Original Article

Treatment with N-acetylcysteine does not alter blood glucose levels and the oxidative stress status in diabetic rats

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Abstract

Objectives: To verify the contribution of N-acetylcysteine (NAC) as an antioxidant drug in the therapy of diabetes, helping to reduce the deleterious effects resulting from oxidative stress associated with the hyperglycemic state. **Materials and Methods:** The animals were divided into normal (saline, 25 mg/kg NAC, and 75 mg/kg NAC) and diabetic rats (saline, 25 mg/kg NAC, and 75 mg/kg NAC) with five rats per group, and were treated for four weeks. Diabetes induction was performed by intraperitoneal injection of alloxan after fasting for 12 hours. Subsequently, glucose solution was used to promote wear of the pancreatic beta cells. Blood parameters such as glucose, glycated hemoglobin, hepatic and renal biomarkers, and butyrylcholinesterase activity were determined by commercial kits. Catalase, glutathione peroxidase, and superoxide dismutase activities were measured using spectrophotometric techniques, while glutathione and malondialdehyde levels were determined by chromatographic techniques. **Results:** NAC had no significant differences on glycemic, hepatic, renal, and oxidative stress biomarkers. Superoxide dismutase activity was significantly higher ($P < 0.05$) in diabetic rats treated with NAC compared to the diabetic saline group, while butyrylcholinesterase activity was significantly lower ($P < 0.05$) in the same groups. There was a negative correlation between superoxide dismutase and butyrylcholinesterase activities. **Conclusion:** NAC supplementation did not re-establish the antioxidant system and consequently the deleterious effects of diabetes did not decrease. Diabetic groups that received NAC demonstrated that superoxide dismutase activity was indirectly linked to the levels of butyrylcholinesterase. More studies are necessary to investigate the action of NAC on superoxide dismutase and butyrylcholinesterase activities in the diabetic state.

Key words: Blood, butyrylcholinesterase, diabetes, N-acetylcysteine, oxidative stress, rats

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action, or both. In diabetes, persistent hyperglycemia may cause high production of a free radical formation, with high production of the reactive oxygen species (ROS) that has been attributed to protein glycation and/or glucose auto-oxidation, due to a hyperglycemic environment. Increased oxidative stress may be the result of a pathological process.[1-3]

Access this article online	
Quick Response Code:	Website: www.pnrjournal.com
	DOI: 10.4103/0976-9234.116755

Literature describes that ROS plays a role in a variety of physiological and pathophysiological processes of diabetes such as nephropathy[1] and non-alcoholic fatty liver disease.[4,5] Therefore, this disease has shown an increase in the renal and hepatic function biomarkers.[1,5,6]

Another common effect in diabetes is the high activity

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of serum butyrylcholinesterase (BuChE) (EC 3.1.1.8) in rats[7] and humans.[8] Despite its physiological importance, BuChE activity is poorly understood.[9] One of the mechanisms that explains the increased BuChE activity in diabetes is the increase of ROS and consequently the increase of oxidative stress.[7,10]

Experimental studies have shown a decrease in the occurrence of complications after supplementation with different antioxidants in diabetic animal models, supporting the role of oxidative stress in the development of diabetic complications.[3,6,11-13] One of these supplements is N-acetylcysteine (NAC), a thiol (sulfhydryl-containing) compound with antioxidant properties, utilized for paracetamol intoxications.

N-acetylcysteine is an oxygen radical scavenger and a precursor for the biosynthesis of glutathione (GSH), replenishing the intracellular stores of GSH and promoting detoxification caused by the free radicals.[14] It has been shown that NAC has beneficial effects on the treatment of HIV infection, cancer, heart disease, nephropathy, and diabetes.[11,13,15,16] NAC treatment against oxidative stress caused by diabetes has been reported, which includes the reduction of lipid and protein oxidation and an increase in antioxidant enzymes.[11,17-20]

In the present study, the effects of NAC administrated intraperitoneally were investigated in normal and alloxan-induced diabetic rats. Blood glucose and glycated hemoglobin (HbA1C) levels, renal, hepatic, and oxidative stress biomarkers, antioxidant enzyme activities, and serum BuChE activity, were analyzed in order to verify the contribution of NAC as an antioxidant drug in the therapy of this disease, especially in helping to reduce the deleterious effects resulting from oxidative stress, usually associated with the hyperglycemic state.

MATERIALS AND METHODS

Chemicals

N-acetylcysteine (NAC), albumin, alloxan monohydrate, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), dinitrophenylhydrazine (DNPH), epinephrine, propionyl choline, glycine, nicotinamide adenine dinucleotide phosphate (NADPH), and malondialdehyde (MDA) were purchased from Sigma (St. Louis, MO, USA). Sodium dihydrogen phosphate (NaH_2PO_4), sodium monohydrogen phosphate (Na_2HPO_4), D-glucose anhydrous, sodium hydroxide (NaOH), trichloroacetic acid (TCA), potassium iron cyanide, potassium cyanide, and copper sulfate were purchased from Vetec (Rio de Janeiro, RJ, BR). Hydrogen peroxide (H_2O_2), methanol, ethanol, ethyl acetate, and acetonitrile were obtained from the Tedia Company (Fairfield, OH, USA). Glycated hemoglobin, butyrylcholinesterase (EC 3.1.1.8), aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (γ-GT), urea, creatinine were used commercials kits from Doles (Goiânia, GO, BR). All other reagents used in the experiments were of analytical grade and of the highest purity.

Animals

Thirty Male albino Wistar rats weighing between 215 and 350 g from the Central Animal House of the Federal University of Santa Maria (Santa Maria, RS, Brazil) were used in this experiment. Diabetes induction was performed according to Lenzen,[19] with minor modifications. After fasting for 12 hours, the animals received a single intraperitoneal injection of freshly prepared alloxan using 2% sodium citrate solution 0,05M (pH = 4,5) as a vehicle, at a dose of 150 mg alloxan/kg body weight. The control animals only received the saline vehicle. After six hours, the diabetic rats received 2 mL glucose solution 20% by gavage. For 24 hours, a 5% glucose solution was the only water source. After 15 days, the blood levels of glucose were measured with the help of a portable glucometer, ADVANTAGE (Roche®, Boehringer Mannheim, MO, USA). The rats with a blood level glucose above 200 mg/dl ($n = 15$) were considered diabetic and selected for the experiment. The animals were maintained at a constant temperature (23 ± 1°C) on a 12 hour light/dark cycle with free access to food and water. All the animal procedures were approved by the Animal Ethics Committee from the Federal University of Santa Maria (protocol under number: 54/2008).

Treatment with N-Acetylcysteine

The animals were randomly divided into six groups (five rats per group): Control/saline; Control 25 mg/kg NAC; Control 75 mg/kg NAC; Diabetic/saline; Diabetic 25 mg/kg NAC; Diabetic 75 mg/kg NAC. After diabetes induction, the animals received NAC and saline solution intraperitoneally, as stipulated to the group. NAC was freshly prepared in 0.9% saline solution and was administered daily between 14 and 16 p.m., for 30 days. After the treatment period, the animals were submitted to euthanasia and the blood was collected by cardiac puncture from diabetic and control rats. Blood was divided in heparinized tubes, ethylenediaminetetraacetic acid (EDTA)-containing tubes, and tubes with no anticoagulant. Plasma-EDTA

and serum were obtained by centrifugation of 1500 g for 10 minutes at 4°C.

Biochemical measurement

Creatinine, urea, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (γ-GT), and glycated hemoglobin (HbA1C) were determined by Doles® commercial kits. The butyrylcholinesterase (BuChE) assay in the serum was determined by Doles® commercial kits, using butyrylthiocoline as a substrate.[21] Serum protein concentrations were determined by following the method of Bradford,[22] using bovine serum albumin as the standard. Serum BuChE activity is defined as the amount of enzyme required to convert 1.0 millimole per liter (mmol) of substrate in one minute per milligram of serum protein (mM enzyme/min/mg protein). Catalase activity was measured using the method of Aebi,[23] which is based on the decomposition of H_2O_2 by catalase in whole heparinized blood and it was expressed as K units per gram of hemoglobin (K/g Hb). Gluthathione peroxidase (GPx) activity was assayed by the method of Wendel[24] through the GSH/NADPH/glutathione reductase system by the dismutation of H_2O_2 at 340 nm. The enzymatic activity was expressed as micromole NADPH in one minute per gram of hemoglobin (μmol NADPH/min/g Hb).

Gluthathione (GSH) assay was measured in an acid derivate with DTNB (5,5-dithio-bis-2-nitrobenzoic acid) by high performance liquid chromatography (HPLC), with a UV detector at 330 nm, using gradient elution, at 39°C.[25] GSH levels were expressed as micromole per gram of hemoglobin (μmol/g Hb). Malondialdehyde (MDA) assay was determined in plasma-EDTA by HPLC.[26] MDA levels were expressed as micomole per liter (μmol/L). Superoxide dismutase (SOD) activity measurement was based on the inhibition of the radical superoxide reaction with adrenalin in whole heparinized blood, as described by McCord and Fridovich.[27] SOD activity was expressed as units of SOD per milligram of hemoglobin (U SOD/mg Hb).

Statistical analysis

The analysis of the data was performed using the Statistica® 6.0 software system (Statsoft Inc., 2001). The statistical analysis used was the Two-way ANOVA, followed by the Duncan multiple range test. The effects were considered significant at values of $P < 0.05$. All data were expressed as mean ± standard error (S.E.). The relationship between superoxide dismutase and butyrylcholinesterase activities was determined via the Pearson correlation. The level of significance was set at $P < 0.05$.

RESULTS

Blood glucose and glycated hemoglobin

The blood glucose levels were determined at the onset and at the end of the experiment. Glycated hemoglobin was determined at the end of the experiment and these results are presented in Table 1. Blood glucose and glycated hemoglobin levels for the diabetic/saline groups ($P < 0.05$) were significantly increased when compared to the control/saline group at the onset and end of the experiment. The treatment with NAC had no effect on the glucose levels in the diabetic groups, which remained increased when compared to the control/saline group at the end of the experiment. Similarly, no significant differences in glucose and glycated hemoglobin levels were observed when NAC was administered *per se* to the control groups at the end of the study, when compared to the control/saline group.

Body weight, hematocrit, and hemoglobin

Body weight levels were determined at the onset and at the end of the experiment. The hematocrit and hemoglobin levels were determined at the end of the experiment. These parameters are presented in Table 2. In relation to body weight, no significant differences among the groups were observed at the onset of the experiment. In the diabetic/saline group a significant decrease ($P < 0.05$) in body weight was observed when compared to the control/saline group at the end of the experiment. The treatment with NAC had no effect on body weight in the diabetic groups at the end of the study, which remained reduced in relation to the control/saline group. NAC supplementation also had no effect on body weight in the control groups when compared to the control/saline group at the end of the study.

Hematocrit and hemoglobin levels did not show significant differences among the groups at the end of the experiment. The treatment with NAC had no effect on the hematocrit and hemoglobin levels in the diabetic groups when compared to the control/saline group. NAC supplementation *per se* also had no effect on the hematocrit and hemoglobin levels in the control groups when compared to the control/saline group.

Hepatic and renal biomarkers

Levels of hepatic (γ-GT, AST, and ALT) and renal biomarkers (creatinine, urea) were determined at the end of the experiment and are presented in Table 3. γ-GT, AST, ALT, and creatinine levels did not show significant differences among the groups. Treatment with NAC had no effect on these parameters in the control groups

when compared to the control/saline group. NAC supplementation also had no effect on these parameters in diabetic groups when compared to the control/saline group. Urea levels for the diabetic/saline group ($P < 0.05$) were significantly increased when compared to the control/saline group. Treatment with NAC had no effect on the urea levels in the diabetic groups, which remained increased, when compared to the control/saline group. Similarly, no significant differences in urea levels were observed when NAC was administered *per se* in the control groups when compared to the control/saline group.

Antioxidant enzymes and oxidative stress biomarkers

Antioxidant enzyme activities (GPx and catalase) and oxidative stress biomarkers (MDA and GSH)

Table 1: Onset and final glucose and glycated hemoglobin levels of normal and alloxan-induced diabetic rats treated with NAC

Group	Onset glucose	End glucose	Glycated hemoglobin
Control	69.50±3.52	69.25±3.40	4.04±0.12
Control+NAC 25 mg/kg	68.60±1.60	60.00±1.76	3.71±0.06
Control+NAC 75 mg/kg	66.11±2.51	65.20±3.00	3.90±0.10
Diabetic	347.66±34.26*	400.33±26.02*	15.57±0.90*
Diabetic+NAC 25 mg/kg	347.00±13.98*	415.42±13.14*	16.10±0.45*
Diabetic+NAC 75 mg/kg	337.00±26.79*	379.60±33.91*	14.85±1.18*

Values are mean±S.E. from five animals in each group. *P<0.05 as compared to control/saline group; Glucose (mg/dL); Glycated hemoglobin (% HbA1C); NAC: N-acetylcysteine

Table 2: Onset and final body weight, hemoglobin, and hematocrit of normal and alloxan-induced diabetic rats

Group	Onset weight	End weight	Hemoglobin	Hematocrit
Control	332.00±8.53	325.00±11.90	13.71±0.23	46.00±0.70
Control+NAC 25 mg/kg	334.00±37.36	370.00±24.89	14.78±0.61	46.00±0.54
Control+NAC 75 mg/kg	332.00±6.63	332.00±5.83	14.45±0.52	47.40±2.29
Diabetic	240.00±5.77*	226.00±8.82*	16.26±1.49	47.33±1.20
Diabetic+NAC 25 mg/kg	228.57±12.03*	194.28±10.87*	15.72±0.89	45.28±1.35
Diabetic+NAC 75 mg/kg	216.00±7.48*	182.00±9.69*	15.57±0.56	49.40±2.25

Values are mean±S.E. from five animals in each group. *P<0.05 as compared to control/saline group; Weight (grams); hemoglobin (g/dL); hematocrit (%); NAC: N-acetylcysteine

Table 3: ɣ-GT, AST, ALT, creatinine, and urea of normal and alloxan-induced diabetic rats treated with NAC

Groups	ɣ-GT	AST	ALT	Creatinine	Urea
Control	2.97±0.42	36.75±4.49	21.75±1.03	0.45±0.02	47.67±5.71
Control+NAC 25 mg/kg	2.53±0.19	36.44±6.34	22.00±1.55	0.46±0.02	42.51±2.87
Control+NAC 75 mg/kg	2.68±0.19	29.50±2.88	22.80±1.06	0.40±0.01	49.56±6.15
Diabetic	3.46±0.23	29.56±3.94	29.33±0.33	0.60±0.05	152.50±0.90*
Diabetic+NAC 25 mg/kg	4.31±0.40	37.69±6.86	25.85±1.62	0.55±0.02	136.54±9.06*
Diabetic+NAC 75 mg/kg	3.70±0.61	46.27±2.04	26.75±2.25	0.52±0.02	172.42±18.06*

Values are mean±S.E. from five animals in each group. *P<0.05 as compared to control/saline group; ɣ-GT, AST, and ALT (UI/L); creatinine and urea (mg/dL), NAC: N-acetylcysteine; GT: Glutamyl transferase; AST: Aspartate transaminase; ALT: Alanine transaminase

Table 4: MDA, GPx, catalase, and GSH levels of normal and alloxan-induced diabetic rats treated with NAC

Group	MDA	GPx	Catalase	GSH
Control	6.11±0.07	34.18±0.44	52.40±2.87	7.34±0.23
Control+NAC 25 mg/kg	8.05±0.41	34.73±1.24	50.97±1.74	6.78±0.22
Control+NAC 75 mg/kg	8.80±0.74	33.92±0.48	55.75±13.96	7.05±0.16
Diabetic	14.03±0.20*	31.45±0.51	30.80±4.05*	5.10±0.51*
Diabetic+NAC 25 mg/kg	13.87±0.48*	34.07±0.96	32.32±4.14*	4.82±0.56*
Diabetic+NAC 75 mg/kg	15.26±3.13*	32.90±1.98	33.34±3.98*	4.75±0.50*

Values are mean±S.E. from five animals in each group. *P<0.05 as compared to control/saline group; MDA (µmol/L); GPx (µmol NADPH/min/g Hb); Catalase (K/g Hb); GSH (µmol/g Hb); NAC: N-acetylcysteine; MDA: malondialdehyde; GPx: Gluthathione peroxidase; GSH: Gluthathione

were determined at the end of the experiment and are presented in Table 4. GPx activities did not show significant differences among the groups. GSH levels and catalase activity for the diabetic/saline group ($P < 0.05$) were significantly decreased when compared to the control/saline group. Treatment with NAC had no effect on the GSH levels and catalase activity in diabetic groups, which remained decreased, when compared to the control/saline group. Similarly, no significant differences in the GSH levels and catalase activity were observed when NAC was administered *per se* in the control groups when compared with the control/saline group.

The MDA levels for the diabetic/saline group ($P < 0.05$) were significantly increased when compared to the control/saline group. Treatment with NAC had no effect on the MDA levels in the diabetic groups, which remained increased, when compared to the control/saline group. Similarly, no significant differences in MDA levels were observed when NAC was administered *per se* in the control groups when compared to the control/saline group.

The results obtained for SOD activity in whole blood is presented in Figure 1. As can be observed, the SOD activity was significantly decreased in the diabetic/saline group ($P < 0.05$) compared to the control/saline group. Treatment with NAC significantly increased ($P < 0.05$) the SOD activity in diabetic groups when compared to the diabetic/saline group. However, treatment with NAC had no effect on the SOD activity in the control groups when compared with the control/saline group.

Serum BuChE activity

The results obtained for BuChE activity are presented in Figure 2. As can be observed, BuChE activity was significantly increased in the diabetic/saline group ($P < 0.05$) compared to the control/saline group. Treatment with NAC significantly decreased ($P < 0.05$) the BuChE activity in diabetic groups when compared to the diabetic/saline group. However, treatment with NAC had no effect on the BuChE activity in control groups when compared with the control/saline group.

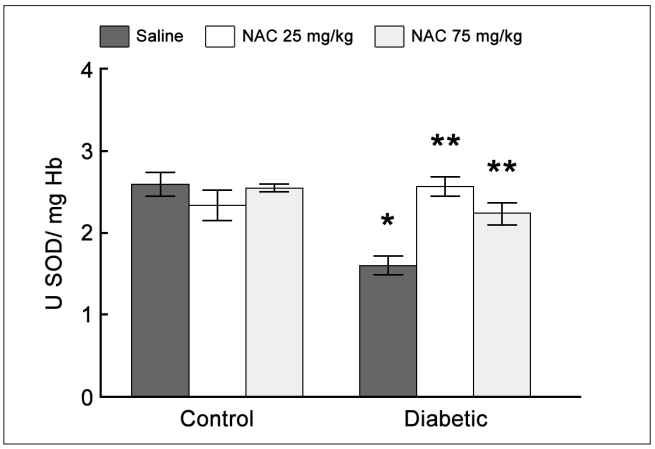

Figure 1: Superoxide dismutase activity in the total blood of rats using epinephrine as a substrate and expressed as U SOD/mg Hb. [Values are means ± S.E from five animals/group; *$P < 0.05$ as compared to control/saline group, **$P < 0.05$ as compared to diabetic/saline group

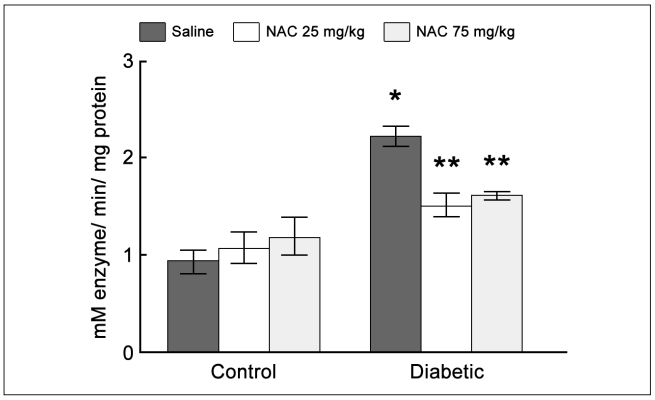

Figure 2: Butyrylcholinesterase activity in the serum of rats using butyrylthiocoline as a substrate and expressed as mM enzyme/min/mg protein. (Values are means ± S.E from five animals/group; *$P < 0.05$ as compared to control/saline group, **$P < 0.05$ as compared to diabetic/saline group)

DISCUSSION

In our experiment, NAC supplementation in diabetic rats was not able to decrease the significant blood glucose and glycated hemoglobin levels [Table 1] when compared to the diabetic/saline group, as has been shown in other studies on humans[18,28] and diabetic animals.[29] Possibly the destruction of pancreatic beta cells by alloxan-induced ROS generation[19] hindered the potentially therapeutic effect of NAC on these cells, and consequently on the hyperglycemia status. In this aspect, perhaps the injection of NAC simultaneously with alloxan, in rats, prevents necrosis of pancreatic beta cells caused by ROS generation,[30] allowing for the proper functioning of the pancreas, and hence, insulin release.

Hyperglycemia promotes increased oxidative stress and lipoperoxidation can be a consequence of this state.[1-3] In this aspect, MDA is a sensitive and specific biomarker of lipoperoxidation and high levels are common in diabetes.[1] Voss and Siems[31] showed that alterations of this biomarker reflect the real redox status *in vivo*. Some studies have shown that NAC treatment is able to decrease the MDA levels in diabetes.[11,20,32] However, Capellini *et al.*,[29] did not find a positive result with regard to the MDA levels after NAC supplement

in diabetes. In our study, the plasma levels of MDA were not altered in diabetic rats treated with NAC, as shown in the Table 4. Our data indicate that NAC had no effect on this specific biomarker in diabetic rats, probably due to uncontrolled hyperglycemia with subsequent H_2O_2 and.OH radical formation, together with an imbalance in the antioxidant system such as failure of GSH synthesis and/or reduced activity of the antioxidant enzymes. Consequently, an increase of lipoperoxidation could have occurred in this situation and high levels of MDA could be formed. However, our results showed a positive effect of NAC on SOD activity in these animals.

Superoxide dismutase offered protection from highly reactive superoxide anions (O_2^-) and converted them to H_2O_2.[1,31] Some authors verified decreased SOD activity in the diabetic state.[2,33-35] Similar behavior was found in our study with this antioxidant enzyme in untreated diabetic rats. This is the effect of excess ROS generation and low ability of SOD in this pathology.[1,2,33] Our work showed that diabetic rats treated with NAC had increased erythrocyte SOD activity when compared to the diabetic/saline group [Figure 1]. We suggest that the increased SOD activity after NAC administration to the diabetic rats was due to the capacity of NAC to directly scavenge the superoxide anions and convert them to H_2O_2 as described by Winterbourn and Metodiewa.[36] Therefore, the therapy with NAC could assist in the increase of SOD activity in these animals. However, there was an increased production of H_2O_2 levels because of O_2^- conversion to H_2O_2 after NAC administration, in diabetic rats, and the catalase activity would not be able to keep pace with the speed of H_2O_2 formation in the detoxification process. This fact could be demonstrated by lower catalase activity in the diabetic groups treated with NAC.

Catalase is responsible for the catalytic decomposition of H_2O_2 to O_2 and H_2O.[1] Studies indicate reduced catalase activity in diabetes,[3,35-37] similar to what we have shown in the Table 4. In our study, diabetic rats treated with NAC have shown lower catalase activities than the control groups ($P < 0.05$) [Table 4], which indicates that NAC supplementation has no effect on catalase activity. This is an unpredictable result because there are reports that indicate simultaneous increase of antioxidant enzymes, especially catalase, after treatment with NAC in a diabetic state,[35,36] and we have just obtained an increase of SOD after NAC supplement in diabetic rats [Figure 1].

We suggest that H_2O_2 formation in diabetic rats treated with NAC was due to the NAC's ability to scavenge O_2^-, which increased the SOD activity in these animals. Probably, the high levels of H_2O_2 inhibited catalase activity allowing for OH formation. Furthermore, the GSH levels in these rats might be insufficient to neutralize the excess OH formation, and these facts prevented an effective detoxification process.

Reduced glutathione (GSH) is the main cellular thiol participating in the cellular redox reaction in which erythrocyte GSH is a physiological free radical scavenger.[12] Our treatment with NAC in the diabetic groups failed to restore erythrocyte GSH levels [Table 4]. This was unexpected, since NAC is a metabolic precursor of GSH and has been used to restore GSH.[14] Possibly these diabetic rats had impaired glutathione synthesis due to decreased gamma-glutamylcysteine synthetase activity, as was described by others studies.[29,38] Patriarca *et al.*,[39] showed that the failure of NAC in the re-establishment of GSH levels could be attributed to the unbalanced ratio between GSH synthesis and consumption, where the high consumption rate could lower GSH, even in the presence of the substrate for the synthesis. Furthermore, poor conversion of NAC to cysteine, excess urinary NAC, and decreased uptake of plasma cysteine by red blood cells could be the cause for the lack of effect of NAC on the GSH levels in a diabetic state.[28]

In other studies, diabetic rats treated with NAC showed lower levels of glycemia than untreated diabetic rats, but hyperglycemia is common to both groups of animals, in spite of the improvement in the GSH status after NAC supplementation.[13,35,40] Darmaun *et al.*,[28] demonstrated high levels of blood glucose after NAC supplementation in diabetes type I, but erythrocyte GSH levels were still decreased, similar to our study [Table 4]. We suggested that NAC transport to the interior of the cells, where it was deacylated and converted to cysteine,[41] could be injured in diabetes type I. Consequently, cysteine was not provided as a substrate for GSH synthesis. Then the NAC levels used in the study would be low, requiring higher concentrations, so as to have some positive effect on the GSH levels. Nevertheless, NAC would possibly remain circulating in the plasma until the excretion of this drug. In this sense, the measurement of plasmatic GSH would not reflect the real GSH status, once red blood cells represent 99.5% of the circulating GSH, being responsible for the erythrocyte homeostasis against oxidative damage.[25,42,43] All these aspects could be involved in the decrease of erythrocyte GSH levels and consequently high levels of MDA, in spite of NAC treatment.

Regarding the activity of SOD, probably the increase of this enzymatic activity after NAC administration

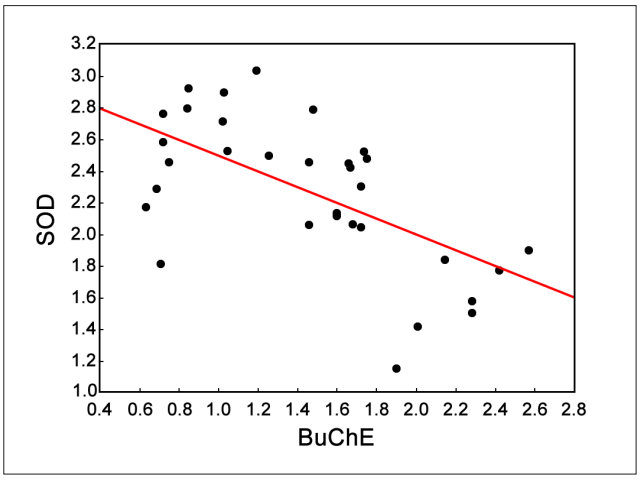

Figure 3: Pearson's correlation between total blood SOD and serum BuChE activities ($r = '0.5997$, $P < 0.05$)

to the diabetic rats allowed the high formation of H_2O_2. However, levels of catalase activity and GSH remained low, and consequently, the MDA levels were elevated, indicating oxidative stress. These results indicate that NAC treatment was not able to avoid the deleterious effects from oxidative stress caused by this disease as can be seen in the final glucose, glycated hemoglobin [Table 1] and urea levels [Table 3], and also body weight [Table 2].

Nevertheless, the BuChe activity in diabetic rats treated with NAC was altered. According with Das,[44] the BuChE activity is a possible marker of low-grade systemic inflammation. High activity of this enzyme in diabetes could be explained due to the increase of ROS and consequently oxidative stress.[7,10] There is a correlation between ROS production and inflammation, in which superoxide anion production is increased *in vivo* during the inflammatory process.[1,45] It has been seen that NAC has anti-inflammatory properties by regulating ROS production.[45] In this regard, Guo *et al.*,[10] have demonstrated that NAC supplementation decreases the inflammatory factors, preventing the increase of anion superoxide production and improving the total antioxidant capacity in diabetic rats. However, in our study, there has been no increment in the antioxidant system in the diabetic rats treated with NAC.

In our experiment, NAC supplementation in diabetic rats scavenged the superoxide anions and increased the total blood SOD activity [Figure 2]. Moreover, there was a negative correlation ($r = -0.5997$) between total blood SOD activity and serum BuChE activity [Figure 3]. We suggested that the increase of SOD activity and possibly reduced levels of superoxide anions could be associated with the decrease of serum BuChE activity in these animals and probably the inflammatory process, independent of the total antioxidant capacity.

In summary, we demonstrated that intraperitoneal NAC supplementation in concentrations of 25 and 75 mg/kg did not lead to significant changes in renal and hepatic biomarkers, blood glucose or glycated hemoglobin levels. Furthermore, treatment with NAC in alloxan-induced diabetic rats was not able to re-establish the antioxidant system, as was anticipated in this study. In this aspect, NAC supplementation did not reduce the deleterious effects resulting from oxidative stress. Nevertheless, the diabetic groups that received NAC demonstrated that SOD activity was indirectly linked to the levels of BuChE, probably due to suppression of the superoxide anions, and this fact could be affecting the inflammatory process. More studies are necessary to investigate the action of this drug on SOD and BuChE activities and their relationship with the diabetic state.

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How to cite this article: de Bairros AV, Roehrs M, Ribeiro G, de Freitas F, Moreira AP, Tonello R, *et al.* Treatment with N-acetylcysteine does not alter blood glucose levels and the oxidative stress status in diabetic rats. J Pharm Negative Results 2013;4:5-12.

Source of Support: National Counsel of Technological and Scientific Development (CNPq), Fund to support research in the state of Rio Grande do Sul (FAPERGS). **Conflict of Interest:** None declared.