



## The study of the influence of *Thlaspi arvense* L. on the characteristics of the reproductive system of male rats

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### Abstract

**Introduction.** The development of new effective and safe herbal medicines capable of having a positive effect on reproductive status is an urgent task of domestic pharmacy. Medicinal plants are considered as alternative therapies aimed at increasing testosterone levels and fertility in men. The arsenal of medicinal plants used in traditional medicine for the treatment of diseases in men is mainly represented by phytoadaptogens, most of which have a small resource potential in Russia, therefore, it is relevant to search for plants with a sufficient raw material base and with the potential for cultivation. *Thlaspi arvense* L. is a promising medicinal plant, as it is widely used in folk medicine as a diuretic, anti-inflammatory, diaphoretic, antihistamine, hemostatic, astringent, has a positive effect on the processes of spermatogenesis and is widely distributed in our country. However, information on the chemical composition and biological activity of *Thlaspi arvense* L. they are insufficient, which shows the relevance of its further more detailed study in order to substantiate the possibility of application in practical medicine and solutions to the issues of standardization of medicinal plant raw materials.

**Aim.** The study of the influence of *Thlaspi arvense* L. on the characteristics of the reproductive system of male rats.

**Materials and methods.** The object of the study was an infusion of herba *Thlaspi arvense* L. The features of the reproductive behavior of male rats after 21-day administration of the infusion of grass yarutka field were studied using tests that allow quantifying the severity of sexual motivation and sexual activity of males. The viability of spermatozoa in the ejaculate of rats was assessed, the total number of spermatozoa (ACS, million), degenerative and immobile forms (%) was calculated. Morphophysiological parameters of spermatogenesis of rats of control and experimental groups of animals were studied using classical histological methods. The concentration of testosterone in the blood serum of experimental groups of animals was determined by the enzyme immunoassay.

**Results and discussion.** The results obtained allow us to conclude that the fertility of rats has increased against the background of the course administration of the infusion of herba *Thlaspi arvense* L., as evidenced by an increase in the testosterone content in the blood, improvement of spermogram indicators and morphophysiological characteristics of spermatogenesis in the testicles of rats. The use of the infusion of herba *Thlaspi arvense* L. has a protective effect on spermatogenesis. An increase in the thickness of the spermatogenic epithelium, the diameter of the cross-section of the convoluted seminal tubules and the index of spermatogenesis compared with the control group of animals was revealed. The positive effect of *Thlaspi arvense* L. on indicators of sexual motivation and sexual activity of male rats is shown.

**Conclusion.** *Thlaspi arvense* L. is a promising medicinal plant, as it is able to have a positive effect on libido, the number and mobility of spermatozoa, the production of sex hormones, spermatogenesis, as well as on the pituitary-gonadal axis, which is associated with the content of a complex of biologically active substances in it.

**Keywords:** herba, sexual behavior, sperm motility, spermatogenesis, testosterone

**Conflict of interest.** The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

**Contribution of the authors.** Rashit G. Farkhutdinov, Kira A. Pupykina, Lucia A. Sharafutdinova developed the design of the experiment. Lucia A. Sharafutdinova and Albina M. Fedorova conducted histological studies and investigated the sexual behavior of animals. Kira A. Pupykina, Ekaterina F. Koroleva carried out the procurement of raw materials and preparation of extracts for research. Rashit G. Farkhutdinov, Lucia A. Sharafutdinova, Kira A. Pupykina A., Ekaterina F. Koroleva, Zuhra R. Hismatullina, Tamara D. Rendyuk participated in data processing and in writing the text of the article. The authors Margarita I. Garipova and Anna A. Yamaleeva. carried out the determination of testosterone content. All the authors participated in the discussion of the results.

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## Изучение влияния ярутки полевой (*Thlaspi arvense* L.) на характеристики репродуктивной системы самцов крыс

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### Резюме

**Введение.** Разработка новых эффективных и безопасных лекарственных растительных средств, способных оказывать положительное воздействие на репродуктивный статус, является актуальной задачей отечественной фармации. Лекарственные растения рассматриваются как средства альтернативной терапии, направленные на повышение уровня тестостерона и фертильности у мужчин. Арсенал лекарственных растений, применяемых в традиционной медицине для лечения заболеваний у мужчин, представлен в основном фитоадаптогенами, большинство из которых имеют малый ресурсный потенциал на территории России, поэтому актуальным является поиск растений с достаточной сырьевой базой и имеющих потенциал к культивированию. Ярутка полевая является перспективным лекарственным растением, так как широко используется в народной медицине как мочегонное, противовоспалительное, потогонное, антигистаминное, гемостатическое, вяжущее средство, оказывает положительное влияние на процессы сперматогенеза и широко распространена на территории нашей страны. Однако сведения о химическом составе и биологической активности *Thlaspi arvense* L. недостаточны, что показывает актуальность ее дальнейшего, более подробного изучения с целью обоснования возможности применения в практической медицине и решения вопросов стандартизации лекарственного растительного сырья.

**Цель.** Изучение влияния травы ярутки полевой (*Thlaspi arvense* L.) на характеристики репродуктивной системы самцов крыс.

**Материалы и методы.** Объектом исследования служил настой травы ярутки полевой (*Thlaspi arvense* L.). Изучены особенности репродуктивного поведения самцов крыс после 21-дневного введения настоя травы ярутки полевой с помощью тестов, позволяющих количественно оценить выраженность половой мотивации и сексуальной активности самцов. Проведена оценка жизнеспособности сперматозоидов в эякуляте крыс, произведен подсчет общего количества сперматозоидов (ОКС, млн), дегенеративных и неподвижных форм (%). С помощью классических гистологических методов изучены морфофизиологические показатели сперматогенеза крыс контрольной и опытной групп животных. Иммуноферментным методом определена концентрация тестостерона в сыворотке крови экспериментальных групп животных.

**Результаты и обсуждение.** Полученные результаты позволяют сделать заключение о повышении фертильности крыс на фоне курсового введения настоя травы ярутки полевой, о чем свидетельствует увеличение содержания тестостерона в крови, улучшение показателей спермограммы и морфофизиологических характеристик сперматогенеза в семенниках крыс. Применение настоя травы ярутки полевой оказывает протекторное действие на сперматогенез. Выявлено увеличение толщины сперматогенного эпителия, диаметра поперечного сечения извитых семенных канальцев и индекса сперматогенеза по сравнению с контрольной группой животных. Показано положительное влияние ярутки полевой (*Thlaspi arvense* L.) на показатели половой мотивации и сексуальной активности самцов крыс.

**Заключение.** Ярутка полевая является перспективным лекарственным растением, так как способна оказывать положительное влияние на либидо, количество и подвижность сперматозоидов, выработку половых гормонов, сперматогенез, а также на гипофизарно-гонадную ось, что связано с содержанием в ней комплекса биологически активных веществ.

**Ключевые слова:** трава, половое поведение, подвижность сперматозоидов, сперматогенез, тестостерон

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Вклад авторов.** Р. Г. Фархутдинов, К. А. Пупыкина, Л. А. Шарафутдинова разработали дизайн эксперимента. Л. А. Шарафутдинова и А. М. Федорова провели гистологические исследования и исследовали половое поведение животных. К. А. Пупыкина, Е. Ф. Королева осуществляли заготовку сырья и приготовление извлечений для исследования. Р. Г. Фархутдинов, Л. А. Шарафутдинова, К. А. Пупыкина, Е. Ф. Королева, З. Р. Хисматуллина, Т. Д. Рендюк участвовали в обработке данных и в написании текста статьи. М. И. Гарипова и А. А. Ямалеева провели определение содержания тестостерона. Все авторы участвовали в обсуждении результатов.

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## INTRODUCTION

In recent years, interest in phytopreparations has been steadily growing worldwide, which is associated with an increase in complications when prescribing synthetic drugs and a change in the pharmacological effect when used together, especially in the treatment of the elderly [12, 17]. It is well known that the concentration of testosterone in men's blood decreases with age [37]. The effects of endocrine disruptors, xenobiotics, obesity, diabetes, concomitant chronic diseases, alcoholism, taking medications have a negative impact on human reproductive health, lead to the development of late hypogonadism in men [20]. Among the adverse effects of exogenous factors caused by androgen deficiency are decreased libido, erectile dysfunction, decreased hairline, increased fatigue, decreased muscle mass and bone mineral density, increased body fat, irritability, and poor quality of life [4, 10]. In general, most of these problems can be solved with the help of modern treatment methods, in particular, surgery, hormone therapy or using assisted reproductive technologies [43]. In addition, alternative therapies using medicinal plants aimed at increasing serum testosterone levels are considered as an effective method of protection, which may help alleviate some of the symptoms. Such therapeutic and preventive tactics are not opposed to practical medicine, but can increase the effectiveness of standard treatment methods [2, 3, 7, 10, 38, 44, 46].

One of the important factors of fertility is the number of spermatozoa, and reducing their number can reduce the likelihood of effective pregnancy [31]. It is suggested that spermatogenesis and maturation of germ cells depend on the degree of cytotoxic and pathological lesions of spermatogenic cells [4, 32, 39]. Cell damage also occurs under the influence of free radicals and oxidative stress, which is associated with a high content of polyunsaturated fatty acids in their plasma membrane and a very low level of cytoplasmic antioxidants [23, 39]. The main effect of lipid peroxidation in all cells, especially in sperm, is a violation of the structure and function of organelles or cell membranes (ion transfer processes, fluidity and permeability, metabolic gradients) [19, 24].

The arsenal of medicinal plants used in traditional medicine for the treatment of diseases in men is mainly represented by phytoadaptogens (*Panax ginseng* C. A. Mey., *Rhodiola rosea* L., *Rhaponticum cathamoides* (Willd.) Iljin., etc.) [2]. However, unfortunately, most of them have little resource potential in Russia [16]. In this regard, it is urgent to search for medicinal plants with a sufficient raw material base and with the potential for cultivation in our country. The literature describes the positive effects of medicinal plants and, in special, *Thlaspi arvense* L., on the processes of spermatogenesis [1, 2, 4, 10, 22, 26, 34, 41]. The *Thlaspi arvense* L. has a wide distribution area in Russia, is found everywhere from Western and Eastern Siberia to the Far East, in the Caucasus on dry meadows, deposits, wastelands, on salt marshes, is one of the most frequently encountered weeds [6, 13, 16, 28].

*Thlaspi arvense* L. is not an official plant and is used only in folk medicine as a diuretic, anti-inflammatory, diaphoretic, antihistamine, hemostatic, astringent, abroad as a food plant, oilseed culture [8, 10, 13, 29]. Research has shown that *Thlaspi arvense* L. has properties that increase fertility in men, antioxidant activity, preventing the formation of free radicals and lipid peroxidation, preventing damage to spermatozoa, enhancing the activity of the hypothalamic-pituitary-gonadal axis at different levels, affecting the secretion of luteinizing hormone and testosterone [1, 2, 9, 18, 19, 25, 33, 35, 36]. It is the flavonoids of *Thlaspi arvense* L. (apigenin, luteolin-7-glucoside, luteolin, etc.) that are powerful antioxidants, cause anti-inflammatory properties, due to their inhibitory effect on cyclooxygenase and lipoxygenase, inhibit the production of hydrogen peroxide and IgE, responsible for inflammation and allergic reactions [21, 27, 30, 40, 42]. Based on a review of the literature, it should be mentioned that the chemical composition of *Thlaspi arvense* L. has not been studied enough. However, it is known that *Thlaspi arvense* L. herb contains flavonoids (glycosides of quercetin, kaempferol, luteolin, apigenin), thioglycosides (sinigrin, glucocapparin), vitamin C, saponins, isothiocyanates, high molecular weight fatty acids (linoleic, linolenic, oleic, palmitic, stearic, tetracosan, eicosene, erucic and etc.), in seeds – fatty oil [6, 11, 13]. So, a more detailed pharmacognostic study of this plant and its biological activity is an urgent task.

**The purpose of the study** – the study of the effect of the herb *Thlaspi arvense* L. on the characteristics of the reproductive system of male rats.

## MATERIALS AND METHODS

*Thlaspi arvense* L. herb used as an object of research, from which an infusion was prepared in a ratio of 1:10, insisted on a boiling water bath for 15 minutes, cooled for 30 minutes, filtered and the resulting solution was used for research.

The experiments were carried out on male rats of the *Wistar* line at the age of 1.5 years (average weight – 300.0–400.0 g), kept in standard vivarium conditions of the Department of Physiology and General Biology of the Ufa University of Science and Technology of the Ministry of Defense of the Russian Federation. The rats of the control ( $n=6$ ) and experimental ( $n=6$ ) groups were kept at 12-hour daylight on a standard diet with briquetted feed, with free access to water and food. Estranging female *Wistar* rats at the age of 6 months used in conducting behavioral tests. During 21 days, an infusion of *Thlaspi arvense* L. herb in a volume of 1 ml/rat (based on the mass of air-dry raw materials 300 mg/kg of weight) administered intragastrically through a probe to the experimental group of animals, the animals of the control group received water. When working with experimental animals, we guided by the decision of the Council of the Eurasian Economic Commission dated 03.11.2016 № 81 «On Approval of the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of circulation of medicines».

Pharmacological research was carried out in accordance with the Interstate standard of the Russian Federation GOST 33647-2015 «Principles of Good Laboratory Practice (GLP)», «Rules adopted by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986). The research design approved by the bioethical commission of the Institute.

*The study of the sexual behavior* of male rats of the control and experimental groups (paired with intact estranging females) performed in an open field installation modified for a «zoosocial preference site» (ZPS). The installation of the ZPS was a circular arena with sides, divided by opaque plastic partitions into 4 compartments, connecting in the center. In the first, second and third compartments placed, respectively: a drinking bowl, a grain feeder, and a plastic house. The fourth compartment of the installation free, the male

subject placed in it 20 minutes before the start of the test. Later, the female test rat placed in the central part. The male monitored for 60 minutes after the female's arrival. Sexual behavior studied using tests that quantify the severity of sexual motivation and sexual activity of males [15]. Sexual motivation assessed by indicators of precopulatory behavior (behavioral reactions that encourage a partner to mate): the latent period of the first «emotional» approach of the male to the female, sniffing, grooming; the number of «emotional» approaches to the female. In the study of actual sexual behavior, the duration of sexual activity estimated (the total period of time spent by a male on «courting» a female, the number of cages with intromissions) [5].

*Sperm viability assessed* in rat ejaculates, which were obtained by anesthesia with a 5 % solution of sodium thiopental administered intramuscularly. Then the animal fixed on a flat surface. Mature spermatozoa were obtained from the appendage of the testis, making a homogenate from it in a 5 % glucose solution (in a volume of 1 ml), preheated to a temperature of 37 °C. Then, the total number of spermatozoa (TNS, million), degenerative and immobile forms (%) calculated in Goryaev's chamber [14].

*Histological research.* The testes of rats fixed in neutral 10 % formalin by Lilly, dehydrated in alcohols of ascending concentration and poured into paraffin. A series of frontal sections with a thickness of 10–12 microns was prepared. Microscopy of the obtained histological preparations carried out using a Micmed-5 light-optical microscope (LOMO, Russia). The photography carried out with the help of a camera Levenchuk C510 (5M pixels). The structural changes of the testes studied in the field of view of the Micmed-5 microscope (LOMO, Russia) at magnification of 100 (eyepiece 10, lens 10) and 400 times (eyepiece 10, lens 40).

The generative function of the convoluted seminal tubules judged on the basis of an estimate of the cross-sectional diameter of the convoluted seminal tubules (microns), as well as the index of spermatogenesis (in 100 tubules, the presence of spermatogonies, spermatocytes of the I and II orders, spermatids and spermatozoa recorded in each tubule according to a 4-point system) [5].

*For enzyme immunoassay determination of testosterone concentration in the blood serum* of male *Wistar* rats, a set of reagents "Testosterone-ELISA-Best" (Vec-



tor-Best, Russia) was used. The determination method based on a single-stage solid-phase competitive enzyme immunoassay using monoclonal antibodies to testosterone. In the wells of the tablet, when the test sample added, there is a competitive binding of serum testosterone and testosterone conjugated with peroxidase, monoclonal antibodies to testosterone located on the inner surface of the wells. Quantity of the conjugate bound determined by a color reaction using a peroxidase substrate, hydrogen peroxide, and a chromogen, tetramethylbenzidine. The intensity of staining is inversely proportional to the concentration of testosterone in the analyzed sample. The optical density measured using the UNIPLAN enzyme immunoassay analyzer (AIFR-01, Russia) at a wavelength of 450 nm.

Statistical processing of the obtained data conducted using the licensed STATISTICA 10 for Windows application software package. The Shapiro-Wilk criterion used to check the normality of the distribution. The comparison of data obeying the law of normal distribution conducted using parametric methods (the Student t-criterion for independent samples).

RESULTS AND DISCUSSION

The results of the study of the peculiarities of animal reproductive behavior against the context of the course administration of the infusion of the herb *Thlaspi arvense* L. presented in Table 1. In the rats of the experimental group, an increase in perceptual and receptive motivations observed. Thus, in males, against the background of the use of the *Thlaspi arvense* L., a

decrease in the latent period of the first «emotional» approach to an intact female rat determined by 27.3 %, an increase in the number of «emotional» approaches by 24.8 % and an increase in the duration of the «courtship» period for females by 42.2 %. The study of sexual behavior itself showed an increase in the number of female coverages by more than two times.

At the next stage, a microscopic analysis of the rat ejaculate performed (Table 2). It was found that against the background of a 21-day course of administration of *Thlaspi arvense* L. herb infusion, the number of spermatozoa in males of the experimental group was significantly higher than in rats of the control group ( $p < 0.05$ ). At the same time, a significant decrease in the number of pathological and immobile forms of spermatozoa found by 38 and 31 %, respectively.

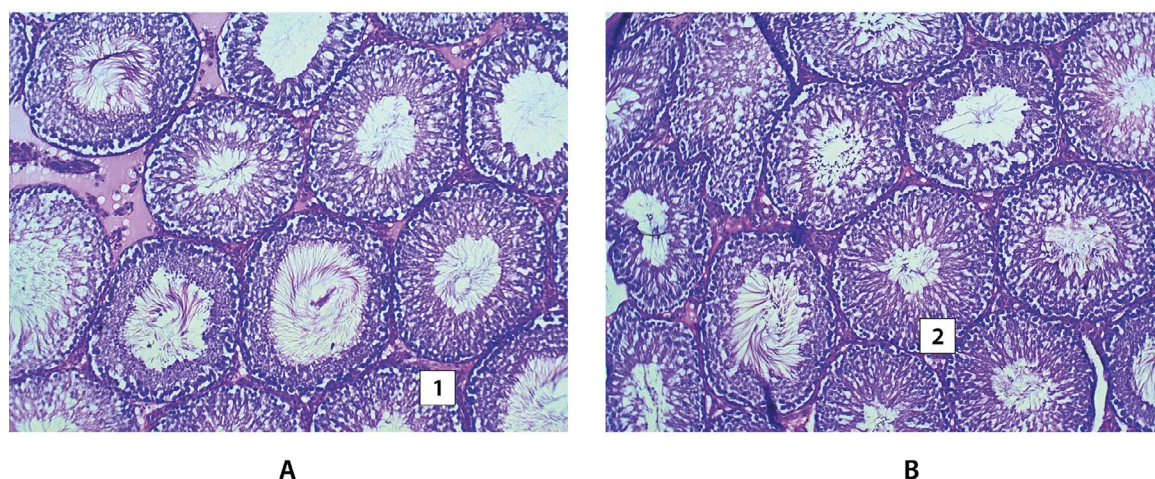
Histological analysis revealed that the structural organization of the testicles of rats in the control group corresponded to the typical one for these animals. Densely arranged convoluted seminal tubules of rounded shape are clearly visualized on histological preparations (Figure 1 A).

Interstitial cloth is located between the loops of the convoluted seminal tubules, in which hemocapillaries are determined, providing metabolism between blood and spermatogenic epithelium, as well as groups of Leydig cells. The spermatogenic epithelium of the tubules is located on the basement membrane and formed by spermatogenic cells at different stages of spermatogenesis. Normal and mature spermatozoa often detected in the lumen of the tubules.

Table 1. Indicators of sexual motivation and sexual activity of male rats against the background of the introduction of an infusion of herba *Thlaspi arvense* L. for 21 days ( $M \pm m$ )

Group	Latent period of the first approach to the female, s	The number of «emotional» approaches to the female, pcs	Total courtship time, s	Number of cages, pcs
Control	6,6 ± 1,8	24,2 ± 1,02	716,4 ± 90,3	0,8 ± 0,1
Experimental	4,8 ± 1,3	29,4 ± 1,3	1022,6 ± 174,4*	2,7 ± 0,2*

Note. \* Statistically significant differences compared to the control group at  $p < 0.05$ .



**Figure 1.** Convoluted seminal tubules of testes of rats of control (A) and experimental (B) groups of animals. Hematoxylin-eosin staining, uv. x100:

1 – interstitial tissue with Leydig cells, 2 – spermatogenic epithelium

**Table 2.** Spermogram indicators of rats before and after the application of the infusion of herba *Thlaspi arvense* L.

Group	Total number of spermatozoa, mln	Pathological forms, %	Fixed forms, %
Control	9,12 ± 2,6	25,8 ± 3,2	45,4 ± 8,9
Experimental	12,5 ± 2,4*	16,1 ± 2,0*	31,3 ± 5,4*

**Note.** \* Statistically significant differences compared to the control group at  $p < 0.05$ .

The morphological picture of the testes of rats of the experimental group (Figure 1 B) characterized by a significant thickness of the epitheliospermatogenic layer due to the presence of cells of all stages of spermatogenesis (spermatogonia, primary spermatocytes, spermatids, spermatozoa). The diameter of the convoluted seminal tubules of the rats of the experimental group was wider.

The results of the morphometric analysis of the testes of the control and experimental groups presented in Table 3. Morphometric parameters (diameter of the convoluted seminal tubules, height of the spermatogenic epithelium, index of spermatoge-

nesis) against the background of the course intake of the infusion of *Thlaspi arvense* L. herb differ significantly from the results of the control group of the analyses.

The width of the spermatogenic epithelium (Table 3) in rats of the experimental group, against the background of intragastric administration of *Thlaspi arvense* L. herb infusion increased by 100 % compared with the control group ( $p < 0.05$ ), and the cross-sectional diameter of the convoluted seminal tubules increased by 15 % ( $p < 0.05$ ). In the testes of experimental rats, compared with the control group, the spermatogenesis index increases ( $p < 0.05$ ). This indicator, reflecting the number of generations of spermatogenic cells in the wall of the convoluted seminal tubules, is the most important quantitative indicator characterizing the generative activity of the testis.

An immuno-enzyme analysis performed to determine the level of testosterone in the blood serum of the control and experimental groups of animals (Table 4).

The results of the analysis showed an increase in the concentration of testosterone in the blood serum of an experimental group of animals under the influence of the infusion of *Thlaspi arvense* L. herb, which is important, since the level of testosterone as the most important androgenic hormone has a direct effect on stem cell division and differentiation of spermatogenic cells. Probably, biologically active substances of *Thlas-*

*pi arvense* L. directly affect Leydig cells, stimulating the secretion of testosterone. At the same time, studies in recent years have shown that the flavonoids, luteolin derivatives, included in the *Thlaspi arvense* L., are natural aromatase inhibitors, which helps to reduce the volume of conversion of the male hormone into estrogen and, consequently, promotes the growth of testosterone [45].

**Table 3. Morphophysiological parameters of rat spermatogenesis after course administration of infusion of herba *Thlaspi arvense* L. ( $M \pm m$ )**

Parametrs	Control group	Experimental group
Thickness of spermatogenic epithelium (mcm)	41,79 ± 3,36	83,76 ± 8,6*
Diameter of the cross-section of the convoluted seminal tubules (mcm)	149,51 ± 6,49	171,97 ± 6,5*
Spermatogenesis index (number)	3,25 ± 0,07	4,69 ± 0,06*

**Note.** \* Statistically significant differences compared to the control group at  $p < 0.05$ .

**Table 4. Enzyme immunoassay indicators of testosterone concentration in the blood serum of animals against the background of the introduction of the infusion of herba *Thlaspi arvense* L.**

Group	Testosterone levels, nmol/l
Control	3,96 ± 0,36
Experimental	4,89 ± 0,43*

**Note.** \* Statistically significant differences compared to the control group at  $p < 0.05$ .

CONCLUSION

In general, the results obtained allow us to conclude that the fertility of rats increased against the background of the course administration of *Thlaspi arvense* L. infusion, as evidenced by an increase in testosterone content in the blood, improved spermogram and morphophysiological characteristics of spermatogenesis in the testicles of rats.

A statistically significant increase in the thickness of the spermatogenic epithelium, the diameter of the cross-section of the convoluted seminal tubules and the index of spermatogenesis revealed compared with the control group of animals. Generalizing the results obtained, it can be noted that the infusion of *Thlaspi arvense* L. herb has a positive effect on the indicators of sexual motivation and sexual activity of males, a protective effect on spermatogenesis, the mechanism of action of which may consist in the ability to inhibit the processes of lipid peroxidation and membrane destruction due to direct and indirect antioxidant and membrane protective effects.

So, *Thlaspi arvense* L. is a prospective medicinal plant for research, as it is able to have a positive effect on libido, sperm count and motility, production of sex hormones, spermatogenesis, as well as on the pituitary-gonadal axis, which is associated with the content of a complex of biologically active substances in it. Therefore, it is advisable to research in more detail the chemical composition and biological activity of *Thlaspi arvense* L. in order to substantiate the possibility of its application in practical medicine and to solve the issues of standardization of medicinal plant raw materials.

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