Original Article
Toxicology International, *May-Aug 2016 / Vol-23 / Issue-2, 153-158*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i2/146694

Modulation of Adriamycin-induced Hepatotoxicity and Genotoxicity by Selective Inhibition of Phosphodiesterase-5 with Sildenafil in Wistar Rats

Anne A. Adeyanju[1*], Adedeji D. Ogunyemi[1], Oluwaseun Owolabi[1], Ololade Adesanya[1], Temilade Omideyi[1], Oluwasanmi O. Aina[2] and Martins Ekor[3]

[1]Department of Biochemistry, Bowen University, Iwo, Osun State, Nigeria;
[2]Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria;
[3]Department of Pharmacology, School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana.
* bukenzo987@yahoo.com

Abstract

This study investigated the effect of selective inhibition of phosphodiesterase-5 on hepatotoxicity and genotoxicity induced by adriamycin in rats. Thirty male Wistar rats (150 – 250 g) were randomly assigned into six groups of 5 rats/group. Negative, positive and sildenafil controls received physiological saline (10 ml/kg, *p.o.*), adriamycin (20 mg/kg, *i.p.*) and sildenafil (20 mg/kg, *p.o.*) respectively. Three separate groups were pretreated with sildenafil (5, 10 and 20 mg/kg respectively) prior to adriamycin injection. Adriamycin increased activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP). This was associated with degeneration and severe central venous congestion in hepatic cells and marked micronuclei formation in erythrocytes. Sildenafil (5 mg/kg) reduced AST and ALP activities by 18.7 % ($p<0.001$) and 14.1 % ($p<0.01$) respectively in the adriamycin-treated rats without any significant change in ALT and GGT activities even at 10 mg/kg. Although, sildenafil (20 mg/kg) raised GGT activity by 77.4% and 51.6% in normal and adriamycin-treated rats respectively, these effects were not significant when compared with control. Similarly, total protein and albumin did not change significantly across the various treatment groups. However, sildenafil significantly ($p<0.05$) increased glutathione levels at all doses and significantly reduced micronuclei formation by 65.5% and ameliorated morphological damage associated with adriamycin toxicity. Our data suggest that low doses of the phosphodiesterase-5 inhibitor, sildenafil, may protect against adriamycin-induced hepatotoxicity and genotoxicity.

Keywords: hepatotoxicity, genotoxicity, adriamycin, phosphodiesterase-5, sildenafil

Received: 25-03-2016 **Revised:** 27-05-2016 **Accepted:** 01-07-2016

1. Introduction

Adriamycin is widely known to be used for the treatment of various type of cancers such as Hodgkin's disease, non-Hodgkin lymphoma, acute leukemia, soft tissue and primary bone sarcomas, breast, ovarian, lung, uterine and cervical cancers [1]. Hepatotoxicity and genotoxicity have been documented in a variety of animal models to limit the clinical use of adriamycin [2,3]. Common mechanisms by which adriamycin induces hepatotoxicity and genotoxicity have been found to be through free radical generation and NO/cGMP pathway.

Sildenafil, a phosphodiesterase (PDE)-5-inhibitor, has demonstrated potentials in the prevention of drug-associated liver toxicity [4] and ROS-induced DNA damage [5] through the enhancement of the NO/cGMP pathway. Dysregulation of nitric oxide-cyclic guanosine monophosphate (NO-cGMP) system has been implicated in liver cirrhosis [6-8]. Guanylate cyclase activation by NO results in the formation of cGMP, which regulates the tonus of stellate cells and sinusoids [9,10]. However, PDE-5 terminates this action by converting cGMP to inactive 5′ - GMP [11,12]. Sequel to the association of PDE-5 with hepatotoxicity, it is worthy of note to explore intervention

* *Author for correspondence*

of phosphodiesterase inhibitors especially the type 5 in drug-induced genotoxicity and hepatotoxicity. In this study, adriamycin was used as the toxicant and the effect of sildenafil at varied doses was investigated.

2. Materials and Methods

2.1 Chemicals and drugs

Adriamycin (doxorubicin hydrochloride) was obtained from Korea United Pharm. Inc. Co. Ltd. (South Korea), sildenafil citrate was obtained from Zurius Lifesciences Pvt. Ltd. (India). Ellman's reagent (5'5'-dithiobis-2-nitro benzoic acid), reduced glutathione (GSH), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Assay kits for determination of aspartate aminotransaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were obtained from Randox Laboratories (Crumlin, U.K.). Other reagents and chemicals used were of analytical grade.

2.2 Animals

Male albino rats of the Wistar strain weighing 150 – 250 g were purchased from a commercial private colony in Ibadan, Nigeria. They were housed in cages under standard laboratory conditions and maintained at ambient temperature and humidity with a 12 h light/12 h dark schedule and fed with commercially available rat pelleted feed and water *ad libitum* during the acclimatization and experimental periods. This study was carried out in compliance with standard guidelines for the Care and Use of Laboratory Animals [13].

2.3 Experimental design and necropsy

Rats were divided into six experimental groups of five rats per group. Rats in group I (negative control) received physiological saline (10 mL/kg, p.o.) while group II (positive control) was treated with adriamycin (20 mg/kg, i.p.). Rats in groups III – V were pretreated with sildenafil (5, 10 and 20 mg/kg/day, p.o.) 1 hour before adriamycin administration and group VI received sildenafil (20 mg/kg/day, p.o.) only. All treatments were given for 7 days and rats were sacrificed by cervical dislocation 24 hours after the last treatment. Blood samples were collected by ocular puncture into plain bottles prior to animal sacrifice and centrifuged at 3000 g at room temperature for 3 minutes to separate serum.

2.4 Micronuclei assay and histopathology

Immediately after euthanasia, bone marrow was flushed from both femurs of each rat using fetal calf serum and spread onto slides. Slides were coded and then air-dried, fixed with methanol and stained with maygrunward stain. Bone marrow cells were then examined microscopically and scored per animal for frequency of micronucleated cells in each of 5 animals per dose group. Similarly, a section of the liver was excised, fixed in formalin and processed for histopathology.

2.5 Biochemical assessment

Liver function was determined by measuring serum activities of AST, ALT, ALP and GGT. The activities of AST and ALT were determined following the principle described by Reitman and Frankel [14]. ALP and GGT activities were determined according to the method of Belfield and Goldberg [15] and Szasz [16] respectively. Total protein (TP) and albumin (ALB) concentrations were measured according to the principle based on Biuret [17] and bromocresol green reactions [18] respectively. The biomarkers of oxidative stress, GSH and lipid peroxidation (estimated by the thiobarbituric acid reactive substance method and expressed in terms of malondialdehyde formed per milligram of protein) were measured according to the method described by Beutler *et al.* [19] and Varshney and Kale [20] respectively.

3. Statistical analysis

Data were expressed as mean ± standard error of mean. Analysis was by one-way analysis of variance using Statistical Package for Social Sciences software for Windows version 17 (SPSS Inc., Redmond, WA, USA) and graph pad prism 4. Post hoc testing for intergroup comparisons was performed using the least significant difference. $P< 0.05$ was considered significant.

4. Results

4.1 Assessment of liver function

4.1.1 AST and ALT

The effect of various treatments on AST and ALT activities

(marker enzymes of liver function) is shown in Figure 1. Adriamycin administered at 20 mg/kg significantly increased AST activity by 16.7% (P<0.01) and ALT activity by 8.1% (P>0.05) when compared with control. Sildenafil at 5mg/kg significantly prevented this increase in AST and ALT activities by 18.7 % and 14.1 % (P<0.001, P<0.01), respectively in the ADR-treated rats. Sildenafil at 10mg/kg reduced both AST and ALT levels by 8.1% and 6.9% respectively (P>0.05) while 20mg/kg of sildenafil reduced AST and ALT levels by 10.6% and 3.1% (P>0.05), respectively, when compared with the group treated with ADR. Sildenafil administered alone at 20mg/kg produced no alteration on the AST activity while non-significant decrease of 6.8% was produced in ALT when compared with control group.

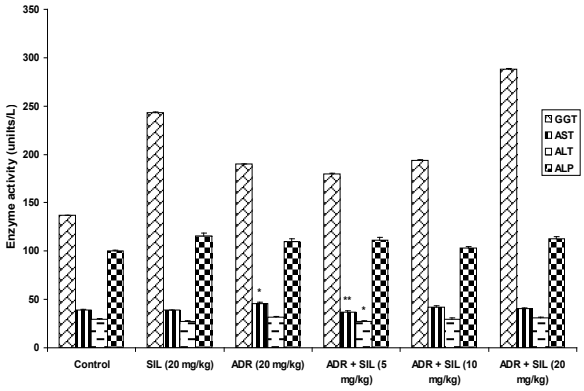

Figure 1. Effect of sildenafil on marker enzymes of liver function in control and adriamycin-treated rats. *p<0.05 when compared with control; **p<0.05 when compared with ADR-treated rats. SIL: Sildenafil, ADR: Adriamycin.

4.1.2 ALP and GGT

Adriamycin increased ALP activity by 10.0% though not statistically significant (P>0.05) when compared with control (saline) group. Treatment with the various doses of sildenafil produced no significant changes in the ALP activity between the groups when compared with ADR-treated group. Treatment with the highest dose of sildenafil (20mg/kg) significantly raised ALP activity by 16.0% when compared with control group. GGT activity was increased by 38.7% (P>0.05) in the ADR-treated group when compared with the control. Treatment with sildenafil (5mg/kg) resulted in a slight decrease (5.3%) in GGT activity while at 10 and 20mg/kg, sildenafil produced 2.1% and 51.6% increase, respectively, when compared with ADR-treated group. Highest dose of sildenafil when administered alone increased GGT activity by 77.4% (P>0.05) when compared with saline-treated group [Figure 2].

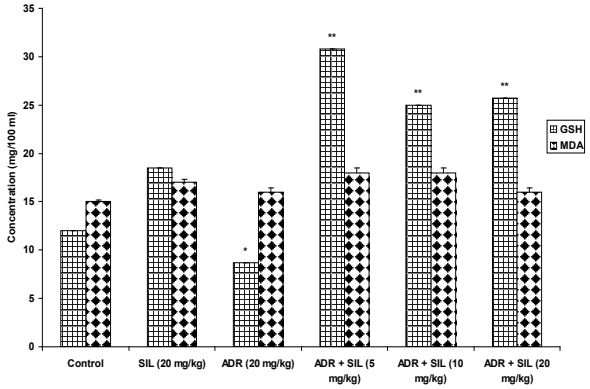

Figure 2. Effect of sildenafil on reduced glutathione and lipid peroxidation in control and adriamycin-treated rats. *p<0.05 when compared with control; **p<0.05 when compared with ADR-treated rats. SIL: Sildenafil, ADR: Adriamycin.

4.1.3 TP and ALB

In this study, administration of single dose of adriamycin (20mg/kg) and treatment with various doses of sildenafil produced no significant alterations on the concentrations of total protein. There was slight decrease of 4.9% (P>0.05) in albumin concentration following adriamycin administration when compared with the control group while sildenafil treatment at various doses had no considerable effect on albumin concentration when compared with ADR-treated group [Figure 3].

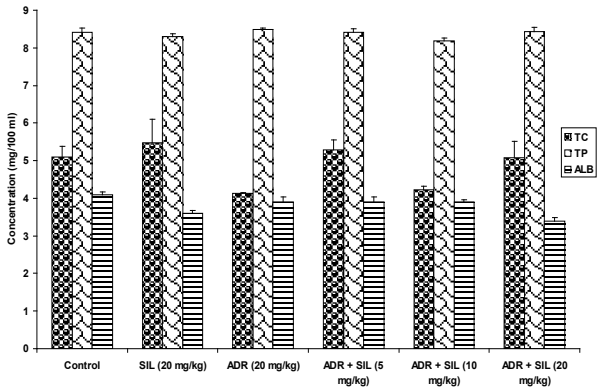

Figure 3. Albumin, total protein and cholesterol levels in control and sildenafil-treated adriamycin-induced nephrotoxic rats. SIL: Sildenafil, ADR: Adriamycin, TC: Total cholesterol, TP: Total protein, ALB: Albumin.

4.2 Markers of oxidative stress

The effect of sildenafil on ADR-induced changes in biomarkers of oxidative stress is shown in Figure 4. The hepatic GSH level was significantly depleted by 27.5% (P<0.05) while hepatic LPO was modestly increased by 6.7% (P>0.05) in the ADR-treated group compared to the control group. ADR-induced decrease in hepatic GSH was significantly elevated by 254.0%, 187.4% and 195.4% at 5, 10 and 20mg/kg of sildenafil, respectively. Sildenafil treatment at 5mg/kg and 10mg/kg both showed slight increase in LPO (11.1%) though not significant, while at 20mg/kg, there was no alteration in LPO when compared with group that received adriamycin alone.

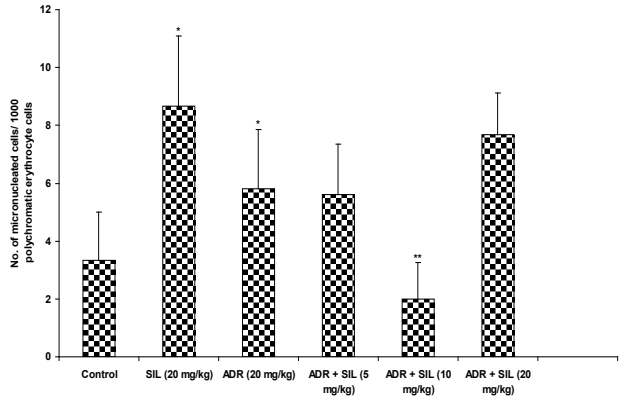

Figure 4. Effect of sildenafil on adriamycin-induced genotoxicity in rats. Values are expressed as mean +standard error of mean. *p<0.001 and **p<0.001 when compared with control and ADR-treated rats respectively. SIL: Sildenafil, ADR: Adriamycin.

4.3 Histopathology

Representative photomicrographs of the liver histology of rats in the various treatment groups are shown in Figure 5. ADR hepatic toxicity was characterized by degeneration and severe central venous congestion of the hepatic cells. ADR-intoxicated rats treated with sildenafil (5mg/kg) showed no visible lesions while those treated with sildenafil (10 mg/kg) exhibited mild portal congestion, with very mild periportal cellular infiltration. Those rats that received sildenafil alone at 20 mg/kg showed periportal cellular infiltration by mononuclear cells

4.4 Assessment of genotoxicity (micronuclei assay)

The effect of sildenafil on adriamycin-induced genotoxicity is shown in Figures 4 and 6. The data presented showed that animals treated with adriamycin (20mg/kg) exhibited a significant (P<0.001) and high frequency of micronucleated polychromatic erythrocytes in bone marrow cells as compared with the control group. There was a slight, non-significant decrease in the number of micronucleated cells formed in the rats treated with sildenafil at a dose of 5mg/kg as compared with the adriamycin-treated group. However, at 10mg/kg of sildenafil, the decrease in the occurrence of micronucleated cells was high and significant when compared with group that received single dose of adriamycin (P<0.001). The treatment of rats with the highest dose (20mg/kg) of sildenafil led to an increase though not significant, in the number of micronucleated cells as compared with adriamycin-treated group. Rats treated with sildenafil only (20mg/kg) exhibited a significant high frequency of micronucleated cells as compared with the control.

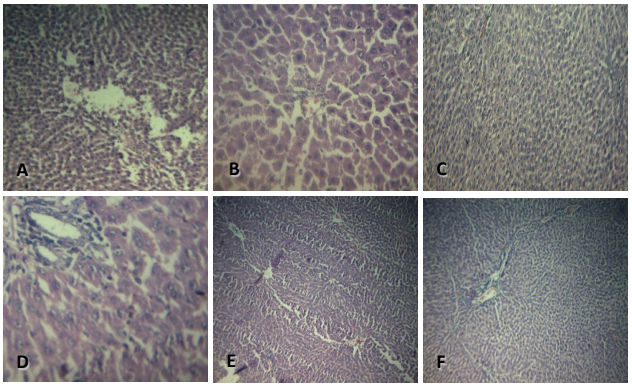

Figure 5. Liver section (×400) of rat treated with [A] normal saline (control, 10 mL/kg) without visible lesion; [B] ADR (20 mg/kg) showing degeneration and severe central venous congestion; [C] ADR + SIL (5 mg/kg) showing no visible lesions; [D] ADR + SIL (10 mg/kg) showing mild portal congestion, with very mild periportal cellular infiltration; [E] ADR + SIL (20 mg/kg) showing no lesions; [F] sildenafil (20 mg/kg) showing periportal cellular infiltration by mononuclear cells.

5. Discussion

Previous studies have suggested a link between PDE inhibition and amelioration or protection against oxidative stress in both experimental models and human subjects. Milani *et al.* [21] reported the inhibitory effect of phosphodiesterase inhibitors against diabetes-induced oxidative stress in rats. In a similar study, Radfar *et al.* [22] observed a reduction in the level of oxidative stress in type II diabetic patients following treatment with pentoxifylline

in a randomized double-blind placebo-controlled clinical trial. Abdollahi et al. [23] also associated increase in intracellular levels of cGMP through PDE inhibition with a reduction in oxidative stress induced by cadmium in experimental model. Also, the ability of sildenafil to raise reduced glutathione level and inhibit lipid peroxidation during hepatic injury induced by paracetamol in rats has been demonstrated in a recent study [4]. These observations seem to support the possible link between PDE-5 inhibition and chemoprevention via antioxidant mechanisms.

Results from the present study suggest protective effect of sildenafil in adriamycin-induced hepatotoxicity rat model. This was evident from the suppression of the increase in serum levels of AST, ALT, ALP and GGT especially at 5 mg/kg dose of sildenafil. The highest dose of sildenafil (20 mg/kg) when administered alone raised the activities of ALP and GGT. Serum transaminases are known markers of liver damage. Mohan *et al.* [24] linked alteration of membrane permeability and transport function to injured hepatocytes. This was described as causing leakage of enzymes from the cells. Decrease in synthetic function of the liver was also demonstrated by decrease in serum ALB concentrations although sildenafil treatment had no considerable effect in preventing this reduction. This protective role played by sildenafil suggests involvement of PDE-5 activity in adriamycin-mediated hepatotoxicity and that sildenafil when administered at a minimal dose could be a useful pharmacological agent in the prevention of liver injury associated with this drug. Subjection of different PDE isozymes to manipulations by various pharmacological inhibitors has been shown to provide great therapeutic benefit in both immune-mediated and inflammatory conditions associated with liver pathology [25]. Since inhibition of PDE-5 by sildenafil contributes to increase in hepatic cGMP and NO levels, we could propose that NO-cGMP signaling pathway is an important mechanism that underlies the protective action of sildenafil against hepatotoxicity induced by adriamycin from our results.

Furthermore, sildenafil exhibited antioxidant potential in this study. The depleted hepatic GSH following adriamycin administration was significantly elevated by sildenafil at all doses used in this study. This hepatoprotection afforded by sildenafil could be related to its antioxidant properties as sildenafil has been shown to have a protective effect independent of the NO/cGMP pathway [26]. This antioxidant property of sildenafil could constitute a mechanism for the decrease of oxidative stress observed. Also, free radical neutralization has been attributed to sildenafil [27]. The slight increase in malondialdehyde level observed with sildenafil treatment at 5 and 10 mg/kg could be an adaptive response to lipid peroxidation induced by adriamycin. Histopathological examination of liver tissues showed that sildenafil at 5 mg/kg and 10 mg/kg doses prevented disruption of the normal architecture, which was distorted following adriamycin administration. Normal liver architecture was observed in control animals whereas degeneration and severe central venous congestion of the hepatic cells were observed in adriamycin-treated group.

The clastogenic effect produced by adriamycin was significantly reduced by pretreatment with sildenafil at 10 mg/kg for 7 days. The antioxidant properties of sildenafil [21] could constitute a key mechanism for the decrease in micronuclei formation observed as exposure of cells to oxidative stress could result in DNA damage. This antioxidant activity could be mediated through inhibition of NADPH oxidase activity which subsequently increases antioxidant enzyme activities and intracellular levels of cGMP [28]. In addition, the inhibition of PDE5 by sildenafil is also a mechanism that results in decrease in genotoxicity [5]. This further lends credence to the involvement of NO/cGMP and phosphodiesterase 5 in genotoxicity. However, sildenafil when administered at 20 mg/kg increased the frequency of micronuclei formation in polychromatic erythrocytes when compared to control. In conclusion, results from the present study suggest that sildenafil citrate at low doses through inhibition of PDE-5 may provide some chemopreventive benefits in adriamycin-induced hepatotoxicity and genotoxicity.

6. Conflict of Interest

None declared.

7. References

1. Priestman T. Cancer chemotherapy in clinical practice. Springer-Verlag London, UK press 2008.
2. Kolarovic J, Popovic M, Mikov M, Mitic R, Gvozdenovic LJ. Protective effects of celery juice in treatments with doxorubicin. Molecules 2009; 14: 1627-1638.
3. Kolarovic J, Popovic M, Zlinská J, Trivic S, Vojnovic M. Antioxidant activities of celery and parsley juices in rats treated with doxorubicin. Molecules 2010; 15: 6193-6204.

4. Ekor M, Odewabi AO, Kale OE, Bamidele TO, Adesanoye OA and Farombi EO. Modulation of paracetamol-induced hepatotoxicity by phosphodiesterase isozyme inhibition in rats: a preliminary study. J Basic Clin Physiol Pharmacol 2013, 24(1): 73 – 79.
5. Rodrigues BP, Campagnaro BP, Balarini CM, Pereira TMC, Meyrelles SS, Vasquez EC. Sildenafil ameliorates biomarkers of genotoxicity in an experimental model of spontaneous atherosclerosis. Lipids in Health and Disease 2013; 12:128
6. Iwakiri Y, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. Hepatology 2006; 43:S121– 31.
7. Iwakiri Y, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. J Hepatol 2007; 46:927 – 34.
8. Malyshev E, Tazi KA, Moreau R, Lebrec D. Discrepant effects of inducible nitric oxide synthase modulation on systemic and splanchnic endothelial nitric oxide synthase activity and expression in cirrhotic rats. J Gastroenterol Hepatol 2007; 22:2195– 201.
9. Sessa WC. eNOS at a glance. J Cell Sci 2004; 117:2427 – 9.
10. Bellamy TC, Wood J, Garthwaite J. On the activation of soluble guanylyl cyclase by nitric oxide. Proc Natl Acad Sci USA 2002; 99:507 – 10.
11. Matsumoto T, Kobayashi T, Kamata K. Phosphodiesterases in the vascular system. J Smooth Muscle Res 2003; 39:67 – 86.
12. Prisant LM. Phosphodiesterase-5 inhibitors and their hemodynamic effects. Curr Hypertens Rep 2006; 8:345 – 51.
13. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals, 8th ed. Washington, DC: National Academy of Sciences, National Academies Press, 2011.
14. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. Am J Clin Pathol 1957; 28:56 – 63.
15. Belfield A, Goldberg D. Colorimetric determination of alkaline phosphatase activity. Enzyme 1971; 12: 561-566.
16. Szasz G. Clin. Chem 1969; 22: 124-136.
17. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the Biuret reaction. J Biol Chem 1949; 177:751.
18. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green reaction. Clin Chem 1971; 22:616 – 22.
19. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882 – 8.
20. Varshney R, Kale RK. Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol 1990; 58:733 – 43.
21. Milani E, Nikfar S, Khorasani R, Zamani MJ, Abdollahi M. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comp Biochem Physiol 2005. 140C: 251 – 255.
22. Radfar M, Larijani B, Hadjibabaie M, Rajabipour B, Mojtahedi A and Abdollahi M. Effect 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 2005; 59: 302 – 306.
23. Abdollahi M, Bahreini-Moghadam A, Emani B, Fooladian F, Zafari K. Increasing intracellular cAMP and cGMP inhibits cadmium-induced oxidative stress in rat submandibular saliva. Comp Biochem Physiol C. Toxicol Pharmacol 2003; 135: 331 – 336.
24. Mohan M, Kamble S, Satyanarayana J, Nageshwar M, Redddy N. Protective effect of Solanum torvum on Doxorubicin- induced hepatotoxicity in rats. Int J Drug Dev Res 2011; 3:131–8.
25. Davies NA, Hodges SJ, Pitsillides AA, Mookerjee RP, Jalan R, Mehdizadeh S. Hepatic guanylate cyclase activity is decreased in a model of cirrhosis: a quantitative cytochemistry study. FEBS Lett 2006; 580:2123 – 8.
26. Elrod JW, Greer JJM, Lefer DJ. "Sildenafil-mediated acute cardioprotection is independent of the NO/cGMP pathway," American Journal of Physiology—Heart and Circulatory Physiology 2007; 292 (1): H342–H347.
27. Beckman JS, Koppenol WH. "Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly," The American Journal of Physiology—Cell Physiology 1996; 271 (5) C1424–C1437.
28. Ebrahimi F, Shafaroodi H, Asadi S, Nezami BG, Ghasemi M, Rahimpour S, Rashemi M, Doostar Y, Dehpour AR. Sildenafil decreased cardiac cell apoptosis in diabetic mice: reduction of oxidative stress as a possible mechanism. Can J Physiol Pharmacol 2009; 87: 556-564.