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Normal Embryos from Mating Albino Male Mice with Hyperprolactinemia and Treated with Peganum Harmala Seed Extract

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Annotation: The Zygophylaceae family includes Peganum harmala (P. harmala). Its medicinal effects make it important in traditional medicine. This research examined how aqueous extracts from Peganum harmala L. seeds affected male reproductive capability in white mice using olanzapine. The research examined how this therapy affected male mating, fertilization, and pregnancy. The average weight of 90 male mice aged 20–25 days was 25 g. The male mice were divided into three groups. The negative control group of 30 male patients got distilled water injections for 15 days. The positive control group of 30 male Olanzapin users received the medicine for the same time. Finally, 30 male subjects got Olanzapine for 15 days and 15 mg/kg Peganum harmala L. Seed aqueous extracts for 7 days in the third cohort. All experimental groups received six female volunteers before collecting male specimens.

The research found that the second group, who underwent medication therapy alone, could not marry or fertilize after 21 days. This was caused by increased prolactin and low testosterone, which altered sperm properties. However, the third group, which got both the medication and the aqueous extract, had successful pregnancies and births. However, each female in this group had six children, and only two got pregnant. Healthy newborns without outward malformations are notable. This research found that Peganum harmala aqueous extracts significantly affect sperm defects and male hormone levels. Testosterone hormone concentration increased significantly ($p<0.01$), whereas prolactin hormone levels decreased significantly ($p<0.01$).

Keywords: Antipsychotic; Capable of mating mice; Piganum harmala; Prolactin; Testosterone.

1. Introduction

Infertility is defined by the World Health Organization as the inability of a couple to conceive after a year of regular sexual activity [1]. According to reports, 15% of couples of reproductive age experience infertility, which is a widespread and complex issue [2], with men accounting for roughly 50% of instances [3]. Inappropriate lifestyle choices and environmental circumstances are the primary causes of this deterioration [4]. Additionally, the average sperm count and seminal volume significantly decreased, according to a scientific evaluation of men's semen quality that used linear regression of data [5]. Natural aphrodisiacs are therefore in high demand, with a focus on enhancing men's sexual performance, including libido and penile erection. Numerous plants are known to have bioactive chemicals in their leaves, blooms, fruits, and seeds that affect physiological organs, especially the reproductive system. Numerous therapeutic plants have been utilized to treat sexual dysfunction and increase human libido [5,6,7]. Peganum harmala is a wild perennial plant belonging to the Nitrariaceae family that grows in arid soils and deserts in the middle and southern parts of Iraq [8,9]. Beta-carbolines like harmaline, harmine, harmalol, and harman, as well as quinazoline derivatives like vassicine and vasicinone, are among the many potent alkaloids found in this plant, particularly in the seeds and roots [10]. In pharmacology, these substances are crucial [11]. Numerous pharmacological actions, including antiviral, antioxidant, antibacterial, anticancer, cardio-protective, antidiabetic, cerebral-protective, anti-proliferative [12], and anti-inflammatory properties, have been demonstrated by prior research on this plant extract. They also have diuretic and antileishmanial properties [13]. Few in vivo studies have examined the impact of P. harmala

seed extracts on male reproductive function, and the findings are debatable, according to the literature [14,15]. These studies did not examine sexual behavior. It should be mentioned that genetic factors, species variants, cultivation or growth circumstances, and geographical differences of the plant can all affect the biochemical substances [16]. Furthermore, the biochemical composition may change depending on the extraction technique [17]. To the best of our knowledge, only testicular histopathology has been documented to exhibit the activity of P. harmala seed alkaloids grown in Algeria [18]. The present investigation centers on the examination of the impact of the aqueous extract derived from Peganum harmala (P. harmala) seeds on sexual behavior, as well as the sequential events that culminate in the mating behavior of pairs of White mice.

2. Material and Method

The study used a sample of 90 adult males with an average weight of 25 g. These individuals were reared in cages that were appropriately sized to facilitate their mobility, and provided with optimal environmental conditions including a temperature range of 20-25°C, enough light, and proper ventilation. The enclosures were constructed using wooden panels, which were regularly updated every two weeks to provide a hygienic environment for the animals. The animals were provided with standard food as described in reference [19], and access to water was unrestricted.

2.1. The plant

The Peganum harmala seeds (P. harmala) were composed from Baghdad's local markets.

2.2. The drugs

The medicine Olanzapin / 5 mg, produced by Microlabs Limited Company in India, was distributed via local pharmacies**.**

2.3. Preparation of Aqueous Extracts of Peganum harmala Seeds L.

A solution was prepared by combining 60 mg of powdered plant seeds with 250 mL of distilled water. It is advisable to let the suspension in the electric stirrer undergo two hours of suspension before filtering. Following this, the suspension should be passed through four layers of gauze. The leached material should be placed into centrifuge tubes and subjected to centrifugation at a rate of 3000 revolutions per minute for 15 minutes. Subsequently, the supernatant should be carefully transferred to Petri plates and subjected to drying in an oven maintained at a temperature of 40 degrees Celsius. Following the drying process, the powder derived from the seed extract was then transferred to a sterile glass container that was tightly sealed to prevent air exposure. The container was then maintained at ambient temperature until it was ready for use. I apologize, but I cannot provide any assistance without any text or information to work with [20-21].

2.4. Make Hyperprolactin

In this study, 60 male mice were injected with olanzapine for 15 days and then divided into two groups at the injection end period.

The initial positive control group, which was solely given the drug, was made up of six females with the aim of mating.

The second group was injected with the medication and then dosed with the aqueous extract of Peganum harmala L for 7 days, and following the dosing period, they were collected with 6 females for mating**.**

2.5. The process of preparing the drug and aqueous extracts

A quantity of 7 milligrams of Olanzapine was measured and subsequently dissolved in drops of hydrochloric acid (HCl) before being diluted with a solution of physiological saline containing 0.9% sodium chloride (NaCl). The resulting solution, with a particle size of 0.2 millimeters, was administered once daily using disposable syringes. The preparation of aqueous extracts involved the measurement of 15 mg of extract from the two preceding stock extracts. This was done to get the

desired aqueous extracts. These extracts were then combined with distilled water at a concentration of 15 mg, resulting in a daily dosage volume of 0.1 ml.

2.6. Experiments Design

The study divided 90 male mice into three groups. The first was a negative control group, in which 30 males were injected for 15 days with distilled water.

The second group was 30 males who were given the Olanzapin drug for 15 days, and the third group 30 males was given the Olanzapin drug for 15 days and then given extracts and aqueous of Peganum harmala L. Seeds (15 mg/kg) for 7 days. Following the treatment, these males were collected in groups of six females. Blood samples were obtained via cardiac puncture from living animals for hormone testing.

2.7. Hormonal Measurements

The experimental animal serum and control group were analyzed for levels of prolactin and testosterone hormones using the Ichroma 2 boditech-KOREA equipment and its Immunofluorescence technique.

2.8. Statistical Evaluation

Using T-test standards, the mean and standard error were extracted for this study's statistical analysis. Using the [22] software, the least significant difference (LSD) test was used to assess the rate differences.

3. Results and Discussion

3.1. Hormonal Measurements:

In the present study, the experimental group's prolactin hormone concentration was statistically significantly (P< 0.01) increased than the control groups. In addition, the experimental group demonstrated significantly (P< 0.01) decreased levels of the hormone testosterone than the control group. The findings of this study's analysis are consistent with those reported by other researchers [23,24]. However, the statements expressed are in disagree with them [25], There is disagreement about the person who first asserted that prolactin has little effect on the male reproductive system. When compared to the control group, the experimental group showed a statistically significant(p< 0.01) drop in testosterone hormone levels. This finding contrasts with the findings of a separate study project [25], although it is consistent with findings reported in earlier studies [23,24].

Table 1: Effect of aqueous extract of P. harmala seeds on the level of PRL and Testosterone

Group	Mean ± SE	
	Prolactin (ng/ml)	**Testosterone (ng/ml)**
Control -	2.45 ±0.07 b	7.60 ±0.14 a
Control +	8.39 ±0.15 a	2.47 ±0.07 c
15 mg/l	177 ±0.05 c	5.41 ±0.11 b
LSD value	0.293 **	0.312 **
P-value	0.0001	0.0001
This means having the different letters in the same column differed significantly**,** ** (P≤0.01).		

3.2. The Effect of Difference Treatments in Epididymis Sperm Parameters

The statistical analysis showed that hyperprolactinemia had a significant detrimental effect on sperm parameters. When comparing the experimental group to the control group, the results show a statistically significant (p< 0.01) decrease in all assessed sperm parameters, such as sperm concentration, proportion of motile sperm, and proportion of morphologically normal sperm.

Furthermore, it has been demonstrated that hyperprolactinemia plays a key part in the emergence of some symptoms linked to abnormalities and reduced sperm motility. However, after giving the experimental group a solution containing plant extracts at a dose of 15 mg/kg, a substantial improvement ($p< 0.01$) was seen in the majority of epididymal sperm parameters, including the percentage of normal and motile sperm as well as the sperm concentration. Table 2 provides a succinct summary of the results described above. With a p-value of less than $p<0.01$, the outcomes showed a statistically significant improvement. The current study gives conflicting findings when comparing the experimental group, which serves as the positive control, to the data supplied by [26] concerning sperm concentration and the proportion of normal and motile sperm. The study's results show that neither the number of sperm nor the proportion of motile sperm decreased in a statistically significant way ($P > 0.01$). The fraction of defective sperm increased somewhat in both the experimental and control mouse cohorts. The results of this study contradict those of a previous study, which found that a 60-day regimen of a Peganum harmala seed extract decreased both the sperm count and motility in rodents [27]. And this study agrees with [28] who concluded that rodents administered chloropromazine had increased sperm counts when Peganum harmala seeds were exposed to evaporation for 7, 14, and 21 days. The observed disparity in results can be attributed to the specific extraction techniques and duration of the treatment period.

Table 2: The Effect of Difference Treatments in Epididymis Sperm Parameters

Group	Mean ± SE		
	Concentration (x 10^6)	**Abnormal (%)**	**Mobility (%)**
Control -	60.80 ±0.69 a	37.20 ±1.38 b	86.90 ±0.55 a
Control +	13.80 ±0.45 c	75.00 ±0.86 a	15.63 ±0.34 c
15 mg/l	47.60 ±0.87 b	44.40 ±1.06 c	82.40 ±0.79 b
LSD value	1.961 **	3.160 **	1.667 **
P-value	0.0001	0.0001	0.0001
This means having the different letters in the same column differed significantly**,** ** (P≤0.01).			

3.3. The effects of P. harmala seed aqueous extract on White mice couples' sexual behavior and mating sequences.

The findings confirm earlier research by indicating that the aqueous extract made from Peganum harmala seeds (P. harmala) has a positive impact on mating behavior and sexual behavior in pairs of White mice .A study by [29] gave proof of the significant effects of giving rats a 0.20g/ml concentration of an ethanolic extract made from C. arabica leaves over seven days on certain performance indicators and sexual drive. Documentation of several sequences, including interactions, intromission, mounting, mating, and licking, was required to observe and study rat nuptial courtship .

The use of aphrodisiac herbs can successfully promote sexual activity, according to some research that has provided empirical data [30,31]. The effectiveness and accessibility of these approaches may be cited as the most plausible explanation for this phenomenon. According to [32,33] studies, there may be a connection between the androgenic and gonadotropic effects of C. arabica ethanolic extract and the sexual behavior displayed by male rats given Macuminata stem.

3.4. Effect of aqueous extract of P. harmala seeds on the embryos of females impregnated by treated males.

The results show that male mice treated with the drug and then the aqueous extract 15g/ml for seven repeated days affected sexual motivation and, specific performance parameters in male mice, and then got pregnant and gave birth, but the number of births was six children for each female, and the number of pregnant females was two out of a total of six females, as shown in figure 1,2,3. This

finding contradicts the findings of [26] who discovered that male mice administered orally aqueous extract of harmala seeds for three weeks did not affect the number of females impregnated by these males, and their fertility rate was 100%. There was no statistically significant difference ($P > 0.05$) in the percentage of malformed and dead embryos between the treatment groups and the control. These findings contradict a recent study that found harmala seed extract reduced the number of impregnated females by treated males, as well as a drop in the number of viable embryos and a rise in the number of dead embryos [33]. This variation could be attributed to the extraction procedure or the treatment period.

(a)

(b)

(c)

Figure -1 (a-b-c) Normal birth

4. Conclusion

We conclude that treated with aqueous extracts of the Peganum harmala deformities that occur in sperm and are capable of mating and giving normal births, as well as male hormone levels. There was a statistically significant rise in the concentration of testosterone hormone ($p<0.01$) and a statistically significant decrease in the prolactin hormone concentration ($p<0.01$).

5. Acknowledgements

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6. Disclosure and conflict of interest

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing

of this manuscript.

7. Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s) and also collected parental written consent for minor patient.

8. Competing interests

Author has declared that no competing interests exist.

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