ISSN 0974 – 5211

Journal of Natural Products

Volume 7 (2014)

www.JournalofNaturalProducts.Com

Research Paper

Comparison of the effects of natural & synthetic antioxidants on altered muscle tension of the rat aorta evoked by activated polymorphonuclear leukocytes

Bauer V.[*], Sotnikova R.

Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

*Corresponding Author

(Received 07 September 2013; Revised 17 Sept. – 03 November 2013; Accepted 10 December 2013)

ABSTRACT

Local inflammation and respiratory burst of polymorphonuclear leukocytes (PMNLs) generate reactive oxygen species. While native PMNLs did not influence muscle tension, those activated by N-formyl-methionyl-leucyl-phenylalanine evoked a biphasic response on KCl-precontracted rat aortal rings. When tissues were precontracted by phenylephrine, i.e. without extensive membrane depolarization, the activated PMNLs caused marked contraction of aortal rings. The aim of our study was to analyze the effect of natural antioxidants (supplements containing arbutin, the mentha villosa extract, rosmarinic acid and curcumine) on the activated peritoneal PMNLs-induced contraction of phenylephrine-precontracted vascular smooth muscle and compare it with that of synthetic antioxidants (pyridoindoles: stobadine and SMe1EC2). Application of the natural antioxidants in the concentrations used reduced the action of activated PMNLs by 75-90% in the following order arbutin < curcumine < rosmarinic acid ≤ mentha villosa extract and the synthetic ones reduced the action of activated neutrophils by 50-70%.

Keywords: Activated neutrophils; Natural and synthetic antioxidants; Rat aorta.

Abbreviation: Superoxide anion radical: $O_2^{\bullet-}$; Hydroxyl radical: $^{\bullet}OH$

INTRODUCTION

Among other tissues, reactive oxygen species (ROS) damage also the vessels. Maugeri et al. (2009), have suggested that processes which activate macrophages in the vessel wall are responsible for systemic and local vascular injury manifestations. Local inflammation and respiratory burst of polymorphonuclear leukocytes (PMNLs) result in progression of vessel damage. While resting PMNLs generate nitric oxide, their activation in the inflammatory process as well as in response to arachidonic acid, phorbol myristate acetate, N-formyl-methionyl-leucyl-phenylalanine (FMLP), or the calcium ionophore A_{23187} mimics the inflammation-induced burst of PMNLs. The burst of PMNLs leads to production and release of superoxide anion radical ($O_2^{\bullet-}$) and subsequently derived ROS: hydrogen peroxide (H_2O_2), singlet oxygen, hydroxyl radical ($^{\bullet}OH$) and hypochlorous acid (Babior, et al., 1976; Bauer and Bauer, 1999; Bauer, et al., 2008; Jančinová, et al., 2011). ROS generated in respiratory burst of PMNLs may remain inside the cells or be released from them to the

extracellular space (Jančinová, et al., 2006; Nosáľ, et al., 2009). They diffuse to the vessel wall and damage the surrounding tissues. Endothelium is the first injured local target of ROS produced by PMNLs and this is at least in part responsible for the development of altered vessel resistance (Akopov, et al., 1992; Bauer, et al., 2011a; b; Csaki, et al., 1991; Tsao, et al., 1992) and consequently for cardiovascular deterioration.

Our recent findings (Bauer, et al., 2011a; b) suggest that the primarily activated PMNLs-released ROS was $O_2^{\bullet-}$. It by itself or after transformation to peroxynitrite can contract the aorta, similarly as can other ROS, including H_2O_2.

The aim of the present study was to compare the effects of natural and synthetic antioxidants on isolated FMLP-activated peritoneal PMNLs-induced contraction of the large conduit artery (rat thoracic aorta).

MATERIALS AND METHODS

The experimental procedures were conform to the Guide for the Care and Use of Laboratory Animals (State Veterinary and Food Administration of the Slovak Republic) and were approved by the Animal Care and Use Committee at the Institute of Experimental Pharmacology and Toxicology of the SAS (IEPT SAS) and by the State Veterinary and Food Administration of the Slovak Republic by Ro-1779/10-221, 2010. The guinea-pigs and rats used in the study were purchased from the breeding station of the IEPT SAS Dobrá Voda. The animals were killed by cervical dislocation and exsanguination.

Chemicals: Acetylcholine (Ach), glycogen, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), percoll, phenylephrine (PhE), sodium citrate tribasic dehydrate, xanthine (X), xanthine oxidase (XO), curcumine, arbutin and rosmarinic acid were all from Sigma and Sigma-Aldrich Chemie (Germany), trypan blue from Fluka (Switzerland), $FeSO_4$, H_2O_2 and the other chemicals were of p.a. purity purchased from Lachema Brno (Czech Republic). Stobadine and SMe1EC2 were synthesized in the IEPT SAS, Bratislava (Slovak Republic), the water mentha villosa extract was prepared in the Department of Pharmacognosy and Botany (DphgBo), Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic (FPh-CU). *Mentha x villosa* Huds. (*Lamiaceae*) was collected at flowering time in The Garden of Medical Plants, FPh-CU. The plants were dried at 32-35°C. Voucher specimen was deposited at the DPhgBo FPh-CU. Chromatographic standards were purchased from Extrasynthese (France). Standards of free radicals were obtained from Sigma-Aldrich (Germany). Water infusion of dried leaves of *Mentha×villosa* (20g) was prepared according to Czecho-Slovak Pharmacopoeia, 4th edition. The infusions were lyophilized; the yield was 10.32%.

Solutions: Phosphate buffer saline A (*PBA*) contained (in mM): NaCl 136, KCl 2.6, Na_2HPO_4 8.0, KH_2PO_4 1.5, pH 7.4; phosphate buffer saline B (*PBB*): NaCl 136, KCl 2.6, Na_2HPO_4 8.0, KH_2PO_4 1.5, $CaCl_2$ 0.6, $MgCl_2$ 0.5 and glucose 5.6, pH 7.4; and the physiological salt solution (*PSS*): NaCl 112.0, KCl 5.0, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11, pH 7.4. The high potassium depolarizing salt solution (*DSS*) was the same as *PSS* with the only difference that the concentration of KCl was increased with equimolar reduction of NaCl concentration to achieve 100mM of KCl.

H_2O_2 (0.01 - 1mM) was added as pure chemical, $O_2^{\bullet-}$ was generated by 0.1mM xanthine (X) with 0.1IU/ml xanthine oxidase (XO), and $^{\bullet}OH$ was produced by 0.1mM $FeSO_4$ with 0.15mM H_2O_2. Stock solutions of stobadine, SMe1EC2, arbutin, mentha villosa extract and rosmarinic acid were in redistilled water and curcumine in redistilled water+0.3µl 1N NaOH/ml.

Isolation and Preparation of PMNLs: PMNLs were isolated from the peritoneal exudate of male Trick guinea-pigs (450-600g; body weight). After intraperitoneal injection of 20ml of

1.2% glycogen in 0.9% NaCl, the animals were sacrificed within 14 to 16h. The abdomen was gently massaged after injection of 20ml of 0.4% trisodium citrate in 0.867% NaCl into the peritoneal cavity and the peritoneal exudate was collected and filtrated. All the following procedures were performed at 4°C. Cell suspension of the peritoneal exudate was washed with *PBA* and centrifuged for 90s at 2500rpm. Erythrocytes were removed by hypotonic treatment. The PMNLs pellets were resuspended in *PBA* and 2ml of their suspension was layered on the discontinuous density gradient of Percoll (1.5ml of 1.095g/ml and 1.5ml of 1.077g/ml). After centrifugation on the Percoll density gradient for 10 min at 2500rpm, PMNLs were collected from the interface and washed with *PBA* and centrifuged two times for 90s at 2000rpm. The PMNLs re-suspended in *PBA* were counted using Coulter Counter Electronics (England), their viability was assessed using trypan blue and the activity was confirmed also by the ability of ROS production (Nosáľ, et al., 2002). FMLP-induced oxidative burst of PMNLs was measured by luminol-enhanced chemiluminiscence in 1ml samples containing 200µl isolated PMNLs (10^6/ml), 20µl luminol (final concentration: 5µM), 770µl *PBB* and 10µl FMLP (final concentration: 0.1µM) (Bauer, et al., 2011a; Drábiková, et al., 2007). PMNLs stored in *PBA* stock solutions at 4°C were used for experiments within the following 2-5hours.

The low chemiluminescence, recorded on the basis of luminol-enhanced chemiluminiscence, of native isolated PMNLs indicated that the isolation procedure was gentle enough to prevent activation and damage of the isolated PMNLs. Upon their activation by introduction of FMLP, a significant "respiratory (oxidative) burst" developed, evidenced by increase of chemiluminiscence (Bauer, et al., 2011a).

Preparation of Rat Thoracic Aorta and Experimental Protocol: The thoracic aorta removed from male Wistar rats (250-300g; body weight) was immersed in *PSS*. Adherent tissues were removed and 2mm long rings were cut. Care was taken not to damage the endothelium.

The rings were mounted between two platinum hooks. The tissue chamber contained *PSS*, bubbled with a mixture of 95% O_2 and 5% CO_2 (pH=7.4) at 37°C (Sotníková, et al., 1994). The rings were stretched passively to the resting tension of 20mN and were allowed to equilibrate for 1hour. During this period of time, *PSS* was repeatedly replaced and the tension was readjusted to 20mN. The isometric tension was recorded using a strain gauge transducer (Experimentria, Hungary).

After the equilibration period, the control contraction was induced by *DSS*. The rings were washed 3times with *PSS* during 30minutes until the tension reached initial values. Then PhE in the concentration of 0.3µM was applied. At the plateau of the contraction, the effect of FMLP (1µM) and PMNLs (10^6/ml) was recorded. The same protocol was repeated in the presence of the antioxidant tested. Based on preliminary experiments, the preincubation period with pyridoindoles was 8minutes and with the natural antioxidants 20minutes. In a different set of experiments, the contraction was induced by *DSS* and the effect of H_2O_2 (0.01-1mM), $^{\bullet}OH$ (0.1mM $FeSO_4$/0.15mM H_2O_2) and $O_2^{\bullet-}$ (0.1mM X/0.1IU/ml XO) was evaluated. At the end of the response to ROS, responses to ACh in the cumulative concentrations of 0.01-10µM were tested as a measure of endothelial function. The same protocol was repeated in the presence of antioxidants. The antioxidants were used in concentrations which in the preliminary experiments reduced the effect of activated PMNLs by more than 50%.

***Analysis of Data*:** All values are given as Mean±SEM of at least 5-7 experiments. The statistical significance of differences between means was established by Student's *t*-test and the values of at least $P<0.05$ were considered statistically significant.

The mechanical responses are expressed generally as percentages of the *DSS*-or PhE-induced contraction measured at the beginning of each experiment.

RESULTS

The effect of activated PMNLs was studied on PhE-precontracted tissues. For these studies PhE in the concentration of 0.3μM was selected because it elevated the basal tension of the rat aortic rings with the highest invariability (n=24) of that evoked by *DSS*. FMLP did not affect the PhE-elevated tension. Injection of PMNLs in the presence of FMLP however markedly and monophasically increased the PhE-elevated basal tension of the rat aortic rings (Table 1).

Development of PhE-induced contraction was delayed and its amplitude was not altered by SMe1EC2 and was reduced by stobadine. Both pyridoindoles (Table 1) reduced the amplitude of contraction evoked by activated PMNLs significantly.

Natural antioxidants: arbutin, mentha villosa extract, rosmarinic acid and curcumine did not affect the development and the amplitude of PhE-induced contraction of the rat aortal rings (Table 2).

Similarly as in the *DSS*-precontracted tissues, in tissues precontracted by PhE (Table 3) X/XO evoked biphasic alteration of the tension (relaxation-contraction). While the mentha villosa extract did not change significantly the effects of X/XO, SMe1EC2 reduced the effect of xanthine but the final muscle tension reached in X/XO did not differ from the control.

The amplitude of relaxation induced by acetylcholine on *DSS*-pretreated aortal rings was significantly attenuated by H_2O_2 and $^{\bullet}OH$. However, it was ameliorated or remained unaltered by $O_2^{\bullet-}$ generated by X/XO and FMLP-activated PMNLs present in the bathing fluid. Except the tissues which were markedly relaxed by the ROS studied, the level of relaxation in the presence of ROS and ACh failed to reach the control level (Table 4).

Under control conditions, ACh reduced also the PhE-elevated muscle tension in a concentration-dependent manner. Under elevated muscle tension by activated PMNLs and in the presence of curcumine, not only the amplitude of ACh-induced relaxation but also the reached relaxation level was enhanced (Table 5).

DISCUSSION

PMNLs are considered to be central cells of acute inflammation. These cells most rapidly reach the site of injury or infection and liberate antimicrobial proteins, proteases and produce ROS (both intra-and extracellularly). ROS might affect not only the resistance size arteries, but as we described recently (Bauer, et al., 2011a; b), also in isolated large conduit artery (rat aortal rings) FMLP-activated PMNLs markedly raised the PhE-elevated muscle tension. The elimination of activated PMNLs-induced contractions as well as reduction of FMLP-activated PMNLs-induced chemiluminescence by superoxide dismutase suggest that $O_2^{\bullet-}$ is the primarily released ROS from activated PMNLs, which may eventually be transformed to other ROS (Bauer, et al., 2011a).

ROS are supposed to contribute significantly to tissue damage, including that of the endothelium and smooth muscle (Bauer, et al., 2011a; b). Compounds with antiradical activity may prove beneficial as they help to reduce the concentration of ROS. Earlier experiments with the synthetic pyridoindole compound stobadine (with α_1-adrenolytic activity) and its derivative SMe1EC2 (without meaningful α_1-adrenolytic activity) revealed a broad spectrum of their antioxidant and scavenging activities (Štolc, et al., 2010). Stobadine was found to decrease $O_2^{\bullet-}$ generation (Drábiková, et al., 2007). There was no difference between the antioxidant effect of stobadine and SMe1EC2 in our study, except the range of effective concentrations which was by one order lower for SMe1EC2, similarly as in the case of their neuroprotective action (Štolc, et al., 2006). The intensity of the antioxidant effect of

pyridoindoles in the present study was comparable to that in ischemia/reperfusion (Brosková and Knezl, 2011; Gáspárová, et al., 2011; Nosál'ová, et al., 2010).

Plant derived phenolics represent a good source of natural antioxidants. Their pharmacological actions stem mainly from their free radical scavenging and metal chelating properties, as well as from their effects on cell signaling pathways and on gene expression. Some of them (arbutin, mentha villosa extract, rosmarinic acid and curcumine) which inhibit ROS production due to their interaction with processes involved in activation of neutrophil oxidative burst (reduced luminol enhanced chemiluminiscence) (Jančinová, et al., 2007; Nosáľová, et al., 2010) were included in our experiments. A significant amount of arbutin was detected in *Arbutus unedo*, species from the *Ericaceae* family. Quantitative analyses and investigation of antioxidant activity of the herb and the dried ethanolic extract of its leafs showed high scavenging activity (Takebayashi, et al., 2010). These findings indicate that the antioxidant activity of arbutin may have been responsible for the reduction of contraction elicited by activated PMNLs in our experiments. Arbutin on molar bases was roughly by one order less effective in this respect than the pyridoindoles.

Mentha x villosa Huds. (*Lamiaceae*) is an aromatic herb widely used in folk medicine. The essential oil of the herb and its major constituent rotundifolone (piperitenone oxide) has a wide range of pharmacological activities (Guedes, et al., 2004; Lahlou, et al., 2001). The water extract of *Mentha x villosa* reduced the intestinal chemiluminiscence in ischemia/reperfusion tissues (Nosáľová, et al., 2010). In our experiments the mentha villosa extract in concentration used reduced the activated PMNLs-induced contraction significantly more effectively as pyridoindoles and arbutin.

Rosmarinic acid is commonly found in species of the *Boraginaceae* and the subfamily *Nepetoideae (Lamiaceae)*. Rosmarinic acid spontaneously penetrates membranes to inhibit lipid peroxidation (Fadel, 2011). It attenuated H_2O_2-induced cell injury by its antioxidant activity (Gao, et al., 2005) and reduced the intestinal chemiluminiscence in ischemia/reperfusion tissues (Nosáľová, et al., 2010). As we presumed, rosmarinic acid exhibited almost the same potency in reducing the activated PMNLs-induced contraction as had the mentha villosa extract.

Curcumine is a natural antioxidant with scavenging activity (Weber, et al., 2005). Reduced formation of ROS in PMNLs may represent another mechanism involved in the proresolving activity of curcumine (Jančinová, et al., 2011; Sharma, et al., 2005). Moreover, curcumine was found to decrease phosphorylation of the signaling enzyme protein kinase C (PKC), namely of its two isoforms PKC_{α} and $PKC_{\beta II}$ (Jančinova, et al., 2009). Since these iso forms directly participate in the activation of the neutrophil NADPH oxidase (Fontayne, et al., 2002), their inhibition could result in reduced oxidant formation and may explain the decreased chemiluminescence observed in the presence of curcumine (Jančinová, et al., 2011). These mechanisms may be responsible also for the reduction of the PMNLs-induced contraction of the aorta in our study, in which we found curcumine roughly equipotent with the mentha villosa extract and rosmarinic acid.

CONCLUSION

Application of antioxidants has been accepted to prevent damage and promote health. Not only synthetic (in our case pyridoindoles) but also natural supplements containing arbutin, mentha villosa extract, rosmarinic acid and curcumine might exert beneficial effects.

Aknowledgements: The authors would like to thank Dr. M. Kourilová for correcting the English and for her critical reading of the manuscript. The work was supported by The Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the Structural Funds of EU, OP R&D of ERDF by

realization of the Project “Evaluation of natural substances and their selection for prevention and treatment of lifestyle diseases” (ITMS 26240220040).

REFERENCES

Akopov, S.E., Grigorian, M.R., Gabrielian, E.S., (1992): The endothelium-dependent relaxation of human middle cerebral artery: effects of activated neutrophils. *Experientia.*, 48: 34-36.

Babior, B.M., Curnette, J.T., McMurick, B.J., (1976): The particulate superoxide-forming system from human neutrophils: properties of the system and further evidence supporting its participation in the respiratory burst. *J. Clin. Invest.,* 58: 989-996 .

Bauer, V., Bauer, F., (1999): Reactive oxygen species as mediators of tissue protection and injury. *Gen. Physiol. Biophys.*, 18: 7-14.

Bauer, V., Sotníková, R., Gergeľ, D., (2008): Comparison of the effects of activated neutrophils with the action of reactive oxygen species (ROS) on rat aortic smooth muscle (RASM). *Prague Med. Rep.,* 109(Suppl.): S13-14.

Bauer, V., Sotníková, R., Drábiková, K., (2011a): Effects of activated neutrophils on isolated rings of rat thoracic aorta. *J. Physiol. Pharmacol.*, 62: 513-520.

Bauer, V., Sotníková, R., Drábiková, K., (2011b): Effects of reactive oxygen species and neutrophils on endothelium-dependent relaxation of rat thoracic aorta. *Interdisciplinary Toxicol.,* 4: 101–107, 2011.

Broskova, Z., Knezl, V., (2011): Protective effect of novel pyridoindole derivatives on ischemia/reperfusion injury of the isolated rat heart. *Pharmacol. Rep.,* 63: 967-974.

Csaki, C., Szabo, C., Benyo, Z., Reivich, M., Kovach, A.G., (1991): Activated neutrophils inhibit cerebrovascular endothelium-dependent relaxations in vitro. *Life Sci.,* 49: 1087-1094.

Fadel, O., El Kirat, K., Morandat, S., (2011): The natural antioxidant rosmarinic acid spontaneously penetrates membranes to inhibit lipid peroxidation in situ. *Biochim. Biophys. Acta,* 1808: 2973–2980.

Fontayne, A., Dang, P.M.C., Gougerot-Pocidalo, M.A., El Benna, J., (2002): Phosphorylation of p47phox sites by PKC α, βII, δ and ζ: effect on binding to p22phox and on NADPH oxidase activation. *Biochemistry*, 41: 7743–7750.

Gao, L.P., Wei, H.L., Zhao, H.S., Xiao, S.Y., Zheng, R.L., (2005): Antiapoptotic and antioxidant effects of rosmarinic acid in astrocytes. *Pharmazie*, 60: 62-65.

Gáspárová, Z., Šnirc, V., Štolc, S., (2011): The new pyridoindole antioxidant SMe1EC2 and its intervention in hypoxia/hypoglycemia-induced impairment of longterm potentiation in rat hippocampus. *Interdiscip*. *Toxicol.,* 4: 56-61.

Guedes, D.N., Silva, D.F., Barbosa, J.M., de Medeiros, I.A., (2004): Endothelium-dependent hypotensive and vasorelaxant effects of the essential oil from aerial parts of Mentha x villosa in rats. *Phytomedicine*, 11: 490–497.

Jančinová, V., Drábiková, K., Nosál', R., Holomáňová, D. (2006): Extra- and intracellular formation of reactive oxygen species by human neutrophils in the presence of pheniramine, chlorpheniramine and brompheniramine. *Neuroendocrinol.Lett*. 27(Suppl 2): 141-143.

Jančinová, V., Petríková, M., Perečko, T., Drábiková, K., Nosál', R., Bauerová, K., Poništ, S., Košťálová, D., (2007): Inhibition of neutrophil oxidative burst with arbutin. Effects *in vitro* in adjuvant arthritis. *Chem. Listy.*, 101: 189–191.

Jančinová, V., Perečko, T., Nosáľ, R., Košťálová, D., Bauerová, K., Drábiková. K., (2009): Decreased activity of neutrophils in the presence of diferuloylmethane (curcumin) involves protein kinase C inhibition. *Eur. J. Pharmacol.,* 612: 161–166.

Jančinová, V., Perečko, T., Nosáľ, R., Mihalová, D., Bauerová, K., Drábiková. K., (2011): Pharmacological regulation of neutrophil activity and apoptosis. Contribution to new strategy for modulation of inflammatory processes. *Interdisc. Toxicol.,* 4: 11-14.

Lahlou, S., Carneiro-Leao, R.F.L., Leal-Cardoso, J.H., Toscano, C.F., (2001): Cardiovascular Effects of the Essential Oil of Mentha x villosa and its Main Constituent, Piperitenone Oxide, in Normotensive Anaesthetised Rats: Role of the Autonomic Nervous System. *Planta Med*., 67: 638-643.

Maugeri, N., Rovere-Querini, P., Baldini, M., Sabbadini, M.G., Manfredi, A.A., (2009): Translational Mini-Review Series on Immunology of Vascular Disease: Mechanisms of vascular inflammation and remodelling in systemic vasculitis. *Clin. Exp. Immunol*., 156: 395-404.

Nosáľ, R, Drábiková, K., Číž, M., Lojek, A., Danihelová, E., (2002): Effect of H_1-antagonist Dithiaden® on human PMN-leukocyte aggregation and chemiluminescence is stimulus-dependent. *Inflamm. Res.*, 51: 557-562.

Nosáľ, R., Drábiková, K., Jančinová, V., Moravcová, J., Lojek, A., Číž, M., Mačičková, T., Pečivová, J., (2009): H1-antihistamines and oxidative burst of professional phagocytes. *Neuroendocrinol. Lett.*, 30: 133–136.

Nosál'ová, V., Sotníková, R., Drábiková, K., Fialová, S., Košťálová, D., Bánášová, S., Navarová, J., (2010): Chemiluminescence response induced by mesenteric ischaemia/reperfusion: effect of antioxidative compounds ex vivo. *Interdisc. Toxicol.*, 3: 105 - 108.

Sharma, R.A., Gescher, A.J., Steward, W.P., (2005): Curcumin: the story so far. *Eur. J. Cancer*, 41: 1955-1968.

Sotníková, R., Kost'álová, D., Vaverková, S., (1994): Effect of bisbenzylisoquinoline alkaloids from Mahonia aquifolium on the isolated rat aorta. *Gen. Pharmacol.*, 25: 1405-1410.

Štolc, S., Šnirc, V., Májeková, M., Gáspárová, Z., Gajdošíková, A., Štvrtina, S., (2006): Development of the new group of indole-derived neuroprotective drugs affecting oxidative stress.*Cell. Mol. Neurobiol.*, 26: 1495-1504.

Štolc, S., Považanec, F., Bauer, V., Májeková, M., Wilcox, A. L., Šnirc, V., Račková, L., Sotníková, R., Štefek, M., Gáspárová, Z., Gajdošíková, A., Mihalová, D., Alfoldi, J., (2010): Pyridoindole derivatives with antioxidant properties: their synthesis and use in therapeutic practice (in Slovak) *Slovak Patent*, No. 287506.

Takebayashi, J., Ishii, R., Chen, J., Matsumoto, T., Ishimi, Y., Tai, A., (2010): Reassessment of antioxidant activity of arbutin: multifaceted evaluation using five antioxidant assay systems. *Free Radic. Res*., 44: 473-478.

Tsao, P.S., Ma, X.L., Lefer, A.M., (1992): Activated neutrophils aggravate endothelial dysfunction after reperfusion of the ischemic feline myocardium. *Am. Heart J*., 123: 1464-1471.

Weber, W.M., Hunsaker, L.A., Abcouwer, S.F., Deck, L.M., Vander Jagt, D.L., (2005): Anti-oxidant activities of curcumin and related enones. *Bioorg. Med. Chem.*, 13: 3811-3820.

Table-1: Effect of activated PMNLs on 0.3µM phenylephrine-induced contraction of isolated rat aorta in the absence and presence of stobadine or SMe1EC2.

Substance applied	Time (min)	Contraction amplitude in % of *DSS*-induced contraction (Mean ± S.E.M.)		
		Control	Stobadine 10µM	SMe1EC2 50µM
	-8	0	0	0
Phenylephrine 0.3µM	0	0	0	0
	2	50.0 ± 5.7	ND	ND
	3	71.0 ± 7.6	ND	ND
	8	74.0 ± 6.5	21.3 ± 2.6^{o}	52.0 ± 5.7^{o}
	16	ND	35.6 ± 3.8^{o}	75.0 ± 8.6
FMLP 0.1µM	20	78.0 ± 7.9	ND	ND
FMLP 0.1µM	25	ND	30.2 ± 4.6	71.0 ± 10.8
PMNLs 10^6/ml	30	76.0 ± 9.0	29.4 ± 4.4^{o}	72.0 ± 11.5
	31	97.0 ± 8.0	ND	ND
	35	117.0 ± 9.5	ND	ND
	38	ND	51.3 ± 5.5^{o}*	88.2 ± 3.8^{o}*
	44	ND	38.1 ± 6.5	89.4 ± 2.7
	47	ND	45.4 ± 6.4	90.0 ± 1.5
	50	112.0 ± 12.5	43.0 ± 6.1^{o}*	91.0 ± 2.0^{o}*

- n = 5-10
- ^{o}P<0.01 compared to control effect of phenylephrine, *P<0.01 compared to control effect of PMNLs.
- FMLP: N-formyl-L-methionyl-L-leucyl-L-phenylalanine, PMNLs: Polymorphonuclear leukocytes, ND: not determined.

Table-2: Effect of synthetic and natural substances on the contraction amplitude evoked by ativated PMNLs of 0.3µM phenylephrine-precontracted isolated rat aorta.

Drug tested	Concentration of drugs	Contraction amplitude induced by 10^6/ml PMNLs in % of phenylephrine–induced contraction (Mean ± S.E.M.)
Control	0	48.2 ± 4.9
Stobadine	10µM	21.2 ± 3.1*
SMe1EC2	50µM	18.5 ± 2.9*
Arbutin	50µM	12.4 ± 2.4*
Curcumine	5µM	8.3 ± 0.7*
Rosmarinic acid	50µM	8.2 ± 0.1*
Mentha villosa extract	13µg/l	4.3 ± 1.7*

- n = 5-7
- *P<0.01 compared to control.

Table-3: Effect of X/XO on phenylephrine-induced contraction of isolated rat aorta in the absence and presence of SMe1EC2 (50 µM) and mentha villosa extract (13µg/ml).

Substance applied	Responses in % of phenylephrine-induced contraction (Mean ± S.E.M.)		
	Control	SMe1EC2	Mentha villosa extract
Phenylephrine 0.3µM	100.0 ± 0	100.0 ± 0	100.0 ± 0
+ X 10µM	105.8 ± 6.7	73.4 ± 6.6*	125.8 ± 18.1
+XO 0.1IU/ml - relaxation	62.3 ± 10.4	58.3 ± 4.0	74.5 ± 11.3
+XO 0.1IU/ml - contraction	150.4 ± 9.4	147.2 ± 11.7	167.0 ± 14.4

- n = 5-7
- *P<0.01 compared to control.
- X – xanthine, XO – xanthine oxidase.

Table-4: Effect of reactive oxygen species on acetylcholine-induced relaxation of *DSS*-precontracted isolated rat aorta.

Substance applied	Responses in % of *DSS*-induced contraction (Mean + S.E.M.)			
	DSS	***DSS*+Ach**	***DSS*+ROS**	***DSS*+ROS+Ach**
H_2O_2 0.1mM	100 ± 0	68.2 ± 3.1 ^{oo}	60.2 ± 6.0 ^{oo}	46.2 ± 2.5**
$FeSO_4$ 0.15mM/H_2O_2 0.1mM	100 ± 0	62.2 ± 5.4 ^{oo}	96.1 ± 2.5	78.6 ± 6.2**
X 10µM/XO 0.1 IU/ml	100 ± 0	70.7 ± 2.5 ^{oo}	121.9 ± 6.6 ^{oo}	78.8 ± 2.1 ^{o}
FMLP 0.1µM/PMNLs 10^6/ml	100 ± 0	76.1 ± 2.4 ^{oo}	116.3 ± 5.2*	92.6 ± 1.3

- n = 5-7
- ^{o} P<0.05; ^{oo} P<0.01 compared to *DSS*, * P<0.05; **P<0.01 compared to reduction amplitude by Ach without ROS treatment
- Ach – acetylcholine (10µM), X – xanthine, XO – xanthine oxidase, FMLP – N-formyl-L-methionyl-leucyl- L-phenylalanine, PMNLs – polymorphonuclear leukocytes.

Table-5: Effect of curcumine on acetylcholine-induced relaxation of phenylephrine-precontracted isolated rat aorta in the presence of activated PMNLs.

Substance applied	Responses in % of *DSS*-induced contraction (Mean ± S.E.M.)	
	Control	**Curcumin (5µM)**
Phenylephrine 0.3µM	72.0 ± 4.9	78.2 ± 5.1
FMLP 1µM + PMNLs 10^6/ml	ND	87.7 ± 6.8
+ Acetylcholine 0.1µM	70.0 ± 6.8	84.0 ± 6.8
+ Acetylcholine 1µM	52.8 ± 7.4 ^{o}	39.8 ± 7.4 ^{oo} *
+ Acetylcholine 10µM	38.5 ± 3.6 ^{oo}	23.1 ± 3.6 ^{oo} *

- n = 5- 7
- ^{o} P<0.05; ^{oo} P<0.01 compared to phenylephrine, *P<0.01 compared to acetylcholine-induced reduction amplitude without treatment by FMLP/PMNLs and curcumine.
- FMLP - N-formyl-L-methionyl-L-leucyl-L-phenylalanine, PMNLs – polymorphonuclear leukocytes.

