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Effect of *Lepidium sativum* Aqueous Crude Extract in Some Fertility Parameters in Mice

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Abstract: ***The uses of traditional plant extract in the treatment of various diseases have been flourished. The present study was aimed to evaluate the effect of Lepidium sativum aqueous extract on the fertility criteria in male mice. Forty-eight mice used in this experiment divided into four groups (12 mice each group). Group 1 (Control), group 2 treated with L. sativum aqueous extract for 2 weeks, group 3 treated with the sulpiride drug to conduct over weight and hyperprolactinemia for 6 weeks and finally group 4 treated with the sulpiride drug for 6 weeks and then with L. sativum aqueous extract for 2 weeks. The results show that the weight does not change over the first three weeks, but there is a significant increase in body weight at the fourth week, especially in the groups (3 and 4). The groups treated with the drug sulpiride showed body weight higher than that of the control group. The group treated with both, drug and LS was showed the higher level of LH, while the group which was treated with LS only showed the higher level of FSH. Prolactin showed its lowest level in the group treated only with LS extract when compared with treated groups. Testosterone showed the higher level in the group treated only with LS extract. The group treated with the drug sulpiride and have a high level of prolactin showed a decline in all the parameters related to infertility. This group had the lowest sperm count (53.33 ± 1.76) sperm/ml, motility (33.33 ± 3.33) % and viability (46.67 ± 1.67) %, in comparison to other groups. In general, there is a significant difference in all the parameters comparison with control group. The group treated with sulpiride drug and has high level of prolactin, showed a decline in all the parameters related with infertility. On the other hand, all the infertility parameters enhanced in the hyperprolactenimic animals which were treated with LS extract. In general there are significant differences in all the parameters comparison with control group. Histological sections for the testis in the group treated with LS only showed a look-like normal appearance of seminiferous tubule with presence of high number of sperms, while sections of hyperprolactinemic mice testis showed partial degeneration and damage of dispersed spermatogonia cells with still presence of sperms inside the lumen with certain morphological abnormality in the shape of the sperms. Sections of treated mice testis showed a look like normal shape and structure of seminiferous tubules with the presence of normal morphology shape sperms in the lumen. The findings of this study highlight the usefulness of using local and easily available plant products and constituents in treating or preventing diseases. The findings are encouraging and warrant further work on the aqueous extract of L. Sativum seeds and its effects on infertility.***

Keywords*:* *Lepidium sativum*, reproductivity, hormones, liver function

1. Introduction

Cruciferous vegetables (Family: Brassicaceae) and their seeds are used in alternative and traditional systems of medicine and healing in many countries. A member of this group of vegetables is the garden cress (*Lepidium sativum* L.) plant and its seeds, which are utilized as nutritional constituents and common ingredients in folk remedies, used mainly in Middle Eastern and Asian countries. *L. Sativum* seeds are re suggested for the treatment of numerous illnesses and they have numerous therapeutic effects [1,2] .

L. sativum known as pepper cress or Rashad belongs to the family Brassicaceae (Cruciferae) and it is an erect, annual herb grows up to 50 cm height. The leaves are variously lobed ,the whole flowers are white small and found in racemes the Fruits are obovate pods, about 5 mm long, with two seeds per pods. Both seeds and leaves contain volatile oils [3].

The plant is consumed and oil of the seeds used in the treatment of dysentery and diarrhea [4], and migraine [5]. The plant was found to contain glucosinolate and glucotropaeolin [6].

Many studies as detailed above have shown that certain constituents of *L. Sativum* and its extracts have chemo-preventive and chemotherapeutic effects, but no studies have been done on the effects of any extracts of *L. Sativum* seeds on the viability and growth of cancer cells. After an extensive search, we aid researchers to study the effects of the aqueous extract of *L. Sativum* seeds on human breast cancer cells [7].

The objectives of this study were to evaluate the activity of *L. sativum* as a treatment for hypert-prolactinemia, which is one of the commonest hormonal disorders, recently in both women and men, and its activity in modulating hormone level in the blood, and increasing fertility of male mice.

2. Materials and Methods

Preparation of *Lepidium stivum* seedsanddosage:
The *L. sativum* seeds were purchased from local markets.The powder of seeds was insoluble in water. So, seeds suspension was prepared directly. The dose used in this research was chosen in view of previous research on *L.sativum*[8,9].

Preparation ofeosin nigrosin stain:
Eosin nigrosin stain prepared by adding 1% eosin (w/v) and 5% nigrosin (w/v) then they were dissolved in 3% Tri-sodium citrate dihydrate solution[10].

Experiment design and biometry:
Forty-eight males albino Swiss mice (*Mus musculus*) their age ranged between 8-12 weeks with an average weight 25 ± 3g were obtained from the National Centre for Drug Control and Research /Baghdad. The mice were acclimatized for two weeks before treatment, they housed in plastic cages containing hard wood chips for bedding, in a controlled animal house at 25± 2C°, 4/10 hour's light / dark cycle, and they were divided mainly into four groups; each group included 12 mice. The mice were given water and fed with the suitable quantity of complete diet. They were housed at the animal house in Biotechnology Research Center/ Al-Nahrain University.

The animals were treated as follows:
Group (1) Control / Treated orally with phosphate buffer saline for 2 weeks.
Group (2) LS / Treated orally with *Lepidium sativum* suspension for 2 weeks.
Group (3) Hyper/ Treated orally with sulpiride suspension for 2 weeks.
Group (4) Treated orally with sulpiride suspension for 6 weeks and then treated orally with *Lepidium sativum* suspension for 2 weeks.

Sulpiride was given for 2 weeks in order to increase weight gain, hyperplasia, hyperprolactinemia, and hypogonadism in the animals according to [11] .

The water suspension of 2g /100 ml D.W (20 mg/ml) of *L. sativum*was prepared as an oral dose. The suspension was shacked before used and gavaged daily to animals using mice gastric tube (0.2 ml/animal/day) [9].

At the end of the experiment, The animals weighted and the blood was obtained by puncture of heart, centrifuged at 3000 r/m. for 10 minutes and the animals were sacrificed.The testis was immediately excised and preserved in 10% formalin for histological study, the serum was stored at -80° C and used to determine the levels of the following parameters:

Hormones: LH, FSH, prolactin, and testosterone (ELIZA kit /Orgmetric/Germany).

Sperm analysis: Included three parameters, sperm concentration estimated according to the method of [12], sperm motility was recorded according to[13], whereas the percentage of sperms were measured according to the method of [14].

- **Sperm concentration:** A drop of sperm suspension was placed on a slide and covered with a coverslip. The concentration of sperms was calculated from the mean number of sperm in five high power microscopy fields under magnification of 400X. This number was multiplied by a factor of one million (× 10^6 sperm/ml).
- **Sperm motility:** Sperm suspension (50 µl) was placed on a slide and covered with a coverslip. By light microscope, several fields were examined to estimate the percentage of individual motility of sperms.
- **Sperm viability:** A drop of sperm suspension was mixed with a drop of eosin stain (1%) and two drops of nigrosin stain. A thin smear of the semen – eosin – nigrosin mixture was done using other slide and left to dry. Then the slide observed under a light microscope, the dead sperms stained with red color while live sperms were not stained. The amount of 200 sperms were counted to calculate the percentage of viability sperms as in the following equation:

$$\textbf{Sperm viability \%} = \frac{\textbf{No.of viable sperms}}{\textbf{Total No.of sperms}} \times \mathbf{100}$$

Histological study: The histological study was conducted according to the method used by[15].
Statistical analysis: The Statistical Analysis System- SAS (2010) [16] was used to study the effect of different factors in studied parameters. Least significant difference –LSD test was used to significant compare between means in this study.

3. Results and Discussion

Body Weight
As shown in the table (1) the weight does not change over the first weeks, but there is a significant increase in body weight at the fourth week, especially in groups (3) and (4). The treated groups with sulpiride showed increasing body weight comparison with control group.

Table 1: Animal weight in the treated groups during five weeks of the experiment:

Week	Mean ± SE				LSD Value
	Group 1 Control	Group 2 LS	Group 3 Hyper	Group 4 Treated	
0	37.08 ± 1.002	35.50 ± 2.60	36.97 ± 0.65	35.91 ± 1.63	4.615 NS
1	37.34 ± 1.02	35.98 ± 1.68	37.03 ± 0.79	36.48 ± 1.60	4.126 NS
2	37.54 ± 1.02	36.38 ± 1.67	37.58 ± 0.41	33.44 ± 2.67	4.240 NS
3	37.76 ± 0.99	37.08 ± 1.74	39.89 ± 0.83	38.10 ± 3.98	5.024 NS
4	38.14 ± 1.03	37.90 ± 1.08	41.93 ± 1.99	40.95 ± 0.70	3.674 *
5	37.08 ± 1.00	38.20 ± 1.07	42.37 ± 0.60	40.47 ± 1.68	3.605 *
LSD Value	2.962 NS	4.886 NS	2.579 *	6.661 *	----
* (P<0.05).					

Excessive body weight gain is often observed during chronic administration of typical and atypical antipsychotic drugs (AP) in psychiatric patients [17]. The sulpiride is a typical antipsychotic drug. So; increasing body weight will appear during administered animals with sulpiride.Prolonged administration of diverse AP also increases body weight in female rats[18]. During sulpiride treatment (a D2–D3 dopamine receptor antagonist[19], the rats display hyperphagia[20], hyperprolactinemia[21]and disruption of the vaginal cycle suggesting drug-induced hypogonadism [22].

It is found that the seeds of *L. sativum* increase body weight as they contain 18-24% of fat and about 34% of the total fatty acids are an alpha linolenic acid which could givenutritional advantages[23]. The primary fatty acids in *L. sativum* oil were oleic (30.6 wt. %) and linolenic acids (29.3 wt. %) and was found to contain high concentrations of

tocopherols. It contains a good amount of lignans and antioxidants, which can balance out the n-3 polyunsaturated fatty acids in seed oil. The essential phytosterols in *L. sativum* were sitosterol and campesterol and avenasterol[24]

Hormones

There is a significant increase in prolactin level (4.20 ± 0.17) ng/ml in the group treated with the drug sulpiride (group 3) comparison with the control which was considered normal since the sulpiride belongs to the antipsychotic drugs.

As knew before, the hyperprolactinemia is one of sulpiride adverse effects caused by the prolonged use of those drugs [18].While the co-use of both sulpiride and*L. sativum*extract in group 4 showed less increasing in prolactin level (3.40 ±0.15) as revealed in the table (2).

L. sativum have hypoglycaemic activity and an aqueous *L. sativum* extract in an acute (single dose) or chronic oral treatments, prompts a significant decrease in blood glucose levels in streptozotocin-induced diabetic rats, there is a marked normalization of glycemia [25] .When there is a decrease in glucose level there is a decrease in prolactin level since the correlation between them is positive [26].

Prolactin influences carbohydrate metabolism and insulin sensitivity, through effects on insulin receptors [27,28]. Elevated prolactin levels are associated with hyperglycemia, hyperinsulinemia and insulin resistance relative to controls in animal studies[29] . Similarly, in humans, hyperprolactinemia secondary to pituitary adenomas is associated with elevated glucose and insulin levels, an9d insulin insensitivity compared with controls. Treatment with bromocriptine to reduce prolactin levels over two months is associated with reductions in glucose and insulin levels [30].

The results in table 2 revealed that the higher level of LH was (0.556 ± 0.037) in the group 4, which is treated both with the drug and LS. While a higher level of FSH was (82.73 ± 1.06) in group 2, which was treated with LS only. Same with testosterone level, the higher value (0.290 ± 0.01) was in the group 2. Prolactin showed the lowest level in the group treated only with LS extract when compared with other treated groups. Prolactin level in group 4 (hyperprolactinemic – LS) was lower than its level in group 3 (Hyper-prolactinemic), this is referred to the role of LS in lowering prolactin level in the blood. The results of testosterone support this role, which showed its higher level in the group treated only with LS extract.

In infertile persons, a positive relationship correlates between prolactin and thyroid stimulating hormone (TSH). At the same time, a negative relationship correlates between prolactin and Leutinizing hormone (LH), Follicle stimulating hormone (FSH) and T3.

Table 2: Hormones levels in the treated groups at the end of the experiment

Hormone	Mean ± SE				LSD Value
	Group 1 Control	Group 2 LS	Group 3 Hyper	Group 4 Treated	
LH (IU/L)	0.306 ± 0.029	0.137 ± 0.008	0.350 ± 0.017	0.556 ± 0.037	0.084 *
FSH (IU/L)	46.33 ± 0.78	82.73 ± 1.06	77.23 ± 1.49	42.13 ± 1.47	4.041 *
Prolactin (ng/ml)	2.10 ± 0.06	2.67 ± 0.09	4.20 ± 0.17	3.40 ± 0.15	0.413 *
Testosterone (ng/ml)	0.039 ± 0.003	0.290 ± 0.01	0.146 ± 0.01	0.113 ± 0.01	0.046 *
* (P<0.05).					

Therefore, chronic hyperprolactinemia and hypothyroidism considered the most important causes of infertility because the high levels of prolactin may develop ovulatory dysfunction. TSH level of all females should be measured at early age todiagnose if there is any subclinical thyroid problem and to prevent later infertility risk [31].

High levels of serum prolactin (PRL) result in decreased kisspeptin expression in Kiss1 neurons in both the hypothalamic arcuate (ARC) and anteroventral periventricular (AVPV) nuclei, So, inhibition of kisspeptin, in turn, decrease the release of GnRH and cause a loss of the ovulatory GnRH surge. This may decrease the ability of pituitary gonadotropin to secrete (LH and FSH) and loss of ovarian stimulation and consequently results in hypogonadism, infertility, and amenorrhea.Hyperprolactin may affect on Gonado-trophic releasing hormone (GnRH) neurons and pituitary gonadotropes, afferent neurons [32].

Seminal parameters:

According to the results in the table (3), the group treated with the drug sulpiride (which had a high level of prolactin) showed a decline in all the parameters related to infertility. This group was had the lowest sperm count (53.33 ± 1.76) sperm/ml, motility (33.33 ± 3.33) % and viability (46.67 ± 1.67) %, comparison to other groups. On the other hand, all the infertility parameters enhanced in the hyperprolactenimic animals were treated with LS extract. Also, there is a remarkable increasing in the motility of sperm in the group treated with LS in comparison with the control. In general, there is a significant difference in all the parameters in comparison with control group.

Lepidium meyenii (Maca) and *L. sativum* , both are a traditional cruciferous vegetable used in the distant past, belongs to the same family (Brassicaceae).Dry Maca hypocotyls contain 59% carbohydrates, 10.2% proteins, 8.5%fiber, 2.2% lipids and a number of other compounds including most of the essential amino acids Arginine, that has been proven to have a good effect on male fertility and increasing sperm production[33].

Maca also contains sterols, such as campesterol, stigma sterol and β-sitosterol and β-Carbolines, it is found that β-Carbolines have an antioxidant properties and inhibits apoptosis of sperms , as a result sperms protected and spermatogenesis improved [34,35].Gonzales *et al.*, 2001,

[36]report that male rats orally administrated an aqueous extract from the roots of *Lepidium meyenii* (Maca) in a dose of 666.6 mg/day for 14 days show an improvement inspermatogenesis by acting on first mitosis stages.Also, it is found that Macaimproves sperm count and sperm motility in normal men without affecting serum testosterone, LH and FSH levels[37].*Lepidium sativum* seeds have high nutritional value and functional ingredient[38].The oil of the seeds contains tocopherol, phenolic compounds, nitrogen compounds, terpenoids, and some other metabolites, that have an antioxidant activity [39]. It has been found that the seeds of *Lepidium sativum*contain two groups of fat-soluble compounds, the tocopherols (Vitamin E), which comprises of (21ppm) alpha-tocopherol and (1422 ppm) gamma-tocopherol and (35ppm) Sigma-tocopherol, and the tocotrienols [40].

Table 3: Seminal parameters values of treated groups at the end of the experiment

Seminal parameters	Mean ± SE				LSD Value
	Group 1 Control	Group 2 LS	Group 3 Hyper	Group 4 Treated	
Count (million)(sperm/ml)	386.67 ± 44.67	365.33 ± 9.27	53.33 ± 1.76	245.0 ± 22.54	85.90 *
Motility (%)	82.67 ± 1.45	91.67 ± 1.67	33.33 ± 3.33	85.00 ± 2.88	804 *
Viability (%)	85.00 ± 2.89	83.33 ± 1.67	46.67 ± 1.67	83.33 ± 1.67	6.65 *
* (P<0.05).					

The human body can not synthesizetocopherol(Vitamin E), therefore it should be obtained from the diet sources asvegetable oil, nuts, and egg yolks[41], and has a beneficial effect on viability, membrane integrity and motility of spermatozoa. Tocopherol administration to male rabbits increases fertility[42].Tocopherol has an antioxidant activity and protect sperms and other body tissues through its ability to inhibitalpha-tocopherol enzyme protein Kinase, and thus reduces the reactive oxygen species[43].

Histological study

Histological sections for the testis for LS group showed a look-like normal appearance of seminiferous tubule with the presence of a high number of sperms (Figure 3,4), while sections of hyperprolactinemic mice testis showed partial degeneration and damage of dispersed spermatogonia cells with the still presence of sperms inside the lumen with a certain morphological abnormality in the shape of the sperms (Figure 5,6). Sections of testis for treated mice showed a look-like normal shape and structure of seminiferous tubules with the presence of normal morphology shape of sperms in the lumen (Figure 7,8).

The histological sections of the testis in the hyperprolactinemic group reflect the morphological changes that may cause by disorders in the reproductive hormones. Hyperprolactinemia causes infertility in around 11% of oligospermic males by inhibiting the pulsatile secretion of the gonadotrophin releasing hormone (GRH), which lead to decrease the releasing of FSH, LH, and testosterone[44].Chronic hyper-prolactinemia in men partly suppresses LH secretion by its inhibitory action on the hypothalamus [45], which in turn causes spermatogenic arrest, impaired sperm motility, and altered sperm quality. It later produces secondary hypogonadism and infertility[44].

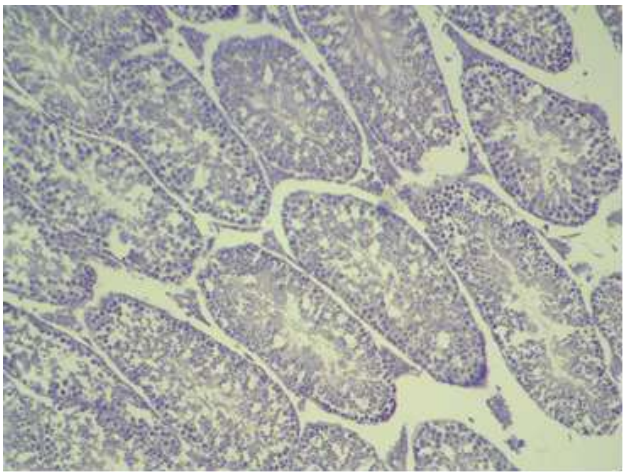

Figure 1: Histological section of control mice testis showing normal structure appearance of seminiferous tubules (X250)

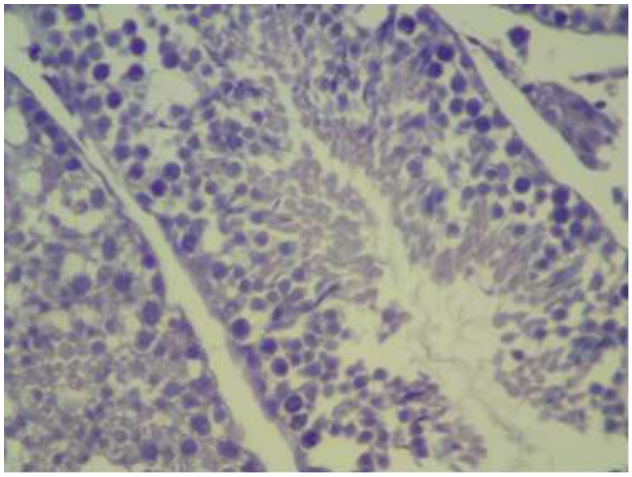

Figure 2: Histological section of control mice testis showing normal development of spermatogonia cells with presence of sperms inside the lumen (X400)

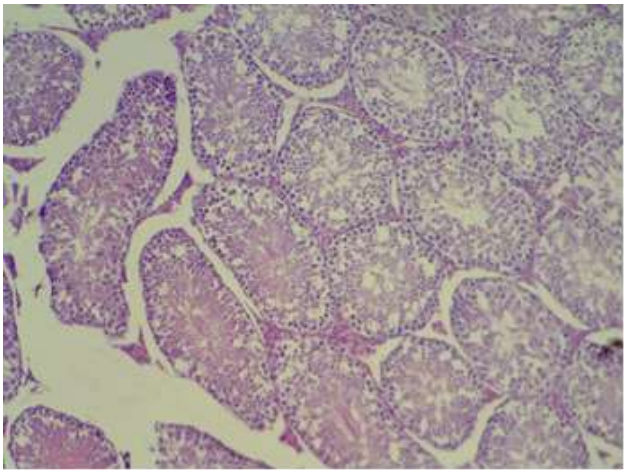

Figure 3: Histological section of SL mice testis showing look-like normal appearance of seminiferous tubule with presence of high number of sperms (X250)

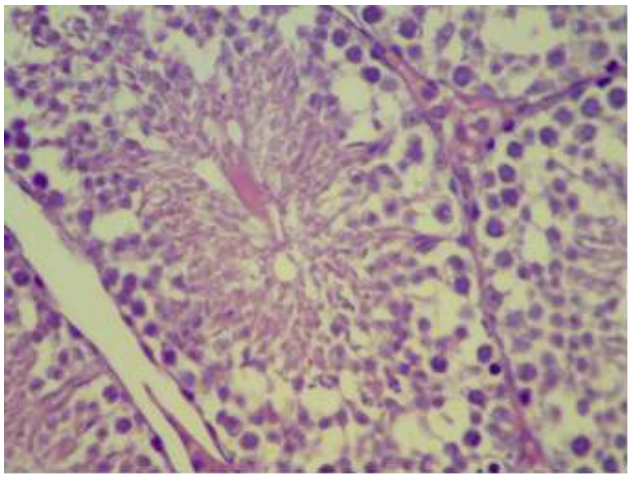

Figure 4: Histological section of SL mice testis showing look-like normal appearance of seminiferous tubule with presence of high number of sperms (X400)

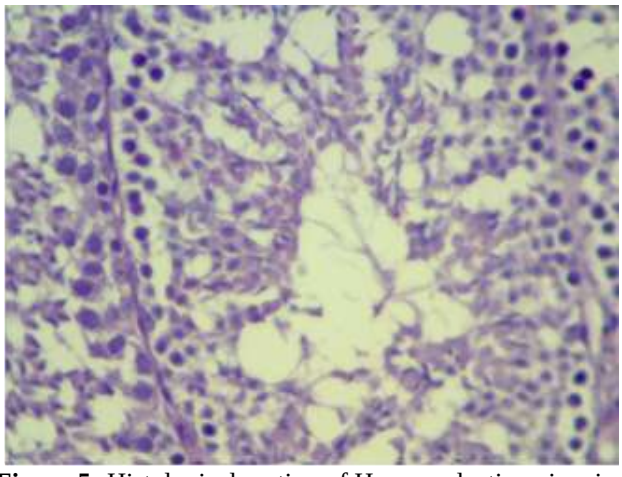

Figure 5: Histological section of Hyperprolactinemia mice testis showing partial degeneration and damage of dispersed spermatogonia cells with still presence of sperms inside the lumen (X400)

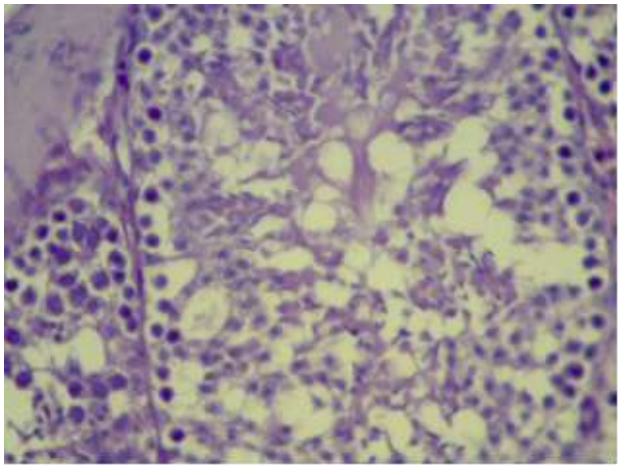

Figure 6: Histological section of Hyperprolactinemia mice testis showing certain morphological abnormality in the shape of the sperm (X400)

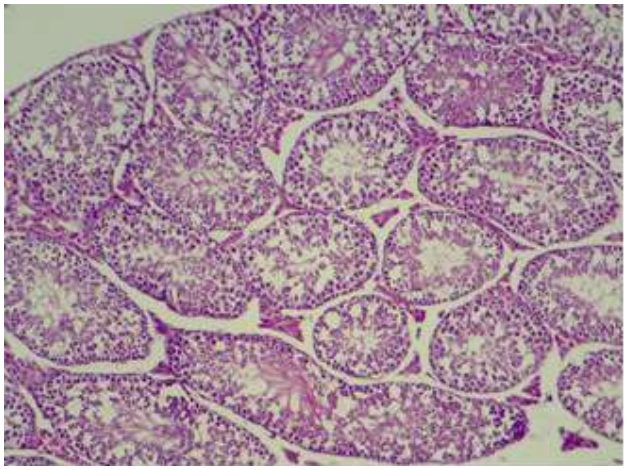

Figure 7: Histological section of treated mice testis showing look like normal shape and structure of seminiferous tubules with the presence of sperms in the lumen (250)

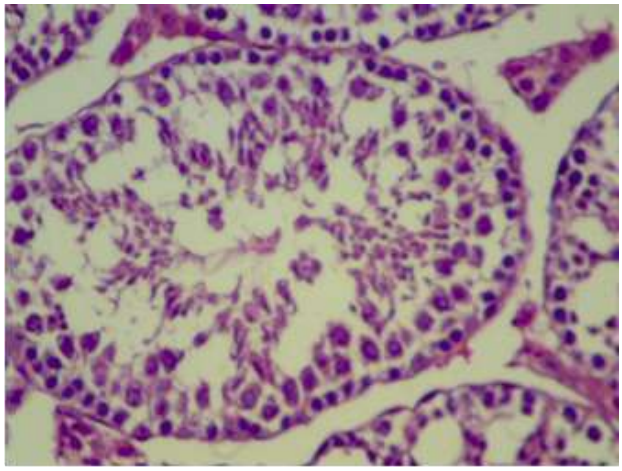

Figure 8: Histological section of treated mice testis showing look like normal shape and structure of seminiferous tubules with the presence of sperms in the lumen (X400)

The histological sections of the testis in the groups treated with only LS show significant improvement in testis structure and sperm functionality criteria, and the hyperprolactinemic group treated later with LS reflect the regression in the side effect caused by hormone disorders and prolactin high levels. Those results confirm the

curability role of *Lepidium sativum* seeds and confirm its ability to reduce risks of infertility. Therefore, according to this study, such treatments are recommended as it may be prepared and administered at home by the patient himself.

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