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**

*A. 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 rigida*, *Ferula alliacea* by incision of the roots or removal of the stems.[1] 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, anthelmintic, 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.[3] 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 asa-foetida*.[4] New pharmacological studies have almost confirmed that asafoetida and its constituents have antiviral,[5] antispasmodic 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. 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. 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 and clastogenicity in mouse spermatocytes. 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.

**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% CO₂). 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.

**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. 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 Arbor, 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 cosin. The stained sections were studied by Olympus light microscopy (Olympus, Japan, magnification ×10 and ×40) to evaluate spermatogenesis and histopathological studies. Johnsen's score 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.

**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.\cite{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 thicker 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 albuginea) 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 spermatocytes 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].

**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,
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. 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. 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. 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 motility. 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. Phytochemistry of Ferula genus showed that this genus is a good source of biological active compounds such as sesquiterpene derivatives. 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. The compounds of asafoetida may cause irritation on this axis and sperm parameters have been enhanced. Phytochemistry 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 feudin and kamolol, farnesiferoles A, B and C. The compositions of its gum fraction are known to be glucose, galactose, L-arabinose, rhamnose and glucuronic acid. 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.\textsuperscript{[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 c\textsuperscript{AMP} and c\textsuperscript{GMP}.\textsuperscript{[22]} These observations seem to impact of asafoetida on such as radical scavenging activity of sulfur-containing compounds, lipoyxigenase inhibition by umbelliprenin, ferulic acid and its derivatives, increase in the activity of endogenous antioxidants and decrease in oxidative parameters.\textsuperscript{[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 albuginea, 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 vacuolized.

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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