malini, T. and Balasubramanian, K. (2005) Effects of streptozotocin-diabetes and insulin replacement on the epididymis of prepubertal rats: histological and histomorphometric studies. Endocr. Res., 31(2):81-98.

Tamburrino, L., Marchiani, S., Montoya, M., Elia, M.F., Natali, I. and Cambi, M.(2012) Mechanisms and clinical correlates of sperm DNA damage. Asian J. Androl., (14):24–31.

Tietz, N.E., ED: Chamical Guid to Laboratory Testes, 3rd ed. Philadelphia, WA Saunders Co.1995.

Valentová, K. and Ulrichová, J. (2003) Small anthus sonchifolius and Lepidium meyenii- prospective Andean crops for the prevention of chronic diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 147(2): 119-130.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M. (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact., 160 (1): 1–40.

Hilczer, J. (2013) The role of oxidative stress and antioxidants in male fertility. Cent European J Urol., 66: 60-67.

Yeni, D., Gundogan, M., Cigerci, I.H., Avdatek, F. and Fidan, AF. (2010) Seasonal variation of oxidative stress parameters in ram seminal plasma. J. Anim. Vet. adv., 9: 49-55.

Yousef, M.I., Abdallah, G.A. and Kamel, K.I. (2003) Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits, Animal Reprod science, 76: 99-111.

Zakaria, A and Al-Busadah, K. (2015) Pentoxifyllin efficiency in protecting testes against cadmium toxicity. J. Anim Vet Adv., 14(1), 18-29.

Zhao, J., Muhammad, I., Dunbar, D.Ch., Mustafa, J. and Khan, I. (2005) New Alkamides from Maca (*Lepidium meyenii*). Journal of the Agricultural and Food Chemistry, 53(3): 690−693.