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Comparative Improvement in Function of Isolated Rat Langerhans Islets by Various Phosphodiesterase 3, 4 and 5 Inhibitors

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ABSTRACT

Increased oxidative stress plays a role in the pathogenesis of cellular death and β -cell failure. The aim of this study was to evaluate the action of different types of phosphodiesterase (PDE) inhibitors including milrinone (PDE-3), rolipram (PDE-4) and sildenafil (PDE-5) on viability, production of Reactive Oxygen Species (ROS) and secretion of insulin from isolated rat pancreatic islets. Pancreatic islets were carefully isolated and incubated in RPMI 1640 for 24 h. After overnight incubation, islets were picked up and divided into ten in each groups. Then, milrinone, rolipram and sildenafil at doses of 0.1, 1, 10 and 100 FM were added to islet groups and incubated for further 24 h. Then static insulin secretion at 2.8 and 16.7 mM concentrations of glucose, was tested. Then the viability of cells, level of ROS and insulin were examined. The results of static experiments showed that secretion of insulin increased significantly in response to glucose at both basic (2.8 mM) and stimulation (16.7 mM) levels by the lower doses of tested PDE inhibitors. The level of ROS at the lower doses of milrinone decreased. The viability of islets at the lower doses of all of PDE inhibitors were increased; however, viability at the higher doses of sildenafil and rolipram reduced significantly. Milrinone was the most effective PDE inhibitors on the function of isolated pancreatic islets. PDE inhibitors show the most significant anti-oxidative effects at lower doses. Concerning improvement of isolated islets function, PDE-3 inhibitor is the best among tested compounds. PDE inhibitors may help management of diabetes and facilitate conditions of islet transplantation.

Key words: Oxidative stress, phosphodiesterase inhibitors, pancreatic islet

INTRODUCTION

Increased oxidative stress plays a role in the pathogenesis of cellular death and β -cell failure. It results from imbalance between production and manifestation of Reactive Oxygen Species (ROS) (Astaneie *et al.*, 2005; Larijani *et al.*, 2011). Free radicals are formed in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the oxidative degradation of glycated proteins. The imbalance in production of ROS and antioxidant defense damages cellular organelles by

peroxidation of cellular lipids and development of insulin resistance. Oxidative stress can accelerate the development of diabetic complications and thus antioxidants have been recently known beneficial in diabetes (Rahimi *et al.*, 2005) and islet transplantation (Mohseni-Salehi-Monfared *et al.*, 2009; Hasani-Ranjbar *et al.*, 2008).

Phosphodiesterases (PDEs) are a class of enzymes that degrade cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The cAMP and cGMP are second messengers responsible for transducing effects of extracellular signals that are formed when adenylyl cyclase and guanylyl cyclase catalyze adenosine triphosphate (ATP) and guanosine triphosphate (GTP). Since cyclic nucleotides have an essential regulatory function and diverse physiological action, PDEs have become recognized as important drug targets for treatment of various disease, such as asthma, erectile dysfunction, depression and heart failure (Rahimi *et al.*, 2010; Salari-Sharif and Abdollahi, 2010; Milani *et al.*, 2005).

Milrinon is a non-selective PDEI-3 inhibitor which increases both cAMP and cGMP and is used in cardiovascular diseases as an inotropic agent. Rolipram is a selective PDE4-inhibitor that increases cAMP and has been found anti-inflammatory in respiratory tract and a possible alternative to current antidepressants. Sildenafil is a selective PDEI-5 inhibitor that increases cGMP and is used in the treatment of sexual dysfunctions (Brunton *et al.*, 2011).

Recent evidences indicate that any increase in intracellular cAMP and cGMP may prevent from induction of oxidative stress (Abdollahi *et al.*, 2003a, b; Azadbar *et al.*, 2009; Ranjbar *et al.*, 2010; Khoshakhlagh *et al.*, 2007; Hoseini *et al.*, 2006). Specifically, there is good evidence on reduction of diabetes-induced oxidative stress by use of PDEIs in rats (Milani *et al.*, 2005). Several agents from natural sources have been tested and showed improvement of islets function in diabetes and islet transplantation (Hasani-Ranjbar *et al.*, 2010; Momtaz and Abdollahi, 2011; Mohseni-Salehi-Monfared *et al.*, 2009). In this respect, some of herbal sources have shown marked PDE inhibitor activities and antioxidant (Rahimi *et al.*, 2010). Studies have indicated that there is a direct relation between production of free radicals and damage to islets (Larijani *et al.*, 2011).

Considering above facts, we aimed to evaluate the action of different types of PDEIs on viability and function of isolated rat pancreatic islets.

MATERIALS AND METHODS

Chemicals: All chemicals were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany) unless otherwise stated. RPMI medium and its supplements were purchased from Invitrogen Co. (Gibco, UK). Rat insulin ELISA kit was purchased from Mercodia Co. (Uppsala, Sweden). Sildenafil and milrinone were obtained from local pharmaceutical companies.

Animals: Male Wistar rats weighing 200 to 250 g were housed in polypropylene cages under standard conditions with free access to drinking water and food, 12 h light: 12 h dark cycle and an ambient temperature of (20 to 25°C). All experiments were performed according to the Animal Welfare Act and the study protocol was approved by the Institute Review Board of Tehran University of Medical Sciences.

Islet isolation and culture: Rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg kg⁻¹) and underwent laparotomy. The common bile duct was ligated at its exit into the liver and was then cannulated at its exit from the duodenum. Then the pancreas was distended by injecting 10 mL of cold collagenase V (1 mg mL⁻¹) prepared in Hanks-[4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (HEPES) buffer (8 g L⁻¹ NaCl, 0.4 g L⁻¹ KCl,

0.2 g L⁻¹ MgSO₄·7H₂O, 0.02 g L⁻¹ Na₂HPO₄·12H₂O, 0.06 g L⁻¹ KH₂PO₄, 0.35 g L⁻¹ NaHCO₃, 2.32 g L⁻¹ HEPES, 0.4 g L⁻¹ glucose·H₂O, 0.186 g L⁻¹ CaCl₂·2H₂O, pH 7.2). After perfusion, the islets were kept in Krebs buffer (8 g L⁻¹ NaCl, 0.27 g L⁻¹ KCl, 0.42 g L⁻¹ NaHCO₃, 0.06 g L⁻¹ NaH₂PO₄, 0.05 g L⁻¹ MgCl₂, 2.38 g L⁻¹ HEPES, 0.22 g L⁻¹ CaCl₂·2H₂O, 0.5 g L⁻¹ glucose·H₂O, pH 7.4), centrifuged, separated from the remaining tissue by hand-picking under a stereomicroscope and incubated overnight in a supplemented RPMI-1640 medium containing 10% BSA, 1% penicillin-streptomycin and 0.1% gentamycin at 5% CO₂ and 37°C.

Static insulin secretion: The islets were washed in Krebs-Ringer buffer and then pre-incubated for 30 min in a water bath with the same buffer at 37°C. The islets were washed again and then dispensed in batches of 10 using a stereomicroscope. In each experimental set, 1 mL of 2.8 mmol L⁻¹ glucose were added and incubated for 30 min. Batches of 10 islets were then divided to two main group; group 1: treated with [Krebs buffer+2.8 mM glucose plus doses (0.01, 0.1, 1 μM) of milrinone or sildenafil or rolipram] and group 2: treated with [Krebs buffer+16.7 mM glucose plus doses (0.01, 0.1, 1 μM) of milrinone or sildenafil or rolipram]. The islets were incubated for 30 min, centrifuged and the supernatant was taken to measure secreted insulin using the ELISA method (Pourkhalili *et al.*, 2009).

MTT assay: Cell viability was assessed by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. To each well (group one), 20 μL of MTT was added and the plates were incubated at 37°C for 4 h. Then to the wells, 100 μL of dimethyl sulfoxide (DMSO) was added and the plates placed on a platform shaker for mixing. Absorbance was measured with an enzyme-linked immunosorbent assay (ELISA) reader at 540 nm.

Measurement of oxidative ability: Oxidative ability was determined in triplicate by conducting the dithiothreitol (DTT) assay, which is used for the quantitative measurement of reactive oxygen species (ROS) formation *in vitro*. Briefly to each well 20 μL of 16 mM DTT in potassium phosphate (0.1 M) buffer at pH 7.4 were added and incubated for 10 min at 37°C in a water bath. Then 40 μL of Tris-HCl, that contains ethylene diamine tetra acetic acid (EDTA, 20 mM) and [5,5'-dithiobis-(2-nitrobenzoic acid)] (DTNB, (16 mM, 25 μL), was added to this mixture to develop the yellow color. After color development, the absorbance was measured at 414 nm with a microplate ELISA reader (Larijani *et al.*, 2011).

Statistical analysis: Data were expressed as Means±SEM of separated experiments and analyzed using one-way ANOVA followed by Newman Keuls posthoc multiple comparison tests. The significance level was set at p<0.05.

RESULTS

Cell viability: The viability of islets at doses (0.01, 0.1, 1) of milrinone, rolipram and sildenafil were significantly increased (p<0.01) as compared to control group, except the dose 0.01 of sildenafil that showed no significant effect on cell viability (Fig. 1).

Formation of ROS: As indicated in Fig. 2, the ROS dropped significantly by exposure to doses (0.1 and 1) of milrinone, rolipram and sildenafil (p<0.01) as compared to control group. ROS decreased to 19 and 40% of control at doses of 0.1 and 1 of milrinone; 14 and 19% at doses (0.1 and 1) of rolipram and 15 and 22% at doses (0.1 and 1) of sildenafil.

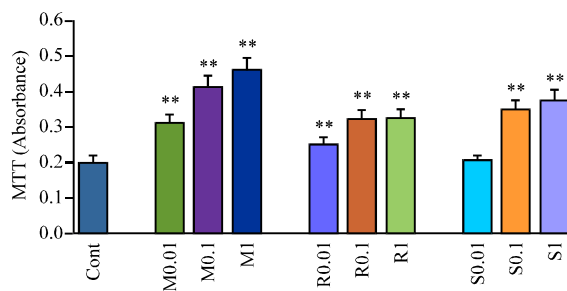


Fig. 1: Analysis of cell viability in isolated Langerhans islets incubated with different PDE inhibitors by MTT assay. Milrinon (M), Rolipram (R) and sildenafil at doses of 0.01, 0.1 and 1 $\mu\text{mol L}^{-1}$ were used. Values are Mean \pm SEM. **Difference between control (Cont) and treated groups is significant at $p < 0.01$

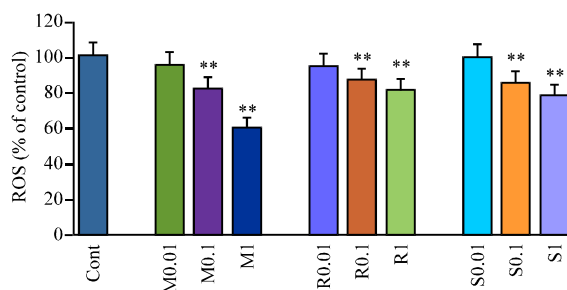


Fig. 2: Generation of ROS in isolated Langerhans islets incubated with different PDE inhibitors. Milrinon (M), Rolipram (R) and sildenafil at doses of 0.01, 0.1 and 1 $\mu\text{mol L}^{-1}$ were used. Values are Mean \pm SEM. **Difference between control (Cont) and treated groups is significant at $p < 0.01$

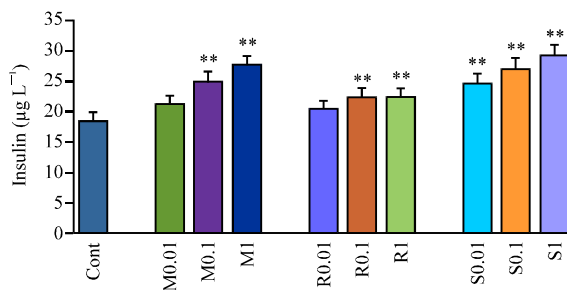


Fig. 3: Release of insulin from in isolated Langerhans islets incubated with different PDE inhibitors. One milliliter of glucose (2.8 mM L^{-1}) was used as secretagogue of insulin. Milrinon (M), Rolipram (R) and sildenafil at doses of 0.01, 0.1 and 1 $\mu\text{mol L}^{-1}$ were used. Values are Mean \pm SEM. **Difference between control (Cont) and treated groups is significant at $p < 0.01$

Insulin secretion: Treatment of incubated islets with doses (0.01, 0.1, 1) of milrinone, sildenafil and rolipram significantly increased insulin secretion after glucose challenge tests ($p < 0.01$). The insulin concentration at the basal 2.8 mM glucose in doses (0.01, 0.1, 1) of milrinone were 21.67 ± 3.97 , 25.254 ± 1.80 and $27.87 \pm 1.58 \mu\text{g L}^{-1}$; of rolipram were 20.83 ± 3.71 , 22.71 ± 1.92 and $22.73 \pm 1.13 \mu\text{g L}^{-1}$ and of sildenafil were 24.97 ± 0.42 , 27.42 ± 1.30 and $29.39 \pm 1.05 \mu\text{g L}^{-1}$, respectively in comparison to control non-treated group ($18.94 \pm 2.45 \mu\text{g L}^{-1}$) (Fig. 3).

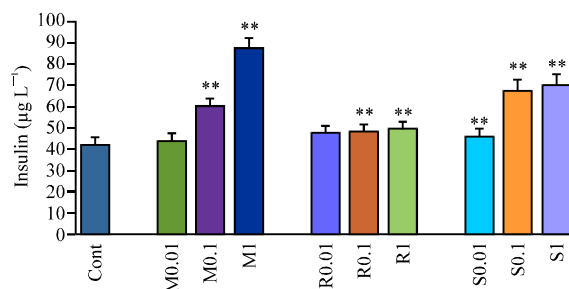


Fig. 4: Release of insulin from isolated Langerhans islets incubated with different PDE inhibitors. One milliliter of glucose (16.7 mM L⁻¹) was used as secretagogue of insulin. Milrinone (M), Rolipram (R) and sildenafil at doses of 0.01, 0.1 and 1 µmol L⁻¹ were used. Values are Mean±SEM. **Difference between control (Cont) and treated groups is significant at p<0.01

When 24 h pre-cultured islets were treated with doses of (0.01,0.1, 1) of milrinone, rolipram and sildenafil, the concentration of insulin post glucose (16.7 mM) were 43.7±3, 59.13±1.68, 85.93±6.38, 46.94±6.20, 47.6±1.30, 48.8±0.15, 45.54±6.76, 66.98±4.59 and 69.70±6.75 µg L⁻¹, in comparison to 41.9±1.98 µg L⁻¹ for the control group. There was a significant increase in insulin secretion after treatment with doses (0.1 and 1) of milrinone and rolipram and doses (0.01, 0.1, 1) of sildenafil (p<0.01) (Fig. 4).

DISCUSSION

The present results indicate that PDE inhibitors increase viability of islets and insulin secretion and decrease formation of ROS. The present positive effects of are most probably return to antioxidant potentials that increasing of cAMP and cGMP give to the cells. Previously, Milani *et al.* (2005) reported that treatment of diabetic rats with PDE inhibitors increase body antioxidant capacity. They reported a better antioxidant activity for milrinone in comparison to sildenafil and theophylline. Therefore, the point is that both cAMP and cGMP activate antioxidant processes that in case of milrinone because of its double PDEI-3 and 4 inhibitory potentials, the antioxidant effect and thus insulin secretion become much better than sildenafil or rolipram.

It is known that biological responses triggered by oxidative products are associated with lipid peroxidation derivatives, which can induce intracellular pathogenic signals such as calcium, G-proteins, cAMP, cGMP and MAP-kinase cascade leading to cellular dysfunction (Leonarduzzi *et al.*, 2000). The mechanism by which increased blood glucose concentrations lead to lipid peroxidation is the reduction of molecular oxygen and production of oxygen free radicals and α-ketoaldehydes, which finally break down phospholipids and accumulate lipid peroxides. Reduction in the activities of antioxidant enzymes like superoxide dismutase and catalase and glutathione peroxidase result in reduced total antioxidant capacity in diabetes (Astaneie *et al.*, 2005). Therefore, by increasing cyclic nucleotides, PDEIs can overcome oxidative stress.

In 1998, it was reported that nitric oxide (NO) donors have antioxidant property due to concerted action of cyclic nucleotides (cAMP and cGMP) (Polte and Schroder, 1998). Then other studies indicated positive effects for NO in protection against cellular damage induced by oxidative stress in vascular endothelium (Motterlini *et al.*, 1996), lung fibroblasts (Wink *et al.*, 1993), salivary glands (Abdollahi and Safarhamidi, 2002; Abdollahi *et al.*, 2000a, b, 2003a, b) and human neuroblastoma cells (Andoh *et al.*, 2003). It was shown that dipyrindamole as a cAMP/cGMP PDE

inhibitor reduces oxidative stress in diabetic nephropathy (Onozato *et al.*, 2003; Michell *et al.*, 2001). Therefore, the second mechanism for the actions of PDE inhibitors can be activation of NO production and promoting positive anti oxidative stress elements. More support come from clinical studies that indicates a higher NO concentration in response to oxidative stress in human illnesses like inflammatory bowel disease (Jahanshahi *et al.*, 2004) and diabetes (Radfar *et al.*, 2005).

These findings suggest that PDEIs may help management of diabetes and facilitate conditions of isle transplantation.

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REFERENCES

- Abdollahi, M., A. Dehpour and P. Kazemian, 2000a. Alteration by cadmium of rat submandibular gland secretory function and the role of the L-arginine/nitric oxide pathway. *Pharmacol. Res.*, 42: 591-597.
- Abdollahi, M., A.R. Dehpour and F. Shafayee, 2000b. L-Arginine/nitric oxide pathway and interaction with lead acetate on rat submandibular gland function. *Pharmacol. Toxicol.*, 87: 198-203.
- Abdollahi, M. and H. Safarhamidi, 2002. Protection by nitric oxide of morphine-induced inhibition of rat submandibular gland function. *Pharmacol. Res.*, 45: 87-92.
- Abdollahi, M., A. Bahreini-Moghadam, B. Emami, F. Fooladian and K. Zafari, 2003a. Increasing intracellular cAMP and cGMP inhibits cadmium-induced oxidative stress in rat submandibular saliva. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, 135: 331-336.
- Abdollahi, M., F. Fooladian, B. Emami, K. Zafari and A. Bahreini-Moghadam, 2003b. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. *Hum. Exp. Toxicol.*, 22: 587-592.
- Andoh, T., C.C. Chiueh and P. Boon Chock, 2003. Cyclic GMP-dependent protein kinase regulates the expression of thioredoxin and thioredoxin peroxidase-1 during hormesis in response to oxidative stress-induced apoptosis. *J. Biol. Chem.*, 278: 885-890.
- Astaneie, F., M. Afshari, A. Mojtahedi, S. Mostafalou, M.J. Zamani, B. Larijani and M. Abdollahi, 2005. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. *Arch. Med. Res.*, 36: 376-381.
- Azadbar, M., A. Ranjbar, A. Hosseini-Tabatabaei, A. Golestani, M. Baeeri, M. Sharifzadeh and M. Abdollahi, 2009. Interaction of phosphodiesterase 5 inhibitor with malathion on rat brain mitochondrial-bound hexokinase activity. *Pestic. Biochem. Physiol.*, 95: 121-125.
- Brunton, L.L., B.A. Chabner and B.C. Knollmann, 2011. Goodman and Gilman's the Pharmacological Basis of Therapeutics. 12th Edn., McGraw-Hill Book Co., New York, USA.
- Hasani-Ranjbar, S., B. Larijani and M. Abdollahi, 2008. A systematic review of Iranian medicinal plants useful in diabetes mellitus. *Arch. Med. Sci.*, 4, 3: 285-292.
- Hasani-Ranjbar, S., N. Nayebi, B. Larijani and M. Abdollahi, 2010. A systematic review of the efficacy and safety of *Teucrium* species; from anti-oxidant to anti-diabetic effects. *Int. J. Pharmacol.*, 6: 315-325.

- Hoseini, S., H. Esmaily, A. Mohammadirad and M. Abdollahi, 2006. Effects of sildenafil a phosphodiesterase 5 inhibitor on rat liver cell key enzymes of gluconeogenesis and glycogenolysis. *Int. J. Pharmacol.*, 2: 280-285.
- Jahanshahi, G., V. Motavasel, A. Rezaie, A.A. Hashtroudi, N.E. Daryani and M. Abdollahi, 2004. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig. Dis. Sci.*, 49: 1752-1757.
- Khoshakhlagh, P., M. Bahrololoumi-Shapourabadi, A. Mohammadirad, L. Ashtaral-Nakhai, B. Minaie and M. Abdollahi, 2007. Beneficial effect of phosphodiesterase-5 inhibitor in experimental inflammatory bowel disease; molecular evidence for involvement of oxidative stress. *Toxicol. Mech. Methods*, 17: 281-288.
- Larijani, B., M. Salimi, N. Pourkhalili, A. Mohammadirad, M. Baeeri, A. Nili-Ahmadabadi and M. Abdollahi, 2011. Positive response of isolated rat pancreatic islets to IMOD; hopes for better transplant outcome and graft function. *Asian J. Anim. Vet. Adv.*, 6: 1019-1025.
- Leonarduzzi, G., M.C. Arkan, H. Basaga, E. Chiarotto, A. Sevanian and G. Poli, 2000. Lipid oxidation products in cell signaling. *Free Radic. Biol. Med.*, 28: 1370-1378.
- Michell, B.J., Z. Chen, T. Tiganis, D. Stapleton and F. Katsis *et al.*, 2001. Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *J. Biol. Chem.*, 276: 17625-17628.
- Milani, E., S. Nikfar, K. Khorasani, M.J. Zamani and M. Abdollahi, 2005. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. *Comparative Biochem. Physiol. Part C Toxicol. Pharmacol.*, 140: 251-255.
- Mohseni-Salehi-Monfared, S.S., B. Larijani and M. Abdollahi, 2009. Islet transplantation and antioxidant management: A systematic review. *World J. Gastroenterol.*, 15: 1153-1161.
- Momtaz, S. and M. Abdollahi, 2010. An update on pharmacology of *Satureja* species; From antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. *Int. J. Pharmacol.*, 6: 346-353.
- Motterlini, R., R. Foresti, M. Intaglietta and R.M. Winslow, 1996. NO-mediated activation of heme oxygenase: Endogenous cytoprotection against oxidative stress to endothelium. *Am. J. Physiol.*, 270: 107-114.
- Onozato, M.L., A. Tojo, A. Goto and T. Fujita, 2003. Effect of combination therapy with dipyridamole and quinapril in diabetic nephropathy. *Diabetes Res. Clin. Pract.*, 59: 83-92.
- Polte, T. and H. Schroder, 1998. Cyclic AMP mediates endothelial protection by nitric oxide. *Biochem. Biophys. Res. Commun.*, 251: 460-465.
- Pourkhalili, N., S. Pournourmohammadi, F. Rahimi, S. Vosough-Ghanbari, M. Baeeri, S.N. Ostad and M. Abdollahi, 2009. Comparative effects of calcium channel blockers, autonomic nervous system blockers and free radical scavengers on diazinon-induced hyposecretion of insulin from isolated islets of Langerhans in rats. *Arh. Hig. Rada. Toksikol.*, 60: 157-164.
- Radfar, M., B. Larijani, M. Hadjibabaie, B. Rajabipour, A. Mojtahedi and M. Abdollahi, 2005. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients: A randomized, double-blind placebo-controlled clinical trial. *Biomed. Pharmacother.*, 59: 302-306.
- Rahimi, R., S. Nikfar, B. Larijani and M. Abdollahi, 2005. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed. Pharmacother.*, 59: 365-373.
- Rahimi, R., S. Ghiasi, H. Azimi, S. Fakhari and M. Abdollahi, 2010. A review of the herbal phosphodiesterase inhibitors: Future perspective of new drugs. *Cytokine*, 49: 123-129.

- Ranjbar, A., M.H. Ghahremani, M. Sharifzadeh, A. Golestani, M. Ghazi-Khansari, M. Baeeri and M. Abdollahi, 2010. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. *Hum. Exp. Toxicol.*, 29: 851-864.
- Salari-Sharif, P. and M. Abdollahi, 2010. Phosphodiesterase 4 inhibitors in inflammatory bowel disease: A comprehensive review. *Curr. Pharm. Des.*, 16: 3661-3667.
- Wink, D.A., I. Hanbauer, M.C. Krishna, W. De Graff, J. Gamson and J.B. Mitchel, 1993. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc. Natl. Acad. Sci. USA.*, 90: 9813-9817.