Research Article

Correction of asymmetric dimethylarginine-like pre-eclampsia in rats by micronized purified flavonoids fraction

Oksana E. Antsiferova, Alesya V. Yurakova*, Yuliya S. Lukyanova, Vladimir V. Gureev, Mikhail V. Korokin, Anastasia V. Gureeva, Tatyana G. Pokrovskaya, Oleg S. Gudyrev

ABSTRACT

Background: About 10% of pregnancies in the world are accompanied by hypertensive disorders, while from 2% to 8% are pre-eclampsia. One of the components of the pathogenesis of pre-eclampsia is placental ischemia. The humoral factors released during it have a pro-inflammatory effect and can contribute to the development of endothelial dysfunction. One of the possible options for reducing the effects of these cytokines may be the use of drugs containing natural flavonoids, one of the positive aspects of which is the reduction of vein-specific inflammation. **The Aim of the Study:** This study aims to study the effectiveness of using purified micronized flavonoid fraction (diosmin+flavonoids expressed as hesperidin) in the correction of functional disorders that occur during pre-eclampsia in the experiment. **Materials and Methods:** The experiment was performed on 150 white female rats of the Wistar line weighing 250–300 g. Asymmetric dimethylarginine (ADMA)-like agent (N-nitro-L-arginine methyl ester) was introduced intraperitoneally at a dose of 25 mg/kg/day from 14 to 20 days of gestation. The purified micronized flavonoid fraction (diosmin+flavonoids expressed as hesperidin) in dosages of 86 mg/kg and 260 mg/kg was administered orally once a day from 14 to 20 days of pregnancy. On 21 days of pregnancy, functional tests and laboratory tests were performed. **Results:** The administration of purified micronized flavonoid fraction to laboratory animals leads to a pronounced correction of pathological changes in experimental ADMA-like pre-eclampsia with the greatest effect in a higher dose of the drug used. A significant decrease in systolic and diastolic pressure was noted, respectively, improved microcirculation in the placenta, restoration of the nitric oxide synthesizing function of the endothelium, and a decrease in proteinuria. **Conclusion:** The results of the study indicate the promise of using a purified micronized flavonoid fraction for the correction of functional changes in pre-eclampsia and substantiate the feasibility of further research in this direction.

KEY WORDS: Endothelial dysfunction, Microcirculation, Pre-eclampsia, Proteinuria, Purified micronized flavonoid fraction, Rats

INTRODUCTION

About 10% of pregnancies in the world are accompanied by hypertensive disorders, with pre-eclampsia accounting for 2–8%.[1] According to Rosstat, hypertensive disorders during pregnancy, childbirth, and the postpartum period from 2013 to 2016 claimed 100 women's lives, and their prevalence in pregnant and parturient women was 164.1 and 81.5/1000 births in 2013 and 2016, respectively. In addition, hypertensive conditions during pregnancy lead to the development of pathological conditions not only in woman but also in the fetus, contributing to the disability of mothers and children. In recent years, much attention in the pathogenesis of pre-eclampsia is given to changes in the functional state of the endothelium.[2] Another component of the pathogenesis of pre-eclampsia is placental ischemia, which attracts a lot of attention. Humoral factors released in it have a pro-inflammatory effect and can contribute to the development of endothelial dysfunction.[3,4] To that end, reducing the release of pro-inflammatory factors of ischemic genesis may be a promising direction for the creation of new drugs for the treatment and prevention of pre-eclampsia. One of the possible options for reducing the effects of these cytokines may be the use of drugs containing natural flavonoids, where the reduction of vein-specific

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Department of Pharmacology and Clinical Pharmacology, Institute of Medicine, Belgorod State University, 85 Pobedy St., Belgorod 308015, Russia

***Corresponding author:** Alesya V. Yurakova, Department of Pharmacology and Clinical Pharmacology, Institute of Medicine, Belgorod State University, 85 Pobedy St., Belgorod 308015, Russia. E-mail: lysenko.av@bk.ru

Received on: 09-07-2019; Revised on: 06-08-2019; Accepted on: 08-09-2019

inflammation can be considered as one of the positive aspects.[5,6]

The Aim of the Study

The aim of this study is to investigate the effectiveness of the use of drug-containing natural flavonoids in disorders arising from asymmetric dimethylarginine (ADMA)-like pre-eclampsia.

MATERIALS AND METHODS

The study was conducted in the Research Institute of Pharmacology of living systems in Belgorod, State National Research University. The experiment was carried out in accordance with the regulations and guidelines governing the conduct of experimental studies in the Russian Federation. Ethical principles of laboratory animals' treatment were consistent "European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes, CETS N170."

The experiment was performed on 150 white female Wistar rats weighing 250–300 g. For the groups' formation of pregnant animals with specified terms, being on separate maintenance, males (two animals) were hooked to females (three animals) for 24 h. Then, the animals were seated and after 10–14 days in the condition of ethereal sleep, the palpation determined the presence of pregnancy. Pregnancy occurred in 30–40% of the experiments. The pregnant rats were then divided into four groups Group 1 – intact, Group 2 – control N-nitro-L-arginine methyl ester (L-NAME administration), Group 3 – L-NAME + purified micronized flavonoid fraction (Detralex [86 mg/kg/day]), and Group 4 – L-NAME + purified micronized flavonoid fraction (Detralex [260 mg/kg/day]). Non-selective nitric oxide (NO) synthase blocker L-NAME ADMA-like agent was administered intraperitoneal at a dose of 25 mg/kg/day for 7 days (14–20 days of pregnancy).[7,8] Detralex at a dosage of 86 mg/kg and 260 mg/kg was administered orally using a probe once a day from 14 to 20 days of pregnancy in animals of Groups 3 and 4.

On the 21st day of pregnancy, the laboratory animal was anesthetized by intraperitoneal injection of chloral hydrate at a dose of 300 mg/kg of body weight. Then, functional tests were performed.[9] The degree of endothelial dysfunction in experimental animals was assessed by the ratio of endothelium-dependent vasodilation and endothelium-independent vasodilation with subsequent calculation of the endothelial dysfunction coefficient.[10-13]

The level of NO metabolites (the total concentration of nitrates and nitrites, [NOx]) was determined by colorimetric method for the development of color in the diazotization reaction with sulfonamide nitrite, which is a part of the Griess reagent.

To obtain data on the state of microcirculation in the placenta on the 21st day of pregnancy under anesthesia, the level of microcirculation at a distance of 1 mm from the edge of the placental disc was measured at four points. The values of the microcirculation were expressed in perfusion units (PU).[14-17]

The collection of urine from the intact and experimental groups of rats was carried out using special metabolic cages. The animal was placed in a cage for 12 h with free access to water. The Brandberg-Roberts-Stolnikov method is based on the Geller ring test.

The large omentum was weighed, dried at 37°C for 24 h, and reweighed to study the fluid content.

Descriptive statistics were applied to all the data: The data were checked for the normality of the distribution. The type of distribution was determined by the Shapiro–Wilk criterion. In case of a normal distribution, the mean (M) and the standard error of the mean (m) were calculated. Intergroup differences were analyzed using Student's t-test or Mann–Whitney *U*-test, depending on the type of distribution. The calculations were performed using statistical programs Microsoft Excel 7.0.

RESULTS

After L-NAME administration in pregnant rats, there was a significant increase in blood pressure: Systolic was 193.6 ± 6.28 mmHg and diastolic 150.8 ± 80 mmHg, while the pressure was 123.4 ± 3.54 mmHg and 83.8 ± 5.47 mmHg, respectively, in the intact animal. In animals with Detralex introduction at a dosage of

Table 1: Effect of Detralex on blood pressure, CED, and microcirculation in the placenta with ADMA-like pre-eclampsia

Group	Indicator			
	SBP, mmHg	DBP, mmHg	CED, cu	Microcirculation, PU
Intact	123.4±3.54^{y}	83.8±5.47^{y}	1.21±0.13^{y}	472.6±22.44^{y}
Pregnant+L-NAME	193.6±6.28*	150.8±80*	2.89±0.25*	215.6±9.29*
L-NAME+Detralex® 86 mg/kg/day	181.6±6.19*	131.5±3.55*^{y}	2.21±0.13*^{y}	318.8±14.27*^{y}
L-NAME+Detralex® 260 mg/kg/day	169.3±5.4*^{y}	125.7±4.91*^{y}	1.79±0.11*^{y}	394.0±9.87*^{y}

SBP, DBP: Systolic and diastolic blood pressure (mmHg.), CED: Coefficient of endothelial dysfunction (cu); PU: Perfusion units; *: $P<0.05$ compared to the group of intact animals, y: $P<0.05$ compared to the control group L-NAME. L-NAME: N-nitro-L-arginine methyl ester

Table 2: Effect of Detralex on diuresis, proteinuria, and NO levels in ADMA-like pre-eclampsia

Group	Indicator		
	The amount of urine, ml/100 g/day	Proteinuria, g/l	Level NO, (μmol/dl)
Intact	5.40±0.22	0.85±0.07^{y}	2.2±0.06^{y}
Pregnant+L-NAME	5.63±0.21	2.34±0.14*	1.27±0.01*
L-NAME+Detralex® 86 mg/kg/day	5.09±0.22	1.71±0.09^{y}	1.56±0.04*^{y}
L-NAME+Detralex® 260 mg/kg/day	5.07±0.20	1.19±0.08*^{y}	1.79±0.03^{y}

*: $P<0.05$ compared to the intact animal group, y: $P<0.05$ compared to the L-NAME control group. L-NAME: N-nitro-L-arginine methyl ester, NO: Nitric oxide

260 mg/kg/day, there was a significant decrease in systolic blood pressure to 169.3 ± 5.4 mmHg and diastolic to 125.7 ± 4.91 mmHg, respectively [Table 1].

Administration of L-NAME to pregnant rats resulted in a violation of the regulatory mechanisms of vascular tone, as evidenced by an increase in coefficient of endothelial (CED) from 1.21 ± 0.13 to 2.89 ± 0.25. In groups of animals with course administration of Detralex from 14 to 20 days of pregnancy at dosages of 86 mg/kg and 260 mg/kg, CED was to 2.21 ± 0.13 and 1.79 ± 0.11 in pregnant animals with ADMA-like pre-eclampsia that indicates an improvement in endothelial function.

Animals with ADMA-like pre-eclampsia showed a decrease in microcirculation from 472.6 ± 22.44 PU to 215.6 ± 9.29 PU. The introduction of Detralex in the studied doses restored microcirculation to 318.8 = 14.27 PU and 394.0 = 9.87 PU, respectively. Modeling of ADMA-like pre-eclampsia did not cause significant changes in daily diuresis in pregnant rats; at the same time, it was characterized by moderate proteinuria which reached 2.34 ± 0.14 g/l ($P < 0.05$).

Administration of Detralex at dosages of 86 mg/kg and 260 mg/kg from 14 to 20 days in pregnant animals with modeling of ADMA-like pre-eclampsia significantly reduced protein parameters in urine compared to the control group, these values practically reached the target values in the group with a higher dosage of Detralex [Table 2].

The study of the endothelium NO synthesizing function was carried out on the basis of nitrite ions (NOx) in blood plasma determination [Table 2]. The introduction of Detralex at doses of 86 mg/kg and 260 mg/kg significantly ($P < 0.05$) increased the value of nitrite ions (NOx) in plasma in animals with ADMA-like pre-eclampsia to 1.56 ± 0.04 mmol/dl and 1.79 ± 0.03 mmol/dl, respectively.

DISCUSSION

The positive effects of the study drug can be explained by a decrease in the effects of pro-inflammatory cytokines.[5] At the same time, there is a decrease in their inhibitory effect on eNOS, a decrease in the content of peroxidation products, and an increase in the bioavailability of NO. It leads to the restoration of the regulatory mechanisms of vascular tone, both systemic and local.

CONCLUSION

Administration of Detralex to animals at dosages of 86 mg/kg and 260 mg/kg/day leads to a marked correction of pathological changes in experimental ADMA-like pre-eclampsia with the greatest effect in a higher dose of the used drug.

REFERENCES

1. Ghulmiyyah L, Sibai B, Maternal mortality from preeclampsia/eclampsia. Semin Perinatol 2012;36:56-9.
2. Krasnyi AM, Gracheva MI, Sadekova AA, Vtorushina VV, Balashov IS, Kan NE, *et al*. Complex analysis of total and fetal DNA and cytokines in blood plasma of pregnant women with preeclampsia. Bull Exp Biol Med 2018;164:721-5.
3. Das UN. Cytokines, angiogenic, and antiangiogenic factors and bioactive lipids in preeclampsia. Nutrition 2015;31:1083-95.
4. Iriyama T, Wang W, Parchim NF, Song A, Blackwell SC, Sibai BM, *et al*. Hypoxia-independent up regulation of placental hypoxia inducible factor-1 alpha gene expression contributes to the pathogenesis of preeclampsia. Hypertension 2015;65:1307-15.
5. Voronkov AV, Gamzeleva OY. Review of modern phlebotrophic preparations based on flavonoids as perspective endothelioprotectors in the treatment of chronic diseases of the veins. Hosp Replacing Technol 2019;1-2:27-33.
6. Lukyanova Y, Kolesnichenko P, Gureev V, Zhilinkova L. Evaluation of pharmacological correction of l-name-induced endothelial dysfunction, platelet aggregation and venous tone with diosmin "detralex 1000 mg". Res Results Pharmacol 2018;44:1-7.
7. Gureev V. New approaches of morfofunctional pharmacological correction of violations of cardiovascular system in experimental preeclampsia. Res Results Pharmacol 2016;2:11-27.
8. Stupakova EG, Lazareva GA, Gureev VV, Dolzhikova IN, Zhilinkova LA, Gureeva AV. L-NAME-induced preeclampsia: Correction of functional disorders of the hemostasis system with resveratrol and nicorandil. Res Results Pharmacol 2019;5:1-12.
9. Gureev VV, Pokrovskii MV, Korokin MV, Gudyrev OS, Philippova OV, Dolzhikov AA, *et al*. Correction of ADMA-induced preeclampsia with use of tetrahydrobiopterin and selective inhibitor of arginase II ZB49-0010. Res J Pharm Biol Chem Sci 2015;6:1538-41.
10. Stupakova EG, Lazareva GA, Gureev VV. Correction of morphofunctional disturbances arising when modelling preeclampsia with resveratrol and nicorandil. Res Results Pharmacol 2018;4:59-71.
11. Soldatov VO, Malorodova TN, Balamutova TI, Ksenofontov AO, Dovgan AP, Urozhevskaya ZS. Endothelial dysfunction: Comparative evaluation of ultrasound dopplerography, laser dopplerflowmetry and direct monitoring

of arterial pressure for conducting pharmacological tests in rats. Res Results Pharmacol 2018;4:73-80.
12. Soldatov VO, Malorodova TN, Pokrovskaya TG, Pokrovskii MV, Kulchenkova TI, Ksenofontov AO, *et al*. Ultrasonic dopplerography for the evaluation of endothelial function in the conduct of pharmacological vascular samples in an experiment. Int J Res Pharm Sci 2018;9:735-40.
13. Pokrovskii MV, Korokin MV, Kudryavtsev KV, Pokrovskaya TG, Gudyrev OS, Gureev VV, *et al*. Study of endothelial protective activity of phenol-derived thrombin and arginase-2 inhibitors KUD-259 and KUD-974. Bull Exp Biol Med 2017;163: 436-8.
14. Gureev VV, Alehin SA, Pokrovskiy MV, Dolghikov AA, Korokin MV, Gudyrev OS, *et al*. Remote ischemic preconditioning correction in ADMA-like gestosis model. Res J Pharm Biol Chem Sci 2014;5:1095-8.
15. Korokin MV, Pokrovsky MV, Novikov OO, Gureev VV, Denisyuk TA, Korokina LV. Effect of L-arginine, Vitamin B6 and folic acid on parameters of endothelial dysfunction and microcirculation in the placenta in modeling of L-NAME-induced NO deficiency. Bull Exp Biol Med 2011;152:70-2.
16. Chernomortseva ES, Pokrovskiĭ MV, Pokrovskaia TG, Artiushkova EB, Gureev VV. Experimental study of cardioprotective and endothelioprotective action of macrolides and azalides. Eksp Klin Farmakol 2009;72:29-31.
17. Gumanova NG, Artyushkova EB, Metel'skaya VA, Kochkarov VI, Pokrovskaya TG, Danilenko LM, *et al*. Effect of antioxidants pQ510 and resveratrol on regulatory function of the endothelium in rats with modeled arterial hypertension. Bull Exp Biol Med 2007;143:678-81.

Source of support: Nil; Conflict of interest: None Declared