

Effect of aqueous and ethanolic extracts of *Tribulus terrestris*, *Phoenix dactylifera* and *Nasturtium officinale* on the reproductive organs in male mice

Amal G.Mattar* and Mohaisen H.Adaay**

*MSc Medical city, Ministry of Health, Baghdad, Iraq.

**PhD Institute of Embryo Researches and Infertility treatment, University of Al-Nahrain Baghdad, Iraq. Corresponding author e-mail: dr_mohsin2004@yahoo.com.

Abstract:

Background

A mixture of three plants was used in this study:

Tribulus terrestris (TT), *Phoenix dactylifera* (Pd) and *Nasturtium officinale* (No) which were believed to have fertility enhancing effects.

Objectives

To study the effects of the aqueous and ethanolic extracts of the mixture of the three plants on body weight and some reproductive organs parameters in male mice

Material and Methods

Extracts of the mixture of the three plants were injected intraperitoneally at doses of 75, 150 and 300 mg/kg/day to male mice for a period of one and two weeks. Body weight and reproductive organs weight were recorded. The height and diameter of the epithelial lining cells of the epididymis and seminiferous tubules were evaluated by the end of the two periods through histological preparations.

Result:

Both extracts showed a significant increase in body weight and reproductive organs weight as well as the diameter and height of the epithelial lining cells of the seminiferous tubules and epididymis.

Conclusion:

A dose dependent pattern of effects was noticed in both extracts with more obvious effects resulted by the ethanolic extract.

Key Words: *Tribulus terrestris*, *Phoenix dactylifera*, *Nasturtium officinale*, male reproductive organs.

Introduction

At present, it is estimated that about 80% of the world population relies on botanical preparation as medicines to meet their health needs (1). *Tribulus terrestris* (TT) is an herb used for its medicinal effects around the world. It has been used for centuries by the Chinese to calm the liver, treat headaches, dizziness, and

premature ejaculation. It also has a beneficial effect on the prostate gland (2). It is believed that in ancient Greece and India, TT was used as a rejuvenation tonic (3). In China, TT is used in a number of conditions affecting the liver and kidney as well as the cardiovascular and immune system (4, 5). *Tribulus terrestris* is now

sold as a standardized pharmaceutical preparation for muscle strength and sexual potency throughout Europe, the Middle East and Asia (6).

This plant is popularly claimed to improve sexual function in humans (7). In Europe, IT has been used in folk medicine throughout history for treating a wide range of conditions such as headache, nervous disorders, constipation and sexual dysfunction (4). In Turkey, IT is commonly used in folk medicine for the treatment of abdominal colic, hypertension and high cholesterol (8).

For centuries, *Phoenix dactylifera* (Pd) has been used in the Middle East as a staple food. About 800 uses were recorded for the date palm. The pollen grains of date palm have been used by Egyptians to improve fertility in women and date pits have been included in animal food to enhance growth (9). Date pits have been included in animal food to enhance growth (9). There are at least 15 minerals in dates; the percentage of each mineral in dried dates varies from 0.1 to 916 mg/100g date depending on the type of mineral. In many varieties, potassium can be found at a concentration as high as 0.9% in the flesh while it is as high as 0.5% in some seeds. Additionally, the seeds contain aluminum, cadmium, chloride, lead and sulphur in various proportions (7).

Nasturtium officinale (No) called "wit- producing food" by the ancient Greeks. Watercress contains calcium, chlorine, cobalt, copper, tannin, fluorine, iodine, iron, manganese, phosphorus, sulfur, vanadium, vitamins A, B1, B2, C, D, and zinc (10). Some of its benefited effects may be due to a general stimulation of metabolism and the nervous system, including autonomous regulation (11). In German pediatric medicine, watercress is used as disinfectant drug for its antibacterial action in the treatment of the lower urinary tract infections (12). The Complete German Commission E Monographs approved watercress for

catarrh of the respiratory tract. In Germany, it is also used to treat urinary tract infections in children. The powdered leaves are used in India as an expectorant to treat bronchitis and a number of conditions affecting human liver. The fresh herb is used in naturopathy as a blood purifier (13).

Since the powder of the mixture of the three plants is used by herbalist in Baghdad to treat sexual impotence in men, the present study was conducted to evaluate its effect on reproductive organs in Albino male mice.

Materials & Methods

Animals:

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines. Two hundred mature Swiss albino male mice, nine weeks old with an average body weight of 27.67 ± 0.52 grams were used in this study. The animals were kept under standard conditions of temperature (25°C) and lighting period (14hrs light/10hrs dark). Food and water were supplied ad libitum. Animals were treated with 75, 150 and 300mg/kg/day intraperitoneally for a period of one and two weeks

Plant materials:

The plants which were collected from Baghdad & identified by the Iraqi National Herbarium staff, consist of a mixture of TT (aerial parts, 40%), Pd (pollen grains, 30%) and No (seeds, 30%). Preparation of the aqueous and ethanolic crude extracts of the plants materials was conducted according to Mattar, 2005 (14).

Treatment:

Intraperitoneal injection (ip) of aqueous or alcoholic extracts in male mice:

Four groups of 25 animals were used for each treatment and given the extracts for two weeks. G1 is the control group given normal saline (with respect to aqueous extract) or olive oil (with respect to ethanolic extract) injection (0.5) ml daily. G2, G3 and G4 given 75, 150 and 300mg/kg/day of aqueous or alcoholic extract respectively. The body weight was recorded once a week. Five animals from each group were killed at the end of the 1st and 2nd week to get the weight of the reproductive organs (testes, epididymis and seminal vesicle), the diameter and height of epithelial lining cells of the seminiferous tubules and epididymis were also recorded.

Preparation of the histological specimens:

Semi-thin sections (1micron thick) were obtained for the seminiferous tubules and epididymis using glass knives on a rotary microtome (Reichert-Jung 2030, Germany), serial sections were prepared and stained with hematoxylin and eosin stain (BDH chemicals Ltd, England and Panreac Quimica SA barcelone, Espana) and were examined microscopically. Various tissue measurements were done using eyepiece micrometer, ocular lens and stage micrometer (15, 16). The following parameters were evaluated:

- 1- Diameter of epithelial lining cells of the seminiferous tubules and epididymis.
- 2- Height of epithelial lining cells of seminiferous tubules and epididymis.

Statistical analysis:

Computerized statistical analysis was performed using the SPSS (statistical package of Social Sciences) version 10 under windows XP-2000(Inc, Chicago, IL, USA) computer software and the use of

excel program. Values reported are means $\pm$ SE. Experimental results were statistically analyzed using the F-test, with P values less than 0.05 considered significant, less than 0.01 considered highly significant (17).

Result:

Effect of ip injection of the aqueous extract in male mice:-

Effect on body weight and reproductive organs weight:

After the 1st week of treatment, at a dose of 75 mg/kg/day (G2), no significant differences in body weight and epididymis weight was recognized whereas a significant ($p < 0.05$) increase in the testes weight was obtained after the 1st and 2nd week and a significant ($p < 0.05$) increase in epididymis and seminal vesicle weight after the 2nd week in comparison with the control group G1. G3 and G4 showed a highly significant increase ($p < 0.01$) in body weight and all reproductive organs weight after the 1st and 2nd week in comparison with the control group G1. Treatment of animals with 150 and 300 mg/kg/day aqueous extract (G3 and G4) showed a highly significant ($p < 0.01$) increase in body weight and all reproductive organs weight after the 1st and 2nd week in comparison with the group treated with 75 mg/kg/day (G2).

Comparison of G4 with G3 revealed a significant ($p < 0.05$) increase in body, epididymis and seminal vesicle weights after the 1st week of treatment with a significant ($p < 0.05$) increase in body and epididymis weight and a highly significant ($p < 0.01$) increase in testes and seminal vesicle weight after the 2nd week of treatment (table 1).

Effect on diameter and height of epithelial lining cells of seminiferous tubules and epididymis:

After the 1st and 2nd week of treatment G2, G3 and G4 showed a highly significant ($p < 0.01$) increase in the diameter of

seminiferous tubules and epididymis compared to G1. However, with respect to the height of seminiferous tubules and epididymis, G2 showed a significant ($p<0.05$) increase in seminiferous tubules after the 1st and 2nd week, a significant ($p<0.05$) and a highly significant ($p<0.01$) increase in epididymis after the 1st and 2nd week respectively. G3 showed a significant ($p<0.05$) increase in the seminiferous tubules after the 1st and 2nd week and a highly significant ($p<0.01$) increase in the height of the epididymis in comparison with G1. G4 showed a highly significant ($p<0.01$) increase in the height of both organs after the two periods of the experiment in comparison with G1. G3 exhibited a significant ($p<0.05$) increase in height of seminiferous tubules and epididymis and a highly significant

($p<0.01$) increase in diameter of the two organs after the 1st week in comparison with G2. After the 2nd week of the experiment, G3 showed, a significant ($p<0.05$) increase in diameter of seminiferous tubules and epididymis and height of epididymis compared to G2. Comparison of G4 with G2 revealed a significant ($p<0.05$) increase in the height of epididymis after the 1st week and a highly significant ($p<0.01$) increase in all other measurements of the two organs after the 1st and 2nd week of treatment. On the other hand, G4 showed a significant ($p<0.05$) increase in height of seminiferous tubules and diameter of epididymes after the 1st week and height of both organs after the 2nd week of the

Table (1) The effects of ip injection of the aqueous extract on body weight (gm), testes, epididymis and seminal vesicles weight (mg/100 gm body weight).

week Group (Dose)	After 1 week				After 2 weeks			
	Body wt	Testes wt	epididymis wt	seminal vesicle wt	Body wt	Testes wt	epididymis wt	Seminal vesicle wt
G1 (control)	25.73± 0.32	1115.1± 0.13	80.64± 0.11	339.24± 0.52	25.92± 0.17	1116.5± 0.21	80.8± 0.25	341.86± 0.28
G2 (75mg/kg)	25.85± 0.45	1126.1± 1.20 ^{a*}	80.86± 0.32	341.3± 0.83	26.14± 0.06	1128.9± 1.01 ^{a*}	82.34± 0.21 ^{a*}	346.24± 0.38 ^{a*}
G3 (150mg/kg)	b**,d** 28.41± 0.25	b**,d** 1238.8± 0.83	b**,d** 85.88± 0.34	b**,d** 364.64± 0.55	b**,d** 30.67± 0.35	b**,d** 1261.3± 1.02	b**,d** 90.18± 0.08	b**,d** 369.18± 0.40
G4 (300mg/kg)	c**,e**,f* 29.90± 0.23	c**,e** 1289.2± 0.91	c**,e**,f* 89.08± 0.34	c**,e**,f* 377.1± 1.02	c**,e**,f* 32.43± 0.36	c**,e**,f** 1320.8± 0.40	c**,e**,f* 92.06± 0.52	c**,e**,f** 386.12± 1.11

Values are mean ±SE. n= 25 male/ group.

* $P<0.05$. ** $P<0.01$. a-G1 vs G2 .

b-G1 vs G3. c-G1 vs G4. d-G2 vs G3. e-G2 vs G4 . f-G3 vs G4

Table (2) The effect of ip injection of the aqueous extract on diameter and height of epithelial lining cells of seminiferous tubules and epididymis (μm).

Weeks Group (dose)	After 1week				After 2weeks			
	Seminiferous tubules		epididymis		Seminiferous tubules		Epididymis	
	Diameter	Height	Diameter	Height	Diameter	Height	Diameter	Height
G1 (control)	181.0 $\pm$ 0.28	90.1 $\pm$ 0.37	150.0 $\pm$ 0.38	25.04 $\pm$ 0.57	184.0 $\pm$ 0.23	92.0 $\pm$ 0.06	153.0 $\pm$ 0.25	25.2 $\pm$ 0.21
G2 (75mg/kg)	a** 185.2 $\pm$ 0.43	a* 91.4 $\pm$ 0.25	a** 169.8 $\pm$ 0.33	a* 26.12 $\pm$ 0.42	a** 193.3 $\pm$ 0.22	a* 93.4 $\pm$ 0.41	a** 190.0 $\pm$ 0.16	a** 27.9 $\pm$ 0.10
G3 150mg/kg	b**,d** 191.6 $\pm$ 0.28	b*,d* 93.2 $\pm$ 0.37	b**,d** 189.2 $\pm$ 1.02	b**,d* 28.4 $\pm$ 0.21	b**,d* 199.0 $\pm$ 0.05	b* 93.8 $\pm$ 0.26	b**,d* 200.6 $\pm$ 0.31	b**,d* 29.8 $\pm$ 0.22
G4 (300mg/kg)	c**,e**,f* 196.0 $\pm$ 0.09	c**,e**,f* 96.1 $\pm$ 0.84	c**,e**,f* 196.0 $\pm$ 0.35	C**,e* 29.14 $\pm$ 0.25	c**,e**,f** 216.1 $\pm$ 0.15	c**,e**,f* 97.8 $\pm$ 0.23	c**,e**,f** 215.8 $\pm$ 0.25	c**,e**,f* 31.7 $\pm$ 0.22

Values are mean $\pm$ SE. n=25 male/group.

*P<0.05. **P<0.01. a-G1 vs G2 . b-G1 vs G3.

c-G1 vs G4. d-G2 vs G3. e-G2 vs G4 . f-G3 vs G4

experiment compared to G3. A highly significant ($p<0.01$) increase was observed in G4 with respect to diameter of seminiferous tubules after the 1st week and diameter of both organs after the 2nd week in comparison with G3 (table 2).

Effect of ip injection of the alcoholic extract on male mice:

Effect on body weight and reproductive organs weight:

As concerning the body weight, G2 showed no significant difference during the 1st week but a significant ($p<0.05$) increase was obtained after the 2nd week, a highly significant ($p<0.01$) increase was found in the testes and seminal vesicles weight after the 1st and 2nd week of treatment and a significant ($p<0.05$) increase in epididymis weight after the 1st and 2nd week in

comparison with G1. G3 and G4 showed a highly significant ($p<0.01$) increase in all parameters after the two periods of the experiment in comparison with G1

Treatment of animals with 150 mg/kg/day ethanolic extract (G3) showed a significant ($p<0.05$) increase in body and epididymis weights after the 1st week of treatment compared to G2, whereas a highly significant ($p<0.01$) increase in testes and seminal vesicle weights was detected after the two periods of the experiment in the same group (G3) compared to G2.

G4 revealed a significant ($p<0.05$) increase in body weight after the 1st week and epididymis weight after the 2nd week in comparison with G2, whereas a highly significant ($p<0.01$) increase was found in the same group (G4) with respect to all other parameters after the 1st and 2nd weeks compared to G2. Comparison of G4 and G3 showed a significant ($p<0.05$) increase in G4 with respect to testes and seminal vesicles weight after the 1st week and epididymis

weight after the 2nd week compared to G3. A highly significant ($p<0.01$) increase was noticed in epididymis weight after the 1st week and body, testes and epididymis weights after the 2nd week of treatment in G4 compared to G3 (table 3).

Effect on diameter and height of epithelial lining cells of seminiferous tubules and epididymis:

After the 1st and 2nd week of treatment, a highly significant ($p<0.01$) increase in the diameter of seminiferous tubules and epididymis were obtained in all groups except the diameter of the epididymis in G2 after the 2nd week in which the difference was only significant ($p<0.05$) compared to the controls. A significant ($p<0.05$) increase in height of seminiferous tubules and epididymis were found after the 1st and 2nd week of treatment in G2 in comparison with G1. G3 and G4 revealed a highly significant ($p<0.01$) increase in the height of the seminiferous

tubules and epididymis after the 1st and 2nd week of treatment compared to G1. G3 and G4 showed a highly significant ($p<0.01$) increase in diameter and height of seminiferous tubules and diameter of epididymis after the 1st week compared to G2. After the same period, the epididymis height in G3 was significantly ($p<0.05$) higher compared to G2 and in G4 it was highly significant ($p<0.01$) compared to G2. After the 2nd week, a significant ($p<0.05$) increase was recognized in the height of seminiferous tubules and epididymis and a highly significant ($p<0.01$) increase in diameter of seminiferous tubules and epididymis in G3 compared to G2.

G4 showed a highly significant ($p<0.01$) increase in diameter and height of the two organs after the 1st and 2nd week compared to G2. A significant ($p<0.05$) increase in diameter of seminiferous tubules was found in G4 compared to G3 after the 1st week and a highly significant ($p<0.01$) increase was obtained in all other measurements of the two organs in G4 compared to G3 after the two periods of the experiment (table 4).

Table (3) The effects of ip injection of ethanolic extract on body weight (gm), testes, epididymis & seminal vesicles weight (mg/100 gm body weight)

week Group (Dose)	After 1 week				After 2 weeks			
	Body wt	Testes WT	epididymis wt	seminal vesicle wt	Body wt	Testes wt	epididymis wt	Seminal vesicle wt
G1 (control)	25.71± 0.09	1115.6± 0.16	81.3± 0.18	340.6± 0.17	25.9± 0.08	1115.7± 0.11	81.5± 0.12	340.7± 0.12
G2 (75mg/kg)	26.5± 0.20	A** 1210.6± 0.29	a* 84.6± 0.16	a** 352.64± 0.15	a* 28.8± 0.24	a** 1251.8± 0.08	A* 85.8± 0.04	A** 358.7± 0.18
G3 (150mg/kg)	B**,d* 29.1± 0.26	B**,d** 1290.2± 0.98	B**,d* 89.4± 0.20	B**,d** 380.7± 0.10	B**,d* 32.4± 0.21	b**,d** 1311.5± 0.19	B**,d* 92.2± 0.22	b**,d** 390± 1.38
G4 (300mg/kg)	C**,e* 30.2± 0.18	c**,e**,f* 1310.5± 0.44	c**,e**,f** 97.1± 0.12	c**,e**,f* 387.6± 0.19	C**,e** 33.4± 0.22	c**,e**,f** 1385.6± 0.15	c**,e*,f* 99.26± 0.13	C**,e** 394.7± 0.75

Values are mean ±SE. n= 25 male/ group. *P<0.05. **P<0.01.

a-G1 vs G2. b-G1 vs G3. c-G1 vs G4. d-G2 vs G3. e-G2 vs G4. f-G3 vs G4

Table (4) The effect of ip injection of the ethanolic extract on diameter and height of epithelial lining cell of the seminiferous tubules and epididymis(μm).

Weeks Group (dose)	After 1week				After 2weeks			
	Seminiferous tubules		epididymis		Seminiferous tubules		Epididymis	
	Diameter	Height	Diameter	Height	Diameter	Height	Diameter	Height
G1 (control)	180 $\pm$ 0.29	79.4 $\pm$ 0.45	148 $\pm$ 0.29	26.4 $\pm$ 0.46	185 $\pm$ 1.48	81 $\pm$ 0.05	166 $\pm$ 1.12	27.4 $\pm$ 0.28
G2 (75mg/kg)	a** 188.8 $\pm$ 0.11	a* 82.4 $\pm$ 0.28	a** 172.2 $\pm$ 0.26	a* 28.4 $\pm$ 0.88	a* 194.7 $\pm$ 0.34	a* 87.2 $\pm$ 1.24	a** 197.4 $\pm$ 0.28	a* 30.3 $\pm$ 0.42
G3 150mg/kg	b**,d** 195.6 $\pm$ 0.25	b**,d** 88.4 $\pm$ 0.29	b**,d** 202.2 $\pm$ 1.42	b**,d* 31.6 $\pm$ 0.28	b**,d** 209.8 $\pm$ 0.43	b**,d* 92.2 $\pm$ 0.52	b**,d** 228.2 $\pm$ 0.43	b**,d* 34.6 $\pm$ 0.68
G4 (300mg/kg)	c**,e**,f* 198.6 $\pm$ 0.27	c**,e**,f** 99.6 $\pm$ 0.45	c**,e**,f** 218.6 $\pm$ 0.29	c**,e**,f** 38.6 $\pm$ 088	c**,e**,f** 221.2 $\pm$ 0.56	c**,e**,f** 107 $\pm$ 0.77	c**,e**,f** 243.6 $\pm$ 0.45	c**,e**,f** 40.8 $\pm$ 0.45

Values are mean $\pm$ SE. n = 25 male/ group. *P<0.05. **P<0.01.

a-G1 vs G2 . b-G1 vs G3. C-G1 vs G4. d-G2 vs G3. e-G2 vs G4 . f-G3 vs G4

Discussion:

A significant increase in body weight of male mice occurred in all groups treated with the plants mixture which may be due to the regulation of fat and carbohydrate metabolism and improvement of appetite, digestion and assimilation (18).

It seems to be a good appetizer and digestion promoter, the relatively high carbohydrate content of this mixture (9) together with proteins may have created along with androgenic activity of the substance (19) favorable conditions for stimulating many male reproductive organs of treated mice. It is well known that amino acids are among the stimulator of growth hormone(GH) secretion from the anterior pituitary gland and also encourage the secretion of insulin like growth factor 1 and II (20). Receptors for growth hormone in the liver are known to increase by glucose (21). Presence of factors IGF-I and II in the plants mixture may reflect an increase not

only in growth hormone secretion but also better stimulation of metabolic activity of the liver (protein and carbohydrate synthesis). These stimulatory effects are potentiated by increased secretion of IGF1 and II. This important metabolic axis i.e. GH-IGFs plays an important role in increasing protein synthesis and decrease protein catabolism, the reproductive effect of these changes is stimulation of spermatogenesis and DNA synthesis, since testis has been shown to contain IGF1 and GH receptors (22).

Concerning the mechanisms through which the significant and highly significant increases in reproductive organs weight of the treated mice (testes, epididymis and seminal vesicles) may be attributed to many factors. One of these probable factors is the well known positive relationship between the increase in testicular weight and the increase in body

weight (23) and since body weight has increased in these animals, it's not surprising that this testicular weight is also increased. Moreover, since the increase in body weight discussed above has been explained on the ground that administered substance because of its high amino acids content may stimulate growth hormone secretion and encourage IGFI and II, these combined hormonal changes has been shown to stimulate Sertoli cells function and finally increase testosterone production and thus stimulation of spermatogenesis (22). Results of the present investigation have indicated not only significant and highly significant increase in testicular, epididymal and seminal vesicle weight of all treated animals, but also an increase in seminiferous tubule and epididymal tissue diameter and height of lining cells associated with presence of more sperms in their lumen. All these changes are indicator of testicular and epididymal tissue growth (24), which may be induced by increased testosterone production and this in turn has brought about stimulation of all male reproductive organs (testes, epididymis and seminal vesicle).

In conclusion, the study demonstrated that the extracts resulted in increased muscle mass, stimulating all male reproductive organs studied, a dose dependent pattern of effect, no adverse effect were noted in any of the studied groups, no evidence of toxicity has been found during the experimental study (on the studied organs) with the ethanolic extract seems to be more effective in most parameters studied.

References:

- 1- Kalpagam P and Nirmala K. Ginger. Its role in xenobiotic metabolism. ICMR Bulletin. 2003; 33(6):57-62.
- 2- Joshi VS, Parekh BB, Joshi MJ and Vaidya ADB. Inhibition of the growth of urinary calcium hydrogen phosphate dehydrates crystals with aqueous extracts of *Tribulus terrestris* and *Bergenia liliolata*. Urol. Res. 2005; 33(2): 80 - 86.
- 3- Ganzera M, Bedir E and Khan IA. Determination of steroidal saponins in *Tribulus terrestris* by reversed- phase high-performance liquid chromatography and evaporative light scattering detection. J. Pharm. Sci. 2001; 90(11): 1752-1758.
- 4- Anand R, Patnaik GK, Kulshreshtha DK and Dhawan BN. Activity of certain fractions *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. Indian J. Exp. Biol. 1994; 32: 548-552.
- 5- De Combarieu E, Fuzzati N, Lovati M and Mercalli E. Furostanol saponins from *Tribulus terrestris*. Fitoterapia. 2003; 74(6): 583-591.
- 6- Nadkarni KM. Indian Materia Medica. Bombay: Popular Prakashan Private Ltd. 1993; pp 947- 948.
- 7- Al-Ali M, Wahbi S, Twaij H and Al-Badr A. *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with *Zea mays*. J. Ethnopharmacol. 2003; 85(2):257-260.
- 8- Sharifi AM, Darabi R and Akbarloo N. Study of antihypertensive mechanism of *Tribulus terrestris* in 2K1C hypertensive rats: role of tissue ACE activity. Life Sci. 2003b; 73: 2963- 2971.
- 9- Al-Qarawi AA, Mousa HM, Ali B El H, Abdel-Rahman H, El-Mougy SA. Protective Effects of Extract from Dates (*Phoenix Dactylifera* L.) and Ascorbic Acid on Thioacetamide-Induced Hepatotoxicity in Rats. Iranian J. Pharmaceutical Research. 2008;7(3):193-201
- 10- Bremness L. Herbs. Eyewitness Handbooks. London: Dorling Kindersley Press. 1994;p304
- 11- Weiss RF. Herbal Medicine. Gothenburg, Sweden: Ab Arcanum and Beaconsfield UK: Beaconsfield Publishers Ltd. 1988; pp 196-197.
- 12- Schilcher H. Phytotherapy in Paediatrics: Handbook for Physicians and Pharmacists. Stuttgart D): Medpharm Scientific Publishers. 1997
- 13- German Homeopathic Pharmacopoeia (GHP) Translation of the German Homopathisches Arzneibuch (HAB 1), 5th suppl. 1991 to the 1st ed. 1978. Stuttgart. Deutscher Apotheker Verlag. 1993
- 14- Mattar AG. Studies on the effect of some plant extracts on the reproduction in mature male Mice High Diploma Thesis, Inst. Emb. Res. Infer Treat, Univ. of Al-Nahrain, Baghdad, Iraq 2005
- 15- Humason GL. Animal tissue techniques 5th edition, the Jons Hopkin University Pres Balmitore and London. 1997.
- 16- Stevens A and Lowe J. Histology techniques used in histology and cell biology, In: Stevens A. Lowe J. Editors. Human histology, 2nd edition, Mosby Co., London, New York. 1997.
- 17- Sorlie DG. Medical biostatistics and epidemiology: examination and board review. 1st edition, Appleton and Lang, Norwalk, Connecticut. 1995.
- 18- Singh B, Sharma SP and Goyal R. Evaluation of Geriforte, an herbal geriatric tonic, on antioxidant defense system in Wistar Rats. Annals N. Y. Acad. Sci. 1994; 717: 170-173.
- 19- Gauthaman K, Genesan A and Parasad R. Sexual effects of puncture vine (*Tribulus terrestris*) extract (protodioscin): An evaluation using a rat model. Altern Compl. Med. 2003; 9(2): 25-26
- 20- Ganong WF. The Gonads-development and function of the reproductive system. In: Ganong WF. Editor. Review of medical physiology. Lang Medical Books/ McGraw Hill. London, Maxico, Sanfrancisco, Chicago, Toronto, Madrid, New Jersey. 2003.
- 21- Brameld J and Butery P. Effect of nutrients on the expression of IGF-1 and G- receptors (GHR) mRNA by cultured pig hepatocytes Proceedings of the 3rd conference on farm animal endocrinology, Brussels-BASE. 1998; 2: 99-100.
- 22- Griffin JE. The physiology of the testis and male reproduction In: Carr BR, Blackwell RE. Editors. Textbook of reproductive medicine Appleton and Lange, Stamford. 1998.
- 23- Murray M and Pizzorno J. Encyclopedia of natural medicine. 2nd ed. Rocklin, CA. Prima Publishing 1998
- 24- Hochereau de Revier MT, Courtens JK, Courrot M and de Reviers M. Spermatogenesis in mammals and birds. In: Lamming GE Editor Marshall's physiology of reproduction: Reproduction in male. 4th ed. Churchill Livingstone, Edinburgh, UK. 1990.