# Study of Asphervon Gum Effect on Diuresis, Spermatogenesis and Its Effect on Testosterone Level in Rat Male Blood

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#### Abstract

The effect of Asphervon resin gum on spermatogenesis, on the level of testosterone in the blood of rats was studied, and the effect of two dosages of the drug on diuresis of rats with an aqueous load was also considered. Data have been obtained on the quantitative change of sperm in the rat ejaculate, on the ability of Asfervon to reduce testosterone levels in the rat's blood, and also, a pronounced dose has been revealed - the dependent diuretic effect of the drug Asfervon.

Keywords: diuresis, testicle, spermatogenesis, pH, urine, testosterone.

#### Introduction

Today, in connection with the need to find effective and safe drugs in medicine, medicines of plant origin are increasingly used. Medicinal plants with diuretic effect accelerate the removal of stagnant fluid from the body during edema, urinary problems, diseases of the heart and urogenital system.

Due to the fact that preparations of plant origin have practically no side effects, and their effect is milder than drugs of chemical origin, they are often prescribed to pregnant women, children and people with chronic diseases.

Basically, swelling, opacity are diverse in nature. It should be emphasized that they occur both in severe pathologies and in healthy people.

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PhD student, department of pharmacology and clinical pharmacy, Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan. Email: samedinov.r@gmail.com Phone number: (+998 93) 386 00 23 Patient conditions such as pregnancy, premenstrual syndrome, obesity, and the use of certain groups of drugs can be accompanied by swelling.

Swelling in most of its cases does not threaten the patient's health, due to the fact that it is associated with congestion, which can be aggravated.

Intercellular fluid in tissues is able to collect almost anywhere, swelling in the face is especially noticeable. A greater manifestation of edema is observed in patients who do not observe a healthy lifestyle and have an unhealthy eating habit. Regardless of the reason for admission from the hospital, patients need help. In most cases, patients can adjust their condition themselves by using mild diuretic drugs.

Diuretics are mainly represented by the following groups:

1) diuretics "loop" and acting on the cortical segment of the Genle loop;

2) potassium-saving diuretics;

3) osmodiuretics.

Diuretics have a different duration of effect

on diuretics, and the force of exposure can also be different. This primarily depends on their physicochemical properties, mechanism of action.

To date, the effectiveness of medicinal plants with diuretic effects has also been proved<sup>1</sup>.

The therapeutic properties of Asafetida are diverse. In treatises on Tibetan medicine, they claim that the plant "eliminates anxiety in the heart and rejuvenates organs that collapse from old age."

According to some authors, the composition of the plant is unique and the main therapeutic component is dried in the air milky juice (gum) obtained from the roots of the plant. Gum resins in the pharmaceutical industry are made by water infusions, emulsions, pills used in nerve diseases, hysteria; asthma, cough, anticonvulsant pills, digestive pills.

Ferula assa foetida L. grows wildly in Central Asia, especially in Iran and Afghanistan. Asafetide, oleo gum resin, is obtained from F. assa-foetida by cutting roots or removing stems<sup>2</sup>.

In Iranian folk medicine, asafetide is used as a diuretic, antispasmodic, windmill and painkiller<sup>3</sup>.

Asafetide normalizes metabolic processes in the body, removes mucus, improves the peristalsis of the gastrointestinal tract, has, calming, antimicrobial and bactericidal, anesthetic and spasmolytic effects, improves potency.

According to literature, asafetide treats many female diseases. Smelly ferula is used for infertility, the threat of miscarriage, painful menstruation and pain<sup>4</sup>.

Our studies also showed antioxidant and antiinflammatory activity of the given drug plant<sup>5</sup>.

Asafetida is also a popular spice in Asian cuisine. It is used in Ayurvedic, Tibetan and many other folk medicine-with its help they successfully treat epilepsy, jaundice, hysteria, seizures, stomach diseases, etc. Speaking of Tibetan medicine, it should be noted that asafetide is considered the best aphrodisiac (effective dose - from 300 to 1000 mg). Asafetide contains essential oil, resin and gum. Oleum Asafoetida has been shown to enhance libido<sup>6</sup>.

In Ayurveda and traditional medicine of various countries, such as Iran, America and Brazil, asafetide was used as an excitatory agent, experiments were carried out proving the effect of the plant on sprermatogenic activity in rats<sup>7</sup>.

A number of researchers also emphasized the effect of Ferula's aphrodisiac assa-foetida, an increase in libido was noted<sup>8</sup>.

AYOUBI A.R. and other co-authors in their studies note the ability of the plant "Ferula assafoetida" to reduce testosterone levels, which is an important factor in the therapy of patients with benign prostatic hyperplasia<sup>9,10</sup>.

This paper presents the results of a study of the effect of Asafetida resin gum on the urinary system, namely the diuretic effect, as well as the ability to affect spermatogenesis and testosterone levels in the blood of male rats.

We obtained gum resin together with employees of the ABDU-S emergency, in the form of a powder obtained during the separation from the upper layer the fat part of the resin. The resin is obtained from the incision of the plant Ferula assa-foetida growing in the Jizzak region of Uzbekistan. Asferwon powder is obtained as a result of simple technological procedures in accordance with patent No. IAP 06453<sup>11</sup>.

In the first part of the experiment, diuretic properties and the ability to affect changes in the level of pH in the urine of rats after the use of Asfervon were studied, since in folk medicine in Iran this plant is used as a diuretic. Also, in the second part of this study, we set ourselves the goal to study the effect of Asferwon, which contains the plant Ferula assafoetida on spermatogenesis and testosterone levels in the blood of male rats. Material, research methods: all studies were carried out on healthy animals quarantined for at least 10-14 days. The object of our study was the drug "Asfervon," which includes: the plant Ferula assafoetida.

Study of diuretic action. Experiments were carried out on an aqueous load model. During the experiment, all animals were kept under standard vivarium conditions. At the same time, the temperature of the room was in the range of 18-25 ° C, the relative humidity in the range of 40-70%.

The experiment was conducted on 24 white, non-fertile female rats weighing 233-246 grams. The animals were divided into 4 groups of 6 rats in each group. A day before the experiment, the animals were placed in separate cells adapted to collect urine with a standard diet. Water was freely available to animals. The animals were kept stationary in vivarium.

Rats 2 hours before the experiment were orally (via a special probe) administered once according to the following regimen:

test group No. 1 - 1 ml of a 1% aqueous Asphervon solution was administered to animals at a dose of 50 mg/kg;

test group No. 2 - animals were injected with 1 ml of a 2% aqueous solution of the preparation "Asfervon" at a dose of 100 mg/kg;

control group (control) - distilled water was introduced in the corresponding volume;

intact group - no water load was produced in intact group.

The experiment was conducted for 5 hours. As a result, the collected volume of urine was studied according to the following criteria: amount of diuresis, odor, transparency, urine response (determined in parallel using a potentiometer and standard universal indicator paper). Data pH collected for 5 hours of urine. **Study of spermatogenic action**. We also studied the effect of the Asferwon preparation containing the plant Ferula assa-foetida on spermatogenesis. The study was conducted at the Med Standard Test Laboratory.

To study the effects of the drug on spermatogenesis, we used data from Seyyed Majid Bagheri and others. The following procedure was used in the work. Extracting rat sperm was performed through a notch and extracting a small portion of the testicular caudal appendage<sup>12</sup>.

Referring to this technique, we also after 2 weeks a small part of the testicular caudal appendage of each rat excised and evaluated the change in sperm count and parameters. The observation was carried out using a light microscope, with an increase of 10Kh times.

To conduct an experiment to study the spermatogenic effect, 12 sexually mature male rats weighing 245-247 gr were selected. During the experiment, all animals were kept under standard vivarium conditions.

At the same time, the temperature of the room was in the range of 18-25  $^{\circ}$  C, the relative humidity in the range of 40-70%. Rats were divided into 2 groups, 6 rats in each group.

The test group - animals for 10 days before testicular separation, orally (by means of a special probe) was administered once 1 ml of 1% aqueous solution of the preparation "Asfervon" at a dose of 50 mg/kg.

Control group (control) - distilled water was introduced in the corresponding volume.

**Study of effects on testosterone levels in rat blood.** The effects of Asferwon on blood testosterone levels were studied using a procedure based on a change in testosterone levels in intact rats. During the experiment, all animals were kept under standard vivarium conditions. At the same time, the temperature of the room was in the range of 18-25 ° C, the relative

humidity in the range of 40-70%. The experiment involved 18 male rats weighing 190-240 g. Rats were divided into 2 groups, 9 rats in each group.

The test group - animals for 10 days before decapitation, orally (by means of a special probe) was administered once 1 ml of 1% aqueous solution of the preparation "Asfervon" at a dose of 50 mg/kg.

Control group (control) - distilled water was introduced in the corresponding volume.

To determine the level of testosterone in the blood of male rats, blood was removed by decapitation for analysis. The seized blood samples were delivered to the laboratory of the joint venture SWISS LAB LLC, where on the equipment Immulite 2000 xpi, Siemens Healthcare Diagnostics Limited, UK using special enzymatic-enhanced chemiluminescence technology, a study of testosterone levels in blood samples of intact and experimental groups was carried out. All the results obtained were processed by the method of variation statistics according to the Student's criterion at  $p = 0.05^{-13,14}$ . The tables show the mean arithmetic values (M), their corresponding standard errors of the mean value (m), Student criterion (t), number of samples (n), confidence boundaries (lower confidence boundary upper confidence boundary).

#### Results

Diurez. From our experiments, we managed to find out that the drug "Asfervon" at a dose of 50 mg/ kg in comparison with the control had a 16.24% more pronounced diuretic effect. At a dosage of 100 mg/ kg, Asferwon compared to the control had 33.58% more pronounced diuretic effect (Table 1). The obtained data are similar to those of S. M. Bagheri, H. Mohammadsadeghi, M. H. Dashti-R, S. M. M. Mousavian, and Z. A. Aghaei, where a comparative study was conducted on the plant "Ferula assafoetida<sup>15</sup>.

Table 1.: Urine volume per 5 hours ( $M \pm tm$ ; p = 0.05; n=6)

N₂	Intact group	Control + H2O (5 ml/100 g)	Group No. 1. Asphervon 50 mg/kg + H2O (5 ml/100 g)	Group No. 2. Asphervon 100 mg/kg + H2O (5 ml/100 g)
Result	3,00 (2,7863÷3,2303)	4,56 (4,2395÷4,8737)	5,05 (4,9342÷5,1590)	5,57 (5,1330÷6,0002)
Increase in diuresis in%	-	+51,46%	+67,7%	+85,04 %

Urine color. In the experiment, the collected urine volume of the test and control groups ranged from thatched yellow to saturated yellow. The experimental group receiving the drug "Asfervon" was more often straw - yellow.

The urine odor in the experimental and control groups was sharp and specific.

Urine reaction. The obtained data on pH

(hydrogen ion concentration) indicators showed that the experimental groups receiving the preparation "Asfervon" in two dosages showed practically no statistically significant changes in the urine pH of rats, compared with the control and intact groups.

In group 1. A 50 mg/kg + H2O (5 mL/100 g) Asphervon preparation, compared with the control group, showed a change in the pH index by 0.43, towards the alkaline medium. As a percentage, this

figure is + 7%.

Group 2. The preparation "Asfervon" at a dose of 100 mg/kg + H2O (5 ml/100 g), compared with the control, showed a change in the urine pH index by 0.36, towards the alkaline medium. The percentage is + 5.93 per cent (Table 2).

Nº	Intact group	Control + H2O (5 ml/100 g)	Group No. 1. Asphervon 50 mg/kg + H2O (5 ml/100 g)	Group No. 2. Asphervon 100 mg/kg + H2O (5 ml/100 g)
Result	6,20(6,0033÷6,3866)	6,07(5,9641÷6,1825)	6,50(6,2471÷6,7595)	6,43(6,1505÷6,6994)
Increase in diuresis in%			+7%	+5,93

Table 2 : Determination of urine pH level ( $M \pm tm$ ; p = 0.05; n=6)

In the literature, there are also no statistically significant changes in the level of rat urine pH when administering the preparation from the plant "Ferula assa-foetida."

Spermatogenesis. Examination of the 50 mg/kg Asferwon effect on rat spermatogenesis showed that the drug improved the quantitative index of sperm presence in the test incision of the caudal appendage of the rat testicle. Compared to the control group by 60.5%. The study of the qualitative index of sperm, occurred under the measurement of the relative length of the sperm. In comparison to the control, the Asferwon group at 50 mg/kg increased the sperm length by 37.03% (Figure 1).



Figure 1.: The amount of sperm in the recovered material in the test and control groups. Experimental "Asfervon" 50 mg/kg Control

In order to obtain comparative data and analyze them, we took a drawing in the control and experimental group, under the same magnification we took an area of 2 cm2. Where we were able to record that the number of sperm in the field of view is an average of 10.16 (8.6216 sound11.7116) pcs in the experimental group and 6.33 (5.2493A 7.4173) pcs in the control group. The ratio of average sperm length to control was 2.22: 1.62. (Table 3).

	Control (H2O)	Experimental "Asfervon" 50 mg/kg		
length	1,62 (1,5187÷1,7378)	2,22 (2,1379÷2,3187)		
Number in view	6,33 (5,2493÷7,4173)	10,16 (8,6216÷11,7116)		
Change in% is long		+37,03		
Change to% Quantity		+60,50		

Table 3. Characteristics of rat sperm ( $M \pm tm$ ; O =; n=6)

In the experimental group, the sperm have a more shaped and mature shape compared to the control.

When studying the results of statistical processing, it was revealed that in comparison with the intact group, the animals to which the preparation from the plant "Ferula Assafoetida" under the trade name "Asfervon" was introduced reduced the level of testosterone in the blood by 4.7 times (Table 4).

Table 4. A study of the effect on total testosterone levels in the blood of rats with Asferwon at a dose of 50 mg/kg (M  $\pm$  tm; p = 0.05; n=9)

№ п/п	Name-group	Blood testosterone level, nmol/l
1	Intact	43,96 (41,0612÷46,8672)
2	Experiment	9,29 (8,5817÷9,9912)
		4.73 times decrease in testosterone level compared to intact group

# Result

The study showed that when the drug Asferwon was administered in experimental animals, testosterone levels were reduced. The results obtained are similar to those obtained by Seyyed Majid Bagheri<sup>16</sup> et al. These results allow us to approach a larger study of the therapy of benign prostatic hyperplasia, without additional prescribing drugs that reduce testosterone levels. Perhaps, in this case, the patient will be able to dispense with two directed pharmacological effects of one drug.

#### **Discussion of the Results**

The results of the studies we obtained showed identity with the given data of the literature. In this regard, there is a need for further deeper study of the various dosages of the drug "Asferwon" on the urogenital system of the rat.

This study clearly showed that the drug has a moderate diuretic effect, as well as improves the quantitative and qualitative indicators of rat sperm. The findings are in line with theoretical expectations and will serve as material for our further research.

The discovered ability to reduce testosterone levels in the blood of rats is necessary in the therapy of benign prostatic hyperplasia and will allow patients to dispense with additional drug therapy.

**Conclusions** in the presence of diuretic activity, Asfervon does not change the level of urine pH of rats, the drug showed spermatogenic activity, and the ability to reduce testosterone levels in the blood of rats.

**Ethical Clearance -** Taken from pharmacological committee

Source of Funding - Self

Conflict of Interest - Nil.

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