International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 6 (4): 413-418, 2010
ISSN 1811-7775

Vasorelaxation and Superoxide Scavenging Activities of Orotic Acid

[1]S. Prachayasittikul, [2]O. Wongsawatkul, [3]A. Worachartcheewan, [4]S. Ruchirawat and [3]V. Prachayasittikul
[1]Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand
[2]Department of Pharmacology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand
[3]Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
[4]Chulabhorn Research Institute and Chulabhorn Graduate Institute, Bangkok 10210, Thailand

Abstract: The aim of study is to investigate effects of orotic acid (OA) on phenylephrine-induced contraction of rat thoracic aorta and its antioxidative activity. Results showed that the OA exhibited maximal vasorelaxation in dose-dependent manner with ED_{50} of 3.16×10^{-7} M, but the effect was less than those of acetylcholine (ACh)-induced nitric oxide (NO) vasorelaxation. Significant reductions of the vasorelaxations were found in the presence of either N^G-nitro-L-arginine methyl ester (L-NAME) or indomethacin (INDO). Synergistic effects were observed in the presence of L-NAME plus INDO that led to loss of vasorelaxation of both the ACh and the OA. In addition, complete loss of the vasorelaxation was manifested under removal of endothelial cells. This implies that the vasorelaxations are mediated by partially endothelium-induced NO and prostacyclin. The OA exhibited antioxidative activity in both DPPH and SOD assays. The significant results reveal novel actions of the OA as vasorelaxants and superoxide scavenger which are benefits as therapeutic uses and health supplements.

Key words: Orotic acid, vasorelaxants, antioxidants, nitric oxide, prostacyclin, L-NAME, indomethacin

INTRODUCTION

Orotic acid (pyrimidine-2,4-dione-6-carboxylic acid **1**, OA), a naturally occurring compound was first isolated from whey. It was found in high content in cow's milk and other sources; liver, yeast, mold, fungi, bacteria including cow serum. The whey protein was reported to be used as a protein beverage/drink composition (Sherwood *et al*., 2007a, b) and as a nutrient medium phase of cosmetic as well as dermatological preparation for physiological wound healing or scar reduction (Monks *et al*., 2005). OA is also known as vitamin B_{13}, produced by the body gut flora, which plays a key role as a precursor of pyrimidine in the biosynthetic pathway of DNA and RNA (Classen, 2004). It is essential for growth and vital activities of animals, plants and microorganisms. In addition, OA is an effective growth stimulating factor participating in carbohydrate metabolism by stimulating the synthesis of glycogen and ATP (Classen, 2004; Rosenfeldt, 1998; Rosenfeldt *et al*., 1998). The OA also showed protective effect of the liver in serious infections and was successfully used for treatment of serious dermatose (chronic eczema, neurodermatitis, ichthyosis and other skin diseases). Furthermore, it was described as cytoprotectant of endothelial cells and antihypoxics in experimental model including pharmaceutic applications (Woerwag, 1997). However, its mode of action was not revealed.

In view of chemical structure, other vitamins like nicotinic acid (vitamin B_3 **2**) (Lai *et al*., 2007) which is a potent hypolipidemic drug and an intense cutaneous vasodilator (Lai *et al*., 2007; Morrow *et al*., 1989) and nicotinamide (**3**, an amide derivative of **2**) is a known vasodilator (Bhattacharyya and Nandy, 1989). The compounds **2** and **3**, pyridine carboxylic acid and its amide (Fig. 1), exhibited their vasodilator properties by different mechanism of actions. Based on the literature, vasorelaxation of pyrimidine carboxylic acid (OA) was not reported. It is of great interest to investigate in details on vascular and antioxidative effects of the orotic acid. In this study, we report the vascular and antioxidative activities of OA conducted under various conditions.

MATERIALS AND METHODS

Chemicals and reagents were of analytical grade; L-phenylephrine hydrochloride (PE), sodium nitroprusside

Corresponding Author: Supaluk Prachayasittikul, Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand Tel: +662-664-1000/8209 Fax: +662-259-2097
Virapong Prachayasittikul, Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand Tel: +662-441-4376 Fax: +662-441-4380

(SNP), N^G-nitro-L-arginine methyl ester (L-NAME), acetylcholine (ACh), ketamine hydrochloride, indomethacin (INDO), α-tocopherol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) disodium salt, ethylenediaminetetraacetic acid (EDTA), phenazine methosulfate (PMS) and bovine erythrocyte superoxide dismutase (SOD) were obtained in 2007 from Sigma Chemical Co. (USA). Orotic acid and dimethyl sulfoxide (DMSO) were purchased in 2008 from Fluka.

Vasorelaxant assay

Isometric tension measurements: The protocols for handling animals were approved by the Animal Care Committee at the Srinakharinwirot University and done at the National Laboratory Animal Centre, Mahidol University. Male Sprague-Dawley rats (170-250 g) were anesthetized with intraperitoneal ketamine hydrochloride (0.05 mL kg^{-1}). The thoracic aorta was quickly removed to cold Kreb-Henseleit buffer (Wongsawatkul *et al*., 2008; Mizuta *et al*., 2008; Guler *et al*., 2006) containing (mM): 118 NaCl; 4.7 KCl; 1.2 KH_2PO_4; 1.2 $MgSO_4 \cdot 7H_2O$; 11.0(+)-glucose; 25.0 $NaHCO_3$ and 2.5 $CaCl_2 \cdot 2H_2O$, pH 7.4, aerated with 95% O_2, 5% CO_2. After removed debris tissue, the vessel was cut into rings, each 2-3 mm-long and hanged in the organ bath containing Kreb-Henseleit solution at 37°C, aerated with 95% O_2, 5% CO_2 and also connected to a force-displacement transducer (Model MLTO50 Force transducer Range: 50, P.R. China) and equilibrated for 50-60 min under a 1 g resting tension. During an incubation period, the Kreb-Henseleit solution was changed every 20 min. After the incubation period, the maximal contraction of the rings was determined with high dose of PE (10^{-5} M) and then washed 5 times until resting tension was recovered. Isometric tension (Woodman *et al*., 2000; Wongsawatkul *et al*., 2008) was recorded by Macintosh MacLab 4E AD Instrument connected to computer hard drive. The endothelial intact was examined using high dose of ACh (10^{-5} M) at a level of submaximal tension. If the relaxation response to ACh was less than 80%, the ring would be discarded. Then, the ring was washed 5 times to remove the residue of ACh. The vessel was again equilibrated for 50-60 min and the responses of vessel were carried out by the following protocols. Submaximal contraction was induced using PE (10^{-7} M), then cumulative dose-response curves to the agonists (10^{-9}-10^{-4} M). Finally, the dose-response curve of SNP was performed in order to test the functional vessel. With inhibitor (L-NAME or INDO) or vehicle, the vessels were pretreated with such compounds prior to submaximal contraction with PE then examine the endothelial response to the tested compounds. After each cumulative dose-response curve, the thoracic aorta preparation was washed and equilibrated 50-60 min before working on the next dose-response curve of tested compounds. The tested compounds were dissolved in DMSO, then the solutions were further diluted by normal saline.

Statistical analysis: The data were expressed as mean±SEM for the number of animals. The unpaired two-tailed Student's t-test and one-way ANOVA were used in the statistical analysis when appropriate. Post-hoc comparisons of individual groups were performed using the Tukey-Kramer test (Wongsawatkul *et al*., 2008; Nagayama *et al*., 1998). The ED_{50} values for the vasorelaxants were calculated using nonlinear regression analyses (GraphPad Prism 4, GraphPad Software Inc., USA). A p-value less than 0.05 was considered significant.

Antioxidative assay: The antioxidative activity of the tested compounds was elucidated by two assays; DPPH and SOD.

DPPH radical scavenging assay: When DPPH (a stable purple color) reacts with an antioxidant, it is reduced to yield a light-yellow colored of diphenylpicrylhydrazine. Color changes can be spectrophotometrically measured. The study (Prachayasittikul *et al*., 2009) was initiated by preparing 0.1 mM solution of DPPH in methanol. One mL of this solution was added sample solution (1 mg mL^{-1} dissolved in methanol, 0.5 mL). After 30 min, absorbance was measured using UV-Visible spectrophotometer (UV-1610, Shimadzu) at 517 nm and the percentage of radical scavenging activity was calculated from the following equation:

$$\text{Radical scavenging (\%)} = \left(1-\frac{\text{Abs.sample}}{\text{Abs.cont}}\right)\times 100$$

where, Abs.cont is the absorbance of the control reaction and Abs.sample is the absorbance in the presence of sample.

SOD activity assay: The assay for SOD activity was performed using slightly modified method (Grey *et al*., 2009). In principle the assay is based on the ability of SOD to inhibit NBT reduction by an aerobic mixture of NADH and PMS, which produces superoxide at nonacidic pH. The complete reaction system (1 mL total volume) consisted of 50 mM phosphate buffer, pH 7.4, containing 0.1 mM EDTA, 50 μM NBT, 78 μM NADH and 3.3 μM PMS (final concentrations). For the assay, 100 μL of sample or standard at various concentrations were added

into cuvettes containing 800 µL of reaction mixture. The reaction was initiated by the addition of 100 µL 33 µM PMS in 50 mM phosphate buffer, pH 7.4. The absorbance at 560 nm was monitored during 5 min as an index of NBT reduction using a UV-Visible spectrophotometer (UV-1610, Shimadzu) and SOD activity was calculated.

RESULTS AND DISCUSSION

Vasorelaxant activity: Effects of orotic acid on vascular function of rat thoracic aorta precontracted with PE were investigated under various conditions; in the presence or absence of inhibitors (L-NAME and INDO) and under denuded endothelial cells. The effects of ACh as a positive control, SNP a negative control and vehicle (DMSO) also were studied. Results confirmed that the vasorelaxation of ACh was related to nitric oxide (NO), meanwhile the DMSO showed no effect on the induction of vasorelaxation (Prachayasittikul *et al*., 2010).

Effect of orotic acid on the vascular function of rat thoracic aorta: The study was conducted in the absence and presence of nitric oxide synthase (NOS) inhibitor; (L-NAME). Results showed that orotic acid exhibited vasorelaxation in a dose-dependent manner (Fig. 2, Table 1). Maximal vasorelaxation (R_{max}) of the orotic acid was 62.88% with ED_{50} of 3.16×10^{-7} M, while the ACh produced 110.32% of R_{max} showing ED_{50} of 5.91×10^{-7} M. In the presence of L-NAME (1 mM), the dose-response curve of OA was shifted to the right giving R_{max} of 34.90% with ED_{50} of 3.16×10^{-7} M. While the ACh displayed its R_{max} 82.13 % with ED_{50} of 4.98×10^{-7} M. This suggested that orotic acid exerted vasorelaxant activity by partially producing NO from the endothelial cells.

Effect of endothelial cells on vasorelaxation of orotic acid: The activity of orotic acid was evaluated with and without intact endothelial cells comparing with ACh. The results (Table 2, Fig. 3) revealed that the vasorelaxation of the orotic acid was abolished under denuded endothelial cells. The similar result was also observed for the control; ACh. This confirmed that the vasorelaxantion of orotic acid was mediated through production of NO from the endothelial cells.

Effect of cyclooxygenase inhibitor on the vasorelaxation of orotic acid: The vasorelaxant activity of orotic acid was investigated in the presence of cyclooxygenase inhibitor (INDO, 1 mM) compared with NOS inhibitor (L-NAME, 1 mM) and L-NAME plus INDO. The results (Table 3) showed that the experiments of OA performed with

Table 1: Vasorelaxant activity of orotic acid on rat thoracic aorta

	Vasorelaxant activity			
	Without L-NAME		With L-NAME	
Compounds	R_{max} (%)	ED_{50} (M)	R_{max} (%)	ED_{50} (M)
OA[a]	62.88±0.98	3.16×10^{-7}	34.90±1.35	3.16×10^{-7}
ACh[b]	110.32±0.43	5.91×10^{-7}	82.13±1.03	4.98×10^{-7}

Data obtained from [a]6 experiments, [b]5 experiments

Table 2: Effect of endothelial cells on vasorelaxation of orotic acid

	Vasorelaxant activity			
	+et[a]		-et[b]	
Compounds	R_{max} (%)	ED_{50} (M)	R_{max} (%)	ED_{50} (M)
OA[c]	62.52±0.86	1.14×10^{-7}	0	0
ACh[c]	104.98±1.33	2.53×10^{-7}	7.00±0.32	3.13×10^{-7}

+et[a]: In the presence of endothelial cells,-et[b]: In the absence of endothelial cells, [c]Data obtained from 5 experiments

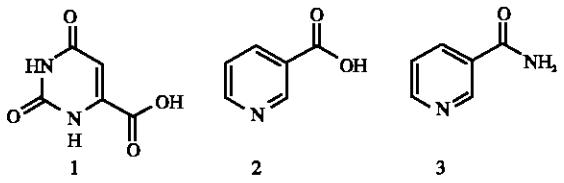

Fig. 1: Chemical structure of orotic acid and related compounds

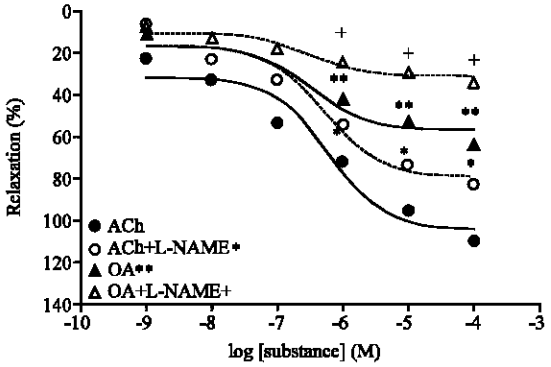

Fig. 2: Effect of OA on the vascular function of rat thoracic aorta in the presence of NOS inhibitor (L-NAME, 1 mM) compared with ACh. Data represent as mean±SEM of 6 experiments, each performed in duplicate. *p<0.05, ACh versus ACh+L-NAME, **p<0.05, ACh versus OA, +p<0.05, OA versus OA+L-NAME

adding L-NAME or INDO produced significant reductions of vasorelaxations (Fig. 4) in dose-dependent manner. Inhibition effect of the INDO on OA was comparable to that of the L-NAME; as seen from R_{max} value of 30.77 and 32.40%, respectively. Moreover, significant reductions of R_{max} were more pronounced in the presence of L-NAME

Table 3: Effect of inhibitors on vasorelaxation of orotic acid

	Vasorelaxant activity							
	-Inhibitor[a]		+L-NAME (1 mM)		+INDO (1 mM)		+L-NAME (1 mM) +INDO (1 mM)	
Compounds	R_{max} (%)	ED_{50} (M)	R_{max} (%)	ED_{50} (M)	R_{max} (%)	ED_{50} (M)	R_{max} (%)	ED_{50} (M)
OA[b]	62.33±0.70	3.27×10^{-7}	32.40±1.05	6.35×10^{-7}	30.77±1.04	2.42×10^{-7}	0	0
ACh[b]	121.70±1.44	9.99×10^{-7}	81.34±0.77	5.44×10^{-7}	68.78±0.92	4.58×10^{-7}	0	0
SNP[c]	120.81±1.18	3.16×10^{-7}	116.70±1.30	3.17×10^{-7}	112.93±0.61	3.16×10^{-7}	104.98±1.41	3.17×10^{-7}

-Inhibitor [a]: In the absence of L-NAME or INDO. Data obtained from [b]5 experiments, [c]6 experiments

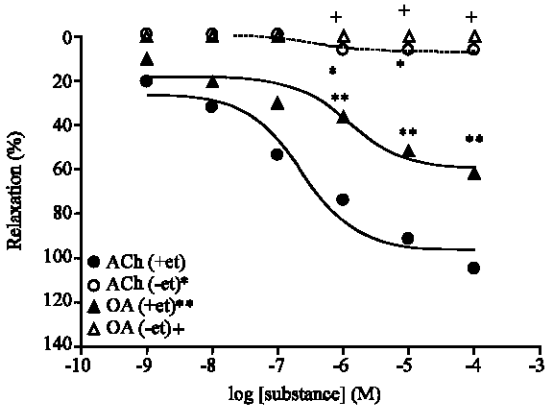

Fig. 3: Effect of OA and ACh on the vascular function of rat thoracic aorta under removal of endothelium (-et) compared with intact endothelium (+et). Data represent as mean±SEM of 5 experiments, each performed in duplicate. *p<0.05, ACh (+et) versus ACh (-et), **p<0.05, ACh (+et) versus OA (+et), +p<0.05, OA (+et) versus OA (-et)

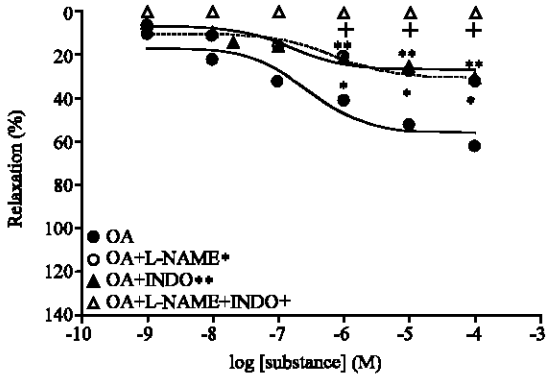

Fig. 4: Effect of OA on the vascular function of rat thoracic aorta in the presence of INDO compared with L-NAME and with L-NAME plus INDO. Data represent as mean±SEM of 5 experiments, each performed in duplicate. *p<0.05, OA versus OA+L-NAME, **p<0.05, OA versus OA+INDO, +p<0.05, OA versus OA+L-NAME+INDO

plus INDO. In such condition it was found that the vasorelaxation of the OA and ACh were completely

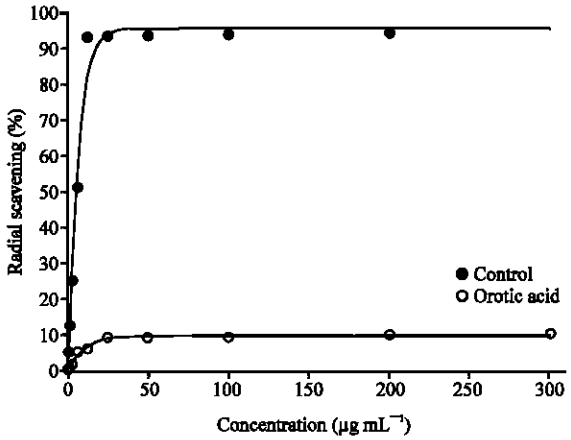

Fig. 5: Radical scavenging activity of orotic acid and α-tocopherol was used as a control

abolished (R_{max} = 0). However, no significant change was observed by the effect of SNP. The results confirmed that the OA exerted partial vasorelaxation via endothelial cells producing NO and prostacyclin (PGI_2).

Antioxidative activity: The antioxidative activity of OA was examined using DPPH and SOD assays. The results of DPPH (Fig. 5) showed that OA was relatively weak antioxidant. The assays were performed at concentrations ranging from 0.781-300 μg mL^{-1}. The maximum of its activity (10.28%) was observed at 300 μg mL^{-1}. However, the OA produced higher activity (Fig. 6) with 41.09% of NBT superoxide scavenging at the same concentration (300 μg mL^{-1}) when compared to the DPPH assay.

The studies have demonstrated that orotic acid exhibited maximal vasorelaxation in a dose-dependent manner, even though its activity is less than those produced by the ACh. Such vasorelaxation is exerted via modulation of NO production from functional endothelial cells, which is observed by a significant reduction of the vasorelaxation in the presence of NOS inhibitor (L-NAME, Table 1). Interestingly, the mode of OA vasorelaxation is as noted for thionicotinic acid analogs which has been recently reported (Prachayasittikul *et al*., 2010).

It is well recognized that the ACh induced vasorelaxation via mediating NO, PGI_2 and endothelium-derived hyperpolarizing factor (Quignard *et al*., 1999;

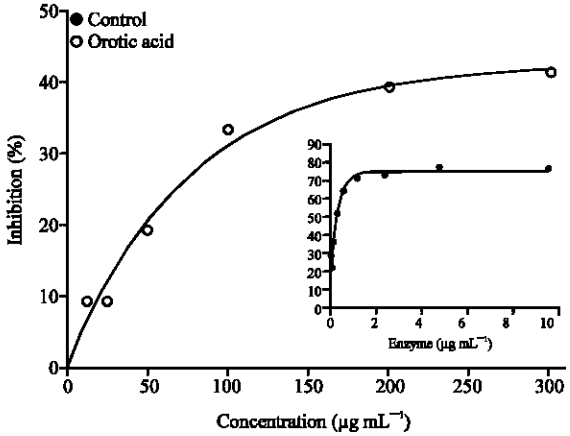

Fig. 6: Superoxide dismutase activity of orotic acid and SOD from bovine erythrocytes was used as a control

Ferrer *et al*., 1999; Edwards *et al*., 1998). Thus, the experiments were designed and carried out in the presence of cyclooxygenase inhibitor (INDO, 1 mM) compared with L-NAME (1 mM). It is noted (Table 3) that the vasorelaxation of the OA and ACh (Fig. 4) is significantly reduced in the dose-dependent manner when compared to that of in the presence of L-NAME or INDO. In particular, the antagonistic effect of INDO was stronger than the L-NAME. Significant reductions of the R_{max} were remarkably observed in the presence of L-NAME plus INDO leading to complete loss of the activity of the OA and ACh. However, there was no significant change of R_{max} generated by the SNP. The data support that the orotic acid exhibits vasorelaxation by partial synthesis of NO and PGI_2 by functional endothelial cells. The former was inhibited by L-NAME, the latter was inhibited by INDO. This is in accord with the literature reported (Ferrer *et al*., 1999; Piccinelli *et al*., 2004; Wongsawatkul *et al*., 2008; Prachayasittikul *et al*., 2010). Accordingly, the most recent study of barakol shows that its vasorelaxation is partly mediated by the endothelium pathway (Busarakumtragul *et al*., 2010). In addition, a number of flavonoids (Villa *et al*., 2005; Norton *et al*., 2005; DalBó *et al*., 2008) and triterpenoids exhibited their vasorelaxations via endothelium induced NO (Rodriguez *et al*., 2004). So far, the mode of vasorelaxation of orotic acid was not reported in the literature. It is known that NO is an important signaling molecule implicated in cardiovascular function such as vascular tone, whereas PGI_2 is a powerful vasorelaxants and antioxidants. PGI_2 is clinically used for treatment of pulmonary hypertension and portopulmonary hypertension (Zardi *et al*., 2007).

The role of NO related to superoxide radical was reported in many studies (Piccinelli *et al*., 2004). Thus, the antioxidative activity of the orotic acid was tested. The SOD assay (Fig. 6) showed that orotic acid was a moderate antioxidant with 41.09% NBT inhibition. It was reported that the antioxidant or protective effect of the OA is mainly due to its ability to increase the synthesis of enzymes which act as free radical scavenger (Zeana, 1999).

CONCLUSION

The study reveals novel actions of the OA as vasorelaxants and antioxidants. Significantly, the OA exhibits vasorelaxation via endothelium producing NO and PGI_2. The results suggest potential uses and impact of the OA as health and therapeutic values.

ACKNOWLEDGMENTS

This project was in part supported by a research grant from Mahidol University (B.E. 2551-2555). A.W. is supported by the Royal Golden Jubilee (Ph.D.) scholarship of the Thailand Research Fund under supervision of V.P.

REFERENCES

Bhattacharyya, M. and P. Nandy, 1989. Vasodilatory effect of nicotinamide on the fluidity of erythrocyte membrane and liposomes. J. Surf. Sci. Technol., 5: 13-20.

Busarakumtragul, P., P. Tep-areenan, S. Chainakul and O. Wongsawatkul, 2010. Effects of barakol on vascular functions in rats. Int. J. Pharmacol., 6: 257-263.

Classen, H.G., 2004. Magnesium orotate-experimental and clinical evidence. Rom. J. Int. Med., 42: 491-502.

DalBó, S., E.G. Moreira, F.C. Brandão, H. Horst, M.G. Pizzolatti, G.A. Micke and R.M. Ribeiro-do-Valle, 2008. Mechanisms underlying the vasorelaxant effect induced by proanthocyanidin-rich fraction from *Croton celtidifolius* in rat small resistance arteries. J. Pharmacol. Sci., 106: 234-241.

Edwards, G., K.A. Dora, M.J. Gardener, C.J. Garland and A.H. Weston, 1998. K^+ is an endothelium derived hyperpolarizing factor in rat arteries. Nature, 396: 269-272.

Ferrer, M.J.M., A. Encabo, M.J. Alonso and G. Balfagón, 1999. Role of K^+ channels and sodium pump in the vasodilation induced by acetylcholine, nitric oxide and cyclic GMP in the rabbit aorta. Gen. Pharmacol., 33: 35-41.

Grey, M., S. Yainoy, V. Prachayasittikul and L. Bülow, 2009. A superoxide dismutase–human hemoglobin fusion protein showing enhanced antioxidative properties. FEBS J., 276: 6195-6203.

Guler, N., O. Hanefi and E. Beyhan, 2006. Vasorelaxant effect of sildenafil on aorta and pulmonary artery in rabbits. Int. J. Pharmacol., 2: 55-59.

Lai, E., I. de Lepeleire, T.M. Crumley, F. Liu and L.A. Wenning *et al*., 2007. Suppression of niacin-induced vasodilation with an antagonist to prostaglandin D2 receptor subtype 1. Clim. Pharmacol. Ther., 81: 849-857.

Mizuta, K., Y. Osawa, F. Mizuta, D. Xu and C.W. Emala, 2008. Functional expression of $GABA_B$ receptors in airway epithelium. Am. J. Respir. Cell Mol. Biol., 39: 296-304.

Monks, M., S. Ibanez, C. Evangelisti and S. Gohla, 2005. Cosmetic or dermatological preparation comprising a nutrient medium phase and uses for physiological wound healing or scar reduction. US Patent 249691, Chem. Abstr., 143: 446260.

Morrow, J.D., W.G. Parsons and L.J. Roberts, 1989. Release of markedly increased quantities of prostaglandin D2 *in vivo* in humans following the administration of nicotinic acid. Prostaglandings, 38: 263-274.

Nagayama, M., F. Zhang and C. Iadecola, 1998. Delay treatment with aminoguanidine decreases focal cerebral ischemic damage and enhances neurologic recovery in rats. J. Cereb. Blood Flow Metab., 18: 1107-1113.

Norton, C., A.Z. Kalea, P.D. Harris and D.J.K. Zacas, 2005. Wild blueberry-rich diets affect the contractile machinery of the vascular smooth muscle in the Sprague-Dawley rat. J. Med. Food, 8: 8-13.

Piccinelli, A.L., S. Arana, A. Caceres, R. Di-villa-Bianca, R. Sorrentino and L. Rastrelli, 2004. New lignans from the roots of *Valeriana prionophylla* with antioxidative and vasorelaxant activities. J. Nat. Prod., 67: 1135-1140.

Prachayasittikul, S., S. Suphapong, A. Worachartcheewan, R. Lawung, S. Ruchirawat and V. Prachayasittikul, 2009. Bioactive metabolites from *Spilanthes acmella* Murr. Molecules, 14: 850-867.

Prachayasittikul, S., O. Wongsawatkul, A. Worachartcheewan, C. Nantasenamat, S. Ruchirawat and V. Prachayasittikul, 2010. Elucidating the structure-activity relationships of the vasorelaxation and antioxidation properties of thionicotinic acid derivatives. Molecules, 15: 198-214.

Quignard, J.F., M. Félétou, C. Thollon, J.P. Vilaine, J. Duhault and P.M. Vanhoutte, 1999. Potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig carotid and porcine coronary arteries. Br. J. Pharmacol., 127: 27-34.

Rodriguez, R.R., M.D. Herrera, J.S. Perona and V.R. Gutiérrez, 2004. Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in orujo olive oil, on rat aorta. Br. J. Nutr., 92: 635-642.

Rosenfeldt, F.L., 1998a. Editorial: Metabolic supplementation with orotic and magnesium orotate. Cardiovasc. Drugs Ther., 12: 147-152.

Rosenfeldt, F.L., S.M. Richards, Z. Lin, S. Pepe and R.A.J. Conyers, 1998b. Mechanism of cardioprotective effect of orotic acid. Cardiovasc. Drugs Ther., 12: 159-170.

Sherwood, S., D.A. Jenkins and S.A. Rittmamic, 2007a. Protein beverage and method of making the same. US Patent 148305, Chem. Abstr., 147: 94678.

Sherwood, S., D.A. Jenkins and S.A. Rittmanic, 2007b. Protein beverage and method of making the same. US Patent 154614, Chem. Abstr., 147: 94663.

Villa, I.C., R. Vera, M. Galisteo, F. O'Valle, M. Romero, A. Zarzuelo and J. Duarte, 2005. Endothelial nitric oxide production stimulated by bioflavonoid chrysin in rat isolated arota. Planta. Med., 71: 829-834.

Woerwag, F., 1997. Use of orotic acid to protect endothelial membrances. Patent No. DE 19530300, Chem. Abstr., 126: 166495.

Wongsawatkul, O., S. Prachayasittikul, C. Isarankura-Na-Ayudhya, J. Satayavivad, S. Ruchirawat and V. Prachayasittikul. 2008. Vasorelaxant and antioxidant activities of *Spilanthes acmella* murr. Int. J. Mol. Sci., 9: 2724-2744.

Woodman, O.L., O. Wongsawatkul and C.G. Sobey, 2000. Contribution of nitric oxide, cyclic GMP and K^+ channels to acetylcholine-induced dilatation of rat conduit and resistance arteries. Clin. Exp. Pharmacol. Physiol., 27: 34-40.

Zardi, E.M., A. Dobrina, A. Amoroso and A. Afeltra, 2007. Prostacyclin in liver disease: A potential therapeutic option. Expert Opin. Biol. Ther., 7: 785-790.

Zeana, C., 1999. Magnesium orotate in myocardial and neuronal protection. Rom. J. Int. Med., 37: 91-97.