PHYTOTHERAPY RESEARCH
Phytother. Res. **23**, 1474–1478 (2009)
Published online 13 March 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2805

The Effect of *Echinacea purpurea* (L.) Moench Extract on Experimental Prostate Hyperplasia

D. Skaudickas[1], A. J. Kondrotas[1], E. Kevelaitis[1] and P. R. Venskutonis[2]*

[1]Department of Physiology, Kaunas University of Medicine, A. Mickevičiaus 9, LT-44307 Kaunas, Lithuania
[2]Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, LT-50254, Lithuania

The aim of this study was to examine the effect of purple coneflower (*Echinacea purpurea* L. Moench) on the prostate gland of rats using an experimental model of benign prostate hyperplasia (BPH). The animals were administered 50 mg/kg of extract preparation for 4 and 8 weeks and the prostate mass and structural degenerative changes were evaluated in the course of the experiment. The administration of *E. purpurea* extract to rats with hyperplasia for 4 and 8 weeks gradually and significantly reduced the prostate mass and reversed the degenerative changes in the structure of the prostate gland. The present investigation suggests extract of purple coneflower prevents the development of BPH. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: Echinacea purpurea; androgens; benign prostate hyperplasia; epithelium; prostate cells.

INTRODUCTION

Easter purple coneflower, a plant rich in polysaccharides, phytosterols, phenolic compounds and caffeic acid derivatives has been reported to possess multifunctional effects, namely immunostimulating, antiinflammatory, antivirus, anticancer and radioprotective, as well as possible antiandrogenic activities (Mishima *et al.*, 2004; Dorsch, 1996; Skaudickas *et al.*, 2003, 2004). In addition, it should be noted that the roots of coneflower contain some other active substances that could affect the prostate function (Bauer, 1996). It was reported that the roots of some other plants, e.g. *Urtica dioica*, contain higher amounts of substances, which could exert an antiandrogenic effect, than the overground parts of the same plant. This fact as well as a lack of comprehensive scientific evidence on the antiandrogenic effects of *E. purpurea* was the most important impetus to investigate possible antiandrogenic effects of the root extract of purple coneflower on experimental prostate hyperplasia in rats. The aim of the work was to determine the influence of *Echinacea purpurea* extract on the prostate size of rats, on structural changes, using the model of benign prostate hyperplasia.

MATERIALS AND METHODS

Plant material and extract preparation. The underground parts (roots) of eastern purple coneflower (*Echinacea purpurea* L. Moench) were collected from the experimental fields of medicinal plants at Kaunas Botanical Garden of Vytautas Magnus University (Lithuania) at the end of vegetation period, in July 2004. Overground parts of *E. purpurea* are usually collected after the flowering period, while the roots are dug out after 3–4 years of its cultivation.

The extract was prepared in the laboratory 'Valentis' (Kaunas, Lithuania) by using a traditional commercial percolation method. According to this method the plant material was divided into several parts and extracted in four consecutive percolators. During the first phase the comminuted roots were macerated with 50% (v/v) ethanol at a ratio to raw material of 1:1 for 6 h and afterwards transferred to a percolator. At the end of the extraction cycle the extract was collected from the fourth percolator by pouring off 5 L of the extract (the amount equal to the weight of *E. purpurea* roots used for the extraction).

The extract was kept at 2–8 °C for not less than 8 days, and afterwards at 15–25 °C for 24 h before subjecting it to decantation and filtration through FibraFix plates AF9-20-50, produced from cellulose and kiselgur fibre. The extract was stored at ambient temperature not exceeding 25 °C. One mL of a standardized extract contained 90 mg of dry matter.

Experimental benign prostate hyperplasia (BPH). YY adult male Wistar rats weighing 250–350 g were used for the *in vivo* experiments (Lithuanian Laboratory Animal Good Practice Certificate, No. 0076). The rats were housed under standard laboratory conditions and fed a standard diet *ad libitum*. The rats in this study cohort were divided into five subgroups: (1) animals with intact prostate glands (n = 6); (2) animals after castration (n = 10); (3) animals with induced BPH (n = 6); (4) animals administered *E. purpurea* extract preparation for 4 weeks (n = 10); (5) animals administered *E. purpurea* extract preparation for 8 weeks (n = 17).

The dose of the extract was 50 mg/kg mass. Ultrasound examination of the rat prostate glands was carried in the course of experiments in order to supplement and specify the parameters under study. The model of BPH was induced on male Wistar rats after performing castration on them. BPH was initiated every 2 weeks

* Correspondence to: P. R. Venskutonis, Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, LT-50254, Lithuania.
E-mail: rimas.venskutonis@ktu.lt
Contract/grant sponsor: Lithuanian State Science and Studies Foundation; contract/grant number: C03.

Received 18 July 2007
Revised 10 July 2008
Accepted 21 January 2009

by intramuscular injection of estradiol depot 1 mL, 10 mg (Jenapharm) and testosterone 1 mL, 250 mg (Omedren-L 250) (Bakteriniai preparatai, Kaunas, Lithuania). The control (intact) rat group ($n = 6$) was followed for 3 months and was not treated with vehicle. The model of Robinette (1988) was used in this study. Clinical death of animals was induced after 4 weeks by administration of sodium phenobarbital. After removal of the prostate it was immediately immersed in a 10% formaldehyde solution for fixation. Later, the preparation was infused in paraffin wax blocks, which were cut into 3–4 μ slices with a rotational microtome. A hematoxylin–eosin technique was applied for staining the preparations, which were further examined with an Olympus BX type microscope using a magnifying power of 10 × 40. Histological pictures were obtained by an Olympus (C 506) digital camera.

The mass of body and organs of the experimental animals were measured by analytical balances. The relative content of the organ mass (*Om*) in the body mass (*Bm*) was calculated by a formula PBR = *Om*/*Bm* × 100 (Vanderschueren *et al.*, 2000; Paubert-Braquet *et al.*, 1996).

In the group with prostate hyperplasia, the size of the prostate was determined using the method of transabdominal echoscopy; the calculations were accomplished according to the established elliptic equation (Wolff *et al.*, 1995): (0.523 × oblique diameter^2 × anterior/posterior diameter).

Statistical analysis. The level of significance at $p = 0.05$ and the power value of 0.80 were used to determine the minimal number of experiments required. The calculations were performed using STATISTICA v.6 power analysis function (StatSoft, Inc. USA).

RESULTS

Ultrasound measurements

Comparisons of the means of the prostate mass and the body mass ratio and their distribution between intact and atrophy groups, as well as between intact and hyperplasia groups were performed using the non-parametric Mann–Whitney test. Statistically significant differences ($p < 0.001$) in the atrophy and hyperplasia groups were established in comparison with the intact group. Statistically significant differences were also obtained for echoscopic characteristics of rats, which were administered *E. purpurea* extract for 4 weeks ($p < 0.03$) and for 8 weeks ($p < 0.001$) in comparison with the results obtained in the intact group (Figs 1, 2). According to these findings the distribution of the average percentage prostate mass as a part of the total body mass between the hyperplasia group and the groups of rats receiving *E. purpurea* extract for 4 and 8 weeks was significantly different ($p < 0.05$) (Fig. 3).

Further studies, performed by the ultrasound method, showed that in the hyperplasia group the distribution pattern was shifted more to the right than in the other studied groups. The indices of the studied characteristics decreased for the rats, which were administered *E. purpurea* extract: $p < 0.032$ and $p < 0.019$ after 4 and 8 weeks of administration, respectively (Fig. 4).

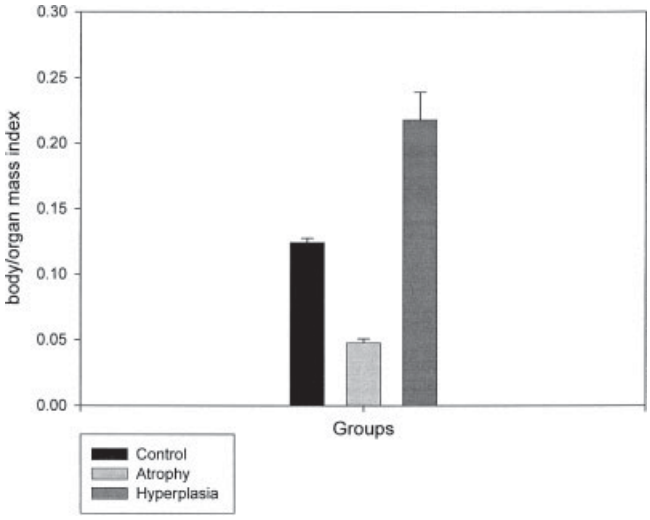

Figure 1. Distribution of the average percentage of prostate mass and body mass ratio in three study groups: in the control group there were six cases when the ratio was 0.10–0.15; in the atrophy group there were four cases when the ratio was 0.05–0.10, and six cases when the ratio was 0.00–0.05; in the hyperplasia group there were three cases when the ratio was 0.15–0.20; two cases with the ratio 0.20–0.25, and only one case with the ratio 0.30–0.35.

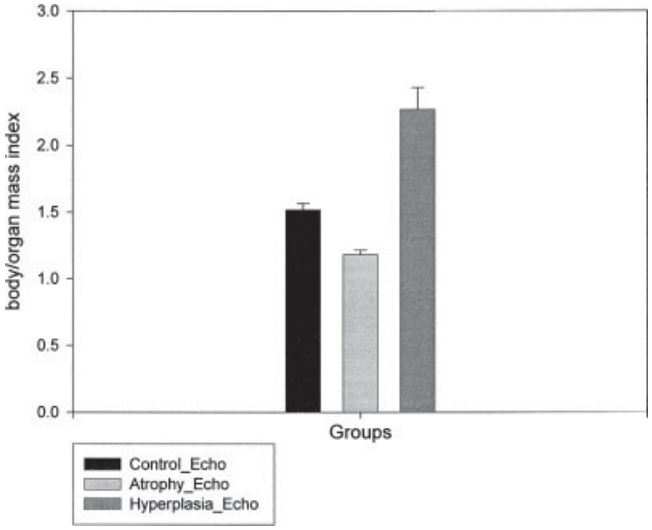

Figure 2. Distribution of the average percentage of prostate mass and body mass ratio in three study groups after ultrasound investigation. In the control group two cases were found when the prostate mass and body mass ratio was between 1.2–1.4, in three cases the ratio was between 1.4–1.6, and there was only one case with the ratio between 1.6–1.8; in the atrophy group there were seven cases with the ratio between 1.0–1.2; two cases with the ratio between 1.2–1.4, and only one case with the ratio between 0.8–1.0; in the hyperplasia group there were three cases with the ratio between 1.8–2.0, one case with the ratio between 2.2–2.4, one case with the ratio 2.4–2.6 and one case with the ratio between 2.8–3.0.

Morphological evaluation

In the case of glandular atrophy, the fibermuscular stroma of the prostate gland was found to be laid out by a single-layer cuboidal or low columnar epithelium (Fig. 5A) showing no vacuoles in the cytoplasm compared with the histological data of the intact group (Fig. 5B). The glands appeared to be of a round-shape or of irregular form, having small and rarely encountered wrinkles directed to the glandular lumen (Fig. 6A).

DOI: 10.1002/ptr

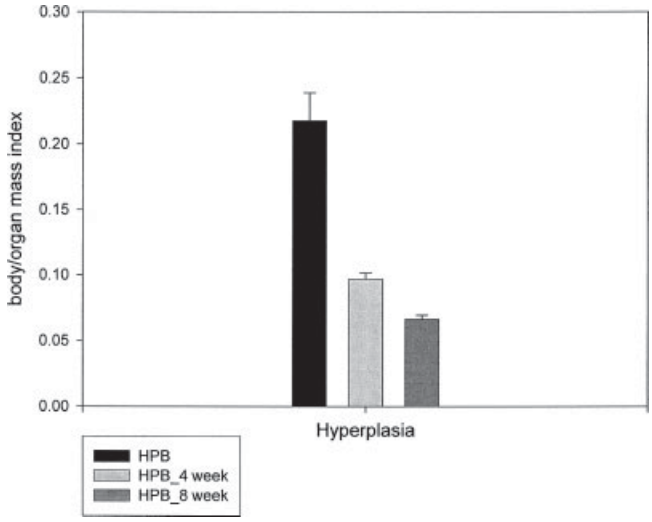

Figure 3. Distribution of prostate mass proportion with relation to the entire body mass of rats in the hyperplasia group and in groups of rats consuming *E. purpurea* extract for 4 and 8 weeks. In the hyperplasia group three cases were found when the prostate mass and body mass ratio was between 0.15–0.20, in two cases the ratio was between 0.20–0.25, and there was only one case with the ratio between 0.30–0.35; after 4 weeks administration there were five cases with the ratio between 0.05–0.10, five cases with the ratio between 0.10–0.15; after 8 weeks administration there was only one case between 0.00–0.05, and 16 cases between 0.05–0.10.

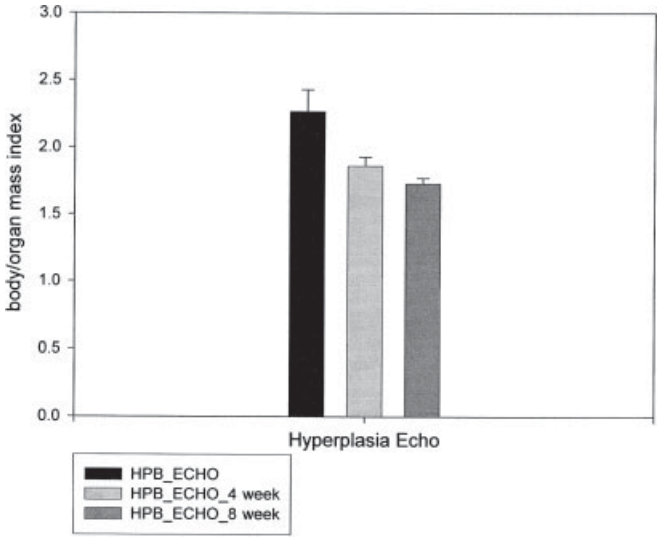

Figure 4. Distribution of prostate mass percentage part in relation to the total body mass in the hyperplasia group and in groups of rats consuming *E. purpurea* extract for 4 and 8 weeks following ultrasound investigation.

In the case of hyperplasia, the epithelium seemed to be obviously flourishing (squamous). The gland itself was laid up with a highly columnar epithelium. The nuclei appeared to be pressed or squeezed up and in some places they were arranged in rows. The cytoplasm was abundant with distinct vacuoles, and the glandular lumen seemed reddish with chromatophilic secretion (Fig. 6A).

Involution changes in the glands were observed by comparing the histological view of prostate hyperplasia in rats with the prostate view of rats in groups which were administered *E. purpurea* extract for 4 and 8 weeks. In spite of the fact that the glands are laid up

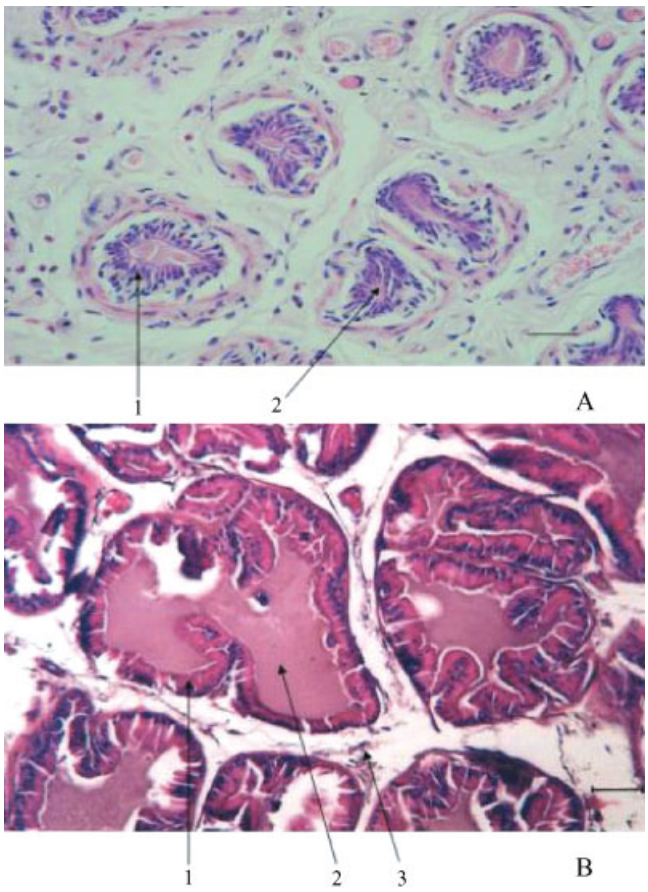

Figure 5. (A) Histological view of samples of rat prostate atrophy. 1, cuboidal epithelium, 2, the glandular lumen (H + E). Scale 20 μm. (B) Histological view of samples of intact group of rat prostate. 1, cuboidal epithelium, 2, the glandular lumen, 3, muscular layer (H + E). Scale 20 μm.

with a high columnar epithelium, the comparison of their secretion function (which is reflected by the vacuoles lodged in glandular epithelium) revealed that the glands, though containing vacuoles, were remarkably lower in number than it had been described previously (Fig. 6A, B). Chromatophilic erythroid liquid present in the lumen of the glandular tubule was different: in the prostate glands of the hyperplasia group, this liquid being more homogenous (Fig. 6A) than in the groups of rats fed with *E. purpurea* extract for 4 weeks (Fig. 6B).

Obvious involution changes were observed in the histological prostate preparations of rats, which were administered *E. purpurea* extract for 8 weeks: the glands appeared to be laid up with a low columnar or cuboidal epithelium (Fig. 6C). The comparison of the secretion function of the glands which is reflected by vacuoles present in the epithelium, indicated that vacuoles in the glands were either invisible or were solitary and rare, while in glands affected by hyperplasia almost every epithelial cell contained vacuoles (Fig. 6C).

By comparing the histological view of prostate samples of rats after 4 and 8 weeks it could be observed that the prostate epithelium was atrophic. The glandular epithelium seemed to be less atrophic, columnar, although containing solitary intracellular vacuoles in the case of administration of *E. purpurea* extract for 4 weeks.

The epithelium of the prostate gland seemed to be squeezed up, containing low columnar epithelial cells

DOI: 10.1002/ptr

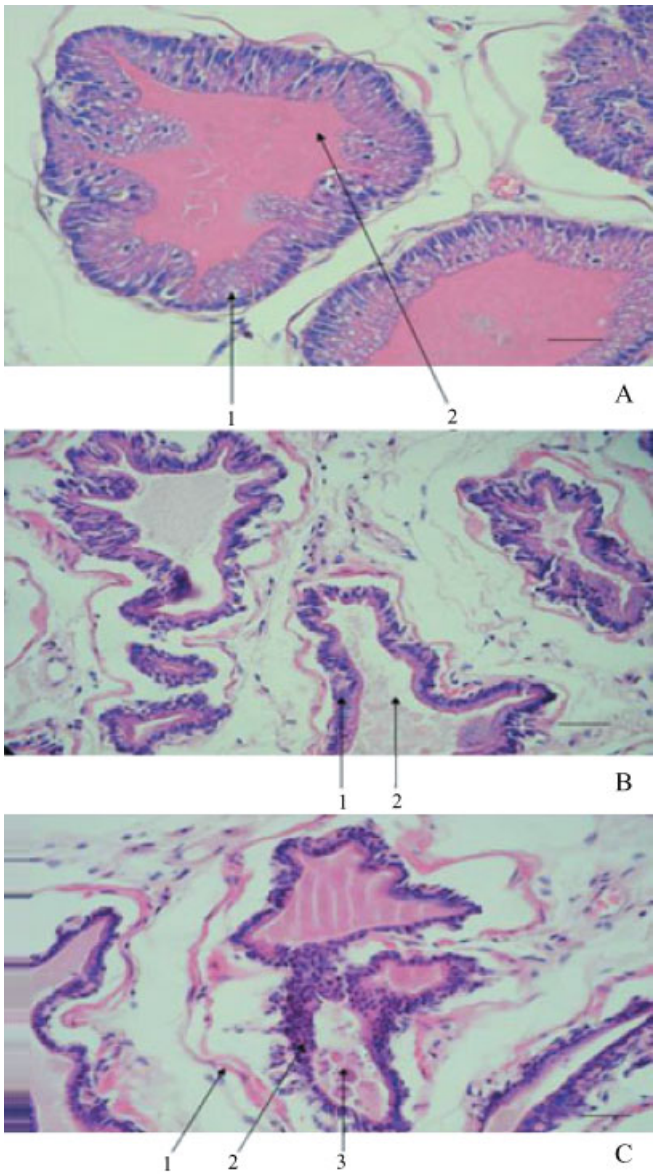

Figure 6. (A) Histological view of prostate hyperplasia in rats. 1, cuboidal epithelium, 2, glandular lumen (H + E). Scale 20 μm. (B) Histological view of prostate samples of rats fed on Echinacea extract for 4 weeks. 1, cuboidal epithelium, 2, glandular lumen (H + E). Scale 20 μm. (C) Histological view of prostate samples of rats on consuming Echinacea extract for 8 weeks. 1, muscular layer, 2, epithelium, 3, glandular lumen (H + E). Scale 20 μm.

without any intracellular vacuoles in the case of administration of *E. purpurea* extract for 8 weeks. Some fragments of degenerating cells could be observed in the lumen of the glands.

In summary, it is possible to assert that after administration of *E. purpurea* extract for 8 weeks histological prostate preparations of experimental animals manifest more conspicuous involution changes in comparison with relevant changes for rats that were administered *E. purpurea* extract for 4 weeks.

DISCUSSION

It is well documented that androgens and estrogens exert a tremendous influence on the development of BPH, however, vigorous thriving of the prostate depends on the interaction of androgens, sexual maturity and age, including a negative impact of chronic infectious diseases of the urogenital system. Therefore, it is expedient to remove testicles while trying to create an experimental BPH model. The development of experimental prostate hyperplasia progresses slower when only male type hormones are being used, compared with the combined hormonal therapy (administering both androgens and estrogens).

The main therapeutic effect of *E. purpurea* extract applied to the model of BPH depends on the presence of sterols, particularly β-sitosterol, in the roots of the plant. The chemical structure of plant origin sterols is closely related to the animal cholesterols. The latter takes part in the synthesis of sex hormones in the body, therefore by changing the amount of cholesterol, it is possible to influence the synthesis of sex hormones.

E. purpurea extract acts not only as an immunostimulatory agent, it also has an antiandrogenic property, therefore, it might be possible to use this preparation only to perform extensive clinical studies on elderly male patients who naturally show immunosuppression signs and micturition disorders. Such disorders are closely related to BPH, and its occurrence increases with the patient's age. At the present time, *E. purpurea* preparations are recommended for prophylaxis and therapy purposes against colds, developing inflammation diseases and in immunosuppressive states. Therefore, the findings in this study might extend the list of indications by prescribing *E. purpurea* preparations in the case of BPH.

However, to obtain an optimal way for conservative treatment of patients with BPH, it is necessary to envisage a tactical approach trying to foresee what type of etiopathogenesis of infravesicular obstruction and what kind of urinary tract symptoms are the most convenient to treat in order to obtain the most effective result.

However, the mechanism of action of *E. purpurea* extract has not been completely elucidated. Further examination of this preparation is needed, especially by using comprehensive clinical studies before recommending it for the patients suffering from BPH.

CONCLUSIONS

It has been established that the administration of *E. purpurea* extract to rats with induced benign prostate hyperplasia had a positive effect on their prostate mass, which significantly decreased after 4 ($p = 0.02$) and 8 ($p = 0.01$) weeks of treatment. Positive effects of the preparations on the development of degenerative changes in the prostate structure (squeezing of epithelium of prostate gland, containing low columnar epithelial cells without any intracellular vacuoles, appearance of fragments of degenerating cells in the lumen of glands) were observed by morphological investigation.

Acknowledgements

The research was supported by the Lithuanian State Science and Studies Foundation, project no. C03

REFERENCES

Bauer R. 1996. Echinacea drugs – effects and active ingredients. *Z Arztl Fortbild (Jena)* **90**: 111–115.

Dorsch W. 1996. Clinical application of extracts of *Echinacea purpurea* or *Echinacea pallida*. Critical evaluation of controlled clinical studies. *Z Arztl Fortbild (Jena)* **90**: 117–122.

Mishima S, Saito K, Maruyama H, Inoue M, Yamashita T, Ishida T. 2004. Antioxidant and immuno-enhancing effects of *Echinacea purpurea*. *Biol Pharm Bull* **27**: 1004–1009.

Paubert-Braquet M, Richardson FO, Servent-Saez N, Gordon WC, Monge MC, Bazan NG. 1996. Effect of *Serenoa repens* extract (Permixon) on estradiol/testosterone-induced experimental prostate enlargement in the rat. *Pharmacol Res* **34**: 171–179.

Robinette CL. 1988. Sex-hormone-induced inflammation and fibromuscular proliferation in the lateral prostate. *Prostate* **12**: 271–286.

Skaudickas D, Kondrotas A, Baltrusaitis K. 2004. The effect of *Echinacea purpurea* extract on sexual glands of male rats. *Medicina (Kaunas)* **40**: 1211–1218.

Skaudickas D, Kondrotas AJ, Baltrusaitis K, Vaitiekaitis G. 2003. Effect of Echinacea (*Echinacea purpurea* L. Moench) preparations on experimental prostate gland. *Medicina (Kaunas)* **39**: 761–766.

Vanderschueren D, Vandenput L, Boonen S, Van HE, Swinnen JV, Bouillon R. 2000. An aged rat model of partial androgen deficiency: prevention of both loss of bone and lean body mass by low-dose androgen replacement. *Endocrinology* **141**: 1642–1647.

Wolff JM, Boeckmann W, Mattelaer P, Handt S, Adam G, Jakse G. 1995. Determination of prostate gland volume by transrectal ultrasound: correlation with radical prostatectomy specimens. *Eur Urol* **28**: 10–12.