

## Effect of Echinacea (*Echinacea Purpurea L. Moench*) preparations on experimental prostate gland

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**Key words:** *Echinacea*, benign prostate hyperplasia, antiandrogenic activity, prostate, immunostimulation.

**Summary.** *This work has investigated the effect of echinacea extract on the weight of prostates in rats as well as on alterations of histological structure and separate blood cells. This preparation was chosen for investigations due to its possible antiandrogenic effect and good immunostimulating features.*

*Experiments were carried out with three-month old male Wistar rats, divided by six into three different groups. The first group was the control one. The rats of the second group were fed for 30 days with the usual food ration plus 50 mg/kg of echinacea extract. The third group was fed for 60 days in the same way as the second one. Clinical death of rats was caused by sodium phenobarbital, later a cervical dislocation was performed. After weighing the rats their prostates were removed and weighed. Pathohistological investigations of the removed organs were carried out. Blood test for Shiling's analysis was taken.*

*The weight of prostates in the first group of rats was 412.0±14.93 mg, in the second group – 403.0±13.33 mg, and in the third group it was 388.0±14.66 mg. Having calculated the proportion between prostates of rats and their body weight it was estimated that in the first group it made 0.125±0.009%, in the second group – 0.105±0.005%, and in the third group – 0.091±0.007%.*

*The percentage of lymphocytes in the first group was 72±1.41; in the second group – 73±0.81; in the third group – 79±1.86. The percentage of segmented neutrophile in the first group was 23±3.31; in the second group – 23±2.25; in the third group – 18±2.33.*

*Having conducted analysis of the experimental results, a significantly important decrease of prostate weight of investigated rats, an increase in the number of lymphocyte as well as the alterations of histological structures after using echinacea extract for eight weeks were observed.*

### Introduction

Benign prostate hyperplasia (BPH) is the disease of middle-aged and elderly men. At the age of fifty the prostate begins to increase, gradually causing dysuria (5). In the normal prostate texture there is 55% of soft muscle fibers, 45% of connective tissue and epithelium. In case of BPH, epithelium as well as connective tissue thrive and hyperplase the cells of prostate soft muscles. While hyperplased prostate presses, urethra contracts, stream of urination weakens, the muscle of urinary bladder hypertrophies and loses tonicity. It is considered that BPH develops when the balance of androgens and estrogens discomfits. Male hormone testosterone in prostate exposed to ferment 5  $\alpha$ -reductase is turned into active dehydrotestosterone and exposed to ferment aromatase is turned into estrogen. If discomfit in the balance of these links occurs, BPH develops.

Increased interest in treatment of symptomatic BPH stimulates investigations of etiology, pathogenesis of this disease, encourages search of new diagnostic opportunities. Lately, increasing possibilities of pharmaceutical development allows expecting that new preparations will be more successful in treating patients, considering etiopathogenetic mechanisms of BPH. The goal of all modern BPH pharmaceuticals intended for treatment is to buffer BPH symptoms and increase maximum urinary stream during urination. Apart from the efficiency requirements of pharmaceutical, its safety is very important as well.

Lately, as a rule, majority of patients having BPH go to see the doctor feeling discomfort of BPH symptoms and only minority go to them having serious complications, such as chronic retention or uremia. Therefore, there is a necessity for such a medication, which could de-

crease symptoms of BPH. While choosing a new medicine it is obligatory to investigate rationality of its usage as well as to know its effect on etiopathogenesis of BPH.

For this work the preparation of *Echinacea purpurea* was chosen. The herb *Echinacea* has immunostimulating effect (1, 6). V.Samorodov and others investigated that in the roots of *Echinacea* apart from polysaccharides and phenol compounds there are also phytosterols ( $\beta$ -sitosterol), which has antiandrogenic effect (2). In other works effect of  $\beta$ -sitosterol on prostate was investigated, however, antiandrogenic effect of this preparation was not widely investigated. That is why it is advisable to investigate this effect in greater detail. Rat males are chosen for this work as they have exclusive urogenital system.

The aim of the work is to investigate effect of *Echinacea* extract on rats' prostate.

Goals of investigation: a) establish prostate weight alterations in investigated rats after using *Echinacea* preparation; b) identify structural alterations in prostates of investigated rats, carrying out histological investigations of prostate preparations; c) estimate blood structure alterations in investigated rats after using *Echinacea* extract.

### Material and methods

For the experiment eighteen 3-month-old male Wistar rats were chosen, divided into 3 groups. The first group was the control one. The rats of the second group together with the usual day ration food were given 50 mg/kg of *Echinacea* extract for 4 weeks (4). The third group of rats was fed for 8 weeks in the same way as the second one.

Extract of *Echinacea purpurea* (*Echinacea Purpurea* L. Moench) was produced in the laboratory of Joint Stock Company „Bakteriniai Preparatai“ from underground part of *Echinacea*, where more active substances having antiandrogenic effect are located (2). The roots of the plants were grained, poured with 50% ethanol solution and tumefied for 6 hours. Tumefied stock was loaded into percolator; percolation was carried out at the speed of 120-150 drops/min.

Later the extract was stored in a cold compartment under plus temperature (2-8°C) no shorter than for 8 days; later it was filtered. Vegetation conditions and introduction of *Echinacea* have been investigated in greater detail in Kaunas Botanical Garden of Vytautas Magnus University since 1991 (3).

Clinical death of all rats was caused intramuscularly overdosing with sodium phenobarbital 1 mg/kg, later a cervical dislocation was performed. Then every rat was weighted, their prostate, epididymis and one testicle were removed. These organs were weighted and their size

was evaluated. Later the removed organs were poured with 10% solution of formalin. Pathohistological preparations were prepared as follows: thickness of histological incision reached 7-10  $\mu$ m, preparations were colored with hematoxylin-eosin dye. After that, histological incisions were evaluated microscopically and pictures were taken. Rats' peripheral venous blood was taken for Shilling's test. Blood swabs were prepared and colored with Romanovski-Gimza's dye. The swabs were overhauled using great (90 times) enlargement with immersion. Experiments were carried out under the guidance of Lithuanian laboratory animals' usage ethic commission permission. (No. 0076).

Relative quantity of rat's body weight and its prostate was calculated, dividing prostate weight by body weight and multiplying by a hundred (5).

For comparison of prostate weight and blood test results among groups t-test was used for independent samples simultaneously comparing dispersions between them. Results are given in format  $x \pm s$ , where  $x$  – is sample mean value,  $s$  – standard deviation. Standard error of the mean ( $s_x$ ) used in the text was indicated.

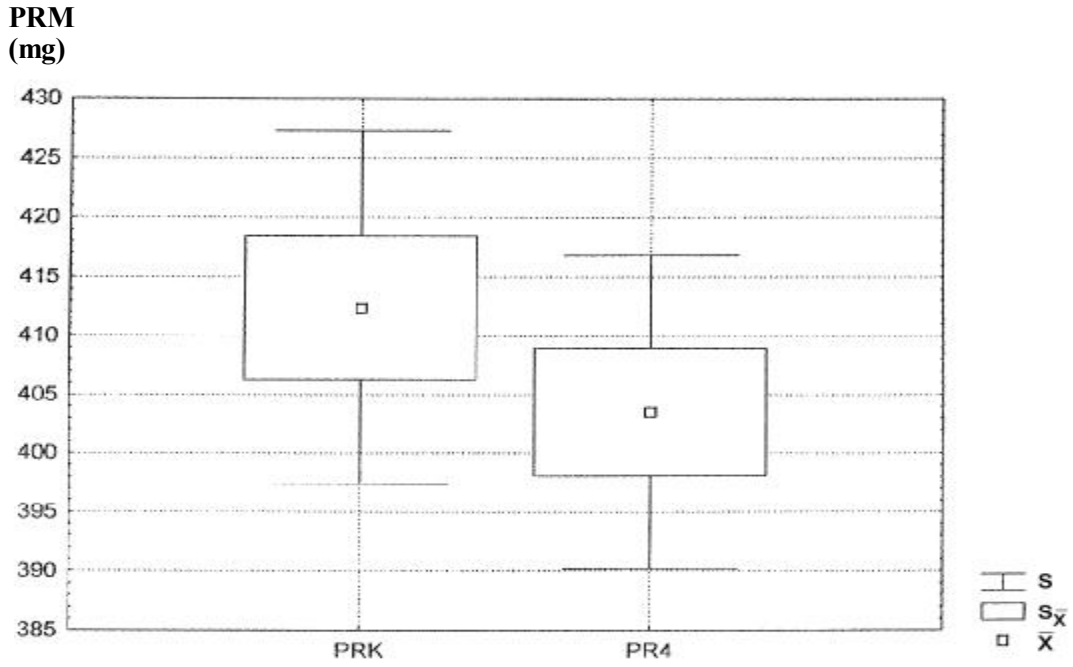
### Results

Having compared prostate weight of rats in control group with the one in the second group, it was established that prostate weight of rats in control group was  $412.0 \pm 14.93$  mg; in the second group it was  $403.0 \pm 13.33$  mg. These data are given in Fig. 1. As it is observed, after using *Echinacea* extract for 4 weeks, prostate weight of rats decreases, but there was not obtained any statistically reliable difference ( $p=0.31$ ) between the first and the second groups.

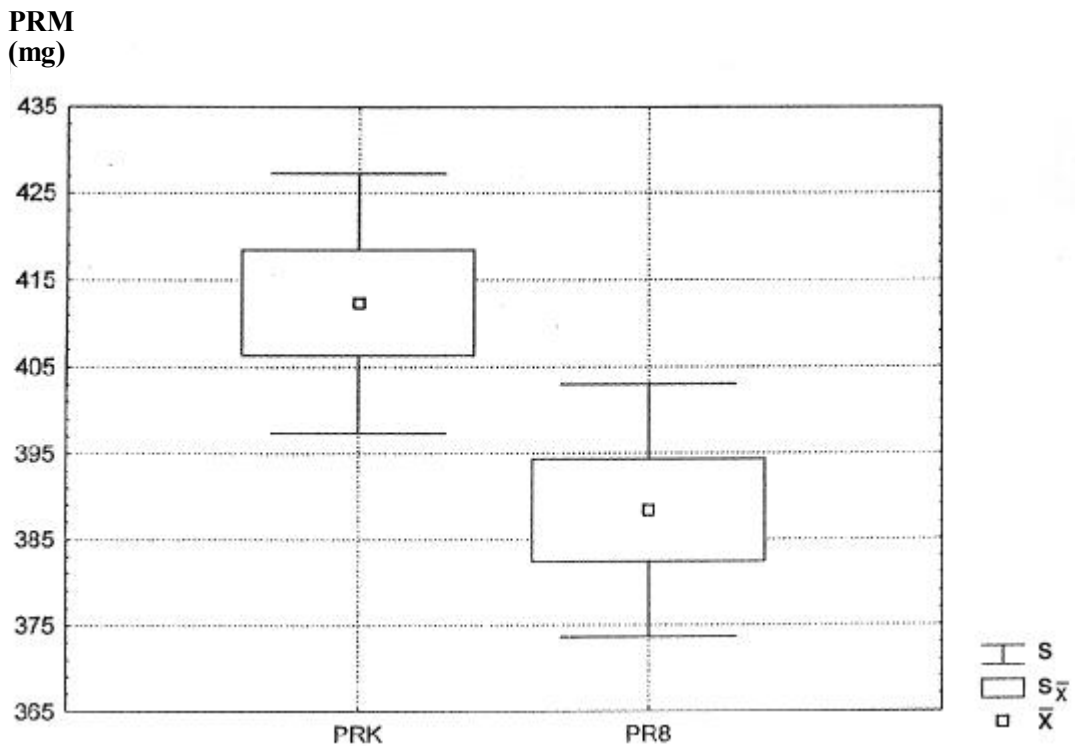
Effect of *Echinacea* extract was also estimated on male rats prostate weight after using it for 8 weeks. Prostate weight of rats in the third group was  $388.0 \pm 14.66$ . The data are given in Fig.2. Having compared this group with the first one (control), an obvious decrease of prostate weight was observed; the difference between the first and the third group was statistically reliable ( $p=0.02$ ). Comparing the data of the second and the third group no statistically reliable difference was obtained ( $p=0.09$ ).

Having calculated relative quantity between rat's body weight and its prostate it was established which percentage part is made up by prostate. In the first group prostate made up  $0.125 \pm 0.009\%$  of body weight; in the second group it was  $0.105 \pm 0.005$ ; in the third group it was  $0.091 \pm 0.007$ . Results are given in the format  $x \pm s_x$ .

Relatively prostate weight was decreasing due to *Echinacea* root extract usage for 4 and 8 weeks (Fig. 3).



**Fig 1. Effect of Echinacea extract on prostate weight after 4-week usage**  
 PRM – rats’ prostate weight in milligrams, PRK – prostate weight in control group,  
 PR4 – prostate weight after using Echinacea extract for 4 weeks.

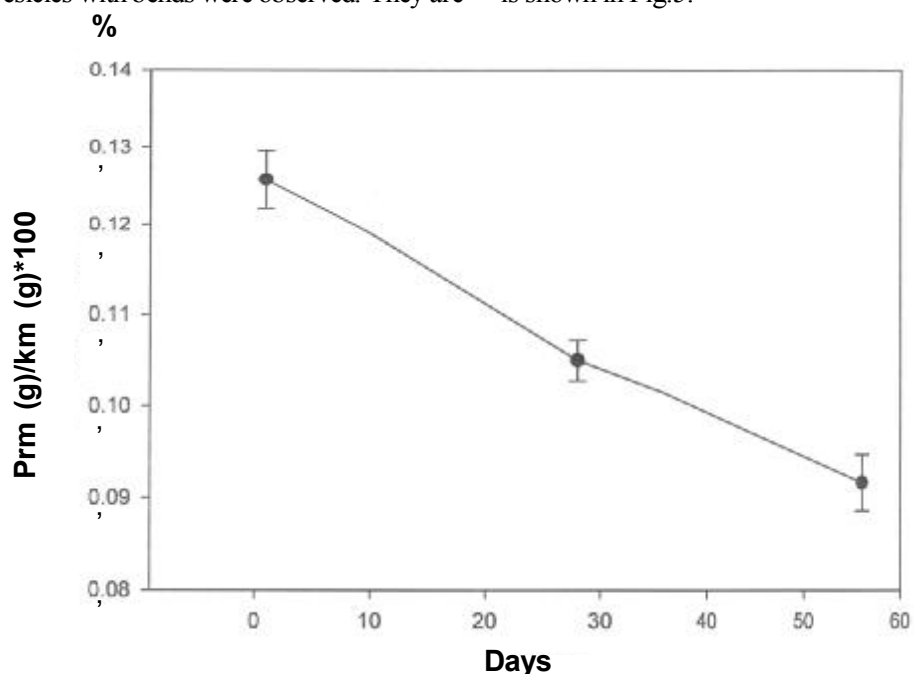


**Fig. 2. Influence of Echinacea extract on prostate weight after 8-week usage**  
 PRM – rats’ prostate weight in milligrams, PRK – prostate weight in control group,  
 PR8 – prostate weight after 8 – week usage of Echinacea extract.

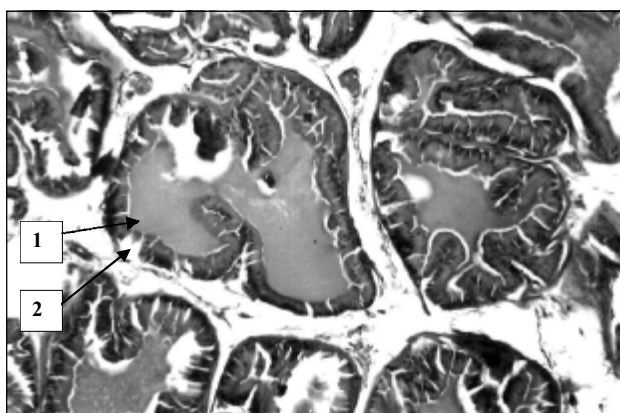
While comparing relative quantities of prostate, statistically reliable decrease was established only comparing the control and the third group of rats ( $p=0.00004$ ).

After analysis of histological preparations of the removed organs, histological alterations of prostates in the control group of rats were established and estimated, the same procedure was carried out with the investigated rats in the second and the third groups.

In the histological preparations of control group male rats' prostates it is observed that part of the secretion gland is covered by thin propria with cells of striped muscles. Considerably more of such muscles are found in multiple part of prostate gland (periphery). In preparations irregular glandular vesicles with bends were observed. They are



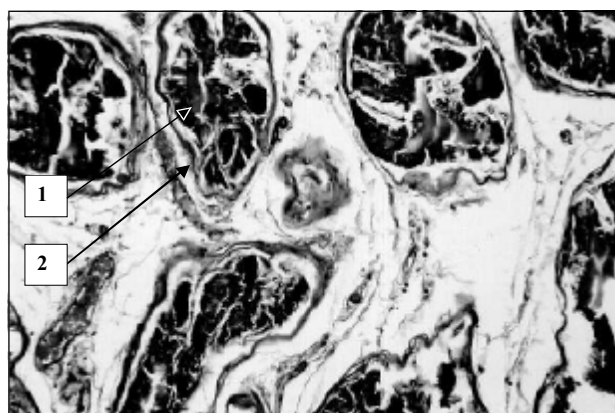
**Fig. 3. Percentage decrease of prostate weight/body weight using Echinacea extract**  
Prm (g) – prostate weight in grams, km (g) – body weight in grams. Results of the figure are given in format  $x \pm s_x$ .



**Fig. 4. Typical histological prostate view in control group of rats**  
1 – secretion inside vesicle, 2 – one-layer cubic epithelium.

lined with one-layer cubic or low columnar epithelium. In a few vesicles some accumulated secretion is seen, which indicates that gland is functioning and is completely mature. Typical view of histological prostate preparation in control group of rats is shown in Fig. 4.

In histological preparations of prostates in the second group of rats glandular vesicles of different forms were observed in the prostate gland. Inside the vesicles there is less secretion in comparison with the control group. Secretion is unevenly spread around all places of secretion vesicles. One-layer cubic epithelium is flattened and initial indications of degeneration appear. Typical view of histological prostate preparation in second group of rats is shown in Fig.5.



**Fig. 5. Typical histological preparation of rat's prostate after using Echinacea extract for 4 weeks**  
1 – secretion inside vesicle, 2 – one-layer cubical epithelium.

In the histological preparations of prostates in the third group of rats a boundary between two lobules of prostate gland is observed. In one of lobules vesicles are slightly changed. They are covered with cubic epithelium and have some secretion inside but here and there epithelium is flattened. Very great changes are seen on the other side of lobule, where epithelium is hardly seen and very flat, and in some alveoli it is indiscernible. There is very little secretion inside the vesicles and this means disorder of secretion function, caused by degenerative alterations of cells. The most typical view of histological prostate preparation in third group of rats is shown in Fig. 6.

Having summarized results of histological preparations, it is possible to claim that in majority of preparations (70%), structural alterations are distinctly observed, which appeared due to Echinacea extract usage; in 20% of histological preparations minimal structural changes were observed. Non-qualitative histological preparations made up 10%.

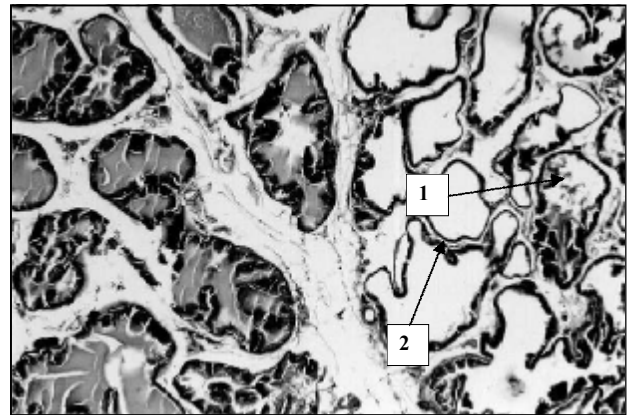
Having conducted blood analysis of investigated rats it turned out that the percentage of lymphocytes in control group of rats was  $72 \pm 1.41$ ; in the second group it was  $73 \pm 0.81$ ; in the third group it was  $79 \pm 1.86$ . The number of segmented neutrophils in control group was  $23 \pm 3.31$ ; in the second group it was  $23 \pm 2.25$ ; in the third group it was  $18 \pm 2.33$ . The percentage of lymphocytes statistically significantly increased only comparing the quantity of lymphocytes in the first and third groups ( $p=0.00001$ ). In the blood of second group rats statistically reliable increase of lymphocytes in comparison with the control group was not obtained. Having compared blood analysis data of rats in all groups there was not observed any increase of segmented neutrophils, eosinophils or monocytes.

### Discussion

Having generalized the results of the investigations, it is possible to affirm that usage of Echinacea extract decreases rats' prostate weight. Statistically reliable prostate weight decrease is only observed when rats are given Echinacea extract for at least 8 weeks.

As prostate weight could alter while body weight of investigated rats was changing, relative quantity was calculated which showed what percentage of rats' body weight was made up by their prostate. Having calculated this parameter, it turned out that in rats, which were given Echinacea extract together with their food for 8 weeks, prostates made up a smaller percentage of their body weight.

Decrease of male rats' prostate weight due to Echinacea extract usage is also substantiated by results



**Fig. 6. Histological preparation of rat's prostate after using Echinacea extract for 8 weeks**

1 – disappeared flat cubic epithelium, 2 – accumulation of secretion inside vesicle.

of histological preparation research. In histological prostate preparations of rats, which were fed for 8 weeks adding Echinacea extract to their food, it was observed that in glandular vesicles epithelium was very flattened or on the verge of disappearing completely. Diminished amount of secretion in vesicles indicates disorder of secretion function due to cells degenerative processes. It is obvious, that it is the cause of prostate atrophy. Therefore, it is possible to affirm that Echinacea extract has a significant antiandrogenic effect.

Apart from that, using Echinacea extract activates rats' immune system. Having tested rats' blood, it was established that the number of lymphocytes increased. However, this increase in the number of lymphocytes can be statistically reliable only if rats were fed with Echinacea extract for at least eight weeks. These data complement other authors' statements about stimulating effect of Echinacea purpurea preparations to cellular immunity (4).

The results allow to expect that Echinacea extract can be useful medication for treatment of benign prostate hyperplasia. It can prevent or decrease enlargement of prostate and in this way abate micturition disorder. Due to its immunostimulating properties Echinacea extract can be used for treatment of immunosuppression in older age. Echinacea extract can also be useful not only in treating benign prostate hyperplasia but also because of its immunostimulating properties, which are vital for elderly people who have immunosuppression. Preparation has not only systemic but also local effect especially for men's urogenital system.

So far, however, function mechanisms of Echinacea extract have not been ascertained, that is why it is expedient to investigate the function of this preparation further.

**Conclusions**

1. Having conducted analysis of male rats' prostates, which were given Echinacea extract together with their food for 8 weeks, it was established that their prostates weight decreased from  $412.0 \pm 14.93$  mg to  $388.0 \pm 14.66$  mg ( $p=0.02$ ).

2. Effect of Echinacea extract for rats' prostate was observed in histological preparations: after 4-week us-

age of Echinacea extract degenerative alterations in rats' prostate were minimal, after 8 weeks atrophy of prostate structures was observed, which confirmed antiandrogenic effect of Echinacea.

3. In peripheral rats' blood tests dominated lymphocytosis, which also manifested itself after using Echinacea extract for 8 weeks ( $p=0.00001$ ). This proves immunostimulating properties of Echinacea.

## **Rausvažiedės ežiuolės (*Echinacea purpurea* L. Moench) preparatų poveikis priešinei liaukai eksperimento sąlygomis**

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**Raktažodžiai:** ežiuolė, gerybinė prostatos hiperplazija, antiandrogeninis veikimas, priešinė liauka, imunostimuliacija.

**Santrauka.** Gerybinė prostatos hiperplazija yra dažna tarp vyresnio amžiaus vyrų liga. Tai skatina gilintis į jos patogenezę ir gydymo taktikos pasirinkimą. Pradinių gerybinės prostatos hiperplazijos stadijų metu rekomenduojamas gydymas augaliniais preparatais, dėl to nuolat ieškoma efektyvesnių.

Tirtas ežiuolės ekstrakto poveikis žiurkių prostatų masės, histologinės struktūros ir atskirų kraujo ląstelių pokyčiams. Toks preparatas tyrimams pasirinktas dėl galimo jo antiandrogeninio poveikio ir gerų imunostimuliuojančių savybių.

Eksperimentai atlikti su Wistar linijos trijų mėnesių žiurkių patiniais, suskirstytais po šešis į tris atskiras grupes. Pirmą grupę buvo kontrolinė. Antros grupės žiurkės 30 dienų maitintos įprastu maistu ir 50 mg/kg ežiuolės ekstraktu. Trečios grupės žiurkės maitintos 60 dienų taip pat kaip ir antros. Žiurkių klinikinė mirtis buvo sukelta fenobarbitalio tirpalu po to atlikta cervikalinė dislokacija. Pasvėrus žiurkes, pašalintos jų prostatos ir jos pasvertos. Atlikti pašalintų organų patohistologiniai tyrimai. Paimta kraujo Šilingo testui.

Pirmos žiurkių grupės prostatų masė buvo  $412,0 \pm 14,93$  mg, antros –  $403,0 \pm 13,33$  mg, trečios –  $388,0 \pm 14,66$  mg. Apskaičiavus žiurkių prostatos ir kūno masės santykį, nustatyta, kad pirmos grupės jis sudarė –  $0,125 \pm 0,009$ , antros –  $0,105 \pm 0,005$ , trečios –  $0,091 \pm 0,007$  proc.

Ryškus histologinių struktūrų pokyčiai, atsiradę po ežiuolės vartojimo, nustatyti 70 proc. histologinių preparatų, minimalūs – 20 proc.

Limfocitų pirmos grupės žiurkių kraujyje buvo  $72 \pm 1,41$  proc.; antros –  $73 \pm 0,81$  proc.; trečios –  $79 \pm 1,86$  proc. Segmentuotų neutrofilų pirmos grupės buvo  $23 \pm 3,31$  proc.; antros –  $23 \pm 2,25$  proc.; trečios –  $18 \pm 2,33$  proc.

Atlikus eksperimentinių rezultatų analizę, nustatytas statistiškai reikšmingas tirtų žiurkių prostatos masės sumažėjimas, limfocitų kiekio padidėjimas, taip pat rasta histologinių struktūrų pokyčių po aštuonių savaičių ežiuolės ekstrakto vartojimo.

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