ORIGINALNI RAD / *ORIGINAL PAPER*

DOI 10.2298/VETGL1604121N UDC 615.322:665.3

# WINE POLYPHENOL RESVERATROL INHIBITS CONTRACTIONS OF ISOLATED RAT UTERUS BY ACTIVATION OF SMOOTH MUSCLE INWARDLY RECTIFYING POTASSIUM CHANNELS*

Novaković Radmila, Radunović Nebojša, Rajković Jovana, Đokić Vladimir, Petrović Aleksandar, Ivković Branka, Ćupić Vitomir, Kanjuh Vladimir, Heinlev Helmut, Gojković-Bukarica Ljiljana**

*Resveratrol is a phytoalexin produced in a number of plant species including grapes. The benefit of resveratrol to health is widely reported. Resveratrol has been found to promote relaxation of non-pregnant and pregnant uterus, but its mechanism of action is unclear. The aims of our study were to investigate the involvement of inwardly rectifying potassium channels (Kir) in inhibitory effects of resveratrol on three models of contractions of non-pregnant rat uterus: the spontaneous rhythmic contractions (SRC), oxytocin-elicited phasic contractions and tonic oxytocin-elicited contractions.Uterine strips were obtained from virgin female Wistar rats in oestrus. Strips were mounted into organ bath for recording isometric tension in Krebs-Ringer solution. Experiments followed a multiple curve design. In order to test the involvement of Kirchannels in a mechanism of action of resveratrol(1-100 μM),$BaCl_2$ (1 mM),a antagonist of inwardly rectifying potassium channels was used. Resveratrol induced a concentration-dependent relaxation of all models of contractions. $BaCl_2$ antagonized the response to resveratrolon SRC and oxytocin-elicited phasic contractions. Relaxation achieved by resveratrolon tonic oxytocin-elicited concentrations was insensitive to $BaCl_2$.The antagonism of resveratrol effects by inwardly rectifying potassium channels antagonist suggests*

* Rad primljen za štampu 30.07.2016.

** Radmila Novaković, Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Serbia; Nebojša Radunović, Institute of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade, Serbia; Jovana Rajković, Vladimir Đokić, Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Serbia; Aleksandar Petrović, Institute of Food Technolgy and Biochemistry, Faculty of Agriculture, University of Belgrade, Serbia; Branka Ivković, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia; Vitomir Ćupić, Institute of Pharmacologyand Toxicology, Faculty of Veterinary Medicine, University of Belgrade, Serbia; Vladimir Kanjuh, Serbian Academy of Sciences and Arts, Belgrade, Serbia; Helmut Heinle, Institute of Physiology, University of Tuebingen, Germany; Ljiljana Gojković-Bukarica, Institute of Pharmacology

*that Kir channels are involved in resveratrol action on phasic contractions of rat uterus. Inhibitory effect of resveratrol on tonic contractions did not include Kir channels.*

*Key words: resveratrrol, uterus, inwardly recifying potassium channels, Kir*

## Uvod / *Introduction*

Resveratrol is a polyphenol, phytoalexin, which is produced in a number of plant species as a secondary metabolite. The rich sources of resveratrol are *Polygonum cuspidatum*, peanuts, grapes and wine as its products (Atanacković i sar., 2012). During the last decade it has been shown that resveratrol has a wide range of effects: anticancer, anti-inflammatory, antioxidant, cardioprotective. The extensive preclinical studies have demonstrated that the many molecules and cellular structures are the targets of resveratrol. Over 20 molecules that directly bind to resveratrol have been identified (Harikumar i Aggarwal, 2008). The relaxant effect of resveratrol on myometrium contractions has been studied in a few papers. It has been demonstrated that resveratrol strongly inhibits the contraction induced by oxytocin, prostoglandin $F_{2\alpha}$, acetylcholine, carbachol, as well as the spontaneous rhythmic contractions (Hsia i sar., 2011; Novaković i sar., 2013; Novaković i sar., 2015). Previously, we have shown that the ATP-sensitive potassium channels ($K_{ATP}$), $Ca^{2+}$-sensitive big potassium channels ($BK_{Ca}$) and voltage-sensitive potassium channels (Kv) are involved in the inhibitory mechanism of resveratrol of the non-pregnant animal and pregnant human uterus (Novaković i sar., 2013; Novaković i sar., 2015). In addition, the numerous studies have shown that resveratrol leads to vasorelaxation via modulation of potassium (K-channels) andcalcium (Ca-channels) ionchannels on the different animal and human blood vessels (Naderali i sar., 2000; Novaković i sar., 2006a; Novaković i sar., 2006b; Gojković-Bukarica i sar., 2008; Li i sar., 2012; Gojković-Bukarica i sar., 2013; McCalley i sar., 2014). However, resveratrol when applied in high concentrations (> 30 μM) has an additional $K_{ATP}$, $BK_{Ca}$ and Kv channels independent mechanism(s) of action (Li i sar., 2012; Novaković i sar., 2013; Novaković i sar., 2015). Then we would like to test the participation of another type of potassium channels, like inwardly rectifying potassium channels (Kir) in inhibitory mechanism of resveratrol. Kir channels have been found in a wide variety ofcells: oocytes, cardiac myocytes, endothelial cells, glial cells, neurons, blood cells and serve important roles in cellular physiology such as cell excitability, $K^+$homeostasis, and insulin secretion (Hibino i sar., 2010). $K_{ATP}$ channels belong to the Kir channel family, but they opened by a decrease in intracellular ATP (Inagaki i sar., 1996). However, the activity of Kir channels is regulated by many intracellular factors and second messengers such as protein kinase C, Gq-coupledreceptor stimulation, pH, intracellular $Mg^{2+}$or G proteins (Du i sar., 2004). McCloskey i sar. (2014) have demonstrated that Kir7.1 current hyperpolarizes uterine smooth muscle cell and promo-

tes quiescence during gestation (McCloskey i sar., 2014).The roles of the different channels are diverse and depend on the physiological environment. The contraction of myometrium is a complex event and a number of potassium channels play a role in shaping the action potential and modulating myometrial relaxation (Brainard i sar., 2007). To date, several types of $K^+$ channels have been identified in the myometrium (Du i sar., 2004; Novaković i sar., 2013; McCloskey i sar., 2014; Novaković i sar., 2015). Kir channels proteins have been identified in pregnant mouse and human myometrium by pharmacological and molecular methods (McCloskey i sar., 2014).

The abnormal contractility might underlie common and important disorders such as infertility, implantation failure, dysmenorrhea, endometriosis, spontaneous miscarriage or preterm birth (Olafsdottir i sar., 2012). The ideal agent for prevention and treatment of uterine abnormal contractility has not yet been found. The mechanism that triggers uterine spasm is currently unclear, although different factors or events seem to be involved. Understanding the processes that lead to relaxation of smooth muscle of the uterus and development of safe and effective tocolytic agents is an important research topic.

The aim of this study is to investigate the participation of Kir channels in inhibitory action of resveratrol on three models of uterus contractility: spontaneous rhythmic contractions (SRC), oxytocin - elicited phasic and oxytocin – elicited tonic contractions of non-pregnant rat uterus.

## Materijal i metode rada / *Material and methods*

### Animals

The experiments were performed on isolated uterus of the virgin female Wistar rats (200 - 250 g), obtained from the animal facilities of the Faculty of Medicine, University of Belgrade. All experiments were conducted in compliance with the Animal Welfare Act of the Republic of Serbia („Službeni glasnik RS“, No. 41/09), the respective regulations („Službeni glasnik RS“, No. 39/10), as well as international legislation (Directive 2010/63/EU and the Guide for the Care and Use of laboratory Animals, 8th ed) with respect to the guidelines of ‘Good laboratory practice’. The study was approved by the Ethical Committee of Faculty of Medicine, University of Belgrade, license number 4211/2.

The estrous cycle phase was daily determined before each series of experiments. Vaginal smear was sampled and transmitted to the microscope slide where the cell staining was performed using haemotoxylin and eosin in the general method described by Marcondes i sar. (2002). Based on the appearance of cells during microscopic examination the estrous cycle phase was determined. Only animals which were in estrous phase (45 animals were used) were taken for the experiments and they were sacrificed by cervical dislocation. Then the abdomen was opened in V-shaped form and the both horns of the uterus were isolated. After separating, the tissue sample was held in the isolated organ bath (TSZ-04 /

1.2, Experimetria, Budapest, Hungary) at a temperature of 37° C in Krebs- Ringer solution through which was bubbled into a mixture of 95% oxygen and 5% carbon dioxide, pH 7.4. Segment of the horn of the uterus 1 cm long was placed in the bath by fixing one end of the sample to a holder at the bottom of bath (10 ml), and the other end was attached to the transducer (TSZ-04 / 1.2, Experimetria, Budapest, Hungary) that registers the change of isometric muscle tension. Before the beginning of experiments, samples were incubated for 45 minutes and then they were gradually stretched to an optimal point of tension of 1 g. The contractions were recording by ISOLAB software (Elunit, Belgrade, Serbia) which was connected to the transducer.

**Experimental procedure**

The inhibitory effects of resveratrol on the contraction of smooth muscles of the rat uterus and the involvement of Kir subtype of K-channel were examined in a separate series of experiments. After the incubation period, 37% of the samples showed a spontaneous rhythmic activity. In these cases, the samples were left for about 30 minutes to stabilize contractions prior to the administration of substances. Phasic contractions were induced by oxytocin (0.2 nM) in the different series of experiments. Tonic contractions were induced by 20 nM of oxytocin. After stabilization of the contractions (~30 minutes) resveratrol was directly cumulatively administered (1 - 100 μM) in the bath. In order to investigate the involvement of Kir channels in the mechanism of action of resveratrol, $BaCl_2$ was used as a non-selective antagonist of Kir channels. After the experiments, concentration - dependent curves with and without the presence of antagonist were designed.

**Drugs and solutions**

Resveratrol was dissolved in 70% (v/v) ethanol, and the further dilutions were made with distilled water immediately prior to use. In that case working concentrations of ethanol were not greater than 0.01% (v / v) in the bath. The Krebs - Ringer solution had the following composition (expressed in mmol/l): NaCl 120, KCl 5, $CaCl_2$2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KH_2PO_4$ 1.2, and glucose 11. All drugs were added directly to the bath in a volume of 50 μl and the concentrations given are the calculated final concentrations in the bath solution. Due to the photosensitivity of resveratrol all the experiments were carried out in the dark. A producer of the substances was Sigma-Aldrich Inc., USA.

**Treatment of data and statistics**

The amplitude of phasic contractions was measured from the baseline to the top of the spike, while the amplitude of tonic contractions was measured from ba-

seline to the plateau. The medium values of the amplitude during the control period were taken as 100%.

The concentration of resveratrol, which produces 50% of the maximum effect was considered for the mean effective concentration ($EC_{50}$) and was calculated for each experiment by using linear interpolation of the graphs. The results were expressed as mean value ± standard error (S.E.M); n indicates the number of experiments. Student's t-test was used for comparison. The probability of $P < 0.05$ was considered statistically significant. Data analysis was carried out by the computer program GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA.

## Rezultat / *Results*

Longitudinal muscle strips of the rat non-pregnant uterus exhibited the SRC of constant amplitude 2.4 ± 0.6 g and frequency 9.5 ± 1.3 contractions per 10 minutes.

Resveratrol (1–100 μM) inhibited SRC in a concentration-dependent manner with a $EC_{50}$ value of 28.2±2.5 μM and maximal response 94.8 ± 5.0 %, n= 8. $BaCl_2$

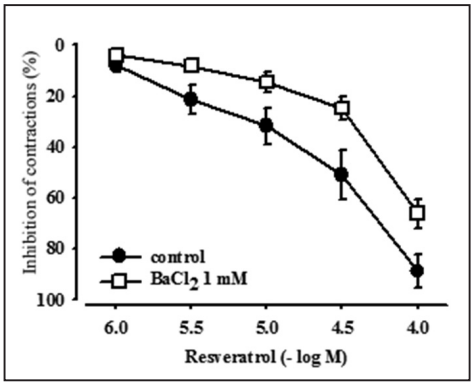


**Figure 1.** Antagonism of the inhibitory effect of resveratrol by $BaCl_2$ (1 mM) on the spontaneous rhythmic contractions (SRC) on the non-pregnant rat uterus. Concentration - response curves for resveratrol in the absence (circle) and presence of $BaCl_2$ (1 mM, square). The points are the means and the vertical lines show the S.E.M. (n = 5–8).

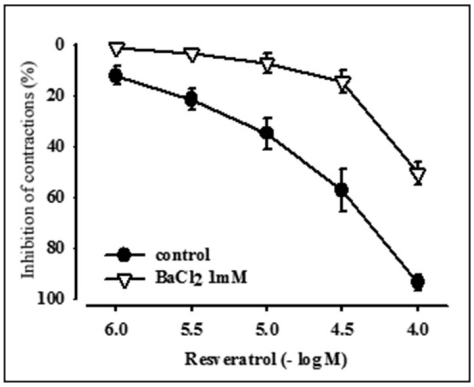


**Figure 2.** Antagonism of the inhibitory effect of resveratrol by $BaCl_2$ (1 mM) on phasic contractions provoked by oxytocin (0.2 nM) on the non-pregnant rat uterus. Concentration - response curves for resveratrol in the absence (circle) and presence of $BaCl_2$ (1 mM, triangle). The points are the means and the vertical lines show the S.E.M. (n = 5–8).

(1 mM, n = 5), a Kir channels antagonist, induced a significant rightward shift of the concentration–response curve for resveratrol ($EC_{50}$= 28.2 ± 2.5μM control vs. $EC_{50}$= 64.6 ± 4.0 μM in presence $BaCl_2$, n=5, P < 0.05, Fig. 1) with significant suppression of the maximal response (94,8 ± 5,0 % control vs. 66.2 ± 3.1 % in presence of $BaCl_2$, n=5, P < 0.05, Fig. 1).

Application of low nanomolar concentration of oxytocin (0.2 nM) to the samples produced phasic contractions of constant amplitude 3.1 ± 0.5 g and frequency 12.0 ± 0.8 contractions per 10 minutes.

Resveratrol (1–100μM) significantly inhibited phasic oxytocin-elicited contractions in a concentration-dependent manner with $EC_{50}$ value of 21.9± 5.2 μM, maximal responses 94.0 ± 5.9 %, n=8 (Fig. 2). $BaCl_2$ (1mM, n=5) induced a significant rightward shift of the concentration– response curve of resveratrol ($EC_{50}$ = 21.9 ± 5.2 μM control vs. $EC_{50}$ = 89.1± 4.0μM in presence of $BaCl_2$, n=5, P < 0.05) and suppression of the maximal response (94.0 ± 5.8 % control vs. 50.8± 3.0 % in presence $BaCl_2$, n=5, P < 0.05, Fig. 2).

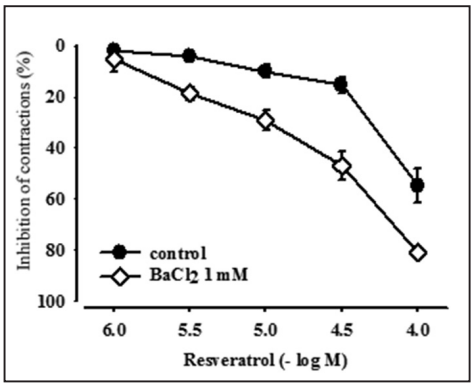


**Figure 3.** Antagonism of the inhibitory effect of resveratrol by $BaCl_2$ (1 mM) on tonic contractions provoked by oxytocin (20 nM) on the non-pregnant rat uterus. Concentration - response curves for resveratrol in the absence (circle) and presence of $BaCl_2$ (1 mM, diamond). The points are the means and the vertical lines show the S.E.M. (n = 5–8)

Application of high concentration of oxytocin (20 nM) to the rat uterus produced tonic contraction with or without phasic contractions in addition of constant amplitude 4.3 ± 0.5 g. Resveratrol inhibited tonic contractions induced by oxytocin in the concentration-dependent manner with $EC_{50}$ value of 87.1 ± 5.6 μM (n=8), but in presence of $BaCl_2$ $EC_{50}$ value was 35.5 ± 6.4 μM (n=5), P > 0.05. Maximal responses were in control 54.8 ± 4.0 %, (n = 8) vs. 81.0 ± 5.7 % in presence $BaCl_2$ (n=5), P > 0.05 (Fig. 3).

There are significant differences between $EC_{50}$ values for resveratrol achieved on SRC, phasic oxytocin-elicited contractions and tonic oxytocin-elicited contractions in presence of $BaCl_2$: 64.6± 4.0 μM vs. 89.1 ± 4.0 μM vs. 35.5 ± 6.5 μM, (n=5, all), P < 0.05.

Used Kir channel antagonist, $BaCl_2$ did not alter the resting tone of the uterus and did not modify the SRC or contractions evoked by oxytocin (n = 12 all, data not shown).

## Diskusija / *Discussion*

$Ba^{2+}$ is often used to examine the physiological roles of Kir channels in cells and tissues. To analyze the contribution of Kir channels to the inhibitory mechanism of resveratrol on contractions of the rat non-pregnant uterus, we used agent that is known to possess a Kir channel-blocking activity – $BaCl_2$. Externally applied $Ba^{2+}$ suppresses Kir currents in avoltage-dependent manner (Oliver i sar., 1998). Although high concentrations of $Ba^{2+}$ can also block Kv channelsat micromolar (< 5 mM) concentrationsthis cation is specific to Kir channels (Inagaki i sar., 1996; Du i sar., 2004; Hibino i sar., 2010). In the present study, $BaCl_2$ used in concentration of 1 mM significantly antagonized SRC and phasic oxytocin - induced contractions. However, the relaxation of tonic component achieved by resveratrol was not reduced in the presence of $BaCl_2$. Accordingly, it seems that Kir channels are not involved in the pathway by which resveratrol induces a relaxation of the tonic contractions. Wang i sar. have published that phasic contractions were more sensitive to resveratrol than basal tension in guinea pig gall bladder (Wang i sar., 2008).The differences between effects of resveratrol on contractions induced by low and high concentrations of oxytocin could come from different sources and routes for initiation and maintenance of phasic and tonic contractions.The phasic contraction of the non-pregnant ratuterus is physiological process with involved $Ca^{2+}$ influx via L-types $Ca^{2+}$ channels. However, oxytocin, at high concentrations produces tonic contraction resulting from additional mechanisms (Novaković i sar., 2013). It has been shown that Kir7.1 is important to maintain a hyperpolarized membrane potential during uterine quiescence in pregnancy. Kir7.1 channels modulate the action potential, modifying the excitation-contraction cycle by participating in key stages of repolarization (McCloskey i sar., 2014). Pharmacological effects of resveratrolon Kir channels could bean explanation for the inhibition of resveratrol's action on phasic contractions. Previous studies conducted on different tissue models documented variable results of influence of different type of K-channels on resveratrol mechanism of action. Studies conducted on human and rat vascular blood vessels show that $BaCl_2$ (Novaković i sar., 2006a; Novaković i sar., 2006b) and tetraethylammonium (Buluc i Demirel-Yilmaz, 2006; Gojković-Bukarica i sar., 2008) did not affect the relaxation induced by resveratrol, but 4-AP (Novaković i sar., 2006a; Novaković i sar., 2006b; Gojković-Bukarica i sar., 2008) and glibenclamide (Buluc i Demirel-Yilmaz, 2006) did affect it. The voltage clamp study on the mouse beta cell line revealed that resveratrol (100 μM) blocked $K_{ATP}$ and Kv, but activated $BK_{Ca}$ channels (Chen i sar., 2007). As opposed, the experiments on the rat hearts suggest that resveratrol inhibits L-type $Ca^{2+}$ channels and enhances activity of the $K_{ATP}$ (Chen i sar., 2008). There are obvious differences in sensitivity to resveratrol among various types of contractions (phasic vs. tonic), species and tissues.

According to our observations, it seems that resveratrol may produce relaxation of SRC and phasic oxytocin-elicited contractions of non-pregnant rat uterus by activation of Kir channel. According to the relative resistance of resvera-

trol's effects to $BaCl_2$ on tonic oxytocin-elicited contractions model indicated that resveratrol exerts inhibition by acting on multiple sites. We need further investigation to elucidate the mechanism of action of resveratrol on tonic contractions induced by oxytocin.

**Acknowledgements**

This work was supported by the Scientific Research Grant from Ministry of Education, Science and Technological Development of the Republic of Serbia (No TR31020). We would like to thank to Mrs. Milena Zabunovic for technical support during this study.

## Literatura / *References*

1. Atanackovic M, Petrovic A, Jovic S, Gojkovic-Bukarica L, Bursac M, Cvejic J. Influence of winemaking techniques onthe resveratrol content, total phenolic content andantioxidant potential of red wines. Food Chem. 2012; 131: 513-518.
2. Brainard AM, Korovkina VP, England SK. Potassium channels and uterinefunction. Semin Cell Dev Biol. 2007; 18 (3): 332-339.
3. Buluc M, Demirel-Yilmaz E. Resveratrol decreases calcium sensitivity of vascular smooth muscle and enhances cytosolic calcium increase in endothelium. Vascul Pharmacol. 2006; 44 (4): 231-237.
4. Chen WP, Chi TC, Chuang LM, Su MJ. Resveratrol enhances insulin secretion byblocking K(ATP) and K(V) channels of beta cells. Eur J Pharmacol. 2007; 568 (1-3): 269-277.
5. Chen YR, Yi FF, Li XY, Wang CY, Chen L, Yang XC, Su PX, Cai J: Resveratrolattenuates ventricular arrhythmias and improves the long-term survival in rats with myocardial infarction. Cardiovasc Drugs Ther 2008, 22(6): 479-485.
6. Du X, Zhang H, Lopes C, Mirshahi T, Rohacs T, Logothetis DE. Characteristic interactions with phosphatidylinositol 4,5-bisphosphate determine regulation of kir channels by diverse modulators. J Biol Chem. 2004; 279(36): 37271-37281.
7. Gojkovic-Bukarica L, Novakovic A, Kanjuh V, Bumbasirevic M, Lesic A, Heinle H. A role of ion channels in the endothelium-independent relaxation of rat mesenteric artery induced by resveratrol. J Pharmacol Sci. 2008; 108(1): 124-130.
8. Gojković-BukaricaLj, Protić D, KanjuhV, HeinleH, Novaković R, Šćepanović R. Cardiovascular effects of resveratrol. Vojnosanitetski pregled. 2013; 70(12): 1145-1150.
9. Harikumar KB, Aggarwal BB. Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle. 2008; 7(8): 1020-1035.
10. Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev. 2010; 90(1): 291-366.
11. Hsia SM, Wang KL, Wang PS. Effects of resveratrol, a grape polyphenol, on uterine contraction and $Ca^{2+}$ mobilization in rats in vivo and in vitro. Endocrinology. 2011; 152: 2090-9.
12. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. Neuron. 1996; 16(5): 1011-1017.
13. Li H, Xia N, Förstermann U. Cardiovascular effects and molecular targets of resveratrol. Nitric Oxide. 2012; 26: 102–110.
14. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol. 2002; 62(4A): 609-614.
15. McCalley AE, Kaja S, Payne AJ, Koulen P. Resveratrol and calcium signaling: molecular mechanisms and clinical relevance. Molecules. 2014; 19(6): 7327-7340.
16. McCloskey C, Rada C, Bailey E, McCavera S, van den Berg H, Atia J, Rand D, Shmygol A, Chan Y, Quenby S, Brosens J, Vatish M, Zhang J, Denton J, Taggart M, Kettleborough C, Tickle D, Jerman J, Wright P, Dale T, Kanumilli S, Trezise D, Thornton S, Brown P, Catalano R,

Lin N, England S, Blanks A. The inwardly rectifying K+ channel KIR7.1 controls uterine excitability throughout pregnancy. EMBO Mol Med. 2014; 6(9): 1161-74.

17. Naderali EK, Doyle PJ, Williams G. Resveratrol induces vasorelaxation ofmesenteric and uterine arteries from female guinea-pigs. Clin Sci (Lond). 2000; 98(5): 537-543.
18. Novakovic A, Bukarica LG, Kanjuh V, Heinle H. Potassium channels-mediated vasorelaxation of rat aorta induced by resveratrol. Basic Clin Pharmacol Toxicol. 2006; 99(5): 360-364.
19. Novakovic A, Gojkovic-Bukarica L, Peric M, Nezic D, Djukanovic B,Markovic-Lipkovski J, Heinle H. The mechanism of endothelium-independent relaxation induced by the wine polyphenol resveratrol in human internal mammaryartery. J Pharmacol Sci. 2006; 101(1): 85-90.
20. Novakovic R, Ilic B, Beleslin-Cokic B, Radunovic N, Heinle H, Scepanovic R, Gojkovic-Bukarica L. The effect of resveratrol on contractility of non-pregnantrat uterus: the contribution of K(+) channels. J Physiol Pharmacol. 2013; 64(6): 795-805.
21. Novaković R, Radunović N, Marković-LipkovskiJ, Ćirović S, Beleslin-Čokić B, Ilić B, Ivković B, HeinleH, Živanović V, Gojković-Bukarica Lj. Effects of the polyphenol resveratrol on contractility of human term pregnant myometrium. Mol Hum Reprod. 2015; 21(6): 545-551.
22. Olafsdottir LB, Gudjonsson H, Jonsdottir HH, Björnsson E, Thjodleifsson B. Natural history of irritable bowel syndrome in women and dysmenorrhea: a 10-yearfollow-up study. Gastroenterol Res Pract. 2012; 2012: 534204.
23. Oliver D, Hahn H, Antz C, Ruppersberg JP, Fakler B: Interactionof permeant and blocking ions in cloned inward-rectifier K+channels. Biophys J 1998, 74: 2318–2326.
24. Wang LD, Qiu XQ, Tian ZF, Zhang YF, Li HF. Inhibitory effects of genistein andresveratrol on guinea pig gallbladder contractility in vitro. World J Gastroenterol. 2008; 14(31): 4955-4960.