

## STUDY THE EFFECT OF ISOFLAVONOID EXTRACT OF *PUNICA GRANATUM* RINDS ON FERTILITY EFFICIENCY AND SEMEN FLUID CHARACTERISTIC IN MALE RABBITS

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### ABSTRACT

The present study was carried out at the animal house of the College of Veterinary Medicine, University of Basrah. This study has been designed to determine whether isoflavonoid extract of *Punica granatum* rinds effect on fertility efficiency and semen fluid characteristic (physical and biochemical properties). Twenty four adult rabbits, six-month-old and 1500-1750 body weight divided into two groups 12 rabbits (6 male and 6 female) each group (male used for treated with extract while female only used for fertility test (untreated with extract)). First group: male rabbits received orally administration of normal saline (3ml) served as control. Second group: male rabbits received orally administration of isoflavonoid extract of *Punica granatum* rinds at the dose of 0.5g/kg B.W dissolve in (3ml) of normal saline. Treatment with isoflavonoid extract of *Punica granatum* rinds 0.5g/kg B.W. caused significant ( $p < 0.05$ ) increases in fertility efficiency such as number of offspring, sperm count, activities of sperm viability, number of sperm live to dead, motility, zinc and testosterone concentrations of treated rabbits in serum and semen plasma compared to control. Isoflavonoid extract of *Punica granatum* rind caused no significant changes in biochemical analysis such as total protein, ALP, ACP, AST and ALT in serum while ALP, ACP, AST and ALT concentrations significant ( $P < 0.05$ ) decrease in semen plasma. Treatment with isoflavonoid extract of *Punica granatum* rinds caused significant ( $p < 0.05$ ) decreases glucose level in serum and abnormal sperm morphology. It caused no visible lesion in the seminiferous tubules and spermatogenesis. These findings revealed that isoflavonoid extract could cause ameliorating effect on the reproductive parameters in male rabbits.

### INTRODUCTION

Semen is a mixture of spermatozoa suspended in a liquid medium, secreted at different locations by the epididymus and various glands, which, at the time of ejaculation, are combined.

Semen evaluation must provide information on the fertilizing capability of spermatozoa. The most important parameters pertaining to fertility are the number of spermatozoa inseminated and their motility [1, 2].

These seminal characteristics are affected by many factors (breed, feeding, health status, rearing condition, season and collection frequency, etc.) and there is thus a wide variety in semen traits [3]. Additionally, semen evaluation is a very difficult topic and differences in laboratory methodologies can introduce substantial variations in the evaluation of sperm parameters (sperm counts, motility and morphology) [4].

*Punica granatum* (Punicaceae) fruit rind [commonly called pomegranate] is rich in antioxidant of polyphenolic class which includes tannins [5] & flavonoids [6]. Antioxidant activity has been proposed to play vital role in various pharmacological activities such as anti-aging, anti-inflammatory, anti atherosclerosis and antiactivities[7,8]. Inhibition of free radical induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases [9]. Several synthetic antioxidants are available, but are quite unsafe and their toxicity is of concern [10]. Natural products with antioxidant activity may be used for human consumption because of their safety. The purpose of the present study was to study the ability of isoflavonoid extract of *Punica granatum* on fertility efficiency and semen fluid characteristic in male rabbits.

## MATERIALS AND METHODS

### 2.1 Experimental Animals

In the present study, a total of twenty four adult male and female local rabbits were obtained from the local market. Rabbits initially weighing 1500-1750 g and six-month-old were used. Animals were acclimated to holding facilities for two weeks prior to the experiment. The rabbits were housed in groups and kept in room under controlled temperature (24°C), humidity (30-70 %) and light (12: 12 hr / light: dark). All animals were provided balanced diet throughout the experimental period. This formed of proteins, fibers, wheat, clover, minerals and many vitamins. Animals were given food and water *ad libitum*.

### 2.2 Experimental Materials

#### Preparation of *Punica granatum* Isoflavonoid Extract

A (50gm) of dried rinds powder was extracted in (500ml) methanol (80%) in water with 3% hydrochloric. The sample was refluxed with solvent for one hour then filtered. The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethylacetate treated with 2% of hydrochloric acid and the ethylacetate layer was concentrated by rotary evaporator at 45°C and dried at room temperature [11]. The resultant extract (7gm) was brown and dry material, the percentage was 15% w/w.

### 2.3 The qualitative Chemical Analysis for

#### 2.3.1 Sepecific Tests

Some chemical tests have been done on *Punica granatum* isoflavonoids extract to find its chemical contains.

**-Carbohydrate test :** (1ml) of *Punica granatum* isoflavonoids extract was treated with 5 drops of  $\alpha$ -naphthol (1%) and then (1 ml) of concentration  $H_2SO_4$  was added to form violet ring[12].

**-Phenoles test :** (1 ml) of *Punica granatum* isoflavonoids extract was treated with drops of folin reagent[13]

**-Flavoniods test :** (1ml) of *Punica granatum* isoflavonoids extract was treated with (1ml) of alcoholic potassium hydroxide (5N)[14].

**-Proteins test :** (1ml) of *Punica granatum* isoflavonoids extract was treated with Biuret reagent (1%  $CuSO_4$ , 10%  $NaOH$ )[12].

**-Amino acids test :**(1ml) of *Punica granatum* isoflavonoids extract was treated with drops of ninhydrine reagent (1%) and the solution was heated[14].

**-Alkaloids test :** (1ml) of *Punica granatum* isoflavonoids extract was treated with (1ml) of Dragendroff reagent ( $\text{O}(\text{BiNO}_3)$ , 1% HCl, KI) [11], Mayer reagent ( $\text{KI}$ ,  $\text{HgCl}_2$ ) and Wagner reagent ( $\text{KI}$ ,  $\text{I}_2$ ) [14].

#### **2.4 Experimental Design**

Twenty four adult rabbits (12 male and 12 female). Twelve males were allocated into two groups- 6 each- and treated with isoflavonoid extract of *Punica granatum* oral administration for 30 successive days while female 6 each group untreated with isoflavonoid extract of *Punica granatum* rinds only used for fertility test

Group (1): Rabbits received orally administration of normal saline (3ml) for 30 days (as served control group).

Group (2): Rabbits received orally administration of isoflavonoid extract of *Punica granatum* at dose 0.5 g/kg B.W. /day dissolve in (3ml) normal saline for 30 days.

#### **2.5 Fertility test**

Females' rabbits were mated with an adult male rabbits (1:1) in separated cages at 4:00 pm.; vaginal smears were obtained in the next early morning for microscopic detection of sperms which indicates the first day of pregnancy. Positive females were isolated and date recorded.

#### **2.6 Sampling**

##### **2.6.1 Blood samples**

At the end of each experimental period, blood samples were collected, from fasted male rabbits (control and treat animals), from the heart by heparinized capillary tubes in plain tubes, and allowed to be clotted at room temperature and put in centrifuge at 5000rpm to obtain serum for hormonal assay and biochemical analysis such as (total protein, glucose, AST, ALT, ACP, ALP and zinc).

##### **2.6.2 Hormonal Assay**

Serum samples and plasma semen were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.

#### **2.7 Semen Collection**

The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen. The semen collected dilution with normal saline and input in tubes and centrifuge for obtain semen plasma and studied physical and biochemical properties of semen such as semen volume, colour.

##### **2.7.1 Semen Analysis**

**Progressive sperm motility:** This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide ( $37^\circ\text{C}$ ) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using 400X magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100) [15].

**2.7.2 Sperm viability (Live/dead ratio):** This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using 400X magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated [16].

$$\text{Live sperm \%} = \frac{\text{live sperm}}{\text{Total sperm count}} \times 100$$

$$\text{Dead sperm \%} = \frac{\text{Dead sperm}}{\text{Total sperm count}} \times 100$$

### 2.7.3 Sperm maturation by aniline-blue:-

Nuclear maturation was evaluated by aniline-blue stain, according to Morel *et al* [17]. Sperm nuclei that stained with blue color were considered to be immature. But nuclear mature sperm was not stained with aniline-blue. The percentage of immature sperm was calculated from the observation of one hundred sperm preparation from each group.

### 2.7.4 Sperm Morphology:-

A drop of Negrosin-Eosin stain was added to the sperm suspension and kept for 5 min. at 37°C. After that a drop of sperm suspension was placed on a clean slide and spread gently to make a thin film. The film was air dried and then observed under a microscope for changes in sperm morphology, according to the method of Feustan *et al* [16]. The criteria chosen for head abnormality was; amorphous, pin and shorthead. For tail, the abnormalities recorded were; coiled flagellum, bent flagellum, bent flagellum tip. The result are the percentage overall abnormal form.

**2.7.5 Sperm count:** This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5ml formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5ml formol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope [16].

## 2.8 Testicular Histology

After removing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 hours in an oven at 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50°C -55°C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1minute before immersed in

absolute alcohol for 1 minute and later in descending grades of alcohol for about 30seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at40X, 100X and 400Xmagnifications [18].

## 2.9 Statistical Analysis:

The mean and standard deviation of mean (S.D.M.) were calculated for all values. Comparisons between the control and the treated groups were done using the student's *t-test*. Differences were considered statistically significant at  $p < 0.05$  [19].

## RESULTS

### 3.1 Effect of Isoflavonoid extract of *Punica granatum* rind on fertility efficiency in male rabbits mating with female untreated

The results in Table (1) observed that the effect of isoflavonoid extract of *Punica granatum* on fertility efficiency in male rabbits mating with female untreated with extract. The results were showed increase pregnancy rate in female mating with male rabbits treated with isoflavonoid extract of *Punica granatum* compared with control and significant ( $p < 0.05$ ) increase in No. of litter size.

**Table (1):-Effect of Isoflavonoid extract of *Punica granatum* rind on fertility efficiency in male rabbits mating with female untrated. Mean  $\pm$  SD N=6**

Parameters Groups	No. of female pregnancy	Pregnancy rate%	No. Litter size	Embryo mortality
Control (Normal Saline) 0.9% NaCl	5	83.33%	3.5 $\pm$ 1.76	-
Isoflavonoid Extract of <i>Punica granatum</i> rinds 0.5 g/kg	6	100%	7.66 $\pm$ 0.81*	-

N=Number of animal, \*= $P < 0.05$

### 3.2Effect of Isoflavonoid extract of *Punica granatum* rind on physical properties of semen analysis in male rabbits

The results in Table (2) observed that the effect of Isoflavonoid extract of *Punica granatum* on physical properties of semen analysis in male rabbits. The results were showed significant ( $p < 0.05$ ) increase in semen volume,sperm motility, sperm concentration, total sperm cell/ ejaculate, live-dead sperm and sperm abnormalities.

**Table (2):-Effect of Isoflavonoid extract of *Punica granatum* rind on physical properties of semen analysis in male Mean  $\pm$  SD N=6**

Parameters Groups	Control (Normal Saline) 0.9% NaCl	Isoflavonoid Extract of <i>Punica granatum</i> rinds 0.5 g/kg
Semen volume(ml)	0.70 $\pm$ 0.05	0.85 $\pm$ 0.03*
Semen colour	Creamy	Creamy
Mass activities	+	++++
Sperm motility%	80.38 $\pm$ 4.72	90.50 $\pm$ 2.59*
Sperm concentration ( $\times 10^6$ /ml)	5.32 $\pm$ 0.12	7.41 $\pm$ 0.37*
Total sperm cell / ejaculate( $\times 10^6$ /ml)	3.74 $\pm$ 0.36	4.98 $\pm$ 0.16*
Live-dead sperm ratio	75:25 $\pm$ 1.58	92:8 $\pm$ 0.24*
Sperm abnormalities	17.56 $\pm$ 2.5	3.67 $\pm$ 1.57*

N=Number of animal, \*=P<0.05

### 3.3 Effect of Isoflavonoid extract of *Punica granatum* rind on biochemical analysis in serum male rabbits.

The results in Table (3) observed that the effect of Isoflavonoid extract of *Punica granatum* on biochemical analysis in male rabbits. The results were showed non-significant (P>0.05) decrease of AST, ALT, ALP levels and ACP in serum and significant (P<0.05) decrease glucose concentration while the results of total protein revealed non-significant (P>0.05) increase in serum male rabbits and significant(P<0.05) increase zinc concentration in serum male rabbits.

**Table (3):-Effect of Isoflavonoid extract of *Punica granatum* rind on biochemical analysis in serum male rabbits Mean  $\pm$  SD N=6**

Parameters Groups	Total protein mg/dl	Glucose mg/dl	AST U/L	ALT U/L	ALP U/L	ACP U/L	Zinc mg/dl
Control (Normal Saline) 0.9% NaCl	68.34 $\pm$ 4.69	100.23 $\pm$ 5.21	80.25 $\pm$ 3.86	56.3 $\pm$ 6.29	26.7 $\pm$ 2.5	29.3 $\pm$ 7.11	0.96 $\pm$ 0.035
Isoflavonoid Extract of <i>Punica granatum</i> rinds 0.5 g/kg	72.5 $\pm$ 2.38	80.26 $\pm$ 2.10*	78.05 $\pm$ 2.19	49.2 $\pm$ 3.15	24.1 $\pm$ 1.2	27.4 $\pm$ 3.18	1.23 $\pm$ 0.027*

N=Number of animal, \*=P<0.05

### 3.4 Effect of Isoflavonoid extract of *Punica granatum* rind on testosterone level in serum and semen male rabbits

The results in Table (4) observed that the effect of Isoflavonoid extract of *Punica granatum* on testosterone in serum and semen in male rabbits. The results were showed significant ( $P<0.05$ ) increase of testosterone concentration in serum and semen rabbits.

**Table (4):-Effect of Isoflavonoid extract of *Punica granatum* rind on testosterone level in serum and semen male rabbits Mean  $\pm$  SD N=6**

Groups \ Parameters	Testosterone in serum ng/ml	Testosterone in semen ng/ml
Control (Normal Saline) 0.9% NaCl	0.67 $\pm$ 0.024	0.88 $\pm$ 0.013
Isoflavonoid Extract of <i>Punica granatum</i> rinds 0.5 g/kg	1.2 $\pm$ 0.017*	1.67 $\pm$ 0.028*

N=Number of animal, \*= $P<0.05$

### 3.5 Effect of Isoflavonoid extract of *Punica granatum* rind on biochemical analysis in plasma semen male rabbits

The results in Table (5) revealed that the effect of isoflavonoid extract of *Punica granatum* on total protein, AST,ALT,ALP, ACP and zinc concentrations. The results revealed significant ( $P<0.05$ ) increase in total protein and zinc concentrations of semen rabbits while the results revealed significant ( $P<0.05$ ) decrease in AST, ALT, ACP and ALP concentrations of semen rabbits.

**Table (5):-Effect of Isoflavonoid extract of *Punica granatum* rind on biochemical analysis in plasma semen male rabbits Mean  $\pm$  SD N=6**

Groups \ Parameters	Total protein mg/dl	AST U/L	ALT U/L	ALP U/L	ACP U/L	Zinc mg/dl
Control (Normal Saline) 0.9% NaCl	71.14 $\pm$ 6.01	84.25 $\pm$ 4.32	59.7 $\pm$ 4.15	58.7 $\pm$ 2.5	49.3 $\pm$ 7.11	1.37 $\pm$ 0.019
Isoflavonoid Extract of <i>Punica granatum</i> rinds 0.5 g/kg	80.13 $\pm$ 2.10*	69.05 $\pm$ 2.19*	38.2 $\pm$ 3.15*	42.1 $\pm$ 6.3*	31.2 $\pm$ 1.52*	2.03 $\pm$ 0.011*

N=Number of animal, \*= $P<0.05$

### Sperm examination

Sperms of rabbits (control). Showing almost of sperms normal, live and decrease number of mature sperm and present large number of sperms dead and large number of different types of abnormalities sperm coiled tail, double-Tail, Only Head when stained with eosin and negrosin while sperms of rabbits treated with isoflavonoid

extract of *Punica granatum*. Showing all sperm normal, increase number of live sperm and mature sperm and decreased in number of dead sperm when stained with aniline-blue stain and showing low number of different types of abnormalities sperms 1-Coiled tail 2-Only Head in sperms of rabbits treated with isoflavonoid extract of *Punica granatum*.

#### **Histological changes:-**

Section of testis of rabbits (control) showing mild vacuolation and widening of inter seminiferous tubules, arrested of spermatogenesis, decrease of interstitial leydig cells while Section of testis of rabbits treated with isoflavonoid extract of *Punica granatum* showing normal seminiferous tubules and spermatogenesis.

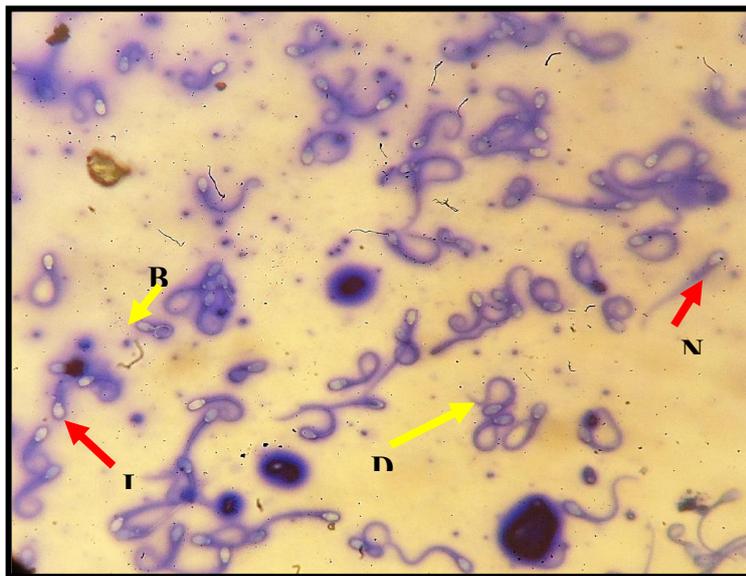


Fig (1):-Sperms of rabbits (control). Showing almost of sperms normal (N), live (L) and mature (M) and present

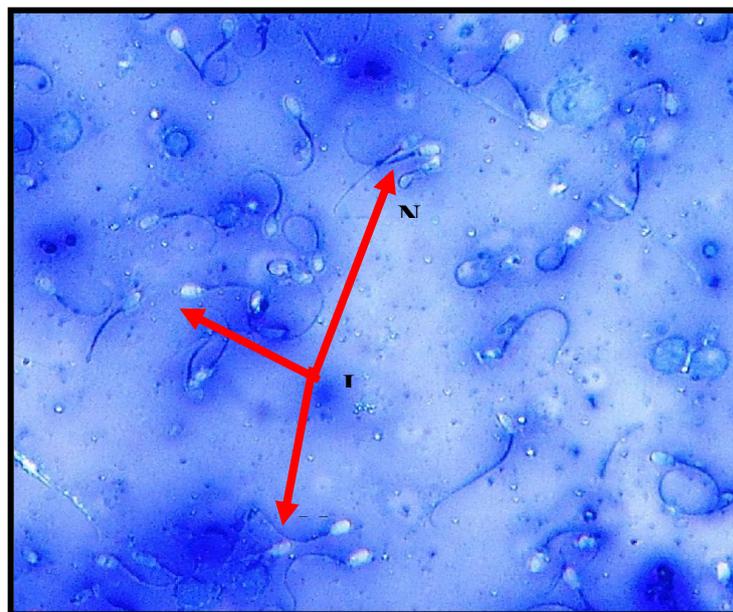
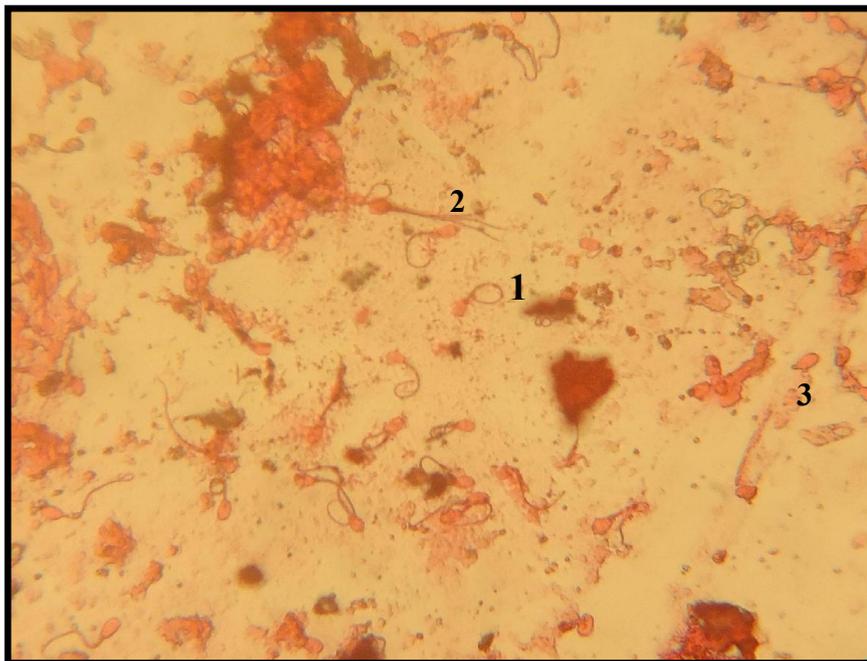
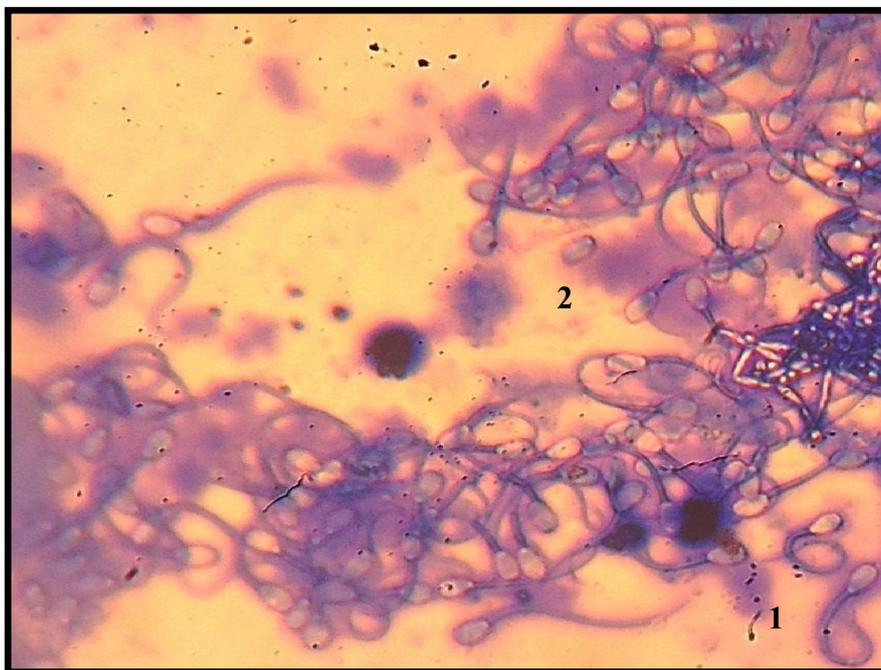


Fig (2):-Sperms of rabbits treated with isoflavonoid extract of *Punica granatum*. Showing all sperm normal (N), live



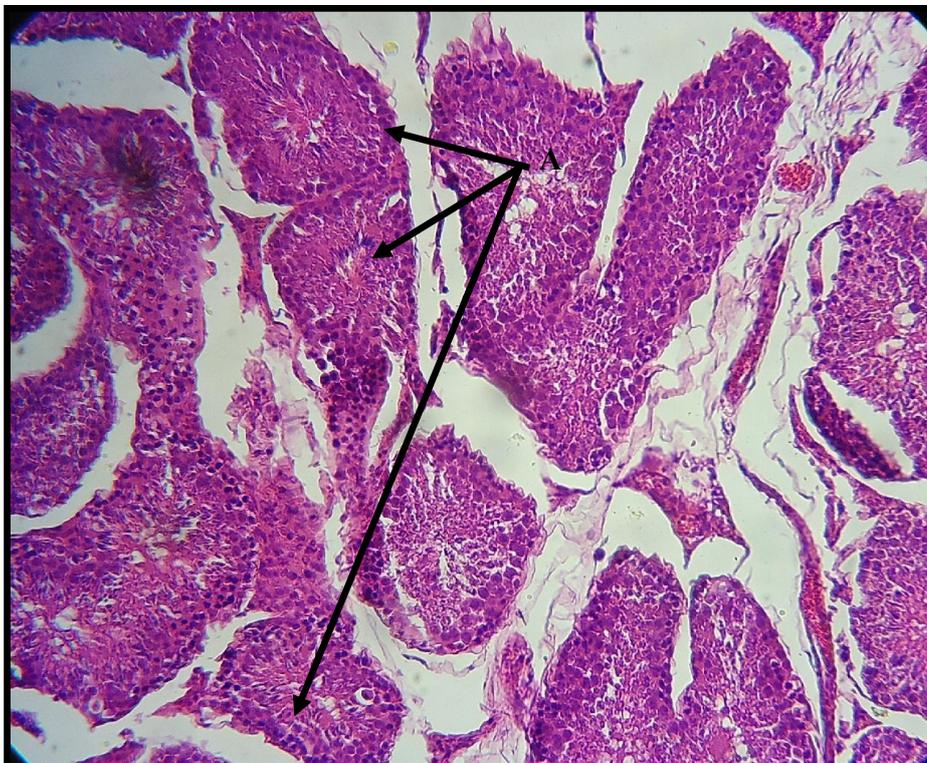
**Fig (3):-Sperms of rabbis (Control). Showing large number of different types of abnormalities sperm1-Coiled tail 2-Double –Tail 3-Only Head.**



**Fig (4):-Sperms of rabbis treated with isoflavonoid extract of *Punica granatum* . Showing low number of different types of**



**Fig(5):-** Section of testis of rabbits (control). Showing (A) mild vacuolation and widening of inter seminiferous tubules, arrested of spermatogenesis(Sp), decrease of interstitial leydig cells (Lc) .  
(H&E stain 100X)



**Fig (6):-** Section of testis of rabbits treated with isoflavonoid extract of *Punica granatum*. Showing (A) normal seminiferous tubules and spermatogenesis (H&E stain 100X)

## DISCUSSION

Isoflavonoid extract of *Punica granatum* caused increase fertility efficiency and improvement of physical and chemical properties of semen rabbits. Isoflavonoid extract of *Punica granatum* caused significant ( $P < 0.05$ ) increase fertility efficiency in female mating with male rabbits treated with isoflavonoid extract, sperm count, sperm viability, testosterone and zinc concentrations in serum and semen plasma of rabbits. This increase in testosterone and zinc concentrations could indicate that isoflavonoid extract of *Punica granatum* rinds active the mechanism intervening in the process of hormone synthesis in the Leydig cells. The andrological results show that treatment of rabbits for 30 days with isoflavonoid extract of *Punica granatum* caused significant increase in sperm motility. This suggests that isoflavonoid extract of *Punica granatum* rinds was able to prevent the blood-testis barrier with a resultant improvement in the microenvironment of the seminiferous tubules, since it has been reported that the increase in sperm motility caused by chemical agents was due to their ability to prevent the blood-testis barrier [20] and thus, creating a suitable microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part [21].

Isoflavonoid extract of *Punica granatum* caused significant increase in sperm viability as well as significant increase in the percentage of morphologically normal sperm cells in the treated rabbits. This could be due to the ability of Isoflavonoid extract of *Punica granatum* to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in activation of spermatogenesis [22, 23].

Isoflavonoid extract of *Punica granatum* rinds caused significant increase in sperm count of the treated rabbits which could be as a result of increase in plasma levels of testosterone since this hormone has been reported to be important in the initiation and maintenance of spermatogenesis [24]. These parameters in male rabbits are regulated by LH and FSH. The spermatogenesis stimulates by FSH which binds with receptors in sertoli cells while the LH stimulates the production of testosterone in leyding cells, which may act on sertoli and pertitubular cells of testosterone [25].

In rabbits treated with isoflavonoid extract of *Punica granatum* lead to decrease in AST, ALT, ALP and ACP concentration in plasma semen because the isoflavonid extract antioxidant activity and prevent damage of germinal and somatic cells of testis and release these enzymes.

Rabbits treated with isoflavonoid extract of *Punica granatum* presented with improvement of seminiferous tubules and spermatogenesis. Testosterone stimulates growth and secretary activity to the reproductive organs[26],so these hormone in this study could increase the number and function of germinal and somatic cells of testis. This suggests that isoflavonid extract of *Punica granatum* rinds had ameliorating effect on the exocrine function of the testes.

## تأثير المستخلص الايزوفلوفونيدات لقشور نبات الرمان على الكفاءة التناسلية والصفات السائل المنوي في ذكور الأرانب المحلية

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### الخلاصة

أجريت هذه التجربة في البيت الحيواني لكلية الطب البيطري- جامعة البصرة، لتحديد تأثير المستخلص الايزوفلوفونيدات لقشور نبات الرمان على الكفاءة التناسلية والصفات السائل المنوي في ذكور الأرانب المحلية. قسمت الحيوانات إلى مجموعتين ( ١٢ ) حيوان في كل مجموعة ( ٦ ذكور و ٦ إناث استخدمت لغرض اختبار الكفاءة التناسلية فقط غير معاملات بالمستخلص). المجموعة الأولى مجموعة سيطرة جرعت ( ٣مل ) من المحلول الفسيولوجي. والمجموعة الثانية جرعت ٠.٥ غم إكغم من وزن الجسم المستخلص الايزوفلوفونيدات لقشور نبات الرمان لمدة ٣٠ يوم.

أظهرت النتائج وجود زيادة معنوية في الكفاءة التناسلية و عدد المواليد وتركيز هرمون التستسترون والزنك وعدد الحيامن ونسبة الحيامن الحية إلى الميتة لذكور الأرانب المعاملة بالمستخلص ونقصان معنوي في الإنزيمات AST,ALT,ACP, ALP في بلازما السائل المنوي وعدم ظهور أي تغير معنوي في هذه الإنزيمات في مصل الدم . ولم يلاحظ أي ضرر في الانابيب المنوية وعملية تكوين النطفولوحظ تحسن في النسيج الخصوي لذكور الأرانب المعاملة بالمستخلص مقارنة بمجموعة السيطرة. لذلك يستنتج ان لمستخلص الايزوفلوفونيدات تأثير ايجابي على الكفاءة التناسلية والصفات السائل المنوي.

### REFERENCE

- 1- **Castellini, C. and Lttaioli, P.** Effect of motile sperms inseminated on reproductive performance of rabbit does. *Anim. Sci.*, 1999, 57: 111-120.
- 2- **Brun,J.M.; Theau-Clement, M. and Bolet, G.** The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. *Anim. Reprod. Sci.*, 2002, 70: 139-149.
- 3-**Alvarino,J.M.R.** Reproductive performance of male rabbits. *In: Proc. 7th WorldRabbit Congr., Valencia Jul., 2000, vol. A, 13-35.*
- 4-**WHO.** Laboratory manual for the examination of human semen and spermcervical mucus interaction. 1999,4th Ed. *Cambridge University Press.*
- 5-**Schubert-SY; Lansky-EP; Neeman.**Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed fermented juice flavonoids; *JEthnopharmacol*; 1999. 66(1): 11-7
- 6- **Negi,P.S. Jayaprakasha G.K.** Antioxidant and antibacterial activities of *Punicagranatum* peel extracts. *J.Food sci.* 2003,68 (4):1473-1477
- 7- **Lansky, E.P.** Physiologically synergistic mixtures of fruit componenas, methods of presentation thereof and method of use thereof. United state patent application Code 50118312 A1. *june 2, 2005.*

- 8- **Gil, M.I.** Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J.Agric Food Chem.* 2000;48(10): 4581-9.
- 9- **Li, Y.** *Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycaemia in Zucker diabetic fatty rats. *Ethopharmacol.* 2005 Jun 3; 99 (2): 239-244.
- 10- **Huang, T.H., et al.** Anti-diabetic action of *Punica granatum* flower extract: activation of PPAR- gamma and identification of an active component. *Appl. Pharmacol.* 2005 Sept. 1; 207 (2):160-9.
- 11- **Wandi, J.; Fomum, T.; Tilequin, F.; Segun, E. and Koch, M.** Two isoflavonoids from *Erythrina senegalensis*. *Phytochem.* 1994, 35:245-8.
- 12- **Saadalla, R.A.** Biochemistry practical manual. Basrah University press. Basrah-Iraq. 54, 1980.
- 13- **Harborn, J.B.** Phytochemical methods. 2<sup>nd</sup> Ed., Chapman and Hall, New York, USA. 1984.
- 14- **Al-Kazraji, S.M.** Biopharmacological Study of Artemisia a herbaalba. M.Sc. Thesis/college of pharmacy/University of Baghdad. 1991.
- 15- **Mohammad – Reza, P.; Farzaneh, D.; Taherch, T.K. and Zoherb, P.P.** The effects of hydroalcoholic extract of *Actinidia chinensis* on sperm count and motility, and blood levels of estradiol and testosterone in male rats. *Achieves of Iranina Medicine*, 2005, 8(3): 211-216.
- 16- **Laing, J.A.** Fertility and infertility in domestic animals. 3rd edition 1979 Bailliere Tindall, a division of Cassell Lt.
- 17- **Morel, B.S.; Mercier, S. and Roux, C.** Inter individual variations in the disomy frequencies of human spermatozoa and their correlation with nuclear maturity as evaluated by aniline blue staining. *Fertil. Steril*, 1998; 69 : 1122-1127.
- 18- **Luna, L.G.** Manual of histology staining methods of the Armed Forces Institute of Pathology. 3<sup>rd</sup> ed., New York, McGraw-Hill. 1993.
- 19- **SPSS Statistical Packages for the Social Sciences.** Statistical software for windows version 13.0 Microsoft. SPSS<sup>®</sup>, Chicago, IL, USA. 2001.
- 20- **Baldessarini, R.** In drugs and the treatment of psychiatric disorders. The pharmacological basis of therapeutics Ed. By Goodman and Gilman. Macmillan Pub. Co. Inc 1980, pp. 301-417.
- 21- **Bloom, W. and Fawcett, D.W.** Male reproductive system. In the textbook of Histology. Saunders Company, Philadelphia. 1975.

- 22-William ,K.W .** Hormones and Hormone antagonists. In: Remington, The Science and Practise of Pharmacy, 2000,vol 11, 20<sup>th</sup> edition 77: 1390-1391.
- 23- Bowman, W.C. and Rand, M.J.** The reproductive system and drugs affecting the reproductive systems. Textbook of pharmacology,1985,2nd edition, 20:1-8.
- 24- Christensen, A.C.** Leydig cell: In: Handbook of Physiology, edited by P.O. Greep and E.B. Astwoods. Washington D C American Physiological Society, 1975.
- 25-O'Donnel, L.; Mc Lanchlan, R.L.; Wreford, N.G. and Robertson, D. M.** Testosterone promotes the conversion of round spermatids between stages vii and viii of the rat spermatogenic cycle. *Endocr.* 1994;135:2608-614.
- 26-Singh,j.; O'Neill,C. and Handelsman,D.J.** Induction of spermatogenesis by androgens in gonadotropin-deficint mice. *Endocr.*1995; 136:5311-5321