

The protective effect of *Fumaria officinalis* against the testicular toxicity of fluoxetine in rat

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Abstract

Background and objective: Extracts of *Fumaria* species have been traditionally used for the treatment of some skin diseases, rheumatism, stomach ache, fever, and male infertility mainly because of the presence of isoquinoline alkaloids. On the other side, fluoxetine is used as an antidepressant induce sexual dysfunction and a decrease in sperm concentration, motility, and morphology. The present investigation dealt with the study of the protective role played by the *Fumaria officinalis* extract against the selective serotonin reuptake inhibitor (SSRI) induced testicular toxicity in the rat. Therefore, the purpose of this work was to determine the benefits of *Fumaria officinalis* extract in animals treated with fluoxetine.

Methods: Thirty six male rats were divided into three following groups, control group, fluoxetine (20 mg/kg/day) treated-group, and the same dose of Fluoxetine plus *Fumaria officinalis* extract (150 mg/kg/day) as a third group. Drugs and extract were administered orally for 30 days. Sections of testes were stained by Haematoxylin and Eosin.

Results: Fluoxetine has caused several structural changes in the rat testis such as vacuolation within germinal epithelium of seminiferous tubules, decrease the diameter of these tubules and decrease number of sertoli and germinal cells. Treating the fluoxetine exposed rats with *Fumaria officinalis* extract has shown to ameliorate the above histological changes.

Conclusion: *Fumaria officinalis* extract can protect testis from histological alterations caused by the toxicity of the fluoxetine.

Keywords: *Fumaria officinalis*; Fluoxetine; Seminiferous tubules; Erbil city.

Introduction

Fluoxetine {FLX} (N-Methyl-γ-[4-(trifluoromethyl) phenoxy] benzene propanamine) is a Selective serotonin reuptake inhibitors (SSRI) and was approved by the US FDA in 1987. It is widely used to treat several disorders, such as major depression, anxiety, and premenstrual dysphoric disorder.^{1,2} Pharmacological evidence demonstrated that fluoxetine is a fluorine – including SSRI drug acting as an antidepressant agent with high absorption after oral administration.³ Fluoxetine inhibits the 5-hydroxytryptamine (5-HT) (Serotonin)

reuptake. Increased synaptic availability of serotonin stimulates a large number of postsynaptic (5-HT) receptor subtypes which lead to complex secondary responses including gastrointestinal disturbances and sexual side effects which include loss of libido, delayed ejaculation, anorgasmia and impaired orgasm, decreased testicular development and decreased sertoli cell population, which may lead to infertility in adults.⁴ Elevation of the cerebral levels of 5-HT affects the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH), for inhibiting the liberation of gonadotrophins

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releasing factor (GnRH) in the hypothalamus, with subsequent effects on the process of spermatogenesis and steroidogenesis in adult rats.⁵ Serotonin plays a role in spermatogenesis, and excess serotonin can lead to sperm dysfunction and its negative effects on semen parameters.⁶ Long-term ingestion of fluoxetine caused a decrease in spermatogenesis, testosterone, follicle stimulating hormone levels, and weights of reproductive organs in rats.⁷ Antidepressant medications can influence levels of sex steroids by altering the metabolism and protein binding of hormones. Additionally, they may influence fertility through peripheral effects as a result of their action on cholinergic and α -adrenergic receptors as well as through their effects on the production of viable spermatozoa.⁸ Fumitory species comprise the genus *Fumaria*, which belongs to the family Fumariaceae. There are around 60 species widely distributed all over the world and especially in the Mediterranean region, seven of which are *Fumaria parviflora*, *Fumaria officinalis*, *Fumaria densiflora*, and *Fumaria capreolata*.⁹ Fumitory is an annual plant of somewhat variable characteristics, often resembling a bush but also growing as a low trailing shrub. The plant is widespread and can be found in gardens, slopes, and wastelands.¹⁰ Previous phytochemical analysis of *Fumaria officinalis* has shown the presence of alkaloids such as protopine, cryptopine, stylopine, N-methylsineactine, fumaritine, fumariline, sanguinarine and polyphenols such as rutin and apigenin.^{11,12} *Fumaria* has been traditionally used for the treatment of rashes, fever, diarrhea, syphilis, and leprosy,¹³ also has aphrodisiac activity¹⁴ as well diuretic, depurative, anti-inflammatory, anti-arrhythmic, antioxidant and antimicrobial activities.¹⁵ The therapeutic effect of different *Fumaria* species for male infertility has been mentioned,¹⁶ but none was *Fumaria officinalis* included. The aim of the present study was to evaluate the

protective effect of aerial parts *Fumaria officinalis* extract grown in Erbil, Kurdistan region against fluoxetine testotoxicity.

Methods

This study was carried out during the period of December 2017 to February 2018. The present work has been achieved on 36 male Wistar rats (6-month) with an average weight of 180-200 g. All the groups of rats have been housed separately in different cages. The rats were divided into three groups (12 rats in each). The 1st group (A) rats received 1ml of normal saline for one month and considered as a control for groups B and C. The 2nd group (B) was injected orally with only 1ml of 20 mg/kg/day of fluoxetine. The 3rd group (C) was injected with both 20 mg/kg/day of fluoxetine and 1ml 150 mg/kg/day of *Fumaria officinalis* orally by gavage. Experimental animals in each group treated daily for 30 days. They were maintained at temperature ($22 \pm 3^\circ\text{C}$) under a 12 h–12 h light–dark cycle with 50% to 60 % humidity for at least one week before starting the experiment. Fluoxetine was dissolved in distilled water and was given orally to animals by gavages at dose 20 mg/kg body weight, equivalent to the therapeutic dose for humans, which is 20-60 mg/day recommended range.¹⁷ All experimental procedures in this study were approved by the Medical Research Ethics Committee of Pharmacy College in Hawler Medical University.

Plant materials

Aerial parts (leaves and stems) were collected from their natural habitats in Kurdistan region, Iraq. The Aerial parts were cleaned, cut into small pieces, air-dried under shade for 5-7 days, and stored in bottles until use. The identity of the plant was confirmed by the Department of Pharmacognosy, College of Pharmacy, Hawler Medical University, Iraq (Voucher No.A3).

Preparation of plant extract

The dried aerial parts (400 g) were powdered in an electric grinder and extracted with ethanol 70% using ordinary reflux for 2hr, then extract was filtered, and the filtrates were evaporated to dryness at low temperature under reduced pressure and the extract left behind (yield was 7 g/kg) was stored at 4^oc. It was dissolved in distilled water whenever needed for experiments.

Histological examination of the testes

After 30 days of exposure, both testes of all rats were dissected out after sacrificing the rats of all groups under ether anesthesia. The tissues were fixed in bouin fluid (picric acid 75 ml + Formalin 25ml + Glacial acetic acid 5 ml), processed, and blocks were made in paraffin wax. 4-5 μ m thick sections were cut by a rotary microtome and stained with Haematoxylin and Eosin (H&E). The sections were examined in the light microscope.

Morphometric analysis

Samples were analyzed using a motic plus2 microscope with an attached camera. All images were acquired digitally using Magna Fire Software (Optronics, Goleta, Calif). The testis net weight in rats was determined by the reduction of 6.5% from its gross weight. The reduction is related to the percentile of albuginea and mediastinum weight in rat testis (Testis Net Weight = Testis gross weight - 6.5%). The germ cell nuclei and sertoli cell nucleolus at stage VII were counted in 10 round seminiferous tubules cross sections, chosen at random for each animal.¹⁸ The number of sertoli nucleus and vacuoles per each section were enumerated. Tubular diameter, epithelium height, were obtained by means of two diametrically opposite measurements. The stained sections were studied under a light microscope to evaluate spermatogenesis. Johnsen's criteria were used to categorize spermatogenesis. This system describes the preservation of spermatogenesis, on a scale from 1 to 10, according to the absence or presence of the main cell types

arranged in order of maturity.¹⁹ A score of 1 to 10 was given to each tubule according to the maturity of the germ cells: a score of 1 indicated no seminiferous epithelial cells and tubular sclerosis. A score of 2 indicated no germ cells, only Sertoli cells. A score of 3 indicated spermatogonia only. A score of 4 indicated no spermatids, few spermatocytes, and an arrest of spermatogenesis at the primary spermatocyte stage. A score of 5 indicated no spermatids and many spermatocytes. A score of 6 indicated no late spermatids, few early spermatids, the arrest of spermatogenesis at the spermatid stage, and disturbance of spermatid differentiation. A score of 7 indicated no late spermatids and many early spermatids. A score of 8 indicated a few late spermatids. A score of 9 indicated many late spermatids and disorganized tubular epithelium. A score of 10 indicated full spermatogenesis.

Statistical analysis:

The statistical analyses were carried out with the statistical package for the social sciences (version 24). Descriptive statistics were used to calculate the means and the standard deviations (SDs). One way ANOVA and Bonferroni were used to compare all groups. A *P* value of less than or equal to 0.05 was considered as statistically significant.

Results

At the end of the experiment, it was observed that groups (B) and (C) rats, which were administrated fluoxetine for 30 days, showed a reduction in testis weight in comparison with the group (A) as a control group (*P* = 0.003). While no significant difference in testis weight was reported in group (C) compared to group (B) as shown in Table 1. The routine histological section showed a normal appearance of the testis in the control group (A) in which seminiferous tubules are lined with stratified epithelium and consists of spermatogenic cells and sertoli cells were seen to be abundant

and healthy. The seminiferous tubule had a germinal epithelium without vacuoles a small lumen filled with sperm tails, and (Figure 1 and 2).

Table 1: Statistical analysis within groups A, B, and C.

P(ANOVA)		P (Bonferroni)
Testis net weight 0.003	AxB	0.003
	AxC	0.052
	BxC	0.871
Seminiferous tubule diameter <0.001	AxB	< 0.001
	AxC	0.151
	BxC	0.006
Epithelium height <0.001	AxB	< 0.001
	AxC	0.119
	BxC	0.003
Sertoli cell number <0.001	AxB	< 0.001
	AxC	0.280
	BxC	0.012
Vacuole number <0.001	AxB	< 0.001
	AxC	1.000
	BxC	<0.001
Johnsen's Score <0.001	AxB	<0.001
	AxC	0.012
	BxC	0.008

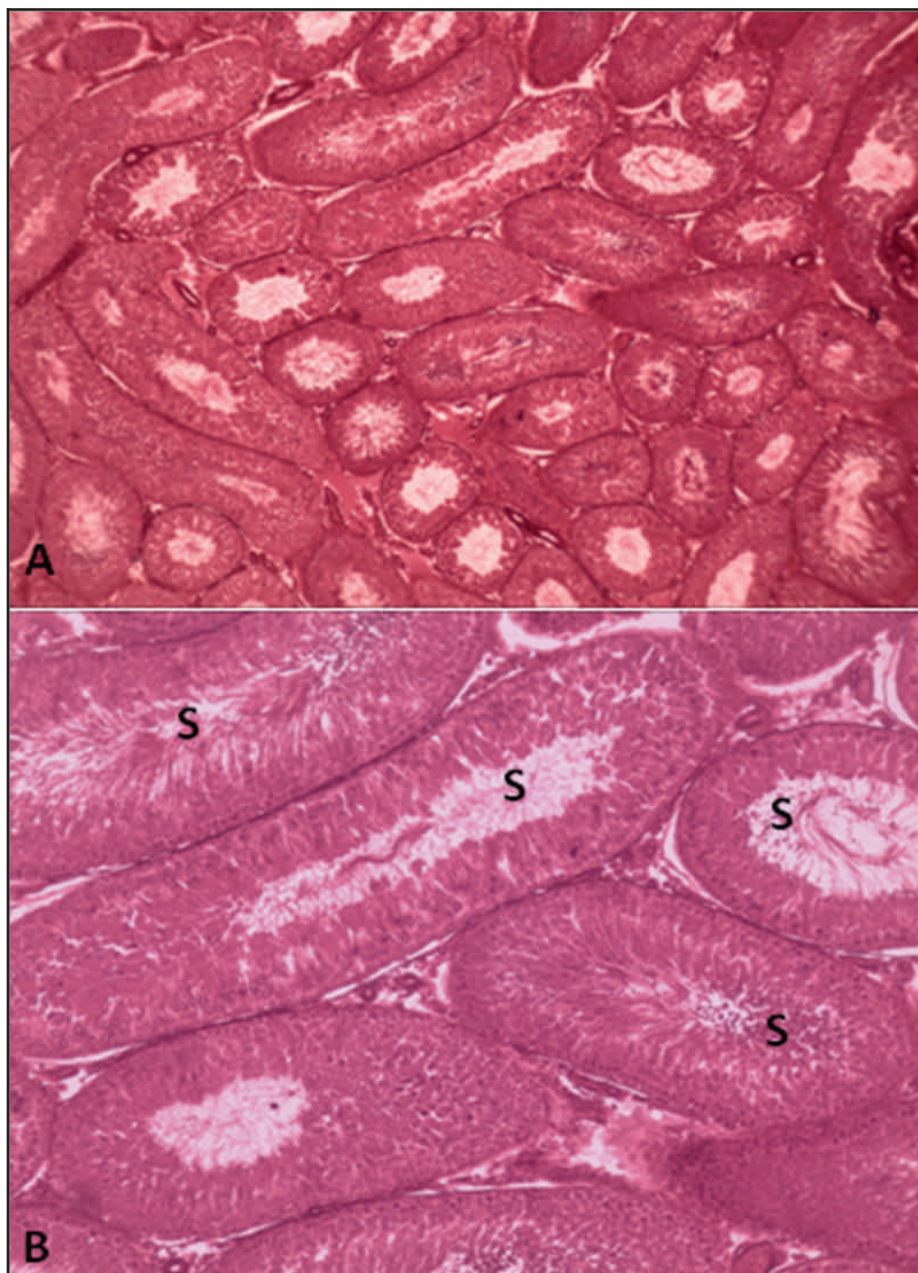


Figure 1: Sections through the testis of rat in the control group showing the normal histological structure of seminiferous tubules (S) with lumen filled with spermatids. H&E, A) 40x,B)100X

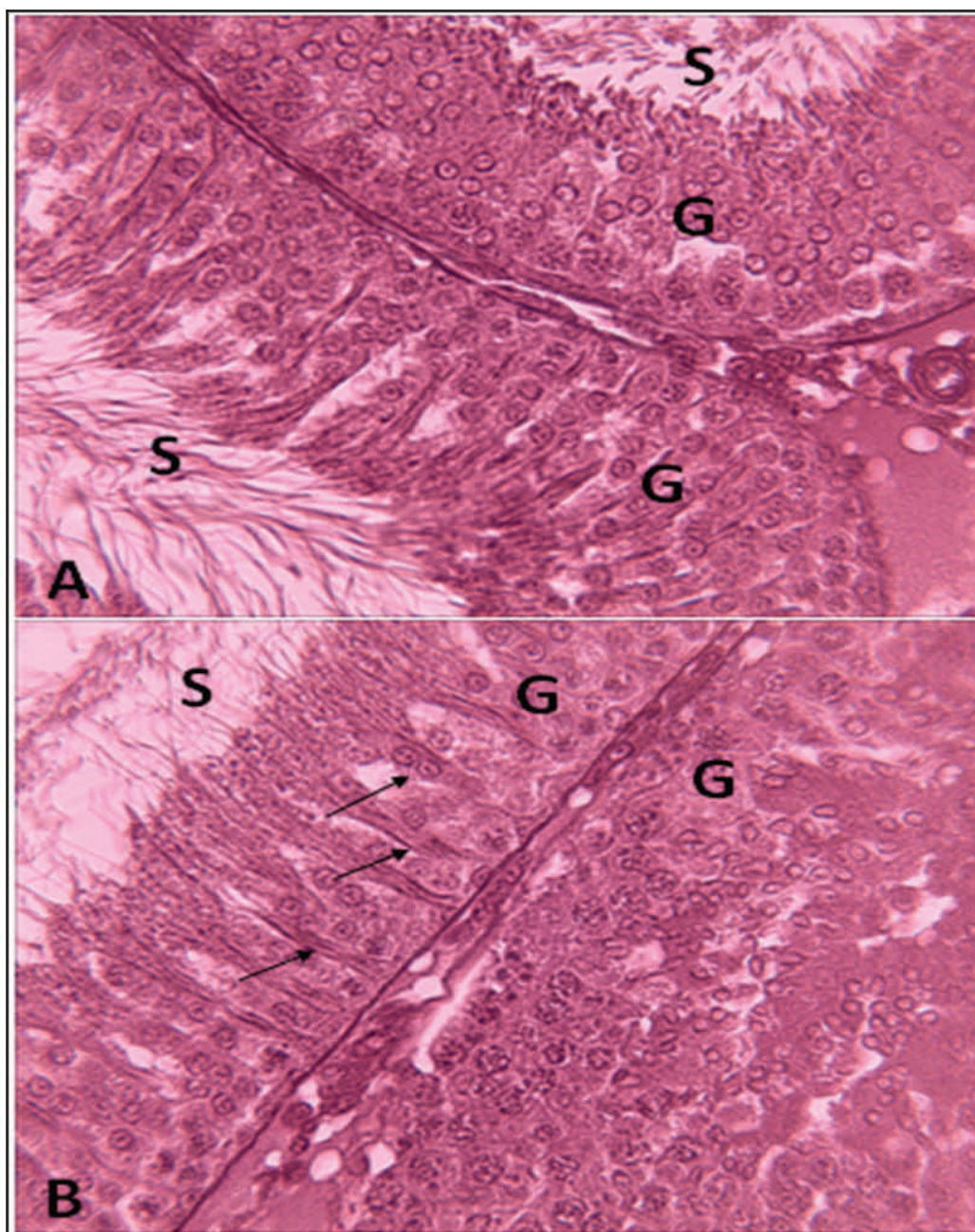


Figure 2: Sections through the testis of rat in the control group showing the seminiferous tubules (S) and the normal appearance of the germinal layer (G), (arrows): Sertoli cells. Both Figures are 400X H&E

In comparison with the control group (A), the testes of the fluoxetine treated group (B) showed several histological alterations, as shown in Table 2, a significant decrease in the diameter of seminiferous tubules and thickness of its germinal epithelium ($P < 0.001$). Signs of distortion of the seminiferous tubules, approximately empty lumen and depleted germinal layer were

observed. Sertoli cells per transversal section were found to be decreased statistically in number ($P < 0.001$). Many large vacuoles within germinal epithelium have also appeared. Furthermore, damaging of boundary and the interstitial tissue which connect the seminiferous tubule was also seen (Figures 3-5).

Table 2: Biometric aspects of the testis in the three groups (mean±SD).

Parameters	Group (A) N=12	Group (B) N=12	Group (C) N=12
Testis net weight (g)	1.14± 0.12	0.89± 0.19	0.96± 0.18
Seminiferous tubule diameter(μm)	302.08 ±7.21	275.91 ± 14.83	292.16± 12.53
Epithelium height (μm)	95.25 ± 4.90	79.08 ± 8.17	89.25± 7.11
Sertoli cell number / cross Section	7.75 ± 0.96	5.41 ± 1.08	6.91± 1.44
Vacuole number / cross Section	0.58 ±0.66	2.25 ± 0.62	0.83± 1.02
Johnsen's Score	9.41 ± 0.66	6.3 3± 1.55	7.91± 1.16

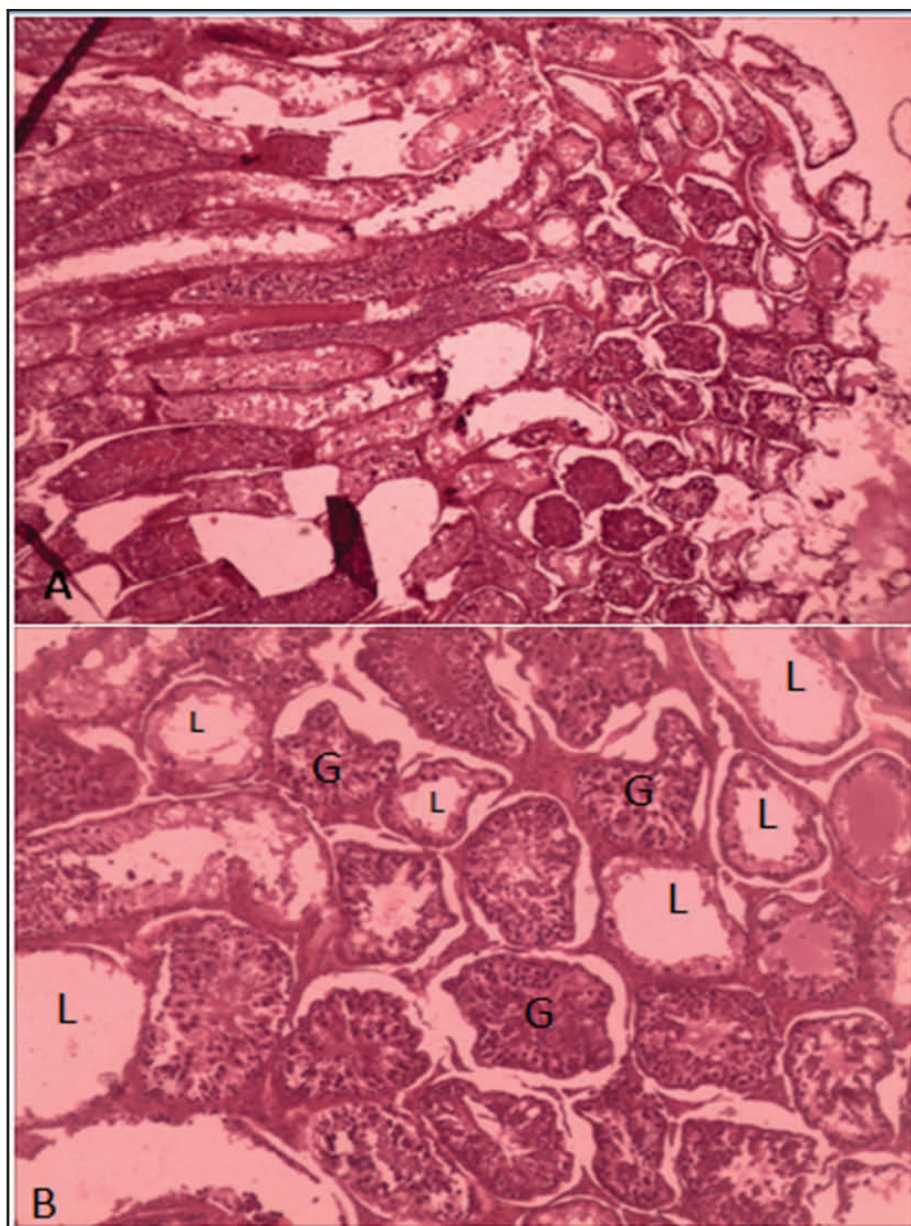


Figure 3: Sections through the testis of rat in fluoxetine treated group showing the Shrunken seminiferous tubules, some of which appear with the empty lumen (L) and the depleted germinal layer (G). Both Figures are 400X H&E

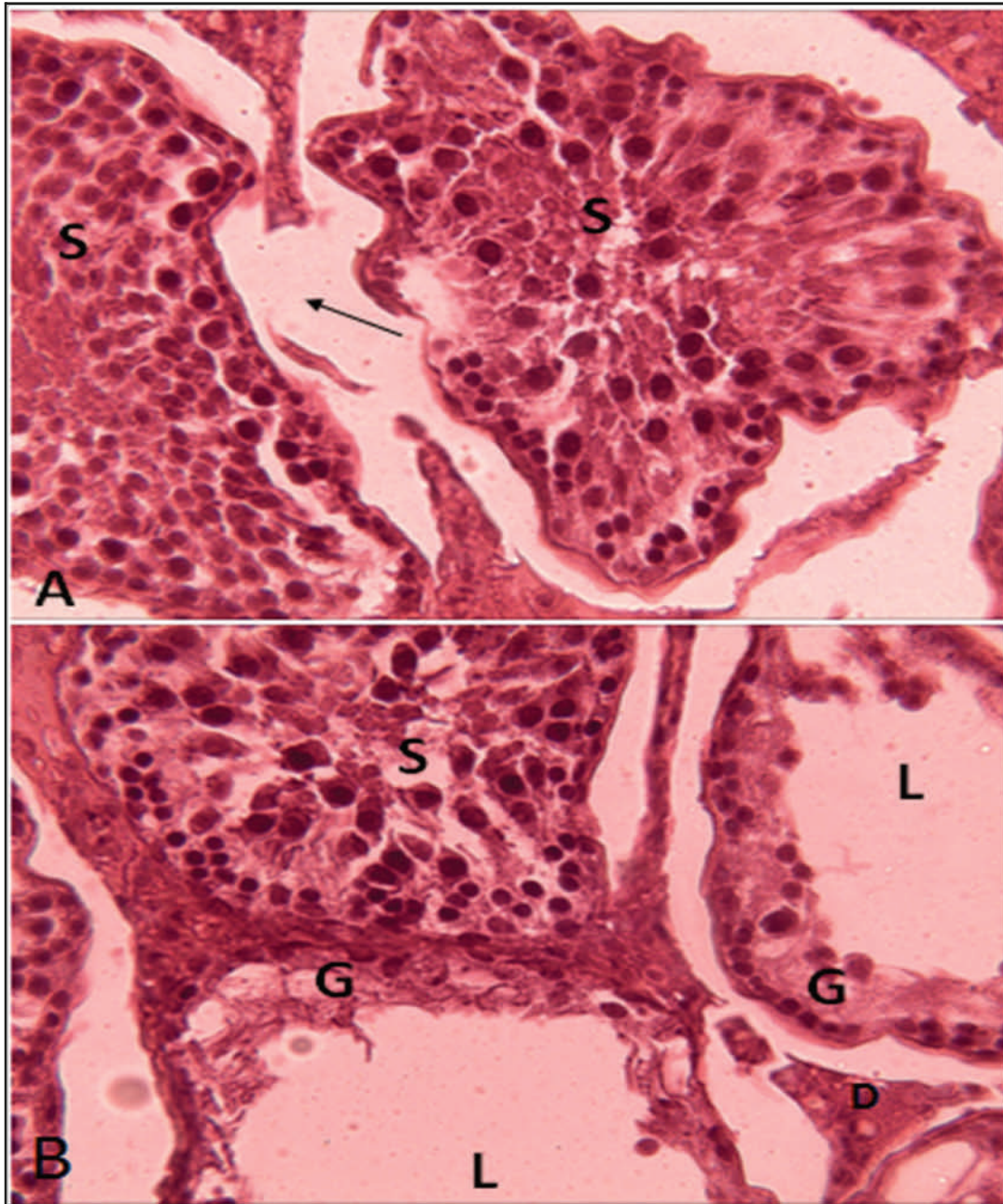


Figure 4: Higher magnification of Figure 3 showing: A) Shrunken seminiferous tubules (S), Notice the spermatogonia arrested in prophase and the damaging of interstitial tissues (arrow), B) No progress in mitosis and empty seminiferous lumen (L) with depleted germinal epithelium (G). Both H&E, 400X

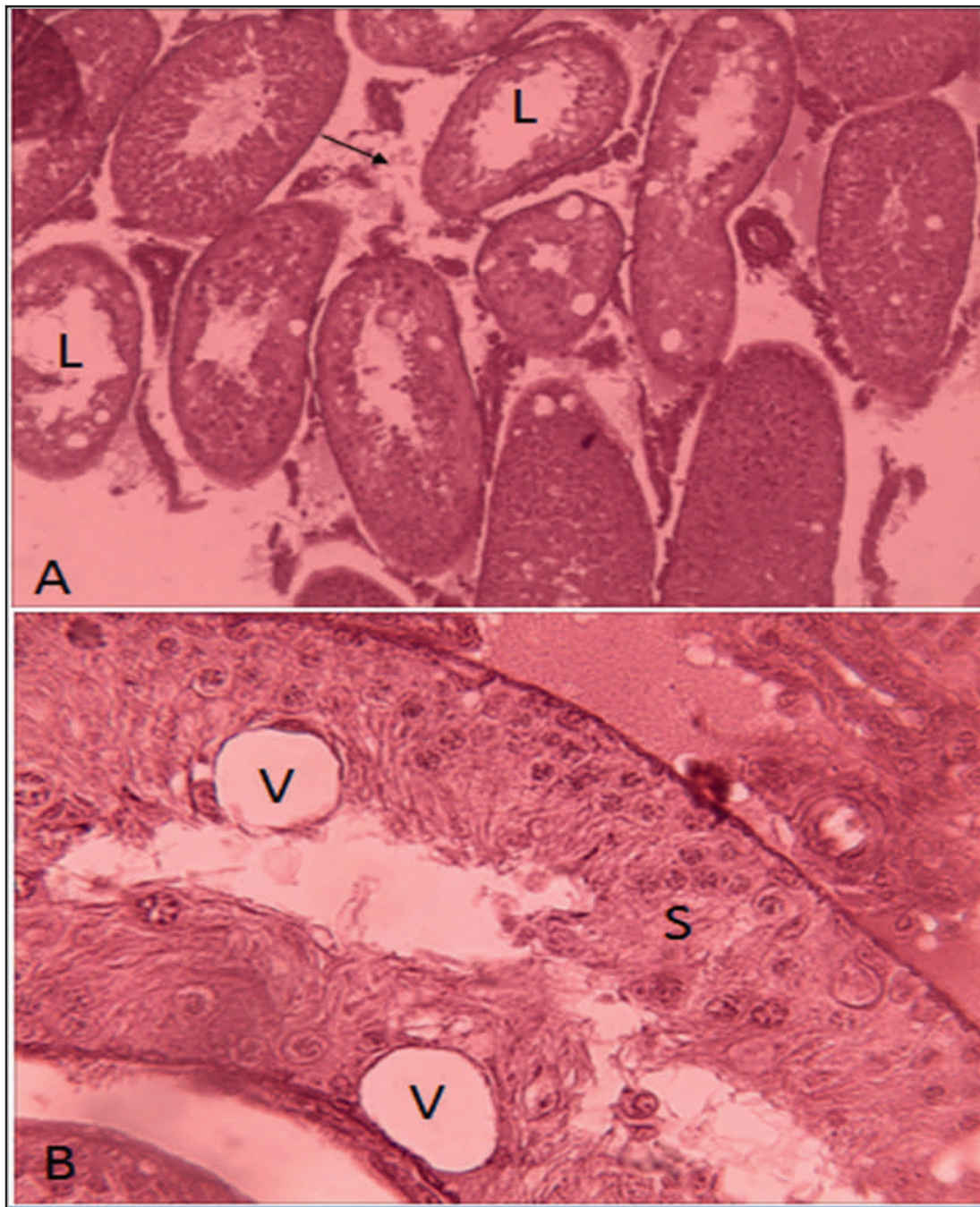


Figure 5: Sections through the Fluoxetine treated rats showing (A) damaging of the interstitial tissues (arrow), vacuolation of the germinal epithelium (S) with empty lumen (L), 100X B) Vacuoles (V), 400X

Several tubules showed shrunk with decrease in the germinal cell population (Spermatogonia, primary and secondary spermatocytes, spermatids, and mature sperm numbers). So, Johnsen's Score showed a significant decrease in group (B) in comparison with control rats ($P < 0.001$). On the other hand, Fluoxetine plus *Fumaria*

-treated rat testis showed approximately normal histological appearance in comparison to fluoxetine treated group. The lumen of the seminiferous tubules were seen full of spermatid with variable mitotic and meiotic stage in the germinal epithelium (Figures 6 and 7).

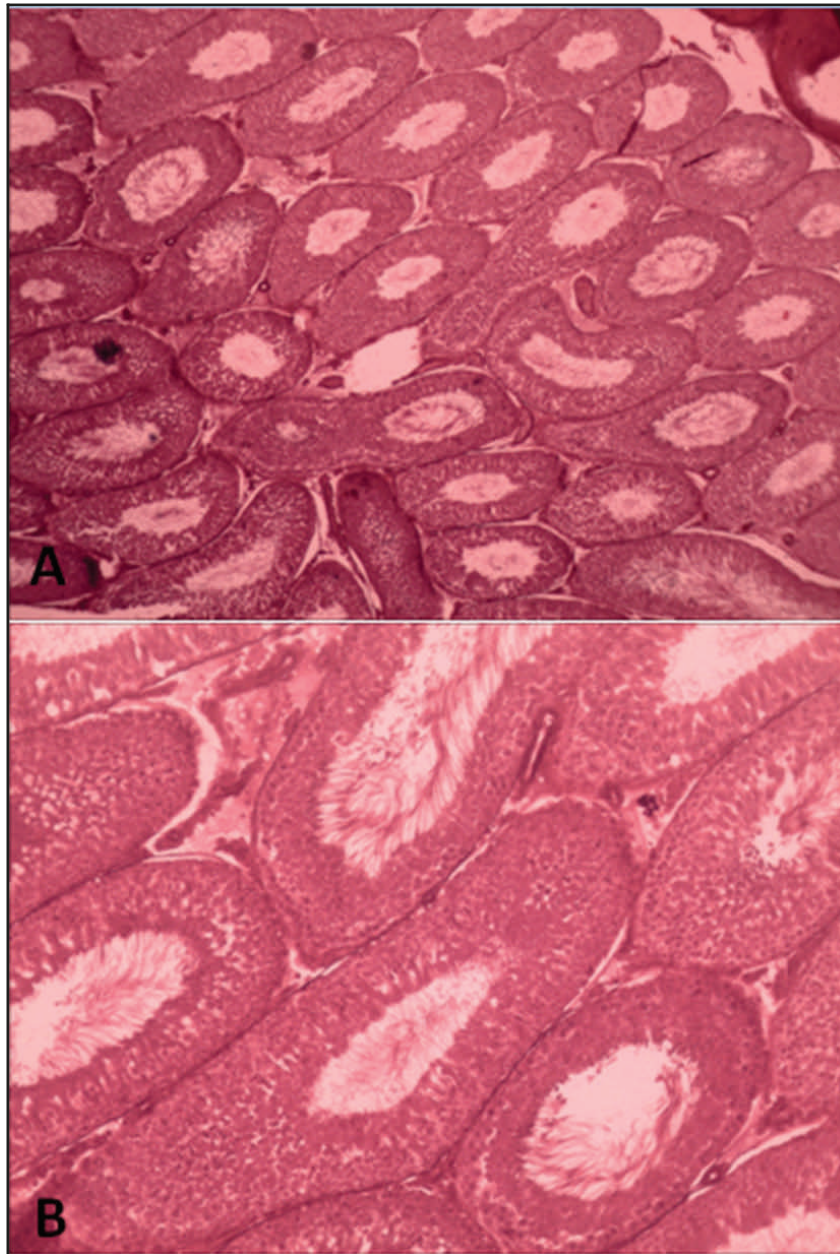


Figure 6: Sections through Fluoxetine plus *Fumaria* treated rat testis showing the approximately normal histological appearance of the seminiferous tubules with lumen filled with spermatids, A) 40X, B) 100X, both H&E

Results in Table 2 show that a statistically significant increase occurred on seminiferous tubule diameter, the thickness of germinal epithelium, and number of sertoli nucleus per cross section of seminiferous tubule in the group (C). While other parameters like the number of vacuoles were decreased significantly (Figure 5). In regard to the cell population of seminiferous tubule, there was a significant increase in Johnsen's Score ($P = 0.008$) as compared to group B.

Discussion

This study included evaluation protective effect of *Fumaria officinalis* against the testicular toxicity of fluoxetine were performed for the first time in Erbil city. The present study demonstrated that the administration of fluoxetine has toxicant effect on the reproductive system. A reduction of testicular weight among animals treated with 20mg/kg of fluoxetine was observed when compared to control

group. Many researchers have shown that any change or alteration in the androgen level results in general decrease in testicular weight.²⁰ In addition, testicular weight is a morphometric parameter directly and positively related to seminiferous tubules total length, sertoli cell population and spermatid production.¹⁸ This results corroborates other findings who reported that in a one -year oral toxicity of 20 mg/kg fluoxetine in beagle dogs was associated with reduction in absolute and relative testis weight by 26% and 33% respectively.²¹ Our experimental design aimed to identify the positive effect of *Fumaria officinalis* extract on testicular development in animals treated with fluoxetine. The results of this study showed an increase in testicular weight in animals treated with extract, however, it was not significant ($P = 0.871$), our findings were similar to findings of previous study²² who used *Fumaria parviflora*. A study confirmed by AlkAgrwal et al.²³ that the distortion of

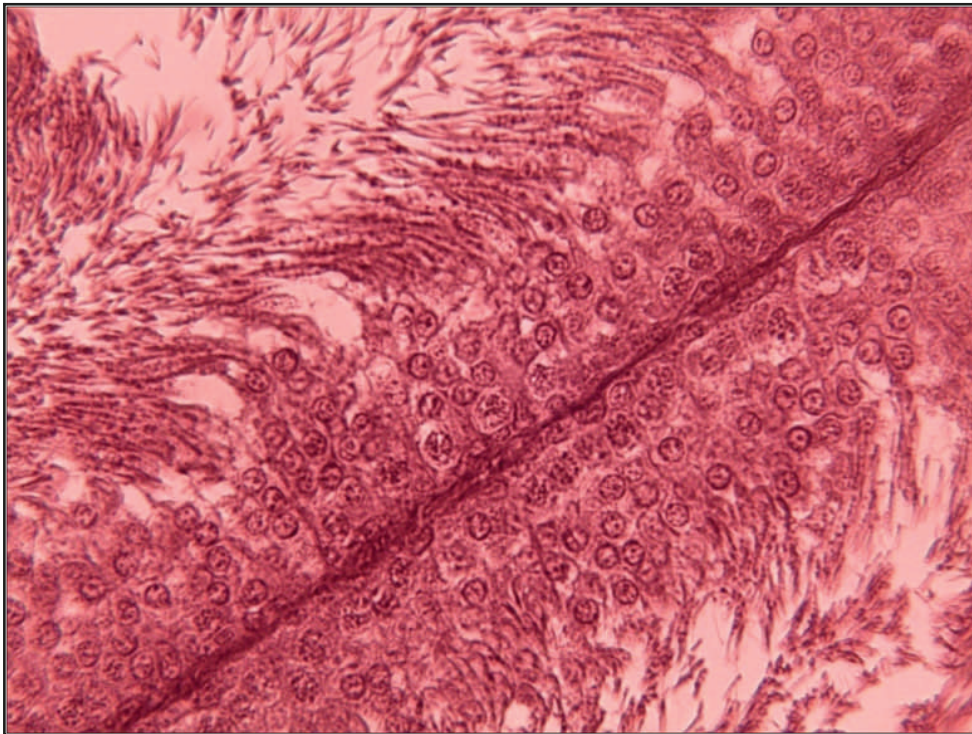


Figure 7: Sections through the testis of Fluoxetine plus *Fumaria* treated rats showing the normal histological appearance of the seminiferous tubules with lumen filled with spermatids, both are 400X, H&E.

seminiferous tubules, decreased thickness of the germinal epithelium, decreased diameters of seminiferous tubules were observed in rat treated with 20 mg/kg of fluoxetine for four weeks. These findings were similar to our results in present study, as well as we found disruption of the seminiferous tubular epithelium with focal damages ranging in severity from increased degeneration of spermatogenic cell profiles to complete loss of the germinal epithelium.²³ According to Ortega Pachco et al.,²⁴ the hormones, neoplasias, irradiation, trauma, and others interrupt the spermatogenic process, initially characterized by germinative cells desquamation in the tubular lumen and by a decrease in seminiferous epithelium height, necrosis and apoptosis of germinative epithelium cells and hyalinization of seminiferous tubules. The present work demonstrated many histological alterations in rats testicular tissue due to fluoxetine exposure. These findings are similar to other many previous studies. Silva et al.⁵ reported a reduction between 30 and 32% in the number of sertoli cells per testis in animals treated with 20 mg/kg fluoxetine. AlkaAgg²³ also observed that as the duration and dose of fluoxetine increased the entire cell lineage (Sertoli cells, Spermatogonia A, pale and dark type, Spermatogonia B, Primary spermatocytes) of the germinal epithelium decreased in number, however, this work used Johnsen's score to categorize spermatogenesis. Fluoxetine caused marked cessation of spermatogenesis via elevating the level of serotonin which stimulates suppression of hypothalamus-pituitary-testis axis mediated by activated hypothalamus-pituitary-adrenocortical axis resulting in decline of plasma luteinizing hormone (LH), Follicle stimulating hormone (FSH) and testosterone normal range. Testosterone and Follicle stimulating hormone act directly upon germinal epithelium.²⁵ Moreover, animals treated with 20 mg/kg of fluoxetine showed lack of spermatogenesis

inside the tubules, shrinkage tubules with wide intertubular space, and degeneration of seminiferous tubules.²⁶ The results in the present study observed that *Fumaria officinalis* increases seminiferous tubule diameter, epithelium height, number of sertoli cells, in rats treated with fluoxetine and extract compared to rats treated with only fluoxetine. Thus *Fumaria officinalis* has positive effects on testicular structure in this work might be due to the existence of antioxidant component. Studies have indicated that *Fumaria officinalis* contain high amount of isoquinoline alkaloids such as protopine, fumaricine, sanguinarine and so on, and polyphenols such as rutin and apigenin, these materials have antioxidative activity.^{11,12} Antioxidant composition can protect cell membrane from damage,²² caused by fluoxetine, which might be lead to atrophy of seminiferous tubule and depletion of the germinal epithelium (Figure 4). The antioxidant capacity of *Fumaria* spp due to the high content of flavonoids has been proved²⁷ and the ethanolic extract of *Fumaria officinalis* was found to be able to inhibit the lipid peroxidation²⁸ and has been found to exert an antioxidant activity.²⁹ This may explain the protective effect of the ethanolic extract of *Fumaria officinalis* against the oxidative stress production by fluoxetine in testis cells and tissue of rats as revealed by the present work. Souriel et al.³⁰ reported that *Fumaria parviflora* protect number of spermatogonium, spermatocytes, spermatozooids and sertoli cells from damage. Therefore histological Examination of testicular tissue in *Fumaria officinalis* treated rats showed marked improvement in histological changes, a little degenerated germ cells with seldom vacuoles of epithelium (Figure 5, 6). In addition, Johnsen's Score was significantly increased (Table 2). These results confirm the protective effect of *Fumaria officinalis*. Other species of *Fumaria* effect on the testis tissue by increasing number of Leydig cell and there

upon increase in testosterone hormone and more blood flow to the testis through angiogenesis increasing led to raising the number of sertoli cells which control spermatogenesis, and improve cells within epithelium²² because the structural and functional probity of reproductive organs depends on the ample bioavailability of testosterone.¹⁶ Mukhtar et al.¹⁴ mentioned the aphrodisiac activities of *Fumaria officinalis*. This action might induce the process of spermatogenesis, leading to increasing of germinal cells number. All of the mechanisms mentioned above give the possibility of *Fumaria officinalis* to preserve the histological structure of seminiferous tubules and its germinal cells.

Conclusion

The study concluded that *Fumaria officinalis* minimize the side effects of fluoxetine on testicular structure. Histological results of the present work confirmed the previous study that fluoxetine caused testicular dysfunctions in rats.

Competing interests

The authors declare no competing interests.

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