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Changes in the Testicular and Preputial Gland Structures of Mice Related to Influence of *Ferula hormonis* Extract

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The effect of ingestion of freshly prepared ethanolic extract of *Ferula hormonis* was investigated on the histology of both testis and preputial gland in intact prepubertal mice. The intra gastric application of 3mg $kg^{-1}day^{-1}$ of this extract for six weeks clearly inhibited the normal growth of both testis and preputial gland. On the other hand, the administration of such extract resulted in a process called tuberculosis and cystic swelling of the testis. Preputial structure was atrophied and has undergone cystic degeneration.

Key words: Preputial, mice, testis, tuberculosis, histology

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Introduction

Large number of medicinal plants possess mild or potent estrogenic activity when assessed in male and female mice (Homady *et al*., 2000) and immature rats (Qureshi and Dixit, 1980; Saxena *et al*., 1985). Owing to their estrogenic characteristics, these extracts may affect the physiology of the reproductive organs (Homady *et al*., 2000).

Ferula hormonis (Family: Umbelliferae) commonly known as shirsh zallouh" has received attention concerning it's antifertility activity. In addition to it's folkloric uses, its aqueous extract inhibit social aggression, reduced weights of both body and other accessory sex glands and also prevents the pregnancy in mice. Additionally, the exposure of male mice to such extract at a dose of 3 mg $kg^{-1}day^{-1}$ resulted a significant decreases in the sperm count, their motilities and a concomitant increase in sperm abnormalities (Homady *et al*., 2000.

Many pharmacological and biochemical studies on the effects of different species of the genus Ferula was conducted. Such as luteolysis in the ovary of cyclic quineapig and isolation of esters extraction of coumarin. Singh *et al*. (1985) have reported that *Ferual jaeschkeana* has various medicinal properties and significant antifertility properties in rats. Many of the antifertility agents of plant origins are known to alter both the histological and biochemical events in varieties organs (Prakash *et al*., 1989). *Ferula hormonis* is being used in traditional medicine for sterility treatment in middle east since two years. However, there is a very strong speculation concerning the importance of its uses i.e., to increase the sexual energy and helps circulation for sexual functions; can be used against frigidity and impotence; as a general stimulant; a nervous activator; a tranquilizer; increase endurance and to cure erectile disfunction.

The present investigation of *F. hormonis* is one of a series which contrast the degree of susceptibility of mice to the physiological and biochemical effects of *F. hormonis* extract. The research work confirms our previous observation that, exposure of male mice to *F. hormonis* is resulted in a significant reduction of their fertility and this was shown by reduced number of pregnant females, number of implantations and viable fetuses in females impregnated by males ingested this extract (Homady *et al*., 2000)

To the best of my knowledge, there was no report which described the histological effects of *F. hormonis*. Therefore, the present findings deals with the effect of its ethanolic extract on the histology of testis or preputial gland in prepubertal male mice.

Materials and Methods

Plant material extracts: Dried material of the *F. hormonis* roots was obtained from local market in Amman, Jordan and pharmacognostical identified in our laboratory before use. The ethanolic extract was prepared by boiling the 500g of powdered material in 97 % ethanol (2L) for 5 min. filtering after 2-3 hr, drying over magnesium sulphate($MgSO_4$)and removing the solvent at 30°C/14 mm of Hg to give oils. The resulting extract (60g) was stored at 4° C and used within 24 hours. In the course of experiment 3g of the residue was dissolved in 100 ml of distilled water immediately before administration to the animals.

Animals: Tuck Ordinary (TO) strain mice were bred and maintained in the animal house unit in the Faculty of Sciences at Mu'tah University under controlled temperature 21 ± 1 °C in 12 hr. lights and 12 hr. darkness schedule. Subjects were housed in type MI plastic cages (North Kent plastics, Erith Kent, U.K.) measuring 30x12x11 cm^3 with wire grid tops. Sawdust bedding was used, food and water were available ad-libitum. Twenty four group-housed intact male mice were allocated to two categories treated at 3 weeks of age for 42 days. No, 1: received *F. hormonis* extract, 0.1 ml $kg^{-1}day^{-1}$ and 2normal saline as controls, 0.1ml $kg^{-1}day^{-1}$

Daily Administrations: Intra-gastric application of plant extract or normal saline were made using animal feeding needles at 10.00 h. each day. The fluids were administered for six weeks. At the end of experiment the mice were killed by cervical dislocation and the testes or preputial glands were used for light microscopy. In the histological methods samples of fresh testes or preputial glands were removed, fixed in freshwater Bouin's fluid for 24 hr. dehydrated in alcohol, cleared in xylene and embedded in the paraffin wax. Routine, 4 μm sections were then cut and stained using Mallory's trichrome method (Homady *et al*., 1986). In addition, seminiferous tubule and preputial acinar diameters were measured in paraffin sections using a micrometer microscope. Five samples were used for each treatment and control groups and 20 sections were taken from each. Three random transverse-section measurements were taken from each of the 100 sections. The total of 300 measurements per treatment was meaned to give an average value in the micrometer (Homady *et al*., 1986).

Results

Tubular and acinar diameter: The mean diameter of seminiferous tubule or preputial acinar are presented in Table 1. A significant suppressant effect was produced by *F. hormonis* administration ($P < 0.0001$) in both seminiferous tubule and preputial acinar diameters (Student's t-test).

Table 1: Mean ±S.E. seminiferous tubule and preputial acinar diameter (μ) in wax sections from intact mice treated daily with saline or *F. hormonis* extract.

Section type	Treatment	Diameter
Seminiferous	Normal saline	287.32 ± 2.14
tubule	*F. hormonis*	239.18 ± 1.36*
Preputial acinar	Normal saline	139.71 ± 1.40
	F. hormonis	42.52 ± 0.38*

* Differs from normal saline treated group ($P < 0.0001$) on Student's t-test.

Testes: The normal morphology of the testis is shown in Fig. 1a, whereas Fig. 1b and c showed the testes of animals treated with the *F. hormonis*. This treatment is resulted in a process called tuberculosis, which being located posteriorly and leads to development of skin sinuses in the posterior part. However, the testis is enlarged but soft and on cut surface it has a uniform greyish pink colour with consistency of dough as compared with control subjects. The administration of extract also caused cystic swelling (a hydrocele) due to accumulation of fluid within the tunica vaginalis.

The histological picture of testis of the control mice displayed a normal features which induced large number of developing and mature spermatozoa (Fig. 2a and c). By contrast, the histology of testis in treated animals (Fig. 2 b and d) showed a thickening of the tubular basement membrane with an increase in the interstitial cells. The seminiferous tubules are small, oval or polyhedral in shapes that are lined by a trophied flattened epithelium. There was no sign of cycles of spermatogenesis. Sertoli cells showed the pyknosis and there was a general tendency for nuclear shrinkage. The mean

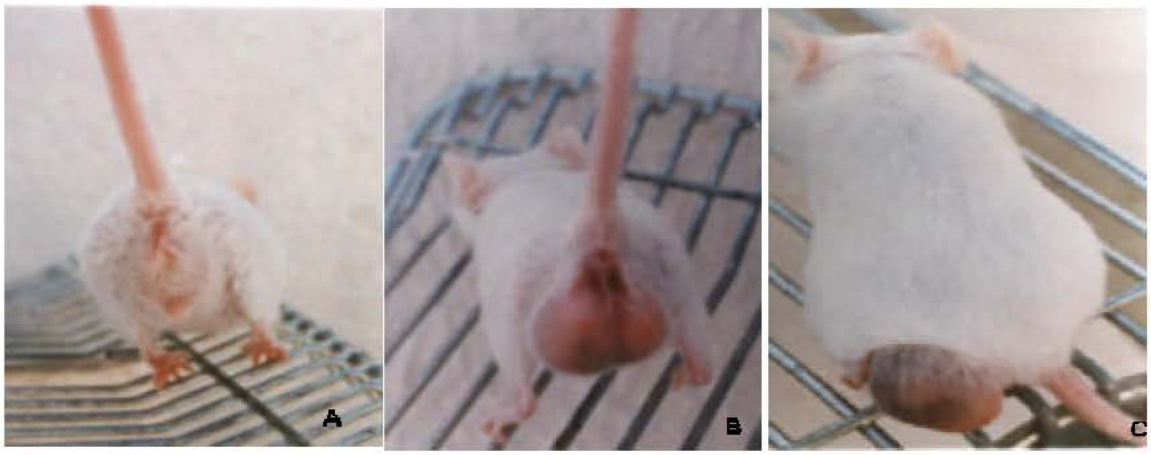

Fig. 1. a, Normal mouse (control); b and c *F. hormonis* –treated animals. Note the caseous tuberculosis of the testis.

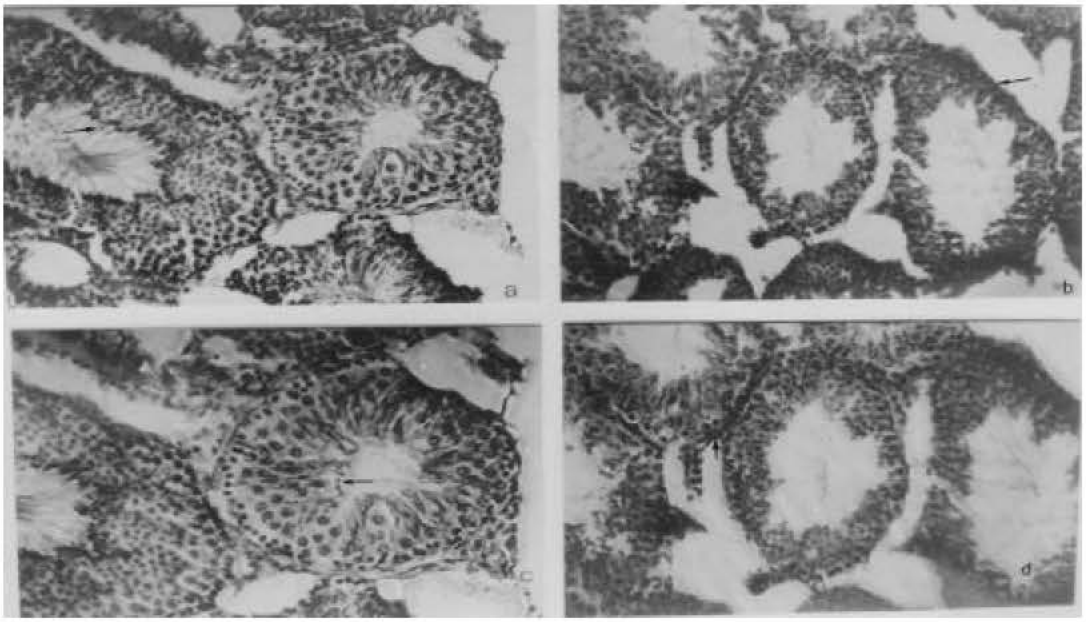

Fig. 2: Thick (6μ) paraffin sections of mouse testes. a, c Control (X 50 and X 100, respectively) with active spermatogenesis (arrow); b, d Ferula hormonis treated animals (X 50 X 100 respectively). Note the thickening of the tubular basement membrane with a tissues (arrow)

seminiferous tubule diameters (Table 1) confirmed the above histological picture.

Preputial gland: The normal histology of the mouse preputial gland was confirmed in the saline treated controls (Fig. 2a and c). Preputial sections from subjects treated with *F. hormonis* (Fig. 2b and d) showed reduced acinar numbers and diameters (Table 1). The normal pattern of the arrangement of acini was lost and the cells showed degeneration. The nuclei were often pycnotic. There was an increased in the connective tissue stroma and the ducts showed dilation, revealing the stratified squamous epithelial lining, which showed keratinization.

Discussion

The morphological differences exhibited by seminiferous tubules throughout the process of *F. hormonis* extract constitute a first point of interest. Cell elongation and extension of cell projections are considered to be signs of active cell behavior (Bell and Reevel, 1980). On the contrary, a polyhedral or oval shape, a thickening of basement membrane with a concomitant increase in the volume of interstitial tissues are suggestive of a more quiescent state. The research work indicated that *F. hormonis* treatment induces a strong histological regression in both testis and preputial gland. These findings are in agreement with the most of previous studies which indicated that spermatogenic

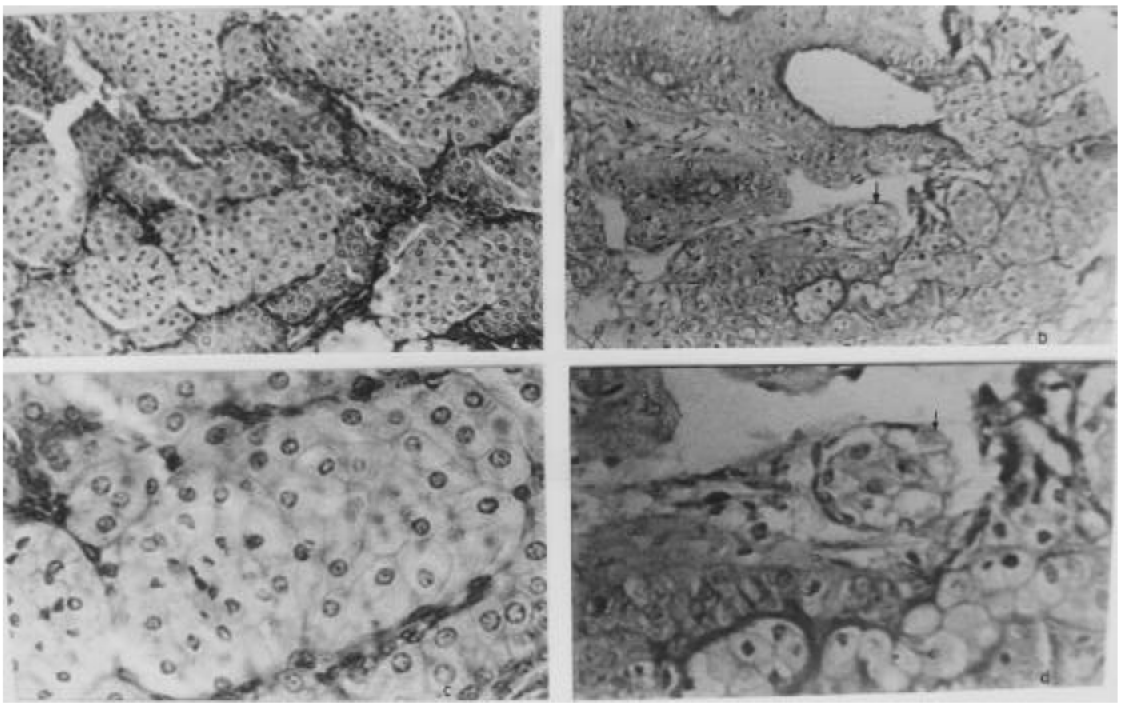

Fig. 3: Thick (6μ) paraffin sections of mouse preputial gland. a, c Control (X 50 and 100, respectively); b, d Ferula hormonis treated animals (X50 and X 100 respectively). Note the cystic formation and progressive degeneration of acini (arrow)

degeneration and preputial atrophy can be induced in mice (Homady *et al*., 1990); rats (Arya and Vanha-Perttual, 1984) and in men (Norse *et al*., 1973) treated with cyproterone acetate (CA) or estrogen. Steinberger *et al*. (1971) reported that, a complete sterility has been obtained in rats after three weeks of CA-treatment. The data presented are agreed with the findings of Chapin and Williams (1989) reported that decreased spermatozoa counts is consistent with a disruption of the normal androgenic control of testis. Testosterone is well known to act on the seminiferous tubules and to play a role in maintaing the spermatogenesis (steinberger *et al*. 1971),and preputial structure (Brain and Homady, 1985).

The investigation demonstrated that *F. hormonis* extract prevent the normal improvement in spermatogenesis and preputial maturation seen during puberty. This may be due to an auto-immune reaction against spermatozoan products. The results also indicated that *F. hormonis* extract resulted in increase of interstitial cells of seminiferous tubules in treated animals. However, Favvcett *et al*. (1973) reported that species like guineapig, rat, chinchilla and mouse have a relatively small volume of interstitial cells and a minimum of interstitial connective tissue. These effects of *F. hormanis* might be due to the direct suppression of endogenous androgen or some other central effect of this extract.

One of the questions posed by this investigation involves the site at which *F. hormonis* extract acts within tissues to produce its toxic effects. One or a combination of the following sites are possible.

1. There may be a direct action of the *F. hormonis* on the tissues or cells within the damaged prepubertal regions.
2. Another potential site of action may involve the disruption of a normal process within either the hypothalamic-pituitary axis or gonads which secondarily produces the observed histological alterations.

At this time the mechanism by which this extract produce its histological damage is speculative and required additional research. In conclusion, the results suggest that chronic *F. hormonis* exposure impairs spermatogenesis and induces multiple effects on both testis and preputial gland.

Acknowledgement

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