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

Merza H. Homady

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⁻¹day⁻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

Merza H. Homady
Department of Biological Sciences, Faculty of Science, Mu'tah University, Al-Karak, 61710, Jordan
Fax:00962-32-372-528
E-mail:merzahh@yahoo.com
Merna H. Homady: Changes in the testicular and preputial structures in relation to Ferula hormonice extract

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 hormonice (Family: Umbelliferae) commonly known as sharh zalboh has received attention concerning its 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 quineaqap and isolation of esters extraction of coumarin. Singh et al. (1985) have reported that Ferula 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 various organs (Prakash et al., 1985). Ferula hormonice 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 fertility and impotence; as a general stimulant; a nervous activator; a tranquilizer; increase endurance and to cure erectile dysfunction.

The present investigation of F. hormonice is one of a series which contrast the degree of susceptibility of mice to the physiological and biochemical effects of F. hormonice extract. The research work confirms our previous observation that, exposure of male mice to F. hormonice 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. hormonice. Therefore, the present findings deals with the effect of its ethanolic extract on the histology of testis or preputial gland a prepubertal male mice.

Materials and Methods
Plant material extracts: Dried material of the F. hormonice roots was obtained from local market in Amman, Jordan and pharmacological 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 [MgSO₄] 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 301x12x11 cm² 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. hormonice extract, 0.1 mg kg⁻¹ day⁻¹ and 2normal saline as controls, 0.1ml kg⁻¹ day⁻¹.

Daily Administration: Intragastric 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 tubules 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 meant to give an average value in the micrometer (Homady et al., 1986).

Results
Tubule and acinar diameter: The mean diameter of seminiferous tubule or preputial acinar is presented in Table 1. A significant suppressant effect was produced by F. hormonice 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 (μm) in wax sections from intact mice treated daily with saline or F. hormonice extract.

<table>
<thead>
<tr>
<th>Section type</th>
<th>Treatment</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminiferous</td>
<td>Normal saline</td>
<td>287.32 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>F. hormonice</td>
<td>239.18 ± 1.36*</td>
</tr>
<tr>
<td>Preputial acinar</td>
<td>Normal saline</td>
<td>139.71 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>F. hormonice</td>
<td>42.52 ± 0.38*</td>
</tr>
</tbody>
</table>

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

Tests: The normal morphology of the testis is shown in Fig. 1a, whereas Fig. 1b and c showed the testes of animals treated with F. hormonice. This treatment is resulted in a process called tuberculosi, which being located posteriorly and leads to development of skin sinusues 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 spermatooza (Fig. 2a and c). By contrast, the histology of testis in treated animals (Fig. 2b and d) showed a thickening of the tubular basement membrane with an increase in the interstitial cells. The seminiferous tubules are small, oval or polyedal in shape that are lined by a trophy 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
Marza H. Homady: Changes in the testicular and preputial structures in relation to Ferula hormonis extract

Fig. 1. a, Normal mouse (control); b and c Ferula hormonis-treated animals. Note the oseous tuberculosis of the testis.

Fig. 2. Thick (5μm parafin 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 tissue (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 Ferula 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 pyknotic. There was an increased in the connective tissue stroma and the ducts showed dilations, revealing the stratified squamous epithelial lining, which showed keratinization.

Discussion

The morphological differences exhibited by seminiferous tubules throughout the process of Ferula hormonis extract constitutes a first point of interest. Cell elongation and extension of cell projections are considered to be signs of active cell behavior (Bell and Reavel, 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 Ferula hormonis treatment induces a strong histological regression in both testes and preputial gland. These findings are in agreement with the results of previous studies which indicated that spermatogenesis...
Fig. 3: Thick (8µm) paraffin sections of mouse preputial gland. a, c Control (X 50 and 100, respectively); b, d Ferula hormonis treated animals (X 60 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., 1980) 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 agree with the findings of Chapin and Williams (1985) that decreased spermatozoa counts is consistent with a disruption of the normal androgenic control of tests. Testosterone is well known to act on the seminiferous tubules and to play a role in maintaining spermatogenesis (steinberger et al., 1971), and preputial structure (Brain and Homady, 1986).

The investigation demonstrated that Ferula hormonis extract prevent the normal improvement in spermatogenesis and preputial maturatation seen during puberty. This may be due to an auto-immune reaction against spermatozoan products. The results also indicated that Ferula hormonis extract resulted in increase of interstitial cells of seminiferous tubules in treated animals. However, Fawcett et al. (1973) reported that species like guinea pig, rat, chinchilla and mouse have a relatively small volume of interstitial cells and a minimum of interstitial connective tissue. These effects of Ferula hormonis 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 Ferula 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 Ferula hormonis on the tissues or cells within the damaged preputial regions.

2. Another potential site of action may involve the disruption of a normal process within either the hypothalamo-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 Ferula hormonis exposure impairs spermatogenesis and induces multiple effects on both testis and preputial gland.

Acknowledgement

This work is supported by a grant from the Deanship of Scientific Research, Mu'tah University, Karak-Jordan.

References


Merza H. Homady: Changes in the testicular and preputial structures in relation to Ferula hormonis extract


