The effect of Echinacea purpurea extract on sexual glands of male rats

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Key words: *Echinacea purpurea, testicle, spermatic duct, epididymis, testosterone.*

Summary. *Fifty percent of men over the age of fifty are diagnosed benign prostate hyperplasia. It is caused by disorders in the balance of androgens and estrogens, depending on the activity of sexual glands; therefore it is advisable to examine the functioning of these organs and to determine the pathogenetic mechanism of effect of this pathology. The antiandrogenic effect of Echinacea preparations was examined in our previous study and hypoplasia of histological structures and the mass reduction of prostate were determined. This encouraged more detailed investigation of the effect of the preparation directly to the organs, participating in the synthesis of the male hormone – testosterone. The effect of Echinacea extract on a testicle and epididymis was examined, the mass of these organs was determined, the proportion between the mass of the organ and the mass of a body was calculated, the changes in histological structures were evaluated in this study.*

Material and methods. Experiments with the Wistar line 3-month-old male rats were carried out. There were three experimental groups of rats. The first one was control group. The rats of the second group were fed on the usual food enriched with the Echinacea extract additive with the proportion of 50 mg/kg for 4 weeks. The rats in the third group were fed equally to the second one for 8 weeks. The clinical death of the animals was caused by overdosage of the solution of phenobarbital (1 mg/kg). The rats were weighed, the testicles and epididymis were eliminated, and pathohistological examinations were carried out.

Results. The average weight of the male rats in the control group was 1530±166.37 mg, in the second group – 1520±164.62 mg, and in the third group – 1499±158.81 mg. Calculations of the relative quantity between the mass of the organs and the body weight were made and it was estimated that the testicles of the rats in the first group made up 0.496±0.399% of a body mass, in the second one – 0.459±0.419%, and in the third one – 0.429±0.410%. The epididymis in the control group made up 0.189±0.332% of a body mass; in the second one – 0.1733±0.328%, and in the third one – 0.1723±0.198%. The histological structural changes were traced after 4 weeks of using the preparation, however they became more obvious after 8 weeks.

Conclusion. Results of the study enabled to determine statistically significant reduction in the percentage of a testicle and the body mass, as well as changes in histological structures after 8 weeks of consuming extract of Echinacea purpurea.

Introduction

In this article we investigate the mechanisms of benign prostate hyperplasia (BPH), the ways of treatment used in the initial stages and the effect of Echinacea on the urogenital system. The effect of Echinacea extract on the prostate was investigated in our previous study; hypoplasia of the histological structures and the reduction of the mass of the prostate were established (1). Preparations of Echinacea Purpurea are rich in polysaccharides, acylamides of non-saturated acids, the substances of coffee acid, flavonoids, glycoproteins, which stimulate the activity of phagocytes, leucopoiesis, encourage T_h (helpers), and the activity of natural killers (NK) (2). These substances provide the features of non-specific immunostimulator; therefore preparations of Echinacea are widely used. In case of BPH urostasis is formed, which favors the formation of infectious complications

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of the urogenital system. While evaluating the possibilities of using these preparations in case of BPH, we attempted to demonstrate that sitosterol, which is found in Echinacea and reduces enlargement of the prostate, also has an antiandrogenic effect.

Male sexual hormone testosterone is produced in a testicle and development of BPH is due to the balance between it and estrogens (3–5), therefore we chose testicles and an epididymis of male rats as the object of study in order to investigate the antiandrogenic properties of this plant. Since functioning of testis and the formation of sexual cells depend on androgens we analyzed spermatogenesis and the effect of Echinacea on it.

Spermatogenesis is a complex of humoral, paracrinic and autocrinic functions, on which proliferation and differentiation of sexual cells depend (6). As long as the functioning of the system of hypothalamus of hypophysis is not unperturbed, normal spermatogenesis is proceeding, with the participation of gonadoliberins and lutropin (LH) and follitropin (FSH), Sertoli, Leydig and stem (germinative) cells.

After FSH and LH get into a systematical blood circulation they both unite with the receptors of the membrane of cell "targets". Lutropin stimulates the secretion of testosterone in Leydig cells and the hormone stimulating the follicles affects Sertoli cells and abates differentiation of germinative cells (5). Testosterone and dihydrotestosterone are the main androgens. For maturing of sexual cells bigger concentration of testosterone is required than the one in general blood circulation. This is ensured by secretion of albumen conveying androgens in Sertoli cells (APB) and biosynthesis of testosterone in Leydig cells (7).

Rats are handy as module animals as they posses a very prominent urogenital system amongst mammal animals. In testicles of rats as in human ones two kinds of spermatogonia can be distinguished (8–10). Due to different distribution of chromatin in cores we can distinguish light and dark spermatogonia. It is believed that light spermatogonia of A type are renewing stem cells. Reserved type spermatogonia of rats stay calm as long as the spermatogenic epithelium is not irritated. Later their fission is inducted and new stem cells are formed (11). In the process of mitotic and two meiotic fissions of spermatocytes, spermatids are being formed, which later become spermatozoids. The cycle from the beginning of stem cells fission to the formation of mature spermatozoids in a rat's sexual system takes 34.5 days. The spermatogonial stage lasts 8, meiosis lasts 13, and spermiogenesis – 13.5days (6). Successful process of these cycles is maintained by condition of spermatic duct epithelium. In case of disorders in the functioning of germinative epithelium the differentiation of sexual cells is violated (12). Maturating spermatozoids from the spermatic ducts of a testicle get into a long spermatic duct - an epididymis, where they finish maturing.

In our research we investigated the effect of the extract of Echinacea on testicle and epididymis. The aims of the investigation were the following: a) to define the alteration of a rat's testicle and an epididymis after consumption of Echinacea extract, b) to estimate the proportion of the eliminated organs (a testicle and an epididymis) and the mass of a body, c) to estimate the possible effect of phytopreparations on spermatogenesis after carrying out the testing of histological preparations.

Material and methods

The experiments were carried out with 18 Wistar line male rats, aged 3 months. Rates were divided into three groups. The first group was a control group. The rats in the second group were given the additives of Echinacea extract with the proportion of 50 mg/kg included in their daily ration. The third group was fed on the same food as the second one. The extract of Echinacea Purpurea was produced in the laboratory of Joint Stock Company "Bakteriniai preparatai" from the roots of the plant. They were grained, poured with 50% ethanol solution and tumefied for 6 hours. The tumefied substance was loaded into a percolator, and percolation was carried out at the speed of 120–150 drops/min. Later the extract was stored in a cold compartment at the temperature of +2–8°C for 8 days and then filtered. The rats' clinical death was caused through overdosing sodium phenobarbital 1mg/kg intramuscularly. After that cervical dislocation was performed. Then each animal was weighed and one of the testicles and epididymis were removed. The weight and size of these organs were evaluated. Removed organs were fixed in 4% formaldehyde solution and pathohistological preparations were prepared. The thickness of histological incisions was 7–10 μm. They were colored with hematoxylin-eosin dye. After that the histological incisions were magnified through a 40x10 objective and photographed. A scale of histological structures was established with the help of an ocular micrometer. The changes of histological structures were evaluated. The proportion between the rat's mass and a testicle and an epididymis was calculated by multiplying the body weight and the mass of the organs by 100 (15). The experiments

were carried out following the order of the commission on the ethics of exploiting animals for laboratory needs in Lithuania (No. 0076).

The data of the removed organs were compared between groups using t-test for independent samples, comparing the dispersions simultaneously. Results were calculated according to the formula x±s, where x – the sample average, s – a moderate square deflection. A standard average error (s_x) was indicated in the article.

Results

The average testicle weight of the rats in control group was – 1530±166.37mg; in the second group – 1520±164.62mg; and in the third – 1499±158.81 mg. The comparison of the testicles mass in the control group with the masses of the rats, which used the extract of Echinacea, did not indicate statistically significant decrease of the testicles.

The average weight of epididymis of the rats in control group was – 585.8±112.4463 mg; in the second one – 578.3±111.43 mg; and in the third one – 601.3±67.33 mg. The comparison of the statistical data of the three groups did not indicate any significant differences.

We calculated what percentage of rat's body is taken up by testis and epididymis. The calculated amounts were compared between separate groups. The average of the testicles in the control group made up 0.496±0.399% of the overall weight, in the second group – 0.459±0.419%, and in the third – 0.429±0.410% (Fig. 1). The average of the epididymis of the rats in the control group made up 0.189+/–0.332% of the body mass; in the second – 0.1733+/–0.328%; and in the third – 0.1723+/-0.198% (Fig. 2).

Percentage of the rats' testicle and the body weight was decreasing, however, statistically significant decrease was established only between the control and the third group of the rats (p=0.01). Percentage of the epididymis weight and the body weight did not decrease statistically significantly, and after evaluation of the average masses of removed organs, there was no significant difference.

After histological research of removed organs and evaluation it was stated that in the testicles of the control group rats the incisions of the spermatic tubes were clearly seen (Fig. 3). Between the tubes thin interstitial tissue with blood vessels and Leydig cells can be seen. The tubes are surrounded by the propria of the connective tissue, from which a thin basic membrane

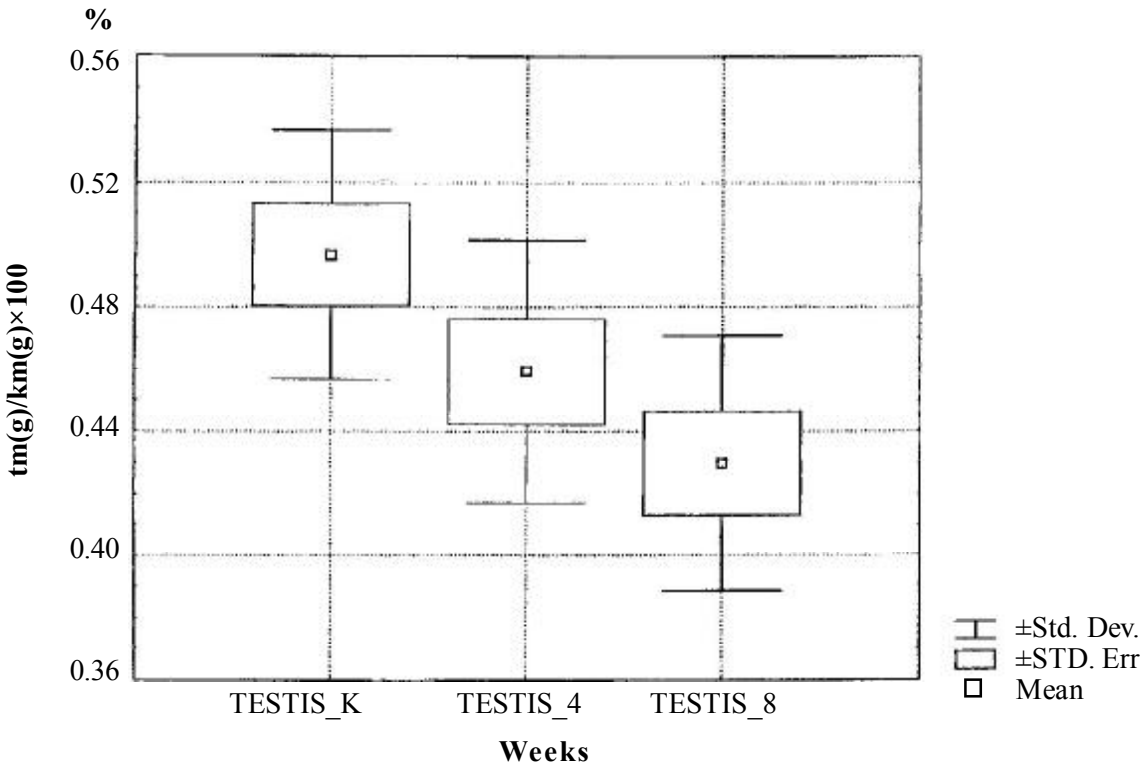

Fig. 1. **Dynamics of proportion between the body weight and the weight of a testicle, consuming the extract of Echinacea for 4 and 8 weeks**

tm(g) – the weight of testis in grams, km(g) – the body weight in grams, TESTIS K – the control group, TESTIS 4 – after 4-weeks consuming of Echinacea extract, TESTIS 8 – after 8-week consuming of Echinacea extract (p 0.01)

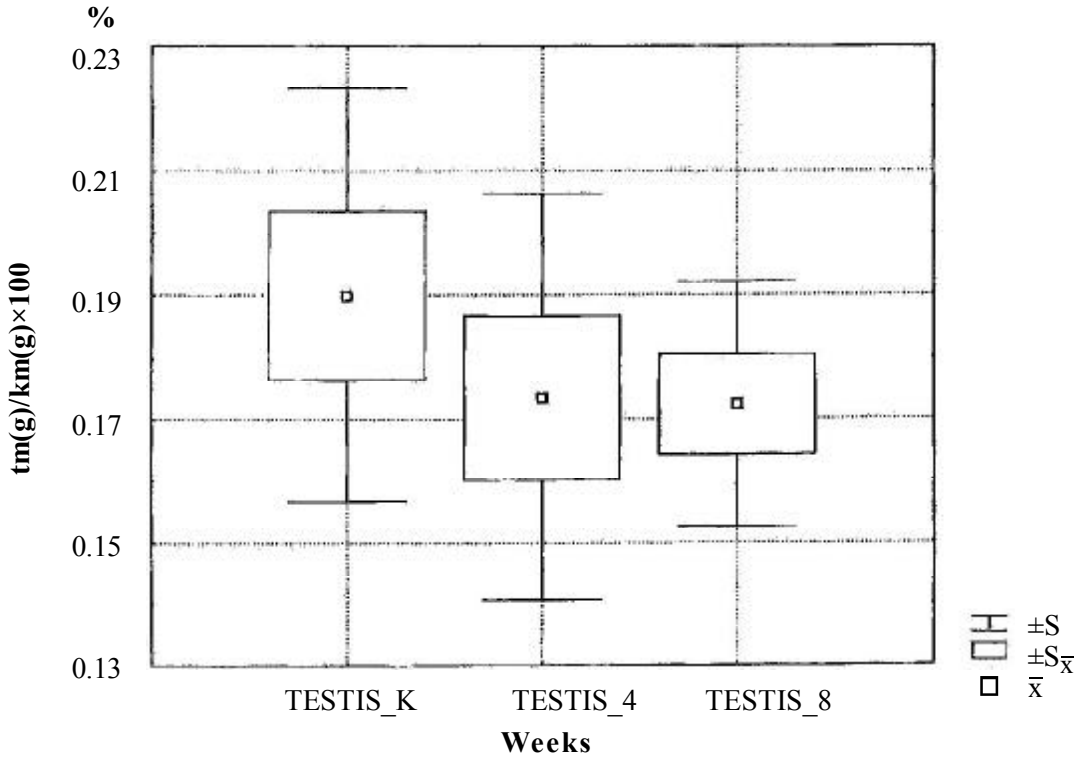

Fig. 2. **Dynamics of the proportion between the weight of the epididymis (EPID) and the body weight, consuming Echinacea extract for 4 and 8 weeks**

epm(g) – epididymis weight in grams, km(g) – the body weight in grams), EPID K – the control group, EPID 4 – after 4-week consuming of Echinacea extract, EPID 8 – after 8-week consuming of Echinacea extract.

stretches inside, on which a spermatogenic epithelium is set out. It can be seen that next to the basic membrane spermatogonia are set out, between which the cores (the lighter ones) of Sertoli cells can be seen. Spermatogonia are small round cells. A narrow stripe of cytoplasm can be seen around their cores. The cores of the spermatogonia are colored rather intensely. It is noticeable that the spermatic tubes are different in their structure depending on the phase of the spermatogenesis. Normally, while penetrating inside the spermatic

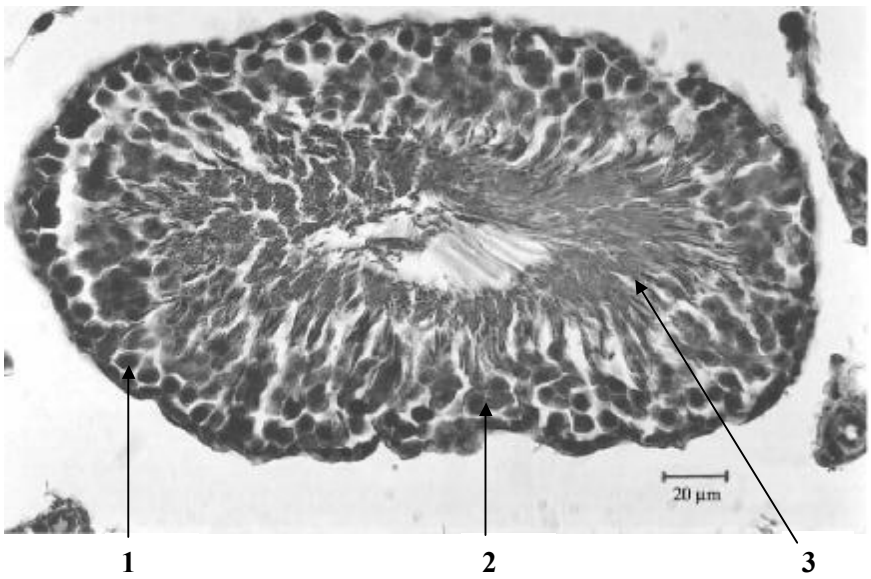

Fig. 3. **Histological picture of testicle in the control group**

1 – spermatogonia, 2 – spermatocytes, 3 – spermatozoids.

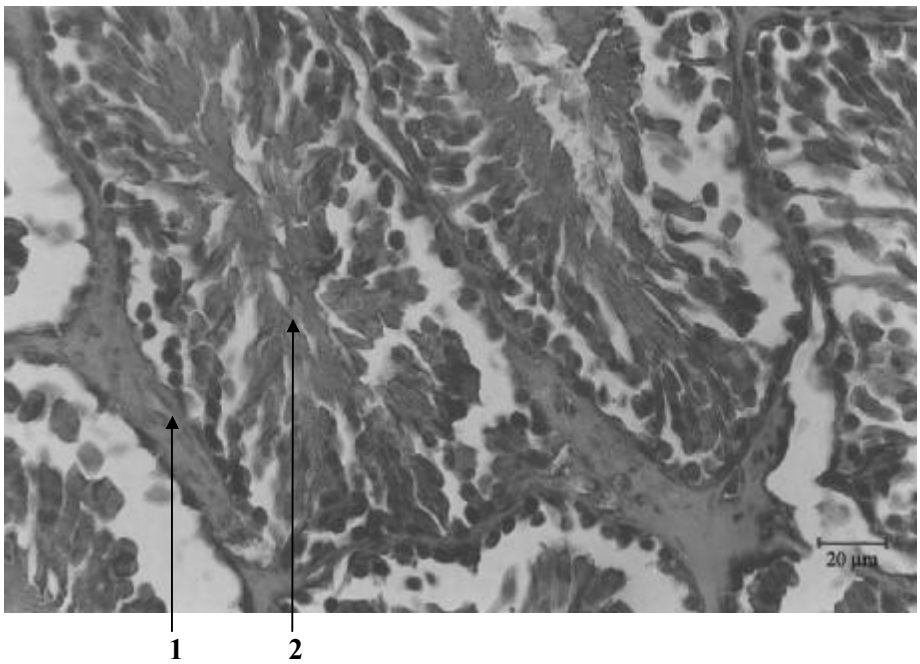

Fig. 4. **Histological picture of rats' testicle after 8-week consuming of Echinacea extract**
1 – nub parries of the spermatic tube, 2 – sporadic spermatozoids.

tubes, behind the spermatogonia in the initial stage of spermatogenesis primary spermatocytes can be well seen. These are the biggest cells of a spermatic tube. Further towards the radius smaller cells are situated – secondary spermatocytes and finally spermatids, which occupy the inner layer of a spermatic tube. The spermatozoids are accumulated in the radius of a tube, with their prolonged heads sticking out from the cytoplasm of the Sertoli cells and their tails directed towards the radius of the tube.

In the histological preparations of rats in the second group after a four-week consumption of Echinacea extract spermatogonia of A type could be distinguished. Other stages of spermatogenesis differentiate poorly. The cell cores color poorly and the cytoplasm frequently homogenizes. Inside the tube ma-

Fig. 5. **Histological picture of the epididymis in the control group of the rats**
1 – the two-line polar epithelium, 2 – spermatozoids.

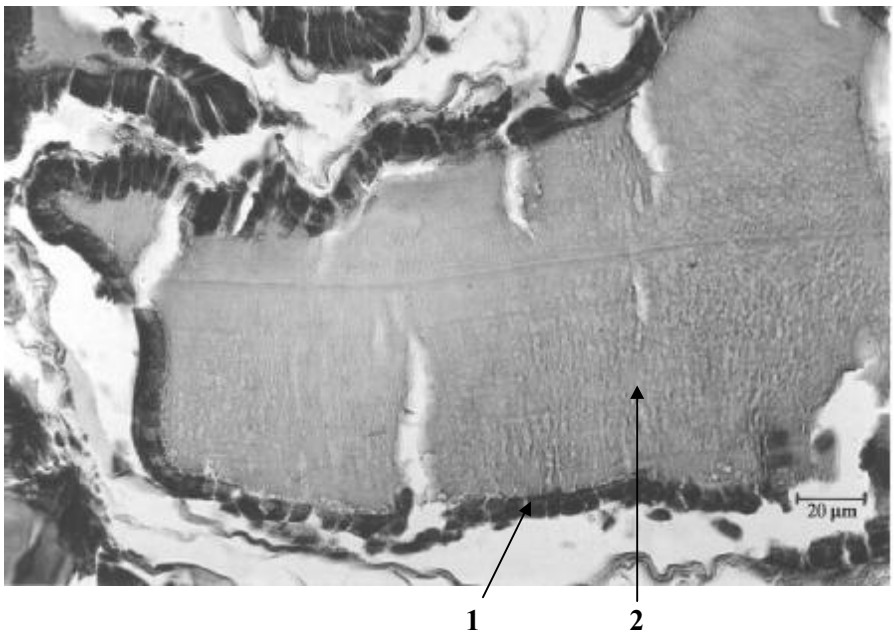

Fig. 6. **Histological picture of the rats' epididymis after 8-week consuming of Echinacea extract**
1 – flat two-line polar epithelium, 2 – extended radius of a duct with no spermatozoids.

ture spermatozoids can also be seen, however, their amount is smaller than that in the regular spermatic tubes.

In histological testicle preparations of rats in the third group spermatic cells are poorly seen, only spermatogonia of A type next to the basic membrane can be distinguished. Mitotic figures are scarce. Sporadic spermatozoids can be distinguished in certain spots of the spermatic tubes. This shows that continuous consumption of the preparation inhibits spermatogenesis and causes variations in the parries of the spermatic tubes (Fig. 4).

Evaluation of the histological preparations of epididymis indicated that a duct of epididymis contained epithelium, the basic membrane and a thin layer of muscles set out circularly. The epithelium of the duct is a two-line pole, which is made up of small basic cells and high poles with stereocilia (Fig. 5). Spermatozoids are plentiful in the radius of the duct. In the histological preparations of the duct of the epididymis in spermatozoids of the second group rats are still plentiful. The radiuses of certain ducts in comparison with the preparations of the control group are a little wider. The epithelium is flatter, thinner, and its cells color more poorly. In the preparations of epididymis in the third group some ducts are extremely wide and thin. Inside them there are no spermatozoids (Fig. 6). The analysis of histological preparations of the removed organs it was indicated that after consuming Echinacea extract for 4 weeks histological variations were not obvious, and after 8 weeks of consumption the variations were evident.

Discussion

Results of the experiments indicate that Echinacea extract affects testicle of rat, i.e. it decreases the weight of this organ and does not affect the weight of epididymis. With rats' body weight and the weight of organs changing proportionally in the course of time (15), we applied a more precise proportion of the organ mass and the body weight and established that the average of testicle weight decreased statistically significantly only after 8-weeks consuming of the preparation. Analysis of the histological preparations of the removed organs partly correlates with the variations in the weight of the organs. Investigating the preparations of testicle after 4-week consumption of Echinacea extract soft variations of spermatogenesis inhibitions can be noticed and after 8 weeks of consumption–disorders in differentiation of cells of spermatogenic epithelium of the spermatic duct are noticed. On the other hand, structural variations of the epididymis, which can disorder maturity of spermatozoids and reabsorption of liquids in the epididymis (12), also occurred consuming the Echinacea extract for longer time.

The variations show that Echinacea extract is distinguished by gradual antiandrogenic effect, which is noticed consuming the preparation from 4 to 8 weeks and affects the organ that produces male sexual hor-

mone testosterone. Answering the question what determines the antiandrogenic effect of the preparations of *Echinacea purpurea* it is possible to claim that it is associated with the vegetative sterols (sitosterol, campesterol, stigmasterol). The chemical structure of these compounds is very similar to cholesterol. In the digestive duct vegetative sterols reduce the absorption of cholesterol, creating a certain competition between sterols and cholesterols. The synthesis of testosterone cholesterol, which is converted into pregnenolone, is vital. From it by a progesteronic or dehydroepiandrosteronic methods testosterone is being synthesized (13, 14), therefore, with the decrease of cholesterol the concentration of testosterone in blood decreases.

Conclusions

Statistically significant decrease of the weight of male rats' testicles and epididymis was not established after consumption of Echinacea extract for 4 and 8 weeks.

After consumption of the Echinacea extract for 8 weeks proportion between testicle weight and body weight decreased statistically significantly from 0.496±0.399% of body weight to 0.429±0.410% (p=0.01).

The effect of Echinacea was also established in the histological preparations. Consumption of the preparation for 4 weeks caused insignificant structural variations in the testicles and an epididymis. Consumption of the preparation for 8 weeks caused antiandrogenic variations of cells of spermatogenic epithelium of a testicle duct. Analysis of the histological preparations showed that Echinacea extract inhibited spermatogenesis.

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Rausvažiedės ežiuolės ekstrakto poveikis žiurkių patinėlių lytinėms liaukoms

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Raktažodžiai: rausvažiedė ežiuolė, sėklidė, sėklinis latakas, prielipas, testosteronas.

Santrauka. Po 50-ties metų gerybine prostatos hiperplazija serga apie 50 proc. vyrų. Gerybinę prostatos hiperplaziją skatina atsirasti androgenų ir estrogenų pusiausvyros sutrikimai, priklausantys nuo lytinių liaukų veiklos, todėl tikslinga ištirti šių organų funkciją ir nustatyti šios patologijos patogenezinį veikimo mechanizmą. Ežiuolės ekstrakto antiandrogeninis poveikis buvo tiriamas ir nustatyta prostatos histologinių struktūrų hipoplazija bei priešinės liaukos masės sumažėjimas. Tai paskatino nuodugniau ištirti tiesioginį šio preparato poveikį organams, dalyvaujantiems vyriško lytinio hormono, t. y. testosterono sintezėje.

Buvo tiriamas ežiuolės ekstrakto poveikis sėklidei ir prielipui, nustatyta šių organų masė, apskaičiuota organo masės ir kūno masės santykinis dydis, įvertinti histologinių struktūrų pokyčiai.

Tyrimo medžiaga ir metodai. Eksperimentai atlikti su Wistar linijos trijų mėnesių žiurkių patinais. Sudarytos trys eksperimentinės grupės. Pirma grupė – kontrolinė. Antros grupės žiurkės keturias savaites maitintos įprastu maistu įpilant ežiuolės ekstrakto 50 mg/kg. Trečios grupės žiurkės aštuonias savaites maitintos taip pat kaip ir antros. Gyvūnų klinikinė mirtis sukelta perdozavus fenobarbitalio tirpalu 1 mg/kg. Žiurkės pasvertos, pašalintos sėklidės bei prielipai pasverti, atlikti patohistologiniai organų tyrimai.

Rezultatai. Kontrolinės grupės žiurkių patinėlių sėklidžių masės vidurkis – 1530±166,37 mg; antros grupės – 1520±164,62 mg; trečios – 1499±158,81 mg. Apskaičiavus santykinį organų masės ir kūno masės dydį, pirmos grupės žiurkių sėklidės sudarė 0,496±0,399 proc. kūno masės; antros – 0,459±0,419 proc; trečios – 0,429±0,410 proc; Kontrolinės grupės prielipas sudarė 0,189±0,332 proc. kūno masės; antros grupės – 0,1733±0,328 proc; trečios – 0,1723±0,198 proc.

Išvados. Histologinių struktūrų pokyčių rasta keturias savaites pavartojus preparatą, tačiau šie pokyčiai buvo ryškesni po aštuonių savaičių.

Įvertinus tyrimo duomenis, nustatytas reikšmingas sėklidės ir kūno masės dydžio mažėjimas bei histologinių struktūrų pokyčiai atsiradę po aštuonių savaičių rausvažiedės ežiuolės ekstrakto vartojimo.

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References

1. Skaudickas D, Kondrotas AJ, Baltrušaitis K, Vaitiekaitis G. Rausvažiedės ežiuolės (*Echinacea purpurea L. Moench*) poveikis priešinei liaukai eksperimento sąlygomis. (Effect of *Echinacea purpurea L. Moench* preparations on experimental prostate gland). Medicina (Kaunas) 2003;39(8):761-6.
2. Jurkštienė V. Preparatai pagaminti iš *Echinacea purpurea L. Moench* – nespecifiniai imunostimuliatoriai. Daktaro disertacijos santrauka. (Preparations produced from *Echinacea purpurea (L.) Moench* – non-specific immunostimulators. Summary of Doctoral Thesis). Kaunas; 2000. p. 16-35.
3. Roehrborn G, Lee M, Meehan A, Waldstreicher J, Pless Study Group. Effects of finasteride on serum testosterone and body mass index in men with benign prostatic hyperplasia. Urology 2003;62(5):894-99.
4. Carson C III, Rittmaster R. The role of dehydrotestosterone in benign prostatic hyperplasia. Urology 2003;61(1):2-7.
5. Re G, Badino P, Odore R, Vigo D, Bonabello A, Rabino S, Capello F, et al. Effects of mepartricin on estradiol and testosterone serum levels and on prostatic estrogen, androgen and adrenergic concentrations in adult rats. Pharmacol Res 2001;44(2):141-7.
6. Meng X. Giall cell line-derived neurotrophic factor and neurturin in the regulation of spermatogenesis. Academic dissertation. Helsinki; 2001.
7. Toshimori K, Kuwajima M, Yoshinaga K, Wakayama T, Shima K. Dysfunctions of the epididymis as a result of primary carnitine deficiency in juvenile visceral steatosis mice. FEBS Letters 1999;446(2-3):223-6.
8. Patil SR, Patil SO, Londorkar R, Patil SA. Effect of pethidine on spermatogenesis in Albino rats. India J Pharmacol 1998;30:249-53.
9. Yang SH, Cheung AP, Han DK, Sim JCh, Lee EJ, Kim JH. Cadmium-induced toxicity on testicular tissue and spermatogenesis in rats and the protective effects of tocohperol. Fertility Sterility 2002;78(1):274-5.
10. Meistrich M, Wilson G, Shuttlesworth G, Porter K. Dibromochloropropane inhibits spermatogonial development in rats. Reproduct Toxicol 2003;17(3):263-71.
11. Rex A. Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biol Reproduct 1990;43:525-42.
12. Hess R, Bunick D, Lee K, Bahr J, Taylor J, Korach K, Lubahn D. A role for oestrogens in the male reproductive system. Nature 1997;390:509-12.
13. Černiauskienė LR, Lukšienė DK. Augaliniai steroliai mažina mažo tankio lipoproteinų, cholesterolio koncentraciją kraujo serume. (Phytosterols decrease small density lipoproteins, cholesterol concentration in serum of blood). Gydymo menas 2003;9(97):75-6.
14. Praškevičius A, Lukoševičius L, Burneckienė J, Rodovičius H, Dūdėnas H. Hormonai. (Hormons). Kaunas; 2002. p. 198-212.
15. Paubert-Braquet M,Richardson FO, Servent-Saez N, Gordon WC, Monge MC, Bazan NG, et al. Effect of Serenoa Repens extract (Permixon) on estradiol/testosteron – induced experimental prostate enlargment in the rat. Pharmacol Res 1996;34(3-4):171-9.

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