

Recommended by Doctor of Biology, professor L.M.Maloshtan

UDC 615.454.1:001.893.54:618.173:582.635.38

THE EFFECT OF A NEW VAGINAL GEL WITH THE HOP EXTRACT ON THE CARDIOVASCULAR SYSTEM IN SPAYED FEMALE RATS

O.S.Sinitsyna, G.V.Zaychenko, I.M.Ryzhenko, Yu.B.Laryanovska

National University of Pharmacy

Key words: spayed rats; gel with the hop extract; estrogen deficiency; cardiovascular system; phytoestrogens

The results of the study of the morphological structure of the aorta, coronary arteries, the myocardium and intramural arteries of the heart in female rats with bilateral oophorectomy after treatment with a new vaginal gel with the hop extract and the reference drug – “Ovestin” suppositories containing estriol are presented. It has been shown that as a result of a persistent hypoestrogenic state in the cardiovascular system of female rats the complex of the morphological changes that can be estimated as endothelial dysfunction is formed. After therapy with the vaginal gel studied there have been no changes in the myofibrillar apparatus of the myocardium in animals; reduction or the absence of signs of endothelial dysfunction have been observed.

Menopause in women is usually accompanied by physiological changes in different systems and organs due to fading and cessation of the ovarian function. One consequence of this process is development of the cardiovascular system (CVS) diseases [3, 11].

It is known that receptors for estradiol are present in the endothelium, smooth muscle cells of blood vessels and in the myocardium [14]. Interacting with endothelial receptors estradiol intensifies production of nitric oxide and blocks vasoconstriction induced by acetylcholine; it also intensifies regeneration of endothelial cells; enhances neovascularization by increasing migration, proliferation and differentiation of vascular endothelial cells [11]. Several studies have found that the presence of endothelial dysfunction in postmenopausal women is a risk factor for atherosclerosis, early coronary heart disease (CHD), hypertension, and peripheral artery lesions. According to the Framingham Study (1986) the incidence of acute coronary events increases by 12 times in postmenopausal women compared to women of the reproductive age [1]. Early natural menopause increases the risk of coronary heart disease by 3 times, while surgical menopause – by 7 times [10].

Currently the problem of prevention of cardiovascular complications caused by hypoestrogen states and in the menopause period in women is of particular relevance. The basis of prevention and treatment of CVD in menopause is the hormonal menopausal therapy [4, 5, 16]. It should be noted that in recent years more and more attention is paid to alternative therapies, particularly herbal medicine with the use of phytoestrogens as a safe and effective method for correction of menopausal cardiovascular disorders [17].

Phytoestrogens have the chemical structure that is close to the endogenous estradiol-17- β , and a similar molecular weight, and therefore, they interact with the estrogen receptors. Plants, which are rich in phytoestrogens

most of all, include soybean (genistein and daidzein), red clover (coumestol), flaxseed (lignans) and hops (8-prenylnaringenin) [5, 2].

Therefore, the aim of our research was to study the effect of the vaginal gel containing the hop extract (*Humulus lupulus L.*) on the morphofunctional state of vessels and the myocardium of female rats with hypoestrogenemia caused by bilateral oophorectomy.

Materials and Methods

The experimental studies were conducted on 30 females of white outbred nonlinear rats weighing 190-195 g. They were divided into 5 groups, 6 animals in each group: intact control; sham-operated female rats; control pathology; spayed rats treated with the vaginal gel with the hop extract; spayed animals treated with the reference drug – “Ovestin” vaginal suppositories containing estriol as an active ingredient manufactured by Organon company, the Netherlands.

The new combined vaginal gel containing the hop extract as the main active ingredient, as well as lactic acid and excipients was developed at the Department of Chemist's Technology of Drugs named after D.P.Salo of the National University of Pharmacy under the supervision of prof. Vishnevskaya L.I.

In animals simulation of the state close to menopausal symptoms in women was caused by bilateral spaying according to Kirshenblat Y.D. [6]. Sham-operated animals were subjected to laparotomy and wound suturing without removal of ovaries. Starting with the 35th day after spaying females were introduced vaginally the drugs studied within 28 days, namely the gel with the hop extract in the dose of 0.06 mg/kg, estriol suppositories – 0.03 mg/kg.

After completing the treatment animals were taken out from the experiment by decapitation under chloroform anesthesia. The aorta, coronary vessels, and the myocardium of female rats were morphologically studied.

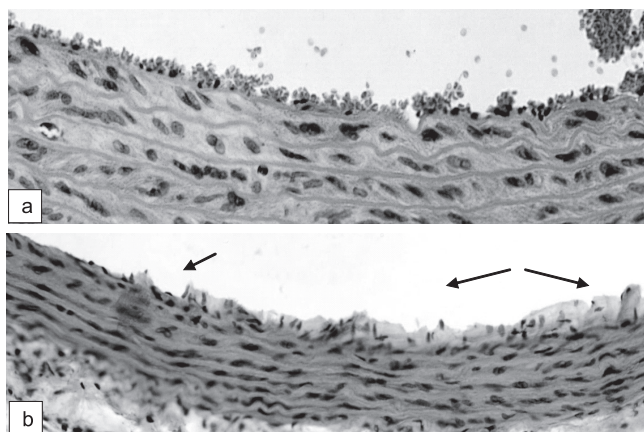


Fig. 1. The abdominal aorta of the spayed female rat: a – red blood cells sticking to the surface of media; b – loosening of the intima and superficial media. Hematoxylin-eosin. x250.

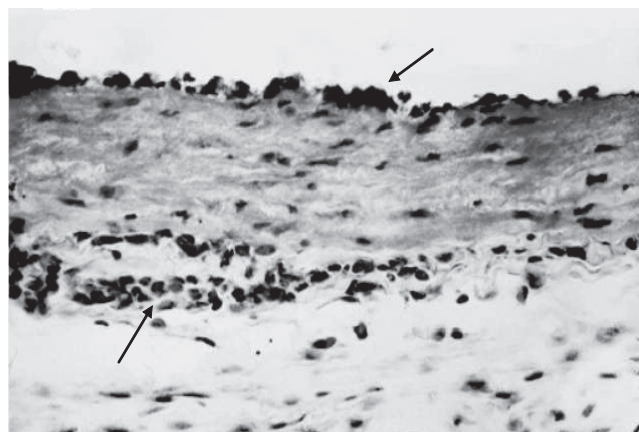


Fig. 2. The abdominal aorta of the spayed female rat. Clusters of white blood cells on the surface of the intima, and on the edge of media and adventitia, loosening of the extracellular collagen matrix. Hematoxylin-eosin. x400.

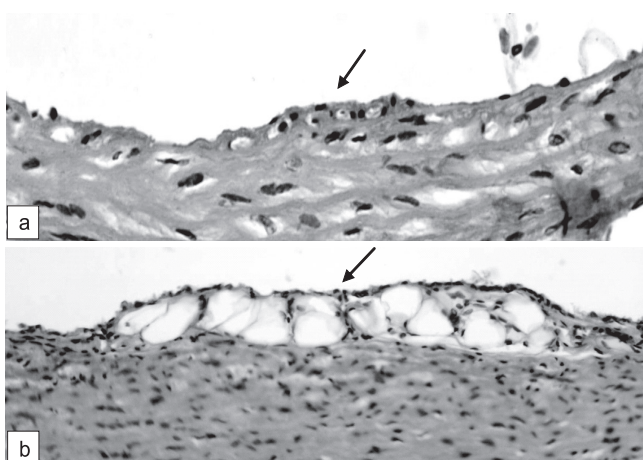


Fig. 3. The coronary vessel of the spayed female rat: a – tiny thickening of intimal surface, loosening of the extracellular collagen matrix of media (x400); b – large accumulations of fat in the area of the wall thickening (x250). Hematoxylin-eosin.

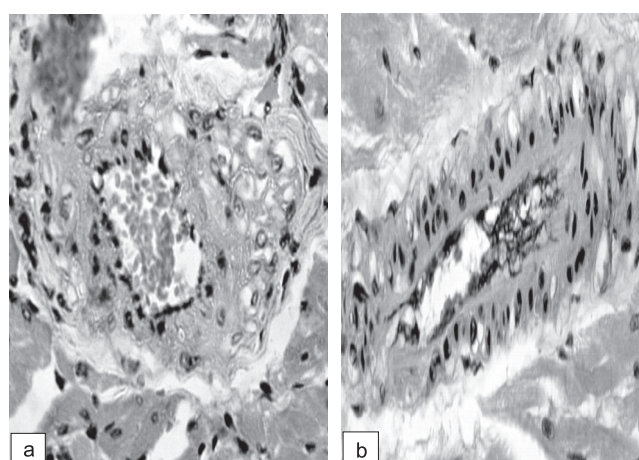


Fig. 4. Intramural arteries of the cardiac muscle of the spayed female rat: a – swelling, loosening of the vascular wall, impregnation with a protein liquid, proliferation of the endothelium, vacuoles in media; b – proliferation of endothelial cells and appearance of vacuoles. Hematoxylin-eosin. x250.

The test material was fixed in 10% formalin solution, dehydrated in alcohols with the increasing concentration, poured to celloidin-paraffin, and sections were stained with hematoxylin-eosin [9]. Microslides were examined using the Granum microscope; the microscopic pictures were taken by a Granum DSM 310 digital video camera. Pictures were processed with Pentium 2,4GHz PC using ToupView software.

The studies were conducted in compliance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [7].

Results and Discussion

The results of the studies conducted show that the histostructure of the aorta, coronary arteries, the myocardium and intramural arteries of female rats of the intact control corresponded to the normal physiological state being typical for these animals [12, 13, 15].

The aorta of ovariectomized female rats showed sticking of red blood cells on the surface of the intima and its focal loosening. There was disorganization of the smooth muscle cells layers and the area of the extra-

cellular collagen matrix loosening. The small clusters of white blood cells on the surface of the intima, and on the edge of media and adventitia were also observed (Fig. 1, 2).

Coronary arteries and media also showed loosening of the extracellular collagen matrix, and in some places there was tiny thickening of the intimal surface (Fig. 3a). One of the females had large accumulation of fat in this wall thickening under the endothelium in the disorganized superficial layers of media (Fig. 3b). Probably, there were atherosclerotic “plaques” at various stages of formation.

The cardiac muscle of the spayed female rats showed changes of the myofibril apparatus, fibre fragmentation accompanied by edema, the loss of striated myofibrils. The vascular wall of intramural arteries is often thickened, swollen, loosened, and sometimes impregnated with a protein liquid. Endothelial cells were proliferated with the picket-fence arrangement. In some cases we managed to trace the appearance of vacuoles in the intima probably of the lipid nature. Vacuoles were also present in media (Fig. 4).

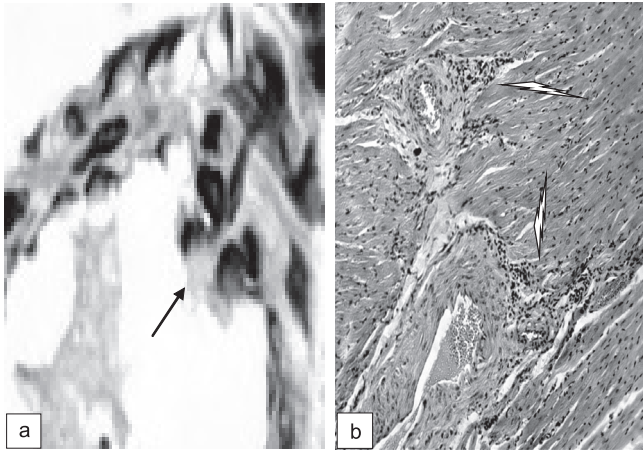


Fig. 5. The intramural artery (a – the endothelial layer defect, its thickening into the lumen of the vessel. Immersion) and the myocardium (b – perivascular cellular infiltrates. x200) of the spayed female rats. Hematoxylin-eosin.

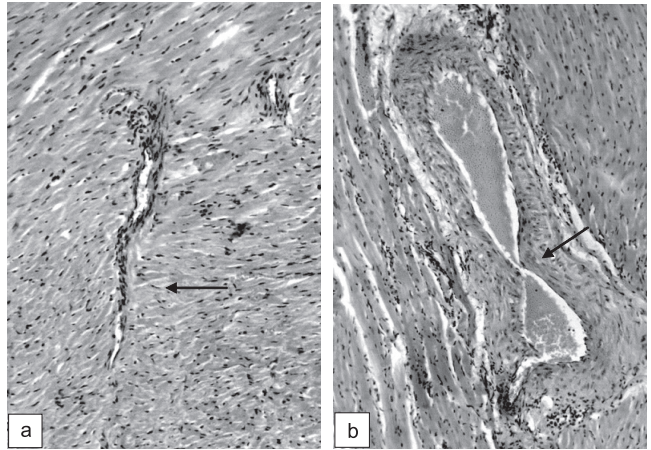


Fig. 6. The myocardium of the spayed female rat. The spasm of blood vessels of different caliber: a – thin-walled vessel; b – large artery. Hematoxylin-eosin.

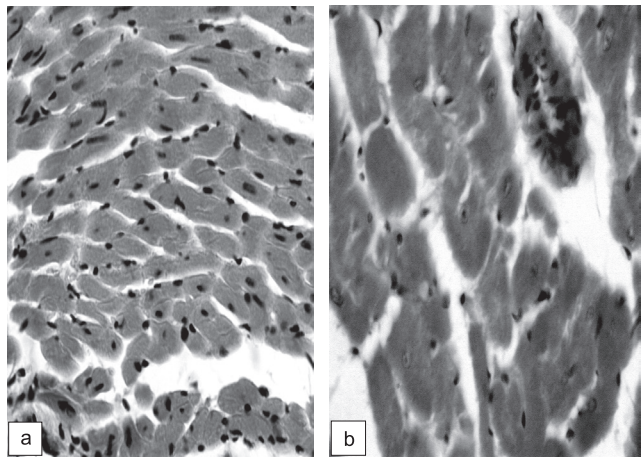


Fig. 7. Different parts of the myocardium of the spayed female rats (cross section): a – normal cardiomyocytes; b – hypertrophic cardiomyocytes nearby vessels in the state of spasm. Hematoxylin-eosin. x200.

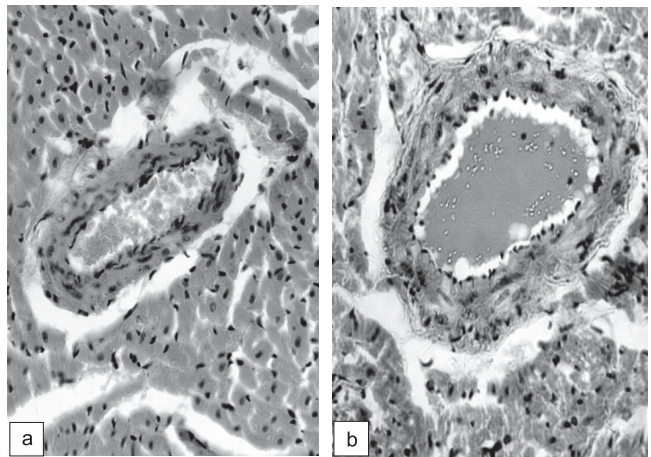


Fig. 8. The myocardium of the spayed female rat after using the gel with phytoestrogens: a – cardiomyocytes with the normal size (cross section); b – the normal state of the vascular wall of the intramural artery. Hematoxylin-eosin. x200.

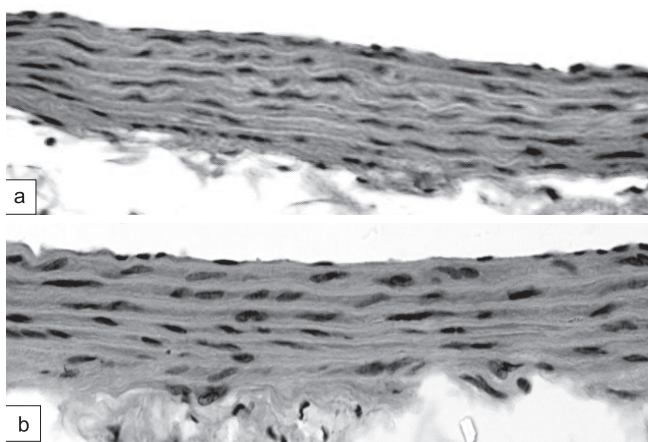


Fig. 9. The vascular wall: a – abdominal aorta; b – coronary artery of the female rat after vaginal application of the gel with phytoestrogens. The absence of changes in the intima and media. Hematoxylin-eosin. x250.

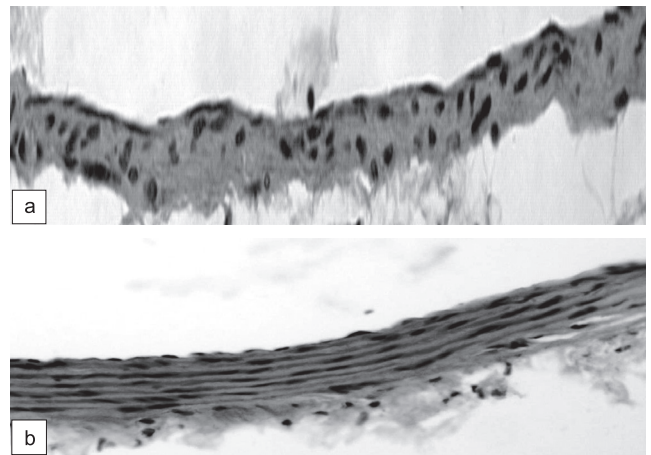


Fig. 10. The vascular wall: a – coronary vessels; b – abdominal aorta of the female rat after application of estriol suppositories. The normal state of the intima and media of blood vessels. Hematoxylin-eosin. x250.

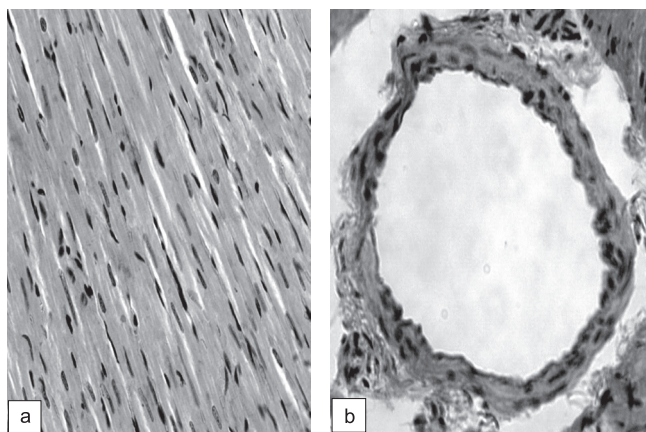


Fig. 11. The state of: a – cardiac muscle; b – intramural arteries of the female rat after application of estriol suppositories. The state of cardiomyocytes and vascular wall is normal. Hematoxylin-eosin. x250.

In one thin-walled artery there was a focal defect of the endothelial layer, namely its thickening into the lumen of the vessel. Sometimes there is moderate perivascular round-cell infiltration was observed (Fig. 5).

Many blood vessels of different caliber were in the state of spasm (Fig. 6). Cardiomyocytes were visually hypertrophied preferably nearby vessels in the state of a distinct spasm (Fig. 7).

The signs of necrotic changes and myocardial cell infiltration were not found. The histological picture described shows development of the complex of morphological changes in the cardiovascular system of the spayed female rats that are typical for deficiency of endogenous estrogens and formation of endothelial dysfunction in them, and the literature confirms this fact [8].

The use of the vaginal gel with phytoestrogens prevents development of degenerative changes of the cardiac muscle (myocytolysis, the loss of striated myofibrils)

manifestations of endothelial dysfunction such as changes in the state of the vascular wall of intramural arteries, coronary vessels and aorta, hypertrophy of cardiomyocytes (Fig. 8, Fig. 9).

There were no apparent disorders of the intima and media in microscopic examination of the abdominal aorta wall and coronary vessels of female rats treated with the reference drug – suppositories with estriol on the background of estrogen deficiency (Fig. 10).

In the cardiac tissue the state of fibres, intramural arterial vessels visually corresponded to the norm (Fig. 11).

CONCLUSIONS

1. Bilateral oophorectomy in female rats leads to degenerative changes of the myofibrillar apparatus of cardiomyocytes by the myocytolysis type, focal fragmentation of fibres, small foci of the myocardial damage. The complex of morphological changes (vasospasm, thickening, swelling, loosening of the vascular wall, focal proliferation, destruction, the picket-fence arrangement of endothelial cells, small foci of fat accumulation in the intima and media) is typical for endogenous estrogen deficiency. This can be interpreted as manifestations of endothelial dysfunction.

2. After application of the vaginal gel with the hop extract in the female rat cardiac muscles there were no changes in the myofibrillar apparatus; the distinct signs of reduction or the absence of endothelial dysfunction was observed.

3. By its effect on the morphological condition of the cardiovascular system the gel with the hop extract under study is not inferior to the reference drug – suppositories with estriol.

4. The vaginal gel with the hop extract is promising for further study for the purpose of its use in complex therapy of cardiovascular diseases associated with hypoestrogenia in women during the menopause.

REFERENCES

1. Аничков Д.А., Шостак Н.А., Журавлева А.Д. // Рациональная фармакотерапия в кардиол. – 2005. – №1. – С. 37-42.
2. Ботоева Е.А. // Бюл. ВСНЦ СО РАМН. – 2010. – №2. – С. 234-238.
3. Бугрим Т.В. // Научные ведомости. Серия Медицина. Фармация. – 2013. – Вып. 24, №25. – С. 90-93.
4. Иванова О.В., Брюхина Е.В., Усольцева Е.Н. // Вестник ЮУрГУ. Серия Образование. Здоровоохранение. Физическая культура. – 2013. – Т. 13, №2. – С. 92-97.
5. Калинин С.Ю., Анетов С.С. // Бюл. ВСНЦ СО РАМН. – 2010. – Ч. 2, №6. – С. 130-135.
6. Кириенко Я.Д. Практикум по эндокринологии. – М.: Высш. шк., 1969. – С.55-57.
7. Кожем'якин Ю.М. Научно-практичні рекомендації з утримання лабораторних тварин та роботи з ними / Ю.М.Кожем'якин, О.С.Хромов, М.А.Філоненко, Г.А.Сайфетдінова. – К.: ВД «Авіценна», 2002. – 156 с.
8. Колодийчук Е.В., Мернова В.П. // Журн. научных статей «Здоровье и образование в XXI веке». – 2012. – Т. 14, вып. 2. – С. 69-73.
9. Меркулов Г.А. Курс патологистологической техники. – М.: Медицина, Ленингр. отд-ние, 1969. – 424 с.
10. Нейфельд И.В., Журняков А.И., Скупова И.Н. // Bul. of Medical Internet Conferences (ISSN 2224-6150). – 2012. – Vol. 2, Issue 12. – P. 994-996.
11. Омеляненко М.Г., Краснова Л.Г., Полятыкина Т.С. и др. // Кардиоваскулярная терапия и профилактика. – 2002. – №1. – С. 47-52.

12. Пискун Р.П. // Труды крымского мед. инст. «Морфология некоторых органов и тканей человека и млекопитающих». – 1986. – Т. 109. – С. 93-96.
13. Смирнов А.В., Панышин Н.Г., Спасов А.А. и др. // Бюл. Волгоградского научного центра РАМН. – 2010. – №3. – С. 14-16.
14. Таинкин А.А., Скворцов Ю.И. // Саратовский науч.-мед. журн. – 2013. – Т. 9, №2. – С. 269-276.
15. Хромова Т.О. // Тез. докл. VI съезда фармакол. «Фармакология: состояние и перспективы исследования». – X., 1990. – С. 328-329.
16. Benson S. // Psychoneuroendocrinol. – 2009. – Vol. 34 (2). – P. 181-189.
17. Erkkola R., Vervarcke S., Vansteelandt S. et al. // Репродуктивная эндокринология. – 2011. – №2. – С. 72-78.

ВПЛИВ НОВОГО ВАГІНАЛЬНОГО ГЕЛЮ З ЕКСТРАКТОМ ХМЕЛЮ НА СТАН СЕРЦЕВО-СУДИННОЇ СИСТЕМИ ОВАРІОЕКТОМОВАНИХ САМИЦЬ ЩУРІВ

О.С.Сініцина, Г.В.Зайченко, І.М.Риженко, Ю.Б.Лар'яновська

Ключові слова: оваріоектомовані самиці щурів; дефіцит естрогенів; серцево-судинна система; гель з екстрактом хмелю; фітоестрогени

Наведені результати дослідження морфоструктури аорти, коронарних артерій, міокарда та інтрамуральних артерій серця у самиць щурів, яким була проведена білатеральна оваріоектомія, а також після лікування їх новим вагінальним гелем з екстрактом хмелю і препаратом порівняння – супозиторіями «Овестин», які містять естріол. Встановлено, що в результаті стійкого естрогендефіцитного стану в серцево-судинній системі самиць щурів сформувався комплекс морфологічних змін, які можна оцінити як ендотеліальну дисфункцію. Після терапії досліджуваним фітопрепаратом у тварин були відсутні зміни у міофібрилярному апараті міокарда, відзначено зменшення або відсутність ознак ендотеліальної дисфункції.

ВЛИЯНИЕ НОВОГО ВАГИНАЛЬНОГО ГЕЛЯ С ЭКСТРАКТОМ ХМЕЛЯ НА СОСТОЯНИЕ СЕРДЕЧНО-СОСУДИСТОЙ СИСТЕМЫ ОВАРИОЭКТОМИРОВАННЫХ САМОК КРЫС

О.С.Синицына, А.В.Зайченко, И.М.Рыженко, Ю.Б.Ларьяновская

Ключевые слова: овариоэктомированные самки крыс; дефицит эстрогенов; сердечно-сосудистая система; гель с экстрактом хмеля; фитоестрогены

Приведены результаты исследования морфоструктуры аорты, коронарных артерий, миокарда и интрамуральных артерий сердца у самок крыс, которым была проведена билатеральная овариоэктомия, а также после лечения их новым вагинальным гелем с экстрактом хмеля и препаратом сравнения – суппозиториями «Овестин», содержащими эстриол. Показано, что в результате стойкого эстрогендефицитного состояния в сердечно-сосудистой системе самок крыс сформировался комплекс морфологических изменений, которые можно оценить как эндотелиальную дисфункцию. После терапии изучаемым фитопрепаратом у животных отсутствовали изменения в миофибрилярном аппарате миокарда, отмечено уменьшение или отсутствие признаков эндотелиальной дисфункции.