Protective effects of N-acetylcysteine on myocardial injury induced by acute fluctuations in blood glucose

Wei Zhang¹/², Guanjing Peng¹, Sheng Zhao³, Yan Li⁴, Ping Han⁵, Yongzhi Li *²

¹Department of Endocrinology, Central people's hospital of zhanjiang, zhanjiang 524000, China
²The Fourth Hospital of China Medical University, Shenyang 110000, China
³Hepatobiliary surgery, Central people's hospital of zhanjiang, zhanjiang 524000, China
⁴Department of Endocrinology, Shengjing Hospital of China Medical University, Shenyang 110000, China
⁵Department of Geriatrics, the First Hospital of China Medical University, Shenyang 110000, China

*Corresponding Author: Yongzhi Li, MD, Department of Urology, the Fourth Hospital of China Medical University, Shenyang 110000, China(Tel.: 86-024-62043470. Email: liyongzhi2324@hotmail.com)

Abstract

Background: To investigate the effect of N-acetylcysteine (NAC) on oxidative stress and inflammation induced by acute fluctuations in blood glucose and the potential mechanism.

Methods: Cannulated Wistar rats (n=6/group) were infused intravenously for 48 h with (1) saline (control), (2) 50% glucose intermittently (IHG), (3) 50% glucose continuously (PHG), (4) IHG plus NAC, or (5) PHG plus NAC. Levels of superoxide...
dismutase (SOD), nitric oxide (NO), nuclear factor-κB (NF-κB), inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF) in cardiac tissues were analyzed. We also evaluated apoptosis in the myocardium using a terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay.

**Results:** The IHG group had higher levels of oxidative and inflammatory markers in the myocardium than the PHG and control groups. Co-infusion with NAC prevented the elevation in inflammatory markers and NF-κB activity in the IHG + NAC and PHG + NAC groups. Apoptosis of myocardial cells was observed in the IHG group but was abolished by co-administration of NAC.

**Conclusions:** Acute fluctuations in blood glucose levels induced more severe oxidative stress, inflammation and apoptosis in the myocardium than persistently high blood glucose levels. The antioxidant NAC may prevent apoptosis of myocardial cells caused by fluctuations in blood glucose levels in vivo, most likely because of its antioxidative and anti-inflammatory properties.

**Keywords**
acute blood glucose fluctuation, N-acetyl cysteine, oxidative stress; inflammation; apoptosis

**Background**

Oxidative stress and inflammatory responses have been considered as a major hallmark for the pathogenesis and development of type 2 diabetes mellitus and is acknowledged to play a role in the development of diabetic complications\(^1\)\(^{-3}\). Chronic inflammatory state is indicated by high plasma levels of numerous pro-inflammatory cytokines notably IL-1β, IL-6, CRP and IL-1β-dependent numerous other cytokines and chemokines\(^4\). N-acetyl cysteine (NAC) is an important potent antioxidant and anti-inflammatory molecule\(^5\) that can suppress oxidative stress and decrease production of free radicals\(^6\). In our previous study\(^7\)\(^{-9}\), we reported that acute blood glucose fluctuations induced myocardial apoptosis through oxidative stress and activation of nuclear factor-κB (NF-κB). We now wanted to determine whether NAC
could prevent the apoptosis of myocardial cells and to further investigate the different effects of acute fluctuations in blood glucose levels and persistent high blood glucose levels on the myocardium.

We used an established in vivo model of blood glucose fluctuation that involves intermittent, or persistent, infusion of high concentrations of glucose in Wistar rats. Co-infusion with NAC was designed to investigate the effects of NAC on apoptosis of myocardial cells and to explore potential mechanisms for the effect of fluctuations in blood glucose levels on myocardial tissues in vivo. For the purpose of our study, we want to investigate the effect of N-acetylcysteine (NAC) on oxidative stress and inflammation induced by acute fluctuations in blood glucose and the potential mechanism.

Methods
Animal models and Surgical Procedures

Normal male Wistar rats (280–330 g) were obtained from the Laboratory Animal Center of China Medical University (Shenyang, Liaoning, China). All procedures were approved by the Animal Care and Use Committee of China Medical University before the study began. This study was conducted in strict accordance with the recommendations in the "Guidelines for the Care and Use of Experimental Animals" of the Liaoning Provincial Institute of Laboratory Animal Surveillance under the National Institute of Experimental Animal Monitoring of the People's Republic of China. The experiment was approved by the animal center of China Medical University without specific pathogen animal protection plan and approved by Animal Ethics Committee of China Medical University Shenyang City (License No.: SYXK (Liaoning) 2008-0008). Wistar rats were adapted to the local animal environment (temperature: 24°C to 28°C, humidity: 60%) and no specific pathogenic conditions. Rats were free to drink water and diet.

The rats were maintained on a standard 12:12 h light-dark cycle and were provided with standard chow and water. Briefly, after acclimatization for 5 days, the rats were anesthetized with 10% chloral hydrate (0.35 mL/100g). Indwelling catheters
(PE-50; Cay Adams, Boston, MA, USA), extended with a segment of silastic tubing (length 3 cm, internal diameter 0.02 in; Care Express Products, Inc., New York, USA), were inserted into the internal jugular vein for infusion and into the carotid artery for blood sampling, as described previously\textsuperscript{7-10}. Both catheters were closed at the end with a metal pin. Before the experiments the rats were permitted to recover from the surgery for 3–4 days.

**Experimental design**

The rats were fasted overnight and randomized to five groups, with six rats in each group. The control, IHG and IHG +NAC groups underwent intermittent intravenous infusion for 1 h every 2 hours, totally for 48 h, with saline, 50% glucose or 50% glucose + NAC (0.35 mg.kg\(^{-1}\).min\(^{-1}\)), respectively. In the IHG group, blood glucose levels fluctuated between 5.5 ± 0.5 mmol/L and 20 ± 0.5 mmol/L [7-10]. The PHG group underwent continuous intravenous infusion for 48 h with 50% glucose (PHG), maintaining a persistently high blood glucose level of 20 ± 0.5 mmol/L. The PHG + NAC group underwent continuous intravenous infusion for 48 h with 50% glucose + NAC (0.35 mg.kg\(^{-1}\).min\(^{-1}\)). At the end of the infusions, plasma samples and myocardia were collected. Part of myocardium was fixed with 4% paraformaldehyde solution and the remainder was flash-frozen in liquid nitrogen and stored at −70°C until analysis.

**Laboratory methods**

Glucose concentrations in plasma were measured using a Beckman Glucose Analyzer II (Beckman, Fullerton, CA, USA). The activities of superoxide dismutase (SOD) and nitric oxide (NO) in myocardium were determined using colorimetric kits. Levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α in myocardium were detected using enzyme linked immunosorbent assay (ELISA) kits. All of the assay kits were purchased from Jiancheng Institute of Biotechnology (Nanjing, China).
Immunohistochemistry for expression of NF-κB and inducible nitric oxide synthase (iNOS) in myocardium

Briefly, myocardium was fixed with 4% paraformaldehyde and embedded in paraffin wax. The samples were then cut into 4-μm-thick sections and placed on microscope slides, as described previously. The expression of iNOS was also evaluated using immunohistochemistry. The primary antibody (monoclonal anti-iNOS) and rat biotinylated secondary antibody were purchased from Santa Cruz Biotechnology, Inc.

Measurement of apoptosis using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)

Briefly, myocardium paraffin sections were analyzed with a TUNEL assay, and myocardial apoptosis was measured using a kit purchased from Boster Biological Engineering Company (Wuhan, China), according to the manufacturer’s instructions, as described previously.

Statistical analysis

Data are presented as means ± standard deviation. Groups were compared using one-way analysis of variance and the Bonferroni–Dunn post hoc test was performed on raw data. Differences were considered to be significant at $P < 0.05$.

Results

NO and SOD level in myocardium

The level of NO was significantly higher, while SOD activity was lower, in the IHG and PHG groups compared with the control group ($p<0.05$). There was also a significant difference in levels of NO and SOD activity between the IHG and PHG groups ($p<0.05$). NAC prevented the increase in oxidative markers in the IHG + NAC and PHG + NAC groups. ($p<0.05$, Table 1).

| Table 1. the levels of SOD, NO, IL-6, MDA and TNF-α

5
| Group   | SOD (U/mg) | NO (umol/g) | IL-6 (pg/mg) | TNF-α (pg/mg) |
|---------|------------|-------------|--------------|----------------|
| Control | 41.23±1.58 | 1.04±0.14   | 5.13±0.85    | 12.17±1.79     |
| IHG     | 10.11±0.92 *▲ | 2.15±0.30 *▲ | 16.26±1.75 *▲ | 26.35±2.45 *▲ |
| PHG     | 23.61±1.03 * | 1.67±0.55 * | 10.83±2.19 * | 19.97±2.66 * |
| IHG+NAC | 38.51±1.67 *# | 1.28±0.17 # | 5.91±0.52 # | 12.15±0.98 # |
| PHG+NAC | 39.28±1.19 *# | 1.19±0.19 *# | 5.45±0.61 *# | 13.02±0.87 *# |

F 627.98 12.82 75.51 65.88
P <0.001 <0.001 <0.001 <0.001

Data are means±SD. *P < 0.05, vs control group, ▲ P < 0.05 vs PHG group, #P < 0.05 vs IHG group.

**TNF-α and IL-6 levels in myocardium**

Levels of TNF-α and IL-6 were markedly higher in the PHG group than in the control group (p<0.05). Levels of TNF-α and IL-6 were much higher in the IHG group than in the PHG and control groups (p<0.05). NAC prevented the production of inflammatory markers in the IHG + NAC and PHG + NAC groups. (p<0.05, Table 1).

**Expression of NF-κB and iNOS in myocardium**

As assessed by immunohistochemical staining, the relative expression of NF-κB and iNOS in myocardium was significantly increased in the group with fluctuating blood glucose levels compared with either the control group or the group with persistently high blood glucose levels (p<0.05). Co-infusion with NAC prevented the elevation of NF-κB and iNOS in the IHG + NAC and PHG + NAC groups (p<0.05, Fig. 1 and Fig. 2).
Figure 1 Expression of iNOS (HE; x400); (a) control group; (b) IHG; (c) PHG; (d) IHG+NAC; (e) PHG+NAC. (f) comparison of optical densities of iNOS immunohistochemical staining. The brown stain in cytoplasm is expressed as iNOS. Data are means±SD. *P < 0.05, vs control group, ▲P < 0.05 vs PHG group, #P < 0.05 vs IHG group.

Figure 2 Expression of NF-κB (HE; x400); (a) control group; (b) IHG; (c) PHG; (d) IHG+NAC; (e) PHG+NAC. (f) comparison of optical densities of NF-κB immunohistochemical staining. The brown stain in cytoplasm is expressed as NF-κB.
Data are means±SD. *P < 0.05, vs control group, ▲ P < 0.05 vs PHG group, #P < 0.05 vs IHG group.

**Apoptosis of cardiomyocytes**

Myocardial cell apoptosis was observed in the IHG group but no apoptosis was observed in the other groups (Fig. 3).

Figure 3 Apoptosis in myocardium in different groups (HE; х400) (a) control group; (b) IHG; (c) PHG; (d) IHG+NAC; (e) PHG+NAC. The brown stain is expressed in the apoptotic myocardial cells.

**Discussion**

It is estimated that more than 150 million people suffer from type 2 diabetes mellitus and its complications and the number of patients with type 2 diabetes mellitus is still growing. Oxidative stress induced by hyperglycemia is an established risk factor for cardiovascular disease. Oxidative stress may alter signal transduction pathways, such as NF-κB, c-Jun N-terminal kinase/stress-activated protein kinase, p38 mitogen-activated protein kinase and protein kinase C, leading to apoptosis.
The glutathione (GSH) precursor NAC is recognized to be an important antioxidant and is commonly administered as a safe way to increase levels of glutathione. Hyperglycemia can cause increased levels of free oxygen radicals in diabetic patients. Lee et al. showed that NAC effectively reduced free oxygen radicals. SOD appears to be essential for maintaining cellular antioxidant capacity and impaired SOD activity has been associated with developmental defects in mice as well as several chronic diseases in humans. An earlier study showed that NAC increased SOD activity in immune cells of septic rats. Levels of iNOS and free radicals such as NO, which are associated with cardiovascular diseases, are also increased in diabetes. In the present study, we showed that myocardial levels of oxidative markers (NO, iNOS) were significantly higher, while SOD activity was lower, in the IHG group than in the PHG and control groups. NAC treatment increased SOD activity and decreased levels of NO and iNOS in the IHG + NAC group.

NF-κB is a redox-sensitive transcription factor, activation of which has been identified as one of the most important causes of diabetes and its complications. Oxidative stress has been implicated in increased activity of transcription factors such as NF-Kb and NF-κB has been identified as a key signal in apoptosis. In present study, the level of NF-κB was increased in the IHG group compared with the other groups. Significant decreases in levels of NF-κB were detected in the groups treated with NAC.

Accumulating evidence suggests that diabetes has features in common with chronic inflammatory states. Circulating levels of IL-6 and TNF-α were both increased in diabetes. Oxidative stressors may increase levels of IL-6, which has a strong relationship with NF-κB activity. In the present study, levels of IL-6 and TNF-α were significant higher in the IHG group, which has fluctuating glucose levels, and were suppressed by NAC. This result is consistent with the findings of Khechaie al. Hsu et al. also showed that NAC prevented the release of inflammatory markers TNF-α and IL-6. Previous studies have suggested that inflammatory cytokines, such as TNF-α and IL-6, can stimulate expression of SOD, further enhancing oxidative
stress. This is a perilous circle. Gül et al. \(^{36}\) showed that reduction of oxidative injury by NAC was strongly correlated with GSH and TNF-\(\alpha\) level.

Our earlier study suggested that apoptosis of myocardial cells was associated with oxidative stress and NF-\(\kappa\)B activation. The antioxidant NAC has been shown to prevent the formation of malondialdehyde and to reduce cardiovascular events by decreasing apoptosis \(^{37,38}\). Most previous studies of the effect of NAC on apoptosis were, however, \textit{in vitro} experiments \(^{39-42}\) and few \textit{in vivo} studies have been described. The uniqueness of our study was that we established the model of acute blood glucose fluctuations \textit{in vivo} and investigated the effect of NAC on the adverse consequences of acute fluctuations in blood glucose \textit{in vivo} and found that NAC could inhibit apoptosis of myocardial cells. More interestingly, we did not see apoptosis in the PHG group, although oxidative stress and inflammatory factors were increased in comparison with the control group. Because the pathway and mechanism of apoptosis may be complicated, and the degree of oxidative stress and increased inflammatory factors may not reach the purpose of inducing apoptosis. Therefore, the pathway and mechanism of apoptosis should be confirmed in the next study.

NAC is a traditional antioxidant. Previous studies have confirmed the role of NAC in anti-oxidative stress and anti-inflammatory responses\(^{43-45}\). However, we had some limitations, the sample size was small, and the time of acute blood glucose fluctuation was further extended. The indexes of observation could be further extended. And the actual mechanism of NAC which prevent the apoptosis of myocardial cells should be needed in the future study.

In the present study, fluctuations in blood glucose levels induced more severe oxidative stress, chronic inflammation and apoptosis in the myocardium than persistently high blood glucose levels. The antioxidant NAC may prevent the apoptosis of myocardial cells induced by fluctuations in blood glucose levels \textit{in vivo}. This effect of NAC can likely be attributed to its anti-oxidative and anti-inflammatory properties. Although we did not use different doses of NAC and did not examine other possible mechanisms, our study has clearly demonstrated that heart injury can be minimized by the administration of NAC.
Conclusions
Acute fluctuations in blood glucose levels induced more severe oxidative stress, inflammation and apoptosis in the myocardium than persistently high blood glucose levels. The antioxidant NAC may prevent apoptosis of myocardial cells caused by fluctuations in blood glucose levels in vivo, most likely because of its antioxidative and anti-inflammatory properties.

Consent for publication
A written consent to publish the information and data of the participants was obtained.

Acknowledgments
We are grateful to Mr. Zuo Zhou for his outstanding statistics assistance.

Funding
This study was supported by the Research Fund from the Bureau of Education of Liaoning Province, China (Grant No. 20060999), the Research Fund for the Doctoral Program of Higher Education of China (Grant No. 20112104110014) and the Outstanding Scientific Fund of Shengjing Hospital (Grant No. 201101).

Authors’ contributions
WZ was responsible for the design conception of the experiments, collection, analysis and interpretation of the data, and drafting of the manuscript. GJP was responsible for the interpretation of the data and drafting of the manuscript. SZ, YL and PH contributed to perform experiments. YZL was principal investigator of the study, contributed to the analysis and interpretation of the results, and produced the final version of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate
All animal experiments were performed with approval from the animal ethics committee of Shengjing Hospital of China Medical University (2016PS026 K).

Competing interests
The authors declare no conflict of interest.
References

1. Baynes, JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-12.

2. Rehman K, Akash MSH. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016; 3:87.

3. Rehman K, Akash MSH. Mechanism of Generation of Oxidative Stress and Pathophysiology of Type 2 Diabetes Mellitus: How Are They Interlinked? *J Cell Biochem* 2017; 118: 3577-85.

4. Albright JW, Albright JF. Soluble receptors and other substances that regulate proinflammatory cytokines in young and aging humans. *J GerontolABiol Sci Med Sci* 2000; 55: B20–25.

5. Cotgreave, IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. *Adv Pharmacol* 1997; 38: 205-27.

6. Lee, EA., Seo, JY., Jiang, Z., et al. Reactive oxygen species mediate high glucose-induced plasminogen activator inhibitor-1 up-regulation in mesangial cells and in diabetic kidney. *Kidney Int* 2005; 67: 1762-71.

7. Zhang, W., Zhao, S., Li, Y., et al. Acute Blood Glucose Fluctuation Induces Myocardial Apoptosis through Oxidative Stress and Nuclear Factor-κB Activation. *Cardiology* 2013; 124: 11-17.

8. Na Wu, Haitao Shen, Henan Liu, et al. Acute blood glucose fluctuation enhances rat aorta endothelial cell apoptosis, oxidative stress and pro-inflammatory cytokine expression in vivo. *Cardiovasc Diabetol* 2016; 15: 109.

9. Han P, Zhang YY, Lu Y, et al. Effects of different free fatty acids on insulin resistance in rats. *Hepatobiliary Pancreat Dis In* 2008; 7:91–96.

10. Lam TK, Yoshii H, Haber CA, et al. Free fatty acids-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am J Physiol Endocrinol Metab* 2002; 10: E682- 691.

11. Khera, A., McGuire, DK. Management of diabetic dyslipidemia: need for reappraisal of the goals. *Am J Cardiovasc Drugs* 2005; 5: 83-91.
12. Nishikawa, T., Edelstein, D., Du, XL., et al. Normalizing mitochondrial superoxide production blocks three pathways of hyper glycaemic damage. *Nature* 2000; 404: 787-790.

13. Cai, L., Kang YJ. Oxidative stress and diabetic cardiomyopathy: a brief review. *Cardiovasc Toxicol* 2001; 1: 181-193.

14. Oka, S., Kamata, H., Kamata, K., et al. N-acetylcysteine suppresses TNF-induced NF-kappaB activation through inhibition of IkappaB kinases. *FEBS Lett* 2000; 472: 196-202.

15. Fulghesu, AM., Ciampelli, M., Muzj, G., et al. N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. *Fertile Sterile* 2002; 77: 1128-1135.

16. Elnashar, A., Fahmy, M., Mansour, A., et al. N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study. *Fertile Sterile* 2007; 88: 406-409.

17. Weisiger, RA., Fridovich, I. Superoxide dismutase. Organelle specificity. *J Biol Chem* 1973; 248: 3582-3592.

18. Oberley, LW. Anticancer therapy by overexpression of superoxide dismutase. *Antioxid Redox Signal* 2001; 3: 461-472.

19. Lebovitz, RM., Zhang, H., Vogel, H., et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Nail Acad Sci USA* 1996;93: 9782-7.

20. Barreiro, E. Barreiro, E., Sánchez, et al. N-acetylcysteine increases manganese superoxide dismutase activity in septic rat diaphragms. *Eur Respir J* 2005; 26: 1032-1039.

21. Pitocco, D., Zaccardi, F., Di Stasio, E., et al. Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud* 2010; 7: 15-25.

22. Demchenko, YN., Kuehl, WM. A critical role for the NFkB pathway in multiple myeloma. *Oncotarget* 2010; 1: 59-68.

23. Bierhaus, A., Schiekofer, S., Schwaninger, M., et al. Diabetes-associated
sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes* 2001; 50: 2792-808.

24. Poligone, B., Weaver, DJ. Jr., Sen, P., *et al.* Elevated NF-kappaB activation in nonobese diabetic mouse dendritic cells results in enhanced APC function. *J Immunol* 2002; 168: 188-96.

25. Morigi, M., Angioletti, S., Imberti, B., *et al.* Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 1998; 101: 1905-15.

26. Droegge, W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; 82, 47-95.

27. Lawrence, T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring HarbPerspect Biol* 2009; 1: a001651.

28. Limb, GA., Chignell, AH., Green, W., *et al.* Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy. *Br J Ophthalmol* 1996; 80: 168-73.

29. Nappo, F., Esposito, K., Cioffi, *et al.* Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002; 39: 1145-50.

30. Beetz A., Messer G., Oppel T., *et al.* Induction of interleukin 6 by ionizing radiation in a human epithelial cell line: control by corticosteroids. *Int J Radiat Biol* 1997; 72: 33-43.

31. Zhang, J., Johnston, G., Stebler, B., *et al.* Hydrogen peroxide activates NFκB and the interleukin-6 promoter through NFκB-inducing kinase. *Antioxid Redox Signaling* 2001; 3: 493-504.

32. Khechai, F., Ollivier, V., Bridey, F.,*et al.* Effect of advanced glycation end product-modified albumin on tissue factor expression by monocytes. Role of oxidant stress and protein tyrosine kinase activation. *ArteriosclerThrombVasc Biol* 1997; 17: 2885-2890.

33. Hsu, BG., Lee, RP., Yang, FL., *et al.* Post-treatment with N-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats. *Life Sci*
2006; 79: 2010-2016.
34. Stralin, P., Marklund, SL. Vasoactive factors and growth factors alter vascular smooth muscle cell EC-SOD expression. *Am J Physiol Heart Circ Physiol* 2001; 281 : H1621-1629.
35. Stralin, P., Marklund, SL. Multiple cytokines regulate the expression of extracellular superoxide dismutase in human vascular smooth muscle cells. *Atherosclerosis* 2000; 151: 433-441.
36. Gül, M., Ayan, M., Seydanoğlu, A., et al. The effect of N-acetyl cysteine on serum glutathione, TNF-α and tissue malondialdehyde levels in the treatment of sepsis. *Ulus TravmaAcilCerrahiDerg* 2011; 17: 293-297.
37. Tepel, M., van der Giet, M., Statz, M., et al. The antioxidant acetylcysteine reduces cardiovascular events in patients with endstage renal failure: a randomized, controlled trial. *Circulation*2003; 107: 992-5.
38. Trimarchi, H., Mongitore, MR., Baglioni, P., et al. N-acetylcysteine reduces malondialdehyde levels in chronic hemodialysis patients: a pilot study. *Clin Nephrol*2003; 59: 441-446.
39. Jin, HM., Zhou, DC., Gu, HF., et al. Antioxidant N-acetylcysteine protects pancreatic β-cells against aldosterone-induced oxidative stress and apoptosis in female db/db mice and insulin-producing MIN6 cells. *Endocrinology* 2013; 154: 4068-4077.
40. Pawlas, N., Malecki, A. Neuroprotective effect of N-acetylcysteine in neurons exposed to arachidonic acid during simulated ischemia in vitro.*Pharmacol Rep* 2009; 61: 743-750.
41. Kumar, S., Sitasawad, SL. N-acetylcysteine prevents glucose/ glucose oxidase-induced oxidative stress, mitochondrial damage and apoptosis in H9c2 cells. *Life Sci* 2009; 84: 328-336.
42. Hung, KY., Liu, etal. N-acetylcysteine-mediated antioxidation prevents hyperglycemia-induced apoptosis and collagen synthesis in rat mesangial cells. *Am J Nephrol*2009; 29: 192-202.
43. Bruno A. Quadros Gomes, Lucio F. D. da Silva, Antonio R. Quadros Gomes, et al. N-acetyl cysteine and mushroom Agaricus sylvaticus supplementation decreased parasitaemia and pulmonary oxidative stress in a mice model of malaria. *Malar J* 2015; 14: 202.

44. Juciano Gasparotto, Alice Kunzler, Mario Roberto Senger, et al. N-acetyl-cysteine inhibits liver oxidative stress markers in BALB/c mice infected with Leishmania amazonensis. *Mem Inst Oswaldo Cruz* 2017; 112: 146–154.

45. Rajat Sandhir, Sandeep Kaur, Saurabh Dhanda. N-acetyl-l-cysteine Prevents Bile Duct Ligation Induced Renal Injury by Modulating Oxidative Stress. *Indian J Clin Biochem* 2017; 32: 411–419.