Effect of Phitofert® on testicular function of vasectomized mature mice

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Abstract

This study was designed to evaluate the role of Phitofert® in the improvement of testes function and attenuating DNA fragmentation in vasectomized and healthy adult mice. Twenty four adult male breed such Albino mice were randomly and equally divided into four groups (G1, G2, G3 and G4). Mice were treated for 35days as follows: the G1 mice were non-vasectomized and given DW and served as controls, mice in G2 were non-vasectomized and given Phitofert® daily with a dose of 0.035mg/kg BW, mice in G3 were vasectomized without treatment while the mice in G4 were vasectomized and given same dose of Phitofert®. At the end of the experiment, fasting blood samples were collected by cardiac puncture for measuring LH and FSH hormones concentrations and section from testes were taken tomeasure the number of Leydig cells and diameter of semniferous tubules. The results of current data showed significant increase of serum LH and FSH concentrations in G2 healthy treated group as compared with control and other groups. Also, the treatment of G4 vasectomized mice with Phitofert® caused significant increase in serum concentration of the above hormones as compared with G3 vasectomized non-treated. The study showed that DNA fragmentation that resulted from the G2 healthy treated mice were lower than that obtained from the control group and other vasectomized mice (G3, G4). The diameters of the seminiferous tubules and the numbers of Leydig cells showed significant increase in healthy and vasectomized treated group (G1, G4) as compared with G3 vasectomized non-treated group. It was concluded that healthy and vasectomized treated of adult male mice with Phitofert® lead to clear improvement of level of reproductive hormones.

Keywords: Phitofert®, testicular function, mice.
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تأثير الفاتيوفرت على وظائف الخصى للفئران البالغة مستأصلة الأسهر

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الخلاصة

صممت الدراسة الحالية لتقييم دور مستخلص عشبة الفاتيوفرت لتحسين وظيفة الخصى وطبيعة الحامض النووي DNA (الدنا) في ذكور الفئران السليمة ومستأصلة الاسهر. تم استخدام أربعة وعشرون من ذكور الفئران البالغة وقسمت عشوائيا إلى أربعة مجاميع متساوية (مجموعة سيطرة G1, G2, G3, G4). تم استئصال الوعاء الناقل لاثني عشر فارا في المجموعتين الثالثة والرابعة. تم معاملة الحيوانات وكما يلي ولمدة 35 يوم، حيوانات المجموعة الأولى اعتبرت مجموعة سيطرة أما حيوانات المجموعة الثانية فقد أعطيت المستخلص فمويا وبجرعة يومية (0.035 ملغم/ كغم من وزن جسم) فيما اعتبرت حيوانات المجموعة الثالثة مجموعة المستاصلة الأسهر وغير معالجة بينما أعطيت حيوانات المجموعة الرابعة والمستأصلة الاسهر جرعة المستخلص نفسها أعلاه. في نهاية فترة التجربة (35 يوم) تم سحب عينات الدم من القلب لغرض حساب تركيز هرموني LH و FSH إضافة إلى

اختبار طبيعة الحامض النووي DNA، بعدها تم التضحية بكل الحيوانات وأخذت مقاطع نسيجية للخصى لحساب أعداد خلايا لايدج وأقطار النبيبات المنوية. أشارت النتائج إلى حصول زيادة معنوية في مستوى هرموني LH,FSH في مصل دم الحيوانات السليمة والمعاملة بالمستخلص (المعاملة الثانية) مقارنة مع مجموعة السيطرة وباقي المجاميع. أدت معاملة الفئران المستأصلة الاسهر بمادة الفاتيوفرت إلى حصول زيادة معنوية في تركيز هذه الهرمونات مقارنة مع الحيوانات مستاصلة الاسهر والغير معالجة. كما وأظهرت الدراسة بأن تشظي الحامض النووي في الفئران السليمة والمعاملة بمستخلص الفاتيوفرت كان منخفضا بالمقارنة مع مجموعة السيطرة والفئران المستأصلة الاسهر. أشارت الدراسة النسيجية إلى زيادة أقطار النبيبات المنوية للخصى وكذلك أعداد خلايا لايدج المجموعة الثانية والرابعة في الفئران السليمة والمستأصلة الأسهر المعاملة بالمستخلص مقارنة بالفئران المستأصلة الاسهر ولغير معاملة بالمستخلص المجموعة الثالثة.

الكلمات المفتاحية: الفاتيوفرت، وظائف الخصى، الفئران.

Introduction

Herbal medicine appears to be the oldest form of healthcare to humankind(1). Such medicine was considered to as herbalist or botanical use of herbs in therapy or medicinal use(2). The WHO gives a definition to herbal medicines as ended labeled medicinal products which have an effective ingredient, above or underground parts of the plant, other substances or its combinations. Report of WHO, showed that more than75% of the world population depend on traditional medicine for their major health care requirements(3). Phitofert® is one of food supplement contain Maca extract and considered as synthetic drug used for the adaptogenic and tonic action due to its content of components of Maca root extract. On the other hand Phitofert® used for man with selenium and for woman with folic acid and useful for improvement the function of reproductive system and fertility(4), but there is no researches about its role in animals, so that we designed the current study on mice as a model of mammals. Maca or (*Lepidiummeyenii*) isherbaceous part of the crucifer family native to the high Andes of Peru (5). It is cultured for its fleshy hypocotyl to be used as a root vegetable and a medicinal herb. Maca was cultivated for more than 1500 years ago(6). Maca used by national Andean people as an important food because of its high nutritional value in addition to its ability to fertility and sexual performance(7). Maca 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 (8, 6, 9). Some athletes have used *Lepidiummeynii* as a substitute to anabolic steroids even though its value is not proven(10). The ethnobotanical studies mentioned the use of maca against cancer, sexual and menstrual disorders(11). Other studies referred to its role in the secretion of hormones, immune stimulation and memory perfection(9). Vasectomy is a common method for fertility control in men that causes several implications as well. However, new surgical techniques could decrease these implications significantly(12). The epididymis has been shown to be responsible for the sustenance, conservation, transport, maturation, and storage of spermatozoa(13). Maturation of sperm within the epididymis needs the interaction of sperm with epithelium and luminal fluid of the epididymis(14). The objective of this study to show the effect of Phitofert® on testicular function in mice as a model of mammals.

Materials and Methods

- **Animals preparation and study design**: Twenty four healthy adult male mice, weighed 25-35g were used. Mice were held in plastic cages and placed 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 equal groups and treated as follows: Control group (G1): Healthy animals of this group received only distilled water. Healthy treated group (G2): Healthy animals of this group received oral administration (0.035 mg/ mice) of Phitofert®once daily. Vasectomized group (G3): Animals of this group received only distilled water. Treated vasectomized group (G4): Animals of this group received oral administration (0.035 mg/ mice) of Phitofert® once daily.
- **Blood collection:** Blood samples were obtained via cardiac puncture technique from each anesthetized animal using disposable insulin needles for hormonal estimation.
- **Hormonal estimation**

A- Serum luteinizing hormone concentration: The LH hormone was determined using kits provide by MonobindInc. USA according to Microplate Enzyme immunoassay (ELISA)(15).

B- Serum Follicle stimulating hormone concentration: The FSH hormone was determined using kits provide by MonobindInc. USA according to Microplate Enzyme immunoassay (ELISA)(16).

- **Acridin Orange test for determination of DNA fragmentation:** The acridine orange fluorescence test was performed according to the method of (17).
- **Histological study of testes:** Histological section was done according to (18). The tissue sections were examined by light microscopy and microphotography with histometrical measurements have been done by using Future Win Joe microscopic camera to measure the diameter of somniferous tubules and the numbers of leydig cells, the images have been analyzed and scored by using Fiji image analyzer system(19).
- **Statistical analysis:** Statistical analysis of data was performed using SAS (Statistical Analysis System-version 9.1). One-way, two way 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 (20).

Results and Discussion

- **Concentration of LH hormone (ng/ml)**: The concentration of LH hormone micewere listed in Table -1 The measurement showed that healthy animals treated with Phitofert® reveled significant ($P<0.05$) increase in serum LH concentration compared to control and other groups (G1), whereas vasectomized mice showed significant ($P<0.05$) decrease in LH hormone concentration compared to vasectomized treated group (G4) and other groups (G1, G2).
- **Concentration of FSH hormone (ng/ml):** Treatment of mice with Phitofert® (G2) showed highly significant ($P<0.05$) increase in FSH hormone concentration compared to healthynon treated mice (control control) and other vasectomized animals. On other hand vasectomized mice treated with Phitofert® showed significant ($P<0.05$) increase of LH hormone concentration compared to vasectomized group (G3) and significant ($P<0.05$) decrease of FSH hormone concentration compared to both healthy mice (G1 and G2) as shown in Table -1.

Table(1) Effect of Phitofrert® and vasectomy on serum testosterone, LH and FSH hormones concentration in mature male mice

Hormone concentration / Groups	LH (m IU/ml)	FSH (m IU/ml)
G1	2.29± 0.06 b	2.93± 0.07 b
G2	3.48± 0.11 a	5.35± 0.22 a
G3	0.55± 0.07 d	0.54±0.06 d
G4	1.30±0.16 c	1.06±0.12 c
L.S.D.	0.33	0.40

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

- **DNA normality (%):** The result of DNA fragmentation % was illustrated in Table - 2 which indicated that healthy mice treated with Phitofert® showed significant (P<0.05) decrease in DNA fragmentation % compared to vasectomized mice (G3 and G4 groups). On the other hand, DNA fragmentation of vasectomized mice (%) were significant (P<0.05) increase as compared with healthy animals, while DNA Fragmentation (%) in vasectomized treated mice (G4 group) showed significant (P<0.05) decrease compared to vasectomized non treated mice (G3 group) and significant (P<0.05) increase compared to healthy mice (G1, G2 groups) (Table-2).

Table (2) Effect of Phitofert® and vasectomyon DNA normality(%) in mature male mice

Groups	G1	G2	G3	G4
DNA Fragmentation	6.50±0.88 c	7.33± 1.35 c	25.50± 1.76 a	11.33± 1.08 b

L.S.D.=3.88 Values are presented as Means ±SE (n=6 mice/ group).
Different capital letters denote significant differences between groups (P<0.05).

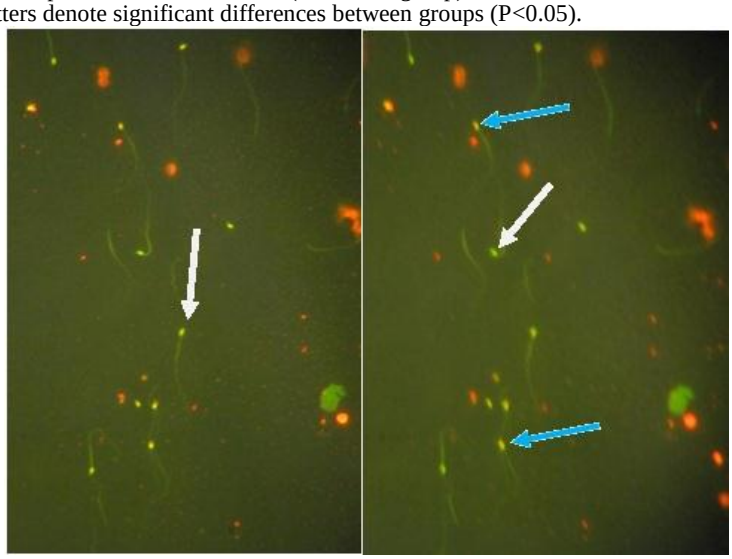

Fig. (1) Normal sperm with green head (white arrow) and sperm with DNA fragmentation with green and yellow head (blue arrow) in G1 group (control group) (Acridin Orange test). X10

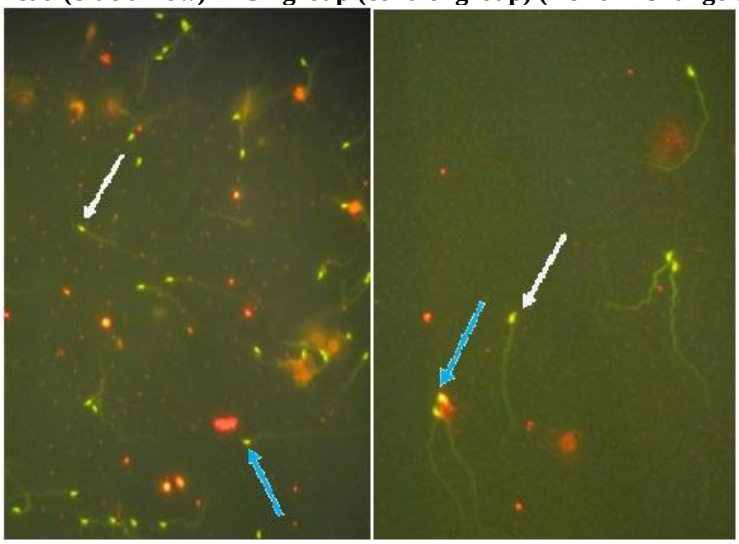

Fig. (2) Normal sperm with green head (white arrow) and sperm with DNA fragmentation with yellow or orange head (blue arrow) in G2 group (healthy treated with Phitofert® group) (Acridin Orange test). X 10

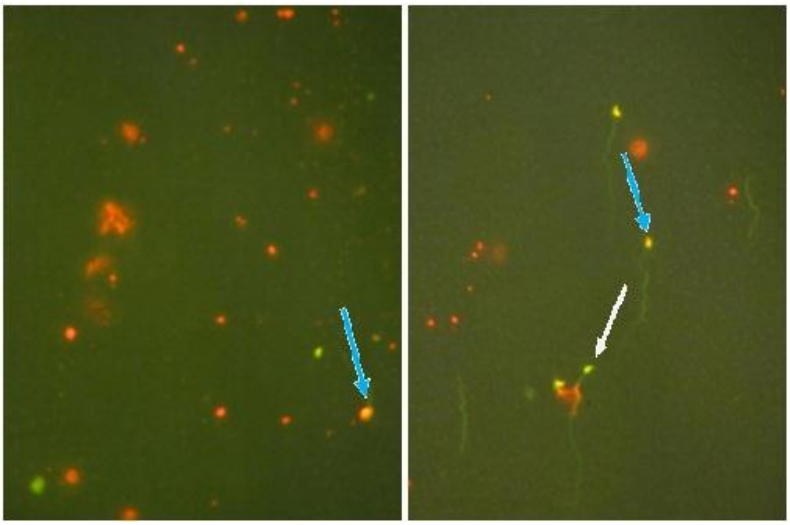

Fig. (3) Normal sperm (white arrow) and sperm with DNA fragmentation (blue arrow) in G3 group (vasectomized without Phitofert® treatment group) (Acridin Orange test). X 10

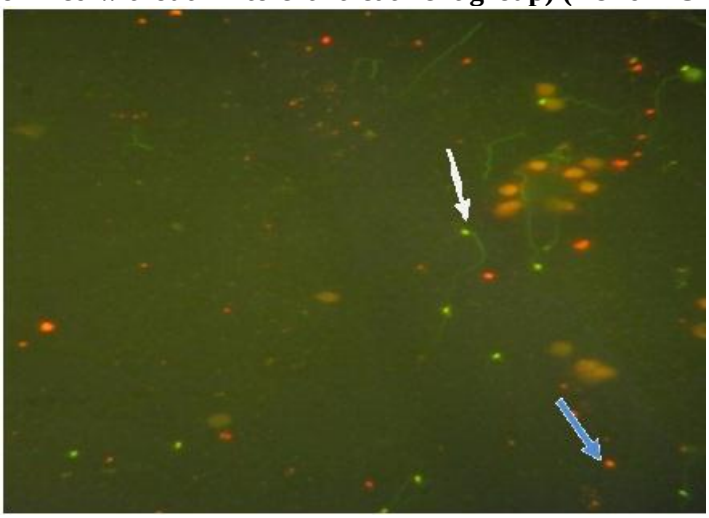

Fig. (4) Normal sperm with green head (white arrow) and sperm with DNA fragmentation with yellow and orange head (blue arrow) in G4 group (vasectomized with Phitofert® treatment group) (Acridin Orange test). X 10

- **Diameter of semniferous tubules:** Table 3- showed that diameters of semniferous tubules in vasectomized mice (G3) was significantly (P<0.05) decrease compared to control and all groups, while the data revealed significant (P<0.05) increase of diameters in mice treated with Phitofert® compared with either in healthy or vasectomized groups (G2 and G4) compared to G1 and G3.

Table (3) Effect of Phitofer® and vasectomy on diameter of semniferous tubule in mature male mice

Groups	G1	G2	G3	G4
Diameter (µm) of somniferous tubules	294.70± 16.88 c	466.97±22.10 a	238.64±26.66 d	356.04±16.12 b

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

- **Numbers of Leydig cells (cell/ µm):** The data illustrated in Table 4 revealed significant (P<0.05) increase in the numbers of Ledyig cells in G2 group which was treated with Phitofert® compared to control and vasectomized mice (G3). The numbers significantly (P<0.05) decreased invasectomized mice compared to vasectomized treated group (G4). The higher number of Leydig cells recorded in G2 while the lower number was observed in G3 compared with other groups.

Table (4) Effect of Phitofert® and vasectomy on numbers of Leydig cellsin mature male mice

Groups	G1	G2	G3	G4
Number (cell/ µm) of Leydig cells	2.30±0.16 b	4.01±0.21 a	1.12±1.02 c	2.00±0.84 b

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

- **LH and FSH concentration:** Table-1 showed the effect of Phitofert® on gonadotropins hormones (LH and FSH). Treatment of mice with Phitofert® causes significant increase in serum gonadotropins levels. Although the treatment contain no hormones have theorized that plants alkaloids may be responsible for balancing the hypothalamus and pituitary glands and supporting function of testes (21) and possible manner to in a stimulate pituitary gland to increase synthesis and release of LH and FSH hormones. On other hand, Phitofert® may achieve such result by improving the response of sertoli cells to FSH and resulted in decrease of inhibin hormones synthesis and subsequently lead to increase of FSH hormone. Also, the antioxidant effect of the components of Phitofert® may be the causes of increscent of gonadotropin especially Flavonoids and alkaloids which act as scavenger to ROS (22) and decrement of LH and FSH in vasectomized mice support this mechanism of extract, thus vasectomy lead to decrease or LH and FSH due to effect of free radicals resulted from oxidative stress in this group of animals. The result on current study indicated significant increase in gonadotropins hormones (LH and FSH) in treated mice. This increment of LH and FSH concentration may be due to effect of Phitofert® to support stimulation of pituitary gland to increase synthesis and release of LH hormone and /or hypothalamus leading to increase the synthesis and release of gonadotropin hormones.
- **DNA normality:** Table-2 showed that treatment of mice with Phitofert® revealed decrease abnormality and fragmentation of DNA. This may be due to productive effect on sperm membranes(23). The Phitofert® has been shown to scavenge hydroxyl group as well as superoxide radicals and inhibit their release(24)**.** Also, the decrease of DNAabnormality in treated groups of mice compared to non-treated mice may be attributed to the quality of CHO in extract which is a great energy source for the sperm active motility with an increase in the percentage of morphological normal sperm and these will effect on number of sperm with normal DNA. On other hand, vasectomy lead to increase DNA fragmentation in G4 and this result may be due to the effect of free radical on sperm genome, causing high frequencies of single and double-strand DNA breaks(25), thus both superoxide (O^{-2}) and the hydroxyl radical (OH^{-}) are known to mutagenic and cause chromosome deletions, dicentrics and sister chromatid exchange(26). Also, vasectomy generally causes the formation of sperm antibodies, a response that is believed to be due to sperm retention and an increased intraluminal pressure in reproductive tract, leading to increased sperm degradation, fragmentation and sperm granuloma formation (27, 28).
- **Diameter of seminiferous tubules:** There is a positive relationship between the tubular diameter and spermatogenic activity of the testis (29). The oxidative stress may be cause damage at the testicular tissues in G3 and reduce the diameter of seminferous tubules. Therefore, the decrease in diameters could reflect defective spermatogenesis as evidenced by reduction in sperm parameters. On the other hand, Phitofert® caused significant increase in tubules diameters may be due to its antioxidant components as scavenger of ROS (22).

- **Numbers of Leydig cells:** The increase of the number of Leydig cells in G2 andG4 may be due to compounds of Phitofert® including Flavonoids and alkaloids which considered as a potent antioxidant activity(30) against oxidative damage induced by vasectomy and improving the pituitary–testes axis function through androgenic activity leading to increase the number of Leydig cells, or could be due to the free radical scavenging effect. Also, the increase of Leydig cells numbers may be due to an increasing the number and/ or the sensitivity of LH receptors on the Leydig cells which led to increase testosterone biosynthesis(31).

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