Effects of Aqueous and Alcoholic Extracts of *Peganum harmala* L. Seeds on Male Fertility of White Mice Treated with Olanzapin Drug

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**Abstract**

This study was designed to study the effects of aqueous and alcoholic extracts of *Peganum harmala* L. Seeds on male fertility of white mice treated with Olanzapin, males were 25 to 30 days old and had a mean weight of 35 g. The mice were divided into two groups, a total of 5 negative control males were injected with distilled water for 15 days, the positive control group (experimental group) injected with Olanzapin drug for 15 days, the was then divided into five subgroups, the first subgroup consists of 5 males were injected with the drug only, while the other four subgroups were dosed with aqueous and alcoholic extracts for 7 days. The first subgroup dosage was with low concentration of aqueous extract (12 mg / kg) and the second with a high concentration of the aqueous extract at a concentration of 24 mg / kg, the third subgroup was dosage with the low concentration of the alcoholic extract at 12 mg / kg and the fourth was dosage with a high concentration of the alcoholic extract at a concentration of 24 mg / kg. The results showed a significant increase (p <0.01) in the level of prolactin hormone in the positive control mice serum, compared to the control group as well as a significant decrease in the level of testosterone hormone as well as the characteristics of the epididymal sperms represented by sperm concentration and percentages of normal and moving sperm. The dosage with aqueous and alcoholic extracts of the *Peganum harmala* led to the occurrence of significantly higher concentration (p <0.01) of the testosterone hormone and significantly lower in the level of the prolactin hormone concentration when compared with the experimental group as well as a significant improvement of p <0.01) in the parameters of epididymal sperms, and the best concentration was for the aqueous plant extract which 12 mg / kg.

**Keywords:** Olanzapin, *Peganum harmala*, Testosterone, Prolactin, Sperms.

**Introduction**

Recent studies on male reproductive health have reported suggestions that sperm counts have decreased significantly during the past 50 years. The suggestions are exposure to chemotherapeutic drugs that can cause unwanted alterations in spermatogenesis [1]. Many investigations concentrated on the effect of different drugs on sex hormones, spermatogenesis, sperm count, and sperm abnormalities in males [2].

Unfortunately, the reproductive system is the target organ for negative effects resulting from the exposure to chemotherapeutic and toxic environmental agents. Chemotherapy is the cause of physiological damage on male sperms in the testis that associated with fertility, indicated by the parameters of semen fluid quality which means that many chemicals used including chemotherapeutics leads to detrimental effects on semen quality [3].

Many hormones including androgens play an important role in initiating and maintenance of male reproductive and testicular function including spermatozoa production. Testosterone which considered the main testicular androgen is produced by leydig cells that stimulated by pituitary LH, the essential for spermatogenesis, fertility in males and spermatogenesis function depends on the action of testosterone [4].

Many researchers found that spermatogenesis failure is a result of treatment with chemotherapeutics [5]. Some studies were conducted to determine the effect of evaporation or extracts of plant seeds like *Peganum harmala* on the tissues of the testes in males of mammalian and its relationship to treating with some drugs.
This information gives a definite indication for the positive reformatory effect of evaporation by seeds of this plant on testicular tissue (6), (7), (8). *Peganum harmala* is one of the most widely known herbal medicinal plants in many countries around the world. It is known as "harmal" in many Middle Eastern countries [9] including Iraq where widely used seed burning for evaporation and some other public traditionally using like uses for some religious believing [10], so this plant take huge spot of researchers in Iraq and many countries to study its components and effects [11].

*P. harmala* seeds reported as widely used in treatment for several diseases, for example, seeds powder used as anti-thelmintic and protozoacidal material, and for treatment of eczema, jaundice, asthma, malaria [12], with anti-inflammatory, and analgesic effects.

The seeds also used by women as flooder of milk lactation, and for regulation of the menstruation cycle [13]. *P. harmala* seeds contain many chemical compounds including: amino acids, polysaccharides, flavinoids and alkaloids compounds. These alkaloids are the active and most effective compounds, because most of the therapeutic properties of the plant seeds are due to the effects of these compounds. Alkaloids of harmala seeds are classified into three main groups: 1st. group is called Harmala indole alkaloids; Second group is called quinazoline [14]. Harmala Indole alkaloids like Harmaline derivatives have many physiological and pharmacological properties [15], [16], [17].

Materials and Methods

The study was conducted on 30 adult males aged 25-30 days at 35 g of weight and were raised in cages in suitable sizes to ensure their freedom of movement as well as providing suitable conditions in terms of temperature (20-25°C), lighting and good ventilation. The cages were laid with wood saws which replaced twice a week to ensure the cleanliness of the animals and the animals have been given standard diet depending on [24] and aqueous was freely available.

The Plant

The seeds were obtained from the local markets in Baghdad.

The Drug

Olanzapin / 5 mg from microlabs limited company / India was obtained from local pharmacies.

Preparation of Aqueous and Alcoholic Extracts of Seeds of *Peganum harmala* L

Aqueous extract was prepared by weighing 50 milligrams of plant seeds powder added to 250 ml of distilled aqueous. Place the suspension on the electric stirrer for 2 hours and then filtered through four pieces of gauze and place the resulting leached material in centrifuge tubes and centrifuged at 3000 cycles per minute for 15 minutes and put the supernatant in Petri dishes and put in the oven for drying at 40°C, after the completion of drying, the seed extract powder was scraped and put in a tightly sealed clean glass container and kept in the room temperature until use.

The alcoholic extract was prepared by weighing of 50 mg of seed powder and adding 250 mL of ethyl alcoholic to the mixture. The suspension was placed in an aqueous bath at 50°C for 24 hours and then placed in the electric stirrer for two hours. The following steps were completed as in the aqueous extract [23]

Make Hyperprolactin

In the study, 30 male mice were used and divided into two groups control and experimental, the control group was injected with physiological saline solution 0.9% NaCl. The experimental group was injected with Olanzzipin drug between fingers for 15 days once per day at the same time as the experimental group injected.

Preparation of the Drug and Aqueous and Alcoholic Extracts

7 mg of Olanzzipin was weighed and dissolved in drops of HCl and then diluted with physiological saline 0.9% Nacl, the size of the injected substance was 0.2 mm between fingers by using disposable syringes once a day. The aqueous and alcoholic extracts were prepared by weighing 12 and 24 mg extract were prepared from the two previous extracts (stock) for aqueous and alcoholic extracts and at the low concentration of 12 mg and the high concentration was 24 mg and were dissolved with distilled aqueous, the volume of the daily dose was 0.1 ml.
Experiments Design

The experimental group that injected with the drug was divided into 5 subgroups, the first subgroup was the positive control that injected with the drug and consisted of 5 mice, the second group was the experimental group and its dosage was the low concentration of the aqueous extract of the plant, 12 mg / kg and for 7 days, the third group of experimental group and its dosage was the high concentration of the aqueous extract of the plant, 24 mg / kg for 7 days, the fourth Experimental group and its dosage was the low concentration of alcoholic extract of the plant, 12 mg / kg for 7 days. The fifth group of experimental group and its dosage was the high concentration of the alcoholic extract of the plant, 24 mg / kg for 7 days.

The animals were then dissected and the blood samples were taken by the method of the heart stab for the purpose of conducting hormonal tests. The right and left tails of Epididymus were separated and placed 0.5 ml of warm physiological saline solution and then cut into small pieces using a sharp scalpel for the purpose of releasing the sperm. The sperm tested under 400x magnification and calculated by counting the sperm concentration and the percentage of the moving and normal sperm.

Concentration of Sperm

The semen was withdrawn to the 0.5 mark using the red blood cell count pipette of the Haemocytometer and then completed the volume to 101 by withdrawing the colored dilution solution (0.9% NaCl with 50 μL of eosin dye 0.1%). the solution was mixed gently in the pipette bulb, then put a drop of it at the edge of the lid of the slide (counting box) of the blood cell scale. Leave the slide on the microscope for five minutes to ensure the stability of the sperm in the squares, and then take the sperm count in five squares on the middle and the center of the divisions and calculated according to the equation:

\[ \text{Concentration of sperm per million / ml} = 10^4 \times 200 \times 400 \times \frac{N}{80} \]

\( N = \) Number of sperm calculated.
\( 80 = \) the number of small squares in five large squares.
\( 400 = \) the total number of small boxes in the counting box.
\( 10^4 = \) Depth of the x103 box for per liter extraction.
\( 200 = \) dilution factor in the absorbent. [25]

Percentage of Sperm Viability

Place a drop of semen on a clean, dry glass slide and add a drop of 0.1% Eosin dye, then leave for the mixture of dye with the sperm. Then spread by using another glass slide to mix droplets together without pressure during spreading. The slide left at room temperature to dry, then the at least 200 sperm counted in order to get the percentage of the living sperm that does not accept dye according to the following equation:

\[ \text{Percentage of living spermatozoa} = \frac{\text{Number of non-stained sperm (live)}}{\text{Total number of sperm (colored in red + not colored)}} \times 100 \] [26]

Percentage of Normal Sperm

To study the normal morphology of the sperm, drops of semen was put on a clean, dry glass slide and add a drop of eosin-negrosin. The tow droplets were mixed gently for half a minute and then spread by using another glass slide to spread the mixed droplets together, with respect to not pressing the slide during the spreading process. Left the slide to dry at room temperature, then the percentage of the normal sperm was calculated according to the following equation:

\[ \text{The percentage of normal sperm} = \frac{\text{the normal sperm count of the sperm}}{\text{Total number of sperm (normal + abnormal)}} \times 100 \]

Marks of deformity have been determined according to changes happened in the morphology of sperms, whether in the head, the midpiece or the tail [27]
Percentage of Sperm Motility
A drop of semen was placed on a warm, clean and dry glass slide and then covered with a cover slide and at least 200 sperm were counted and recorded. The percentage of moving sperm was calculated as follows:

\[
\text{The percentage of mobile sperms} = \frac{\text{The number of mobile sperms}}{\text{Total number of sperms (mobile+ not mobile)}} \times 100
\]

Microscopic Examination and Photography
The sperm smears were examined using the Altay Biolabline Microscope using various magnification powers. Photographs of some smears were taken using a digital camera, DigitalCameraDCE-2, 0.3M Pixel

Hormonal Measurements
I chroma Kit was used for the purpose of determining the level of the prolactin and testosterone hormones in the experimental animal serum and the control group and using the Ichroma 2 boditech-KOREA device using its Immunofluorescence method.

Statistical Analysis
The statistical analyzes of this study were performed by extracting the average and standard error using T-test standers and comparing the differences between the rates by testing the least significant difference of LSD (Least significant difference) by using the SAS 2012 program.

Results and Discussion
Hormonal Measurements:
The results of the present study showed a significant increase in in the concentration of the prolactin hormone (P <0.01) in the experimental group compared to the control group as well as a significant decrease in the level of testosterone hormone (P <0.01) in the experimental group compared with the control group and that agree with [18 and 19] and disagree with [20],because they said that there is no significant effect of prolactin in male reproductive system.

The differences were lower for the high concentration of aqueous extract of the plant and the high and low concentration of the alcoholic extract of the plant, and when compared between the four concentrations of aqueous and alcoholic extracts by proportions Level of testosterone hormone observed that there was a significant increase p <0.01 in the level of testosterone hormone ( compared with positive control) in the aqueous extract of low concentration dose group and no significant differences in relation to the level of hormone between the groups of high concentration of aqueous and high and low concentrations of the alcoholic extract .

The best concentration among the four groups is the low aqueous concentration 12 mg / kg and 24 mg / kg of aqueous and alcoholic extracts respectively.(Table 1)

<table>
<thead>
<tr>
<th>The Group</th>
<th>Prolactine (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control -</td>
<td>2.18 ± 0.23 bc</td>
<td>8.04 ± 0.34 a</td>
</tr>
<tr>
<td>Control +</td>
<td>7.76 ± 0.45 a</td>
<td>2.82 ± 0.11 e</td>
</tr>
<tr>
<td>12 mg/kg Aqueous ext.</td>
<td>1.72 ± 0.18 c</td>
<td>5.64 ± 0.18 b</td>
</tr>
<tr>
<td>24 mg/kg Aqueous ext.</td>
<td>2.64 ± 0.37 b</td>
<td>4.40 ± 0.16 c</td>
</tr>
<tr>
<td>12 mg/kg Alcoholic ext.</td>
<td>2.94 ± 0.10 b</td>
<td>3.50 ± 0.17 d</td>
</tr>
<tr>
<td>24 mg/kg Alcoholic ext.</td>
<td>2.78 ± 0.14 b</td>
<td>4.16 ± 0.17 c</td>
</tr>
<tr>
<td>LSD value</td>
<td>0.805 **</td>
<td>0.586 **</td>
</tr>
</tbody>
</table>

Means with different letters in the same column indicated significant differences. ** (P<0.01).

The Effect of Hyperprolactin (Hyper PRL) in Epidydimal Sperm Parameters
It is obvious from Table 2 that hyperprolactin has a negative effect on the sperm parameters and there is a significant decrease in (p <0.01) in all sperm parameters represented by sperm concentration and percentage of the mobile and normal sperms when comparing the experimental group with the control group and the hyperprolactin leads to the emergence of many signs of motility and deformity on the sperms, which
is characterized by the enlargement of the head and the curved tail and the adhesion of the head with the tail and the broken tail with the emergence of the cytoplasmic droplet as well as the emergence of the adhesion of the heads and the adhesion of the tails between the sperms as shown forms (1-4). Those results agreed with (Huang et al. 2008) who concluded that hyper PRL influence the motility of epididymal sperms [21].
Effect of Dosage with Aqueous and Alcoholic Extracts on the Parameters of Epididymal Sperm

The results of the statistical analysis showed a significant improvement (p < 0.01) in most parameters of the epididymal sperms, represented by the percentage of the normal and mobile sperms, as well as the concentration of sperm during the dosage of aqueous and alcoholic extracts of the experimental group as shown in Table 2.

There was a significant improvement (p < 0.01) in both the sperm concentration and the percentage of the normal and mobile sperms when compared to the experimental group (positive control) when using the low concentration of the plant aqueous and alcoholic extracts 12 mg / kg. When comparing between the four concentrations of aqueous and alcoholic extracts that related to the concentration of sperms, observed that the best concentration was the low concentration of aqueous extract of the plant. For the percentage of the abnormal sperms it was observed that the best concentration is 12 mg / kg aqueous low concentration and the least effect is 24 mg / kg. There are no any significant differences between Concentrations 12 and 24 mg / kg were found to be significant for the percentage of abnormal sperms when compared the four concentrations of the mobile sperms, significant differences were observed in the percentage of the mobile sperms (p < 0.01).

The best concentration was the low concentration of the aqueous extract, then the high concentration of the aqueous extract respectively, because the aqueous extracts of Peganum harmala have some adverse effects on all processes of spermatogenesis made effects on testicular tissues including seminiferous tubules directly or indirectly [18]. And we did not notice any significant differences in the percentage of the mobile sperms between the concentrations of 12 mg / kg and 24 mg / kg extract of alcoholic extract of the plant. The results showed that the aqueous extract of the plant is more effective physiologically as reported by many papers as having an antioxidiant effect against reactive oxygen [22]. Other papers mentioned that prolactin was markedly higher, whereas testosterone level was lower compared to those treated with olanzapine [19].

Table 2: Effect of difference treatments in parameters study

<table>
<thead>
<tr>
<th>The Group</th>
<th>Concentration (x 10^6)</th>
<th>Abnormal sperm (%)</th>
<th>Motility sperm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control -</td>
<td>60.60 ± 1.69 a</td>
<td>31.80 ± 3.65 d</td>
<td>86.80 ± 1.15 a</td>
</tr>
<tr>
<td>Control +</td>
<td>12.00 ± 0.95 f</td>
<td>73.20 ± 1.56 a</td>
<td>15.60 ± 1.50 e</td>
</tr>
<tr>
<td>12 mg/kg Liquid ext.</td>
<td>49.80 ± 1.65 b</td>
<td>34.80 ± 1.59 d</td>
<td>79.00 ± 1.30 b</td>
</tr>
<tr>
<td>24 mg/kg Liquid ext.</td>
<td>41.80 ± 1.07 c</td>
<td>43.20 ± 0.92 c</td>
<td>60.80 ± 1.24 c</td>
</tr>
<tr>
<td>12 mg/kg Alcoholic ext.</td>
<td>22.80 ± 1.02 e</td>
<td>51.80 ± 1.07 b</td>
<td>45.00 ± 1.22 d</td>
</tr>
<tr>
<td>24 mg/kg Alcoholic ext.</td>
<td>30.20 ± 1.65 d</td>
<td>46.00 ± 1.22 bc</td>
<td>50.20 ± 0.58 d</td>
</tr>
<tr>
<td>LSD value</td>
<td>5.582 **</td>
<td>6.794 **</td>
<td>5.367 **</td>
</tr>
</tbody>
</table>

Means having with the different letters in same column differed significantly. ** (P<0.01).

References


First Scientific Conference of the College of Agriculture and Forestry, Anbar University (7-8): 1-15.

