Effect of *Ferula assa-foetida* oleo gum resin on spermatic parameters and testicular histopathology in male wistar rats

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ABSTRACT

Background: In Ayurveda and traditional medicines of different countries such as Iran, America and Brazil, asafoetida has been used as an aphrodisiac agent. **Objective:** The present study was aimed to evaluate the effectiveness of asafoetida on spermatic and testicular parameters in treated rats. **Materials and Methods:** A total of 30 male Wistar rats divided equally to five groups (one control and four test groups receiving 25, 50,100 and 200 mg/kg asafoetida respectively). After 6 weeks, a small part of the cauda epididymis of each rat was dissected, and the spermatic parameters were evaluated for at least 200 spermatozoa of each animal. Testis of all rats was harvested for pathologic examination. The testosterone concentration of serum was also determined. Data were statistically assessed by one-way ANOVA and value of $P < 0.05$ was considered as the level of significance. **Results:** This study indicated that the asafoetida significantly increased the number and viability of sperms ($P < 0.05$). Histological study showed that spermatogenesis process and numbers of Leydig cells were increased with increasing the dose, but the Leydig cells become vacuolated. Johnsen score in experimental groups was increased compared to control although this difference was not significant ($P > 0.05$). **Conclusion:** Asafoetida showed a positive effect on spermatic parameters although the histopathological effects on the testis were observed, particularly at high doses.

Key words: Asafoetida, spermatic motility, testes, testosterone, toxicity

INTRODUCTION

Ferula assa-foetida L. grows wildly in central Asia especially in Iran and Afghanistan. Asafoetida, an oleo-gum-resin, is obtained from *F. assa-foetida* and some others *Ferula* species such as *Ferula foetida, Ferula rubricaulis, Ferula rigidula, Ferula alliacea* by incision of the roots or removal of the stems.[1]

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Asafoetida has been used as a folk phytomedicine for centuries. In Iranian traditional medicine, it has been used as antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelminitic, aphrodisiac and antiseptic agent.[2] In the ancient Indian ayurvedic system and other traditional medicines such as America and Brazil, Asafoetida is considered as an aphrodisiac agent.[1] In Nepal, it is regularly consumed in daily diets and mainly considered as an aphrodisiac agent.[3] Ibn Sina (Avicenna) and Al-Antaki have also emphasized the aphrodisiac effect of *Ferula assa-foetida*.[4] New pharmacological studies have almost confirmed that asafoetida and its constituents have antiviral,[5] antispasmolitic action.[6] The researchers also showed that sulfur compounds potently activated TRPA1.[7] Effects of some *Ferula* species on reproductive activities have been investigated in some previous studies. Khleifat *et al*.[8] reported that methanolic extract of *Ferula hermonis* reduced the fertility in female mice and decreased numbers of epididymal sperm and their motility and increased sperm abnormalities in male mice. In other study, the researchers showed that the ethanolic extract of *F. hermonis* inhibited growth of testis and preputial gland.[9] This extract also decreased the number

of mated females; the total number of implantation and the number of viable fetuses.[10] There are a little study about effects of *F. assa-foetida* on the reproductive system and sexual appetite. Kassis *et al.* examined ethanolic extract of seeds and roots of *F. assa-foetida* that was called "Masculine" on male fertility and sexual functioning in rats and humans.[4] They showed that Masculine exhibits a high level of safety in rats, humans and cultured human fibroblasts and increases erection in rats. Their results also showed that consumption of one tablet of Masculine daily for 3 months could increase sperm number and sperm motility in men who had no sexual complains and azospermia. Number of old studies mentioned that the asafoetida has a weak sister chromatid exchange-inducing in spermatogonia[11] and clastogenicity in mouse spermatocytes.[12] Although in different traditional literatures has been emphasized on aphrodisiac of asafoetida, to our knowledge, there is no comprehensive study on the aphrodisiac and reproductive potency of asafoetida. To the best of our knowledge, there were no scientific reports available in the literature in support of the traditional claims of the aphrodisiac and reproductive potency of asafoetida. The present study is, therefore, an attempt to assess the effect of asafoetida on spermatic parameters, blood testosterone level and testis tissue.

MATERIALS AND METHODS

Animals and experimental design

Thirty male Wistar rats were bred and maintained in the animal house unit of the faculty of medicine under controlled temperature 21°C ± 1°C in 12 h light: 12 h darkness schedule. Animals were housed in plastic cages and food and water was made available *ad libitum*. The rats were divided into five groups. One group received saline as control, and experimental groups were treated by asafoetida (25, 50, 100 and 200 mg/kg) orally every day for 6 weeks. Ethical approval for this study was obtained from the Ethics Committee of the Shahid Sadoghi University of Medical Sciences.

Plant oleo-gum resin

Asafoetida was collected from Tabas region (Yazd province, Iran) during the summer, and the plant species was botanically identified by a botanist in Yazd Agricultural Research Center and voucher number of the specimen was 2365. The dried powder of asafoetida was dissolved in distilled water overnight at room temperature, and the yielded suspension was used orally. Concentrations and dosages of the extract were expressed as crude amount of the dried oleo-gum-resin used in preparing the stock solution.[11]

Epididymal sperm preparation

After 6 weeks, animals sacrificed in the histology laboratory of the faculty of medicine and a small part of the cauda epididymis of each rat was dissected and located in 1 mL of prewarmed Hams F10 medium (37°C, 5% CO_2). Gentle tearing of the tissue was done to make spermatozoa swim out into the culture medium. The dishes were placed in an incubator at 37°C for 15 min.[13]

Sperm analysis

The sperm motility, normal morphology, viability and sperm count were evaluated for at least 200 spermatozoa of each animal. Sperm movement analysis was done by Makler Chamber and light microscopy (Olympus Co., Tokyo, Japan). Motility was expressed as a percentage of progressive motility including rapid (Grade a) and slow (Grade b) spermatozoa, nonprogressive (Grade c) and immotile (Grade d) spermatozoa. The morphologically normal spermatozoa and the percentage of viable sperm cells were assessed by Papanicula staining and Eosin test respectively.[14] The light microscope was set at ×10 and ×40 eyepiece magnifications. All analyses were performed by one experienced technician blinded to the study. Double checking of results was also done for each specimen.

Hormone assay

Blood was collected from the orbital sinus of rats. Serum was prepared by centrifugation (3000 r.p.m., 20 min) and stored frozen until testosterone assay. The testosterone concentration was determined in duplicate using the Testosterone Enzyme Immunoassay kit (Assay Design Inc., Ann Arbour, USA) according to the manufacturer's instructions.

Histopathology of testes

Both testes of all rats were harvested for pathologic examination, and each testis were fixed in Bouin's fixative, processed by routine histological methods and embedded in paraffin blocks. The sections were cut by a rotary microtome and stained with Ehlrich's hematoxylin and eosin. The stained sections were studied by Olympus light microscopy (Olympus, Japan, magnification ×10 and × 40) to evaluate spermatogenesis and histopathological studies. Johnsen's score[15] was used to categorize the spermatogenesis. This method applies a score of 1–10 for each tubule cross section examined [Table 1]. The germinal epithelium of at least 40–50 tubules was assessed for each testis and the mean Johnsen's score per rat was calculated.[16]

Acute toxicity

At the end of experiments, the rats under study were observed for symptoms of short and long-term toxicity and

finally mortality recorded. Then, animals were kept under observation for up to 10 days to rollout the behavioral changes (tremor, paralysis), weight loss and mortality.[17]

Statistical analysis

Statistical data were assessed with one-way ANOVA, followed by *post-hoc* Tukey test using Graph pad prism version 5. Results were expressed as mean ± standard error (SEM). A value of $P < 0.05$ was considered as significant.

RESULTS

Effect of asafoetida on sperm production, viability, motility and morphology

The results of sperm examinations are summarized in Table 2. Epididymal sperm counts were significantly increased in all doses ($P < 0.05$). Sperm morphology and viability were significantly improved in asafoetida treated rats except asafoetida 25, 50 mg/kg. Total motility was significantly decreased in treated rats with asafoetida 25 and 50 mg/kg compared to the control group ($P < 0.05$).

Histological changes

Histological findings were examined in different groups. No histopathological changes were seen in the control specimens [Figure 1a]. In extract group (25 mg/kg), an increase in germ cells was found, and the spermatogenesis process was clearly seen. The spermatogenic cells formed a thick layer. An increase in epithelial height of the seminiferous tubule was observed and the interstitial space between tubule was decreased [Figure 1b]. An increase in thickness in the capsule (tunica alboginea) of the testes and sub capsular edema was observed in extract group (50 mg/kg), and blood vessel expansion was seen [Figure 1c]. An increase in thickness in the capsule of the testes and sub capsular edema was found in extract group (100 and 200 mg/kg). Blood vessel expansion and a decrease in epithelial height of the seminiferous tubule were observed in both groups. The thickness of the basal membrane of the tubule was also increased significantly [Figure 1d and e]. In dose 200 mg/kg extract, some cells were vacuolated in the seminiferous tubule epithelium, and the number of germ cells in the seminiferous tubules was decreased but spermatozoa were clearly observed in the lumen of the tubule. The number of Leydig cells was decreased with increase the extract dose and these cells became vacuolated. By increasing the extract dose (50, 100, 200 mg/kg) the interstitial space between the seminiferous tubules was increased but displacement of sertoli and germinal cells were not found among these groups. In the pathologic evaluation, Johnsen score was increased in experimental groups compared to control group, but this difference was not significant [Table 2].

Table 1: Modified Johnsen score

Score description
Complete spermatogenesis and perfect tubule
Many late spermatids present but disorganized tubular epithelium
Only a few late spermatids
No late spermatids but many early spermatids
Few early spermatids, arrest of spermatogenesis at the spermatid stage, disturbance of spermatid differentiation
No spermatids, many spermatocytes
Few spermatocytes, arrest of spermatogenesis at the primary spermatocyte stage
Only spermatogenia
No germ cells, sertoli cells only
No germ cell or sertoli cells, tubular sclerosis

Testosterone assay

The result of serum testosterone analysis was shown in Figure 2. There is no significant difference between the control and treated animals with asafoetida. The results showed that blood concentration of testosterone decreases with increasing asafoetida dosage.

Acute toxicity study

Asafoetida in concentrations used did not show any short or long-term toxic effect. This was evidenced by the absence of tremor, paralysis, weight loss and autonomic behavioral changes as compared to control group. Furthermore,

Table 2: The results of semen analysis in controls and treatment rats with asafoetida

Variables	Control	Asafoetida 25	Asafoetida 50	Asafoetida 100	Asafoetida 200
Count ($\times 10^6$)	3.1±0.9	3.6±0.9*	4.2±1.4*	4.5±1.1*	5.1±1.6*
Rapid motility (%)	12±1.8	9±1.8	10±1.2	14±0.3	17±2.9*
Slow motility (%)	9±1.4	8±0.6	22±2.9*	9±1.5	14±2.6*
Nonprogressive motility (%)	41±1.8	10±0.6*	28±1.9*	22±3.5*	25±0.8*
Immotile sperm (%)	37±3.3	75±6.1*	48±3.2*	46±8.0	43±6.7
Total motility (%)	62±12	27±91*	52±11.8*	54±9.8	57±13.3
Normal morphology	92.6±18	94.4±19.2	95.2±16.2	96.3±17	98.7±19.9*
Viability (%)	71±15.8	74±14.3	81±17.5*	86±19.2*	87±18.6*
Johnsen score	8.66±1.6	8.14±1.3	8.25±1.5	8.25±1.1	8.99±1.6

*Represent significant increases and decreases respectively at $P<0.05$ when compared to control values. Values are means±SEM. $n=6$ in each group. SEM=Standard error of mean

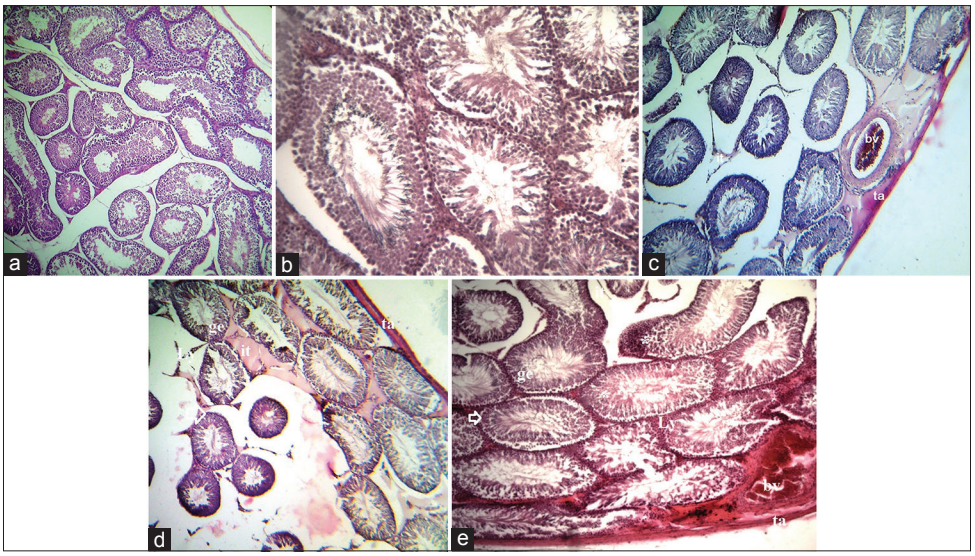

Figure 1: Histological architecture of the testis in different groups. No histopathological changes were seen in control (a) and in treatment group 25 mg/kg (b) In group 50 mg/kg, an increase in thickness in the capsule (tunica alboginea), sub capsular edema and blood vessel expansion was seen(c). In 100 and 200 mg extract, a decrease in epithelial height of the seminiferous tubule and an increase in thickness in the basal membrane of the tubules (arrows) were found

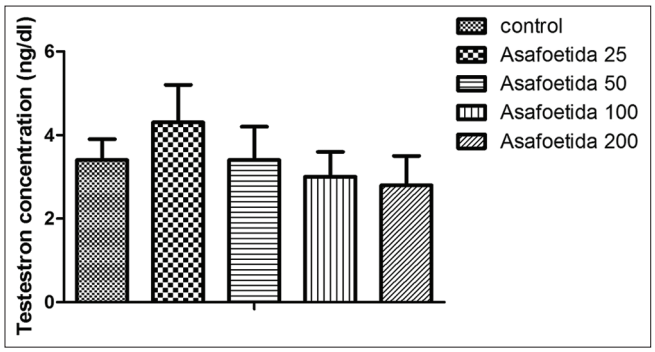

Figure 2: Serum level of testosterone was measured and expressed as individual values in the control group and asafoetida in different doses groups. There is no significant difference between the control and treatment groups

there was no mortality in treated animals during 6 weeks of observation.

DISCUSSION

The data of the current study showed that the asafoetida improved the number, motility, morphology and viability of sperms. Zanoli *et al.* reported that acute or repeated ingestion of *F. hermonis* impairs female sexual behavior and has antiestrogenic effects.[18] They also showed that long-term administration of high doses of *F. hermonis* reduced testosterone and copulatory performance in rats, although, acute administration improved sexual functioning.[19] Our results showed that asafoetida could increase number and viability, however, the testosterone level in the treated groups declined, and this decrease was dose dependent. The results obtained from this study are consistent with the results of Ayoubi *et al.*[20] They showed that taking high doses (300 mg/kg) of asafoetida reduced testosterone level. In our study, histological evaluation showed with asafoetida in the highest dose (200 mg/kg) the numbers of Leydig cells decreased, and these cells also become vacuolated. Kassisa *et al.* showed that Masculine (ethanol extract seeds and roots of *F. assa-foetida*) increased sperm number and motilility.[4] They mentioned that the mechanism of action probably was encouraged endothelial cells to release nitric oxide that stimulates the synthesis of cyclic guanosine monophosphate in the penile corpus cavernosum. The mechanism action of *Ferula* extracts and components on the reproductive system are not clear. Phytochemestry of *Ferula* genus showed that this genus is a good source of biological active compounds such as sesquiterpene derivatives.[2] Physiological changes in the reproductive system regulated by the hormones of hypothalamo-hypophyseal origin, which are inhibited or stimulated by number of endocrine and exocrine factors.[8] The compounds of asafoetida may cause irritation on this axis and sperm parameters have been enhanced. Phytochemestry of asafoetida showed that this oleo gum resin contains about 40–64% resin, 25% endogeneous gum, 10–17% volatile oil and 1.5–10% ash. Its resin fraction consists of ferulic acid esters, free ferulic acid, umbelliferone and coumarin derivatives such as fetidin and kamolonol, farnesiferoles A, B and C. The compositions of its gum fraction are known to be glucose, galactose, L-arabinose, rhamnose and glucuronic acid.[1] Maybe asafoetida sesquiterpene coumarines have similar effects on reproductive system similar to some sesquiterpenes

such as ferutinin and teferdin. These compounds have been shown to have androgenic activity and may contribute to its aphrodisiac activity.[21] This oleo-gum resin also has beneficial compounds to sperm viability and motility and reduces damage of sperm membranes lipid peroxidative through increase of intracellular cAMP and cGMP.[22] These observations seem to impact of asafoetida on such as radical scavenging activity of sulfur-containing compounds, lipoxygenase inhibition by umbelliprenin, ferulic acid and its derivatives, increase in the activity of endogenous antioxidants and decrease in oxidative parameters.[1] To obtain a pattern of asafoetida effects on testis tissue, for the first time, we have used histopathological evaluation and compare these changes to biochemical results. Histopathological changes with dose (25 and 50 mg/kg) were negligible. The increase in thickness of tunica alboginea, sub capsular edema, blood vessel expansion and an increase in thickness in the basal membrane of the tubules was found in extract group (100 and 200 mg/kg). In dose 200 mg/kg, Vacuoles are seen between germ cells. This character may represent a loss or reduction of cell adhesion molecules like cadherin and can be considered as a sign of apoptosis. The results of biochemical assessment of the present study have been shown that by increasing the doses of asafoetida the blood concentration of testosterone was decreased. This may be concluded that although the number of Leydig cells increased in response to a higher dose of asafoetida, these cells were vacuolized. In addition, Johnsen score improved with increasing asafoetida dosage, and this result showed that the asafoetida in high doses induced some histopathological changes, but spermatogenesis did not decrease. Although with biochemical and histological evaluation asafoetida in low doses showed a beneficial effect on the sperm count and viability, testosterone levels and testes structure (with low doses the testis tissue had normal structure and cells) but some adverse histological effects such as sub capsular edema, an increase in thickness of the basal membrane are seen at 200 mg/kg. High doses of asafoetida reduced testosterone level and this was also agreement with histological assessment that showed with asafoetida in the highest dose (200 mg/kg) the numbers of Leydig cells decreased and these cells was vacuolated.

CONCLUSION

In conclusion, the results indicated that the asafoetida in low doses can improve sperm parameters (the sperm count and viability, testosterone levels) and preserve the normal structure of testis tissue and its cells, although in high dose maybe have an adverse effect on testis tissue but spermatogenesis did not decrease. Therefore, asafoetida in low doses showed a beneficial effect on testis and spermatogenesis. In general, further studies are required to isolate the active principals of asafoetida on spermatogenesis.

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REFERENCES

1. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)-a review. J Ethnopharmacol 2011;134:1-10.
2. Bagheri SM, Sahebkar A, Gohari AR, Saeidnia S, Malmir M, Iranshahi M. Evaluation of cytotoxicity and anticonvulsant activity of some Iranian medicinal Ferula species. Pharm Biol 2010;48:242-6.
3. Eigner D, Scholz D. Ferula asa-foetida and Curcuma longa in traditional medical treatment and diet in Nepal. J Ethnopharmacol 1999;67:1-6.
4. Kassisa E, Fuldera S, Khalilb K, Hadiehc B, Nahhasb F, Saad B, *et al*. Efficacy and safety assessments of ferula assa-foetida L., traditionally used in greco-arab herbal medicine for enhancing male fertility, libido and erectile function. Open Complement Med J 2009;1:102-109.
5. Ghannadi A, Fattahian KH, Shokoohinia Y, Behbahani M, Shahnoush A. Antiviral evaluation of sesquiterpene coumarins from Ferula asssa-foetida against HSV-1. Iran J Pharm Res 2014;12:523-30.
6. Sadraei H, Ghannadi A, Malekshahi K. Composition of the essential oil of asafoetida and its spasmolytic action. Saudi Pharm J 2003;11:136-40.
7. Shokoohinia Y, Chianese G, Appendino G, Di Marzo V, De Petrocellis L, Ghannadi A, *et al.* Some like it pungent and vile. TRPA1 as a molecular target for the malodorous vinyl disulfides from asafoetida. Fitoterapia 2013;90:247-51.
8. Khleifat K, Homady MH, Tarawneh KA, Shakhanbeh J. Effect of Ferula hormonis extract on social aggression, fertility and some physiological parameters in prepubertal male mice. Endocr J 2001;48:473-82.
9. Homady MH. Changes in the testicular and preputial structures of mice related to influences of Ferula hermonis extract. Science 2001;1:108-12.
10. Homady MH, Khleifat KM, Tarawneh KA, Al-Raheil IA. Reproductive toxicity and infertility effect of Ferula hornonis extracts in mice. Theriogenology 2002;57:2247-56.
11. Abraham SK, Kesavan PC. Genotoxicity of garlic, turmeric and asafoetida in mice. Mutat Res 1984;136:85-8.
12. Walia K. Effect of asafoetida (7-hydroxycoumarin) on mouse spermatocytes. Cytologia (Tokyo) 1973;38:719-24.
13. Mangoli E, Talebi AR, Anvari M, Pourentezari M. Effects of experimentally-induced diabetes on sperm parameters and chromatin quality in mice. Iran J Reprod Med 2013;11:53-60.
14. Talebi AR, Khalili MA, Nahangi H, Abbasi A, Anvari M. Evaluation of epididymal necrospermia following experimental chronic spinal cord injury in rat. Ir J Reprod Med 2006;5:171-6.
15. Erdemir F, Atilgan D, Markoc F, Boztepe O, Suha-Parlaktas B, Sahin S. The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters. Actas Urol Esp 2012;36:153-9.
16. Mohamadghasemi F, Faghani M, Khajehjahromi S, Bahadori M, Nasiri E, Hemadi M. Effect of Melatonin on proliferative

activity and apoptosis in spermatogenic cells in mouse under chemotherapy. J Reprod Contracept 2010;21:79-94.
17. Bagheri SM, Dashti-R MH, Morshedi A. Antinociceptive effect of Ferula assa-foetida oleo-gum-resin in mice. RPS 2014;9:207-12.
18. Zanoli P, Zavatti M, Rivasi M, Baraldi M. Ferula hermonis impairs sexual behavior in hormone-primed female rats. Physiol Behav 2005;86:69-74.
19. Zanoli P, Benelli A, Rivasi M, Baraldi C, Vezzalini F, Baraldi M. Opposite effect of acute and subchronic treatments with Ferula hermonis on copulatory behavior of male rats. Int J Impot Res 2003;15:450-5.
20. Ayoubi A, Arshami J, Valizadeh R, Mousavi Z, Mousaie A. The Effect of asafetida Gum Extract on Blood Parameters and Histopathology of Testes in Male Wistar Rat. Inrte J Arts Sci 2013;4:310-5.
21. Zanoli P, Rivasi M, Zavatti M, Brusiani F, Vezzalini F, Baraldi M. Activity of single components of Ferula hermonis on male rat sexual behavior. Int J Impot Res 2005;17:513-8.
22. Zheng RL, Zhang H. Effects of ferulic acid on fertile and asthenozoospermic infertile human sperm motility, viability, lipid peroxidation, and cyclic nucleotides. Free Radic Biol Med 1997;22:581-6.

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