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Lipid Peroxidation and Antioxidant Enzymes Assessment in Alloxan Monohydrate Induced Hyperglycaemic Male Wistar Rats Following Oral Administration of Theophylline

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DCE, YT and AM designed the study. Authors EAA, CNC and MO managed the experimental work. Authors RAA, MY AEO managed the literature review. Author ECU performed the statistical analysis. Author NSE wrote the protocol and author HDM wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Lipids are referred to as one of the primary targets of reactive oxygen species (ROS). The main sources of oxidative stress in diabetes mellitus comprise various enzymatic pathways, non-enzymatic pathways and mitochondrial pathways. This study was designed to evaluate the effect of theophylline and treatment on lipid peroxidation (serum malondialdehyde concentration) and some

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antioxidant enzymes (superoxide dismutase, glutathione peroxidase and Catalase) in alloxan-induced hyperglycaemic male Wistar rats.
Study Design: Thirty apparently healthy male wistar rats weighing between 160-180 g were grouped into five of six animals each (n=6) and treated for a period of fourteen days (14) after induction of hyperglycaemia using alloxan monohydrate.
Methodology: Group 1: (Normoglycaemic) Group 2: Diabetic control (DC), Group 3: Glibenclamide, 5mg/kg, Groups 4 and 5; theophylline 5 mg/kg and 10mg/kg respectively. At the end of the fourteen (14) days, rats were anaesthetized using ketamine and diazepam at 75 and 25 (mg/kg). Blood samples were taken from all treated groups for evaluation of serum MDA, SOD, GPx and CAT level.
Results: The result on serum MDA concentration was significantly decreased ($P< 0.05$) in glibenclamide treated group compared to diabetic control; 1.14±0.03 vs 1.32±0.06. Although a decrease was observed in the theophylline treated groups, the difference was however not statistically significant compared to diabetic control. There was also a significant increase ($P< 0.05$) in serum SOD and CAT level in the glibenclamide and theophylline treated group (5 mg/kg) compared to DC; 2.02±0.04 and 1.92±0.24 vs 0.92±0.05 respectively for serum SOD and 53.20±0.58 and 52.80±1.07 vs 46.00±0.84 respectively for CAT. However, serum GPx increased significantly ($P< 0.05$) only in the theophylline treated groups compared to DC.
Conclusion: In conclusion, Theophylline and Glibenclamide decreases lipid peroxidation while increasing serum antioxidant levels in alloxan induced hyperglycaemic male Wistar rats after 14 days oral administration.

Keywords: Lipid peroxidation; antioxidant enzymes; theophylline; glibenclamide; hyperglycaemia.

1. INTRODUCTION

Diabetes mellitus is implicated in reduced life expectancy and diminished quality of life as it is the commonest metabolic disorder with typical complications the likes of retinopathy, nephropathy and neuropathy. Despite efforts aimed at managing this metabolic complication, it however still remains a globally increasing burden and public health problem [1,2]. Theophylline mostly found in cocoa and coffee belongs to the xanthine family [3,4]. It has also been reported to possess antioxidant activity. As a phosphodiesterase enzyme inhibitor, it has evidences of a possible effect on reverting induction of oxidative stress [5,6,7]. Glibenclamide as one of the most frequently prescribed oral hypoglycaemic agents is known to stimulate insulin secretion with a corresponding reduction in hepatic glucose synthesis precipitating reduced blood glucose level [8,9]. However, the use of glibenclamide is limited due to prolonged hypoglycaemia, high secondary failure rate and other adverse events [10,11]. Oxidative stress is implicated in cellular injury caused by hyperglycaemia from an increased free radical formation. Weak defence system of the body becomes unable to counteract the ROS generation and as a result, the condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress [12,13]. Therefore this study was aimed at evaluating lipid peroxidation and antioxidant enzymes status in alloxan monohydrate induced hyperglycaemic male Wistar rats treated with theophylline.

2. MATERIALS AND METHODS

2.1 Experimental Site

This study was carried out in the Physiology Laboratory of the Department of Human Physiology, Faculty of Medical Sciences, College of Health Sciences, Ahmadu Bello University. Zaria is located between latitudes 11^0 and 3´ N, and between 7^0 and 42´ E, at an altitude of 670 m above the sea level and 664 km away from the sea, in the Northern Guinea Savanna zone [14]

2.2 Chemicals and Reagents

Chemicals which were used include; alloxan monohydrate, formalin, normal saline, they were of analytical grade and purchased from Sigma Aldrich chemical Company (St. Louis U.S.A.). Glibenclamide tablets, 5mg (Clamide, Hovid Pharmaceuticals Ltd, Malaysia) NAFDAC Reg. no. 04-4015) and Theophylline, 100mg (Theolair, Mancare Pharmaceuticals ltd, India NAFDAC Reg. no. A4-9071) were purchased from the Pharmacy section of Ahmadu Bello Teaching Hospital, Zaria, Kaduna state Nigeria.

Table 1. Showing animal grouping and treatment. Route of drug administration: Oral gavage. Animals were grouped into five (5) groups consisting of six (6) animals and treated as shown below

Groups	No. of Rats (n)	Treatment (mg/kg b.w)
I	6	Distilled water (1 ml/kg) Normoglycaemic
II	6	Diabetic +Distilled water (1 ml/kg)
III	6	Diabetic +Glibenclamide (5 mg/kg)
IV	6	Diabetic +Theophylline (5 mg/kg)
V	6	Diabetic +Theophylline (10 mg/kg)

2.3 Experimental Design

Thirty (30) adult male Wistar rats weighing between 160-180 g were purchased from theAnimal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria. The rats were housed in the Animal house of the Department of Human Physiology under laboratory conditions with access to food and water *ad libitum.*

2.4 Induction and Confirmation of Hyperglycaemia

Diabetes (Hyperglycaemia) was induced by single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution into the Wistar rats of groups III, IV and V after 16-18hrs fasting of the Wistar rats [15]. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin secretion; the induced Wistar rats were treated with 20% glucose solution orally within 6 hrs. The Wistar rats were kept for the next 24 hrs on 5% glucose solution in their cages to prevent hypoglycaemia [16]. After 72 hours of alloxan treatment, blood was collected from tail vein of each rat and assessment of blood glucose level was carried out using a digital Glucometer. Rats having fasting blood glucose level 180 mg/dl and above were considered diabetic [17].

2.5 Animal Grouping and Treatment

2.5.1 Estimation of serum Malondialdehyde (MDA) concentration

Lipid peroxidation in this study was estimated based on the spectrophotometric measurement of the optical density of the colour of MDA in a thiobarbituric acid environment at 532 nm. Concentration of the thiobarbituric acid reactive substance was calculated by the absorbance coefficient of malondialdehyde thiobarbituric acid complex as described by Placer, (1966).

2.5.2 Estimation of Superoxide Dismutase level (SOD) Glutathione peroxidase (GPx) and Catalase (CAT)

The above mentioned antioxidant enzymes were assayed using respective assay kits from North West Life Science Specialties according to the manufacturer's manual. The enzyme specific monoclonal antibodies were pre-coated onto microplates. Standards and samples were pipetted into the wells and gently mixed, then covered with closure plate membranes and incubated for 30 mins at 37ºC. These were allowed so that the respective enzyme present would be bound by the immobilized antibody. After incubation, the wells were washed with a prepared 'wash solution'. The microplates were inverted and thereafter blot-dried by hitting onto absorbent papers to remove the moisture. An enzyme-linked monoclonal antibody specific for the respective enzymes was added to the wells, sealed and, again incubated for 30 mins at 37ºC. It was washed to remove any unbound antibody-enzyme reagent, by adding substrate solutions (tetramethylbenzidine and hydrogen peroxide) to the wells and a colour was developed in proportion to the amount of enzyme bound in the initial step. This was covered and incubated for 15 mins at 37ºC. Their Colours which was developed was stopped by addition of 'stop solution' and the intensity of the colours for each respective enzyme was then measured with a microplate reader.

2.6 Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. Statistical analysis was carried out using version 20 of SPSS with the aid of one way analysis of variance (ANOVA) and Tukey's post-hoc test. Values with ($P< .05$) were considered significant.

3. RESULTS

3.1 Effect of Fourteen (14) Days Oral Administration of Theophylline and Glibenclamide on Serum Malondialdehyde (MDA) Level

In Table 2. below, the serum levels of malondialdehyde (MDA), in the theophylline treated groups; 1.30 ± 0.10 µmol/L and 1.30 ± 0.10 µmol/L (5 mg/kg and 10 mg/kg respectively) were not statistically significant when compared to the diabetic control group. However, there was observed a significant decrease in the glibenclamide treated groups.

Table 2. Serum malondialdehyde concentrations in Alloxan induced hyperglycaemic male wistar rats

Treated Groups	MDA(µmol/L)
Normal control (NC)	1.08 ± 0.04
Diabetic Control (DC)	1.32 ± 0.06
Glibenclamide (5 mg/kg)	1.14 ± 0.03[b]
Theophylline (5 mg/kg)	1.30 ± 0.10
Theophylline (10 mg/kg)	1.30 ± 0.10

3.2 Effect of Fourteen (14) Days Oral Administration of Theophylline on Serum Superoxide Dismutase (SOD) Level

In Fig. 1, the serum level of superoxide dismutase (SOD), in the theophylline (5 mg/kg) treated group; 1.92 ± 0.24 IU/L was significantly increased when compared to the diabetic control group (P< 0.05), there was an insignificant increase in the 10 mg/kg theophylline treated group when compared to the diabetic control. In the glibenclamide treated group; 2.02 ± 0.04 IU/L recorded a statistical significant increase when compared with the diabetic control (P<0.05).

3.3 Effect of fourteen (14) days Oral Administration of Theophylline on Serum Catalase (CAT) Level

In Fig 2 showing the serum level of catalase below was significantly increased in the Glibenclamide (5 mg/kg) and theophylline (5 mg/kg) treated groups; (53.20 ± 0.58 IU/L and 52.80 ± 1.07 IU/L) respectively, when compared to the diabetic control group (P< 0.05).

3.4 Effect of Fourteen (14) days Oral Administration of Theophylline on Serum Glutathione Peroxidase (GPx) Level

In Fig. 3, the serum level of GPx was significantly increased in the theophylline (5 mg/kg and 10 mg/kg) treated groups; (46.50 ± 1.34 IU/L and 46.50 ± 1.21 IU/L) respectively, when compared to the diabetic control group (P< 0.05).

4. DISCUSSION

Oxidative damage is precipitated from not only increase level of reactive oxygen and nitrogen species but also reduced antioxidant defences [18]. The increase in serum MDA concentration observed in this current study, in the diabetic control would be from the pro peroxidative activity of alloxan monohydrate administration. This action of alloxan is mediated by reactive oxygen species which are formed under such conditions. Formation of superoxide radicals are associated with alloxan treatment which is reduced to dialuric acid and subsequently results in the formation of highly reactive hydroxyl radicals by the Fenton reaction. During these cascades of events, the action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells [19]. The mechanism by which hyperglycaemia leads to lipid peroxidation is the reduction of molecular oxygen and production of oxygen free radicals and alpha-ketoaldehydes which finally breaks down lipid peroxides [20]. The action of theophylline on serum MDA concentration could be attributed to its antioxidant capacity due to its affiliation with coffee. Theophylline mostly found in cocoa and coffee possesses antioxidant activities which have been attributed to their enhancing effect on cellular cyclic nucleotides contributing to protection against hyperglycaemia and oxidative stress in diabetic complications. The activity of theophylline in this study could also have been through direct mopping out of the reactive oxygen or nitrogen species, serving as an impediment to progressive lipid peroxidation. This antioxidant activity is also represented in the result of serum SOD level, which was increased following theophylline administration. This activity could have been due to theophylline pro-activity on endogenous SOD synthesis. Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutase of superoxide anion into hydrogen peroxide and molecular oxygen [21, 22]. In endothelial cells, it has been reported that

hyperglycaemia increases hydrogen peroxide production causing a deficiency in catalase enzyme [23]. This is in concert with the decrease in serum CAT level observed in the diabetic control group. The depletion could also have been from consistent usage in mopping out the ROS and RNS. However, and increase was observed in the glibenclamide and theophylline treated groups which suggest that these substances possess antioxidant activities. This result is also in concert to the result of serum glutathione peroxidase (GPx) which shows an

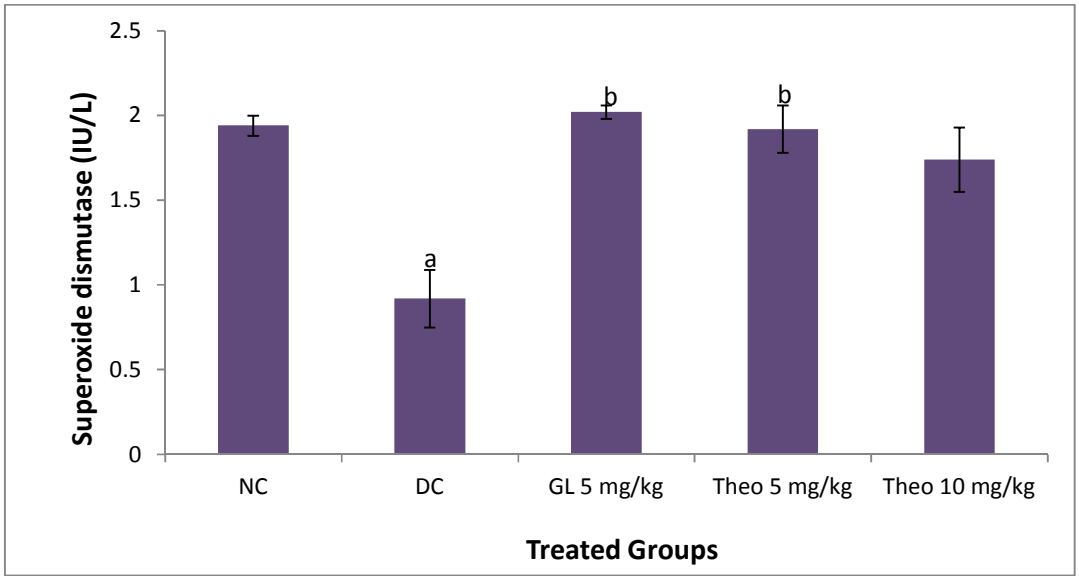

Fig. 1. showing serum SOD level following treatment with glibenclamide and theophylline.
NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant (P< .05) compared to normal control (NC), a= statistically significant compared to NC, b= statistically significant (P< .05) compared to diabetic control DC

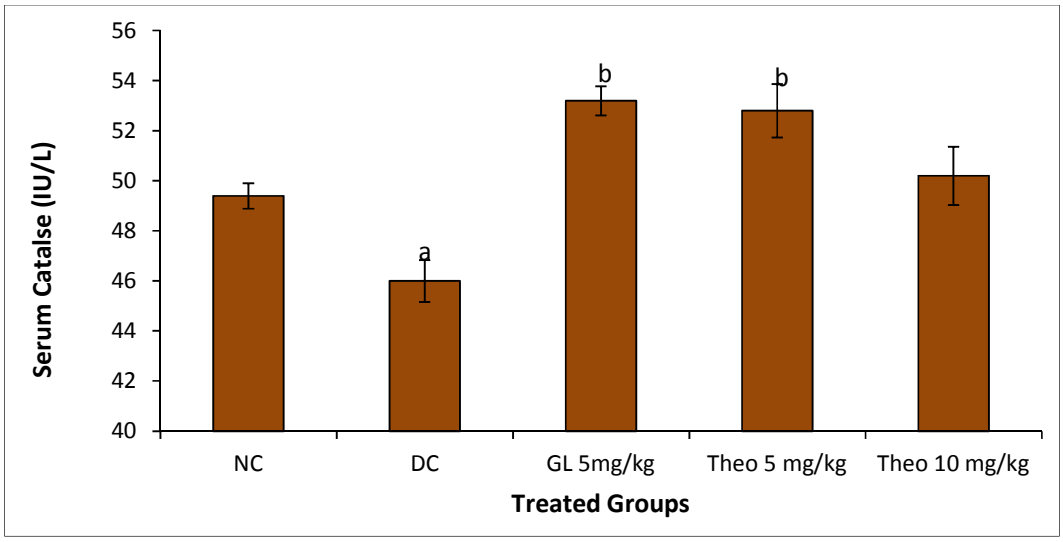

Fig. 2. showing serum SOD level following treatment with glibenclamide and theophylline
NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant (P< .05) compared to normal control (NC), a= statistically significant compared to NC, b= statistically significant (P< .05) compared to diabetic control DC

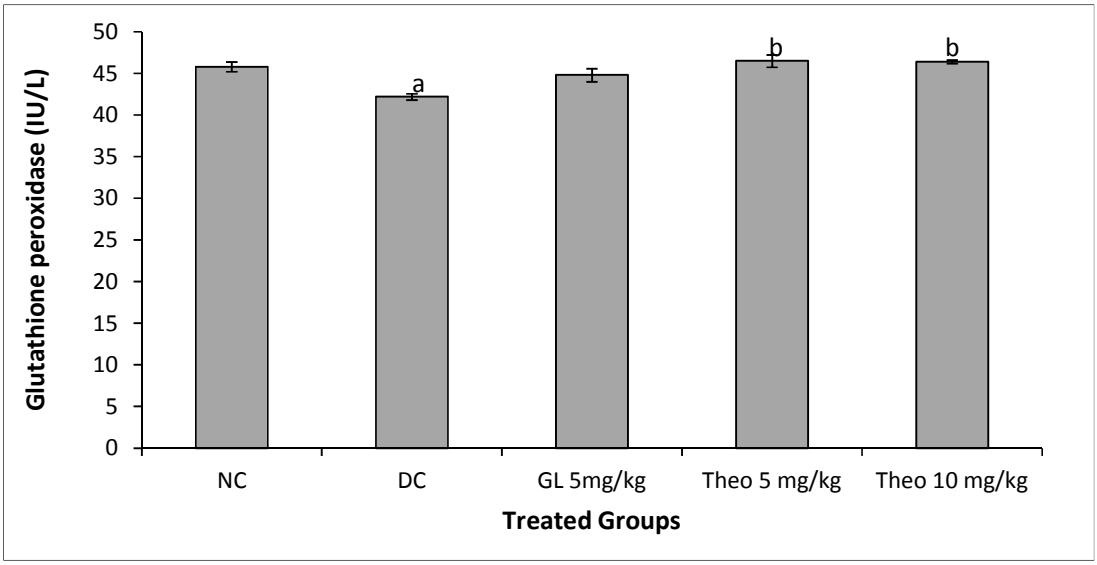

Fig. 3. Showing serum SOD level following treatment with glibenclamide and theophylline.
NC= Normal control, DC= Diabetic control, Theo= theophylline and GL= glibenclamide. Superscripts a= statistically significant (P< .05) compared to normal control (NC), a= statistically significant compared to NC, b= statistically significant (P< .05) compared to diabetic control DC

increase in the serum level of GPx following theophylline and glibenclamide treatment. Glutathione disulfide is recycled back to glutathione by glutathione reductase; using the cofactor NADPH generated by glucose 6-phosphate dehydrogenase [24].

5. CONCLUSION

Theophylline and glibenclamide possess potent antioxidant activity and also decrease lipid peroxidation by decreasing serum malondialdehyde concentration in alloxan monohydrate induced hyperglycaemic male Wistar rats treated for fourteen days.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King, H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27:1047-1053.
2. Shaw J, Sicree R, Zimmet P. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clinical Practice. 2010;87:4-14.
3. Henderson-Smart D, De Paoli A. Methylxanthine treatment for apnoea in preterm infants. Cochrane Database of Systemic Reviews. 2010;(3):140-142.
4. Milani E, Shekouefeh N, Reza K, Mohammed J, Mohammed A. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comparative Biochemistry and physiology Part C: Toxicology & pharmacology. 2002; 140(2):251-255.
5. Abdollahi M, Fooladian F, Emami B, Zafari K, Bahreini-Moghadam A. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. Hum. Exp. Toxicol. Pharmacol. 2003;22(11):587-92.
6. Arkkila P, Koskinen P, Kantola I, Ronnemaa T, Seppanen E, Vilkar J. Diabetic complications are associated with liver enzyme activities in people with type 1 diabetes. Diabetes Res Clin Pract. 2001; 52(2):113-8.
7. Radfar M, Larijani B, Hadjibabaie M, Rajabipour A, Mojtahedi A, Abdollahi M. Effects of pentoxifylline on oxidative stress and levels of EGF and NO inbloodof diabetic type 2 patients: A randomized double-blind placebo controlled clinical trial. Biomed. Pharmacother. 2005;59:302-306.

8. Nathan D, Buse J, Davidson M, Ferrannini E, Holman R, Sherwin R, Zinman B. Medical management of hyperglycaemia in type 2 diabetes mellitus: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia. 2009;52(1):17-30.
9. Rendell M. The role of sulfonylureas in the management of type 2 diabetes. Drugs. 2004;64(12):1339-1358.
10. Harrower D. Comparison of efficacy, secondary failure rate, and complications of sulfonylureas. Journal of Diabetes Complications. 1994;8:201-203.
11. Mukai J, Tada H, Watanabe Y, Miura M, Katsuyama S, Shoji T, Mohri K, Eto M, Suzuki T. Lipids behavior and adverse effects for oral antidiabetic agents in patients with Type 2 diabetes treated with sulfonylureas alone based on systematic review. Yakugakuzasshi. 2007;127(10): 1747-1756.
12. Halliwell B, Chinco S. Lipid peroxidation: Its mechanism, measurement and significance. American Journal of Clinical Nutrition. 1993;57(5):715s-724s.
13. Pandey K, Mishra N, Rizvi S. Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clinical Biochemistry. 2010;43(45):508-511.
14. Marthins MI. Zaria and its regions. Department of Geography Ahmadu Bello University, Zaria. 2006;41.
15. Dhandapani S, Ramasamy SV, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. Pharmacological Research Journal. 2000;46(3):251-255.
16. Milani E, Shekouefeh N, Reza K, Mohammed J, Mohammed A. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comparative Biochemistry and physiology Part C: Toxicology & Pharmacology. 2002; 140(2):251-255.
17. Nikfar S, Khorasani R, Zamani J, Abdollahi, M. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comp Biochem Physiol C Toxicol Pharmacol. 2005;140(2):251-5.
18. Bandedeira S, Fonseca L, Guedes S. Oxidative stress as an underlying contributor in the development ofchronic complication of diabetes mellitus. Int. J Mol Sciences. 2013;14(2):3265-3284.
19. Szkudelski T. The mechanism of alloxan and streptozotocin action on β cells of the rat pancreas. Physiological Research. 2001;50(6):537-546.
20. Abdollahi M, Sima G, Ghias S. A review on the potential benefits of phosphodiesterase enzyme inhibitors in various models of toxicities in animals. Asian Journal of Animal and Veterinary Advances. 2011;7: 210-224.
21. Faraci F, Didion P. Vascular protection: Superoxide dismutase isoforms in the vessel wall. Arteriosclerosis, thrombosis and Vascular Biology. 2004;24(8):1367-1373.
22. Wang X, Tao L, Hai X. Redcox regulating role of insulin: The essence of insulin effect. Molecular and Cellular Endocrinology. 2012;349(2):111-127.
23. Patel H, Chen J, Das K, Kavdia M. Hyperglycaemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. Cardiovascular Dialectology. 2013;12(1):142-146.
24. Bisht S, Sisodia SS. Diabetes, dyslipidemia, antioxidant and status of oxidative stress. International Journal of Research in Ayurveda & Pharmacy. 2010; 1(1):33-42.

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