PharmacologyOnLine

Archives • 2021 • vol.3 • 227-234

PHARMACOLOGICAL STUDY OF HERBAL MEDICINAL PRODUCTS FOR THE COMBINED TREATMENT OF MENOPAUSAL SYNDROME

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Abstract

This article represents the results of combined herbal medicinal products pharmacological study under the conditions of experimental surgical ovariectomy in rats.

Materials and methods. All ovariectomized rats have had disorders of carbohydrate and lipid metabolism, which is correlated with clinical signs of menopausal syndrome in patients. The main task was to study the effect of herbal medicinal products on metabolic disorders in menopause. Markers of lipid and carbohydrate metabolism were measured by enzymatic method in operated animals.

Results. All herbal medicinal products studied corrected the pathological manifestations of hypoestrogenic conditions in rats. The ADMH test sample in this therapeutic regimen restored the fasting serum glucose level, reduced the serum triglycerides and total serum cholesterol to normal levels, significantly reducing the low-density lipoprotein cholesterol content by 23.2%, and did not cause hyperproduction of sex hormones in female rats under ovariectomy. The IMH test sample significantly reduced the total cholesterol content by 12.7 %, and did not cause hyperproduction of sex hormones in female rats under ovariectomy.

Conclusions. The use of combined alcohol drops of medicinal herbs contributed to the significant correction in fasting serum glucose, triglycerides, total and low-density lipoproteins cholesterol levels. Herbal medicines do not directly affect the serum level of sex steroids in rats.

Keywords: *ovariectomy, herbal medicines, pharmacological studies, menopause*.

Introduction

Menopausal syndrome is a pathological condition that occurs in every female patient over the age of 50. Symptoms of the menopausal syndrome include a number of disorders of the organs and systems of the body caused by the suppression of the ovaries hormonal function [1, 2]. Patients with the menopausal syndrome have cognitive and psycho-emotional disorders; cardiovascular diseases which are associated with changes in the serum rheology and lipid metabolism and metabolic syndrome [3].

Replacement hormone therapy with estrogens is often used for the treatment of menopausal syndrome in clinical practice. It is currently the most effective measure for the treatment of this pathology [4]. Replacement hormone therapy does not completely compensate secondary symptoms of menopause like dyslipidemia and obesity [5].

The efficacy of medicinal products for the menopausal syndrome correction should be evaluated by the impact on the above-mentioned pathological conditions as they are mostly associated with the risk of premature mortality in female patients with the menopausal syndrome [6]. The study of potential pharmacologically active agents for correcting this symptom complex is a promising task of modern pharmaceutical science.

Thus, the development of herbal medicinal products for menopausal syndrome therapy of the is a topical issue for the current pharmaceutical technology and gynecology.

Methods

The objects of the study were test samples of plant origin provided and developed at the Drug Technology Department of the National University of Pharmacy (Kharkiv, Ukraine):

1) 70% alcohol drops of medicinal herbs of the combined composition made of cones of hops, sage herb and nettle herb;

2) infusion of medicinal herbs of the combined composition made of clover grass, linden flowers, yarrow and thyme herb.

Tazalok (LLC "Universal Agency "Pro-Pharma", Ukraine) was used as a reference herbal medicinal product authorized in Ukraine. Tazalok oral drops contain a tincture of the following medicinal plant raw material : ethanol (1:10): *Filipendula camtschatica* roots – 0.28 g, fresh parsley roots – 0.225 g, fresh celery roots – 0.17 g, *Galium verum* herb– 0.135 g, yellow toadflax herb – 0.11 g, calendula flowers – 0.08 g per 10 ml of drops.

The second reference drug in the experimental study was the herbal medicine Klimapin (CPhP Red Star, Ukraine) registered at the Ukrainian market. Klimapin oral drops contain a tincture of a mixture of the following medicinal plant raw material : ethanol (1:10) (40% ethanol as an extractant): *Crataegi fructus* (hawthorn fruits) – 3 g; *Lupuli strobili* (cones of hops) – 2 g; *Leonuri cardiacae herba* (common motherwort herb) – 1.5 g; *Urticae folia* (nettle leaves) – 1 g; *Salviae officinalis folium* (sage leaves) – 1.5 g; *Origani vulgaris herba* (oregano herb) – 0.5 g; *Belladonnae folia* (belladonna leaves) – 0.5 g per 100 ml of drops. This OTC drug is under the ATC code N05CM "Sleeping drugs and sedatives".

Animals

The experiment was carried out on 60 white outbred female rats of the same age (approximately 7 months) with the body weight of 205-235 g. The experimental animals were kept in the vivarium according to standard sanitary standards and recommended conditions on the necessary diet [7]. All studies were carried out in accordance with the EU Council Directive 86/609 of the EEC dated November, 24, 1986 on the compliance with the laws, regulations and administrative provisions of the EU Member States on the protection of animals used for experimental and other scientific purposes [8].

Induction of menopausal syndrome

For the animal simulation of a condition equivalent to the menopausal syndrome in women, a surgical technique for removing the ovaries – Kirshenblat bilateral ovariectomy was used. The experimental ovariectomy in experimental animals was performed under aseptic conditions using chloroform anesthesia, in non-operated animals the incision and suture of the wound were without removal of the ovaries [9].

Experimental Design

The animals were weighed and divided into groups. Each group contained 10 animals. All groups were coded:

Group 1 – non-ovariectomized animals (NOA);

Group 2 – operated animals with the model pathology, in which experimental ovariectomy was reproduced (OP);
Group 3 – the animals with the experimental ovariectomy pathology treated with the alcohol drops of medicinal herbs of the combined composition (ADMH);
Group 4 – the animals with the experimental ovariectomy treated with infusion of medicinal herbs of the combined composition (IMH);
Group 5 – animals with the experimental ovariectomy treated with the reference-sample containing Tazalok drops (RS 1).
Group 6 –animals with the experimental ovariectomy treated with the reference-sample containing Klimapin drops (RS 2).

To facilitate dosing of liquid dosage forms of the combined composition it was decided to express the doses used in this study in ml/kg. When determining the doses for the reference sample the animal equivalent dose (AED) was calculated taking into account the average therapeutic daily dose of the drug for humans and interspecific differences in the body mass and surface area, and this dose for rats appeared to be 0.27 ml/kg [10]. It was decided to use the same dose (0.27 ml/kg) for the objective evaluation of the effectiveness of the test samples and the expediency of their further studies and the relevant interpretation of the results obtained.

According to the literature data, a stable change in the hormonal status of animals was observed on day 35 after the experimental ovariectomy; therefore, that day the pharmacological agents under research were introduced for correcting the menopausal syndrome manifestations [11]. The test samples and reference medicinal products were daily administered intragastrically using a special probe in the morning for 21 days [12, 13].

All animals were measured on the diestrus part of cycle [14, 15]. In 72 hours after the last intragastrical administration the experimental animals were euthanized by decapitation under chloroform anesthesia; the animals' blood was collected for further production of the serum [16].

Measurement of biochemical parameters

Glucose in the rat serum was measured by the glucose oxidase method using a standard reagent kit – Glucose F HP009.05 (LLC Research and Production Enterprise "Felicit Diagnostics", Ukraine) [17]. The lipid content (triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol) was measured by the enzymatic method using the following standard reagent kits: Triglycerides F HP022.02, Cholesterol F HP026.02, Cholesterol–HDL F HP026.04 and Cholesterol-LDL F HP026.05 (LLC "Felicit Diagnostics", Ukraine) according to the appropriate instructions for use [18].

The analysis of the content of sex hormones (estradiol, progesterone) in the blood serum of rats was performed by enzyme immunoassay on a Stat Fax 303 Plus enzyme-linked immunosorbent analyzer (Awareness Technology, USA) using standard reagent kits – Estradiol-IFA and Progesterone-IFA (LLC "XEMA", Ukraine) [19].

Statistical analysis

The results obtained were statistically processed using the one-way ANOVA test with the standard software package STATISTICA 7.0 and the 4PL statistical and logistic method using the MyAssays Internet service for free use [14].

Results

After surgical removal of the ovaries the value moderately potentially increased in the serum of female rats by an average of 1.8 mmol/L (Fig. 1).

The animals with the ovaries removed showed a significant increase in the content of TG, TC and LDL-C in the serum. At the same time, the HDL-C index decreased slightly, but the change was not significant ($p > 0.05$) (Table 1).

No significant changes were observed in IMH group with exception of a statistically significant decrease in the total cholesterol level by 12.7% without a significant change in its various fractions (Table 1).

The administration of the reference sample RS 1 did not alter the TG and TC levels, but in a certain way it affected the distribution of cholesterol fractions, significantly reducing the LDL-C level by 18.6% compared to this value in the OP group ($p \leq 0.05$). There was also an insignificant tendency to increase the HDL-C level, but it was not statistically significant ($p \leq 0.05$) (Table 1). When using the reference sample RS 2 the content of TG and TC did not also change, there was a slight increase in the HDL-C level (Table 1).

The pharmacological activity of the combined herbal medicinal products on the experimental ovariectomy model in rats has been studied. Thus, the data obtained during this stage of the study indicate the presence of a positive effect on the serum glucose level only in the ADMH test sample in the dose of 0.27 ml/kg in intragastric administration for 3 weeks. In this regard, the further search for the effect of a different IMH sample on the glucose content indicator in this work is not promising. In further studies, it may be advisable to combine it with plant components having the proven antipyretic activity.

All herbal medicinal products studied corrected the pathological manifestations of hypoestrogenic conditions in rats. The ADMH test sample in this therapeutic regimen restored the fasting serum glucose level, reduced the serum triglycerides and total serum cholesterol to normal levels, significantly reducing the low-density lipoprotein cholesterol content by 23.2%, and did not cause hyperproduction of sex hormones in female rats under ovariectomy. The IMH test sample significantly reduced the total cholesterol content by 12.7 %, and did not cause hyperproduction of sex hormones in female rats under ovariectomy. The reference sample RS 1 reduced the cholesterol low-density lipoprotein cholesterol level by 18.6 %; and did not cause hyperproduction of sex hormones in female rats under ovariectomy. The reference sample RS 2 did not affect the low-density lipoprotein cholesterol level by 18.6%, and also did not cause hyperproduction of sex hormones in female rats under ovariectomy.

Discussion

Fasting serum glucose levels in female rats after treatment with ADMH was decreased; moreover, the value of this indicator in this group was not significantly different from the same indicator in non-operated animals. Other test samples in this treatment regimen showed no statistically significant effect on fasting serum glucose levels in animals.

As part of the study of the effect of test samples on the correction of metabolic disorders the lipid profile of the serum was also studied in ovariectomized female rats. Within this step, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein (HDL-C) cholesterol were measured in animals.

After removal of the ovaries the levels of estradiol and progesterone in the serum of rats were likely to decrease, indicating that surgical manipulations were performed unmistakably, and the experimentally relevant model was reproduced (Table 2).

Data of the enzyme-linked immunoassay concerning sex hormone levels in the blood of ovarioctamized rats treated with experimental and reference samples showed that none of the pharmacological agents had significantly altered estradiol and progesterone compared to the similar indicators in the control pathology group ($p>0.05$) (Table 2).

The metabolic disorders that occur in the menopausal syndrome are primarily the consequences of the impaired hormonal regulation on the lipid and carbohydrate metabolism. The menopausal syndrome in women is most commonly associated with dyslipidemia and impaired glucose tolerance, and it is virtually consistent with the pathogenesis of the metabolic syndrome and increases the risk of death from cardiovascular diseases in women [17]. The fasting serum glucose levels of the female rats were measured in order to assess the effect of the medicinal products developed on the carbohydrate metabolism values.

A definite moderate effect of each test sample was shown on manifestations of dyslipidemia in ovariectomized female rats. However, the highest efficacy was demonstrated by ADMH in ovariectomized animals in the dose of 0.27 ml/kg as a partial normalization of the serum lipid profile was observed. The use of ADMH sample caused a practical normalization of TG and TC levels in the blood serum of the operated animals, and the corresponding indicators were not statistically different from the content of the similar serum markers of animals in the NOA group ($p >0.05$). The LDL-C level was likely to decrease by 23.2% compared to OP without a significant effect on HDL-C (Table 1).

Primarily, surgical removal or ovarian dysfunction affects the reduction of estradiol and progesterone in women [18]. However, some drugs that are not related to full-analog hormone replacement therapy may cause a significant increase in the levels of these hormones in the serum under suppressed or completely non-ovarian production [20]. Such a fact is more negative than positive since it is likely that these drugs cause an increase in the peripheral aromatase activity (including those in the adipose tissue) and overload the hormone-synthesizing adrenal systems, being a kind of manifestation of toxicity.

Thus, the determination of sex hormone levels is not the main method of verification in our study. However, such determination provides us with the information about the reproduction of the experimental model and a possible mechanism of the pharmaceutical activity and side effects of the herbal medicinal products studied.

Data from this phase of the study indicate that none of the test samples increased the content of the sex hormones measured in the blood serum of ovariectomized animals. This may indicate that the experimental herbal medicinal products do not cause an increase in the peripheral aromatase activity and adrenal hyperfunction, which is a positive toxicological characteristic.

Taking into account the literature data on the phytochemical composition of the plant raw material (phytoestrogens) [21] used in the development of drugs, as well as the moderate positive results of the previous stages of the study it can be hypothesized that these test samples correct manifestations of the hypoestrogenic state (the experimental ovariectomy) and do not significantly affect the synthesis of estrogens in the body.

It has been determined that ADMH is the most effective. Therefore, it can be recommended for further pharmacological studies as a promising medicinal product for the correction of pathological manifestations of the menopausal syndrome.

The use of combined alcohol drops of medicinal herbs contributed to the significant correction in fasting serum glucose, triglycerides, total and low-density lipoproteins cholesterol levels. Herbal medicines do not directly affect the serum level of sex steroids in rats.

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ISSN: 1827-8620

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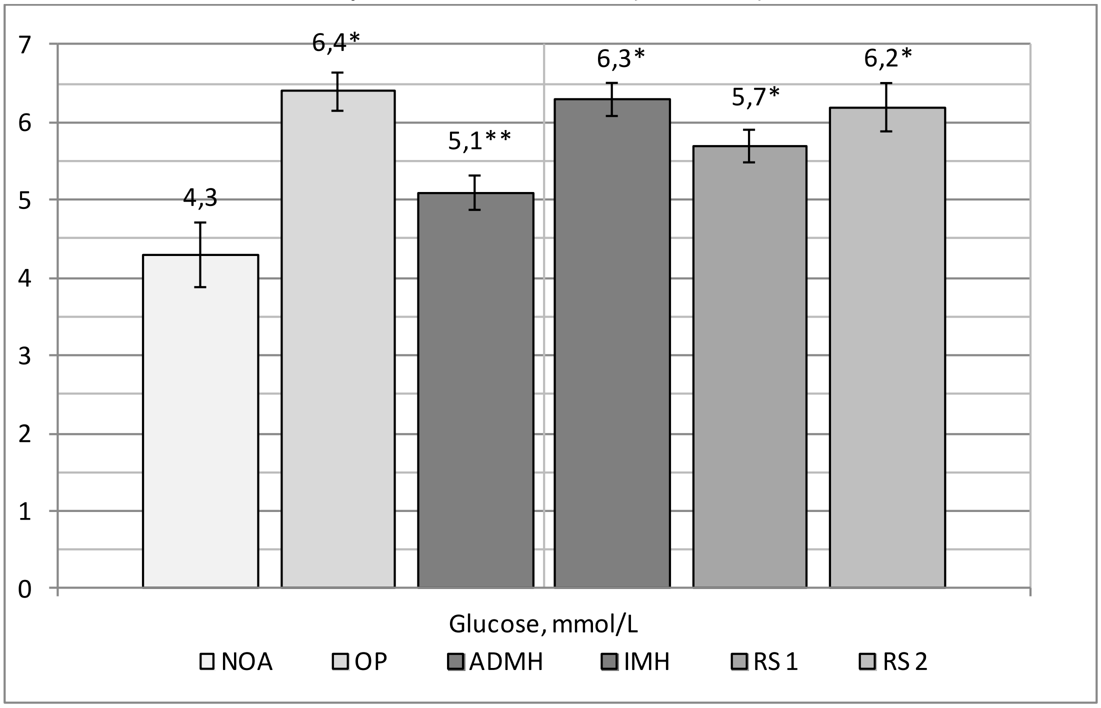
Figure 1. The mean fasting serum glucose in female rats after the treatment with the test samples under experimental ovariectomy for 21 days



Notes:
1) * – differences are likely in relation to animals in the intact control group (p≤0.05);
2) *#* – differences are likely in relation to animals in the OP group (p≤0.05).

Table 1. Markers of the lipid metabolism in the serum of female rats after the treatment with the test samples under experimental ovariectomy for 21 days ($\bar{X} \pm S_X$, n=10)

Experimental group	TG (mg/dL)	TC (mg/dL)	LDL–C (mg/dL)	HDL–C (mg/dL)
NOA	79.3 ± 5.6	152.5 ± 8.3	69.8 ± 8.1	58.1 ± 5.7
OP	112.5 ± 9.4*	205.7 ± 10.2*	130.4 ± 9.5*	47.3 ± 4.9
ADMH	87.2 ± 7.2**	172.5 ± 7.7**	100.1 ± 7.2*/**	49.6 ± 4.8
IMH	95.8 ± 3.8*	179.8 ± 6.4*/**	111.4 ± 8.6*	47.6 ± 6.2
RS 1	97.6 ± 6.7*	198.3 ± 11.3*	106.1 ± 6.5*/**	64.2 ± 8.5
RS 2	99.3 ± 1.3*	199.9 ± 6.4*	108.1 ± 5.5*/**	66.2 ± 7.7

Notes:
1) * – differences are likely in relation to animals in the intact control group (p≤0.05);
2) *#* – differences are likely in relation to animals in the OP group (p≤0.05).

Table 2. Sex hormone levels in the serum of female rats after the treatment with the test samples under experimental ovariectomy for 21 days ($\overline{X}\pm S_X$, n=10)

Experimental group	Estradiol level, pg / ml	Progesterone level, ng / ml
NOA	49.6 ± 4.8	22.8 ± 4.4
OP	22.3 ± 3.2*	8.3 ± 1.4*
ADMH	24.2 ± 3.5*	8.7 ± 1.8*
IMH	20.8 ± 3.7*	7.9 ± 1.1*
RS 1	22.9 ± 3.7*	10.1 ± 2.1*
RS 2	19.8 ± 3.7*	7.4 ± 1.3*

Notes:
1) * – differences are likely in relation to animals in the intact control group (p≤0.05);
2) *#* – differences are likely in relation to animals in the OP group (p≤0.05).