Beneficial effects of N-Acetyl-Cysteine on hepatic oxidative stress in streptozotocin-induced diabetic rats

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| Complete List of Authors: | Rosa, Lucas; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu
Kaga, Anderson; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu
Barbanera, Pedro; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu
Queiroz, Priscila; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu
do Carmo, Nágilla; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu
Fernandes, Ana Angélica; São Paulo State University, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu |
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Beneficial effects of N-Acetyl-Cysteine on hepatic oxidative stress in streptozotocin-induced diabetic rats

Lucas Rodolfo de Oliveira Rosa¹*, Anderson Kiyoshi Kaga¹, Pedro Octavio Barbanera¹, Priscila Manfio Queiroz, Nágilla Orleanne Lima do Carmo, Ana Angélica Henrique Fernandes¹

¹ - Department of Chemistry and Biochemistry, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil. Zip-Code: 18618-970

*Corresponding Author:
Lucas Rodolfo de Oliveira Rosa
Email: lucasrodolforosa@gmail.com
Professional Address: Department of Chemistry and Biochemistry, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil. Zip-Code: 18618-970
Telephone: +55 14 3880-0600
Contribution: Experimental design, conduction of the experiment, data analysis and write-up of the paper.

All authors can be reached through the postal address and telephone above.

Anderson Kiyoshi Kaga:
Email: anderson.kaga@hotmail.com
Contribution: Experimental design, Conduction of the experiment, data analysis

Pedro Octavio Barbanera
Email: pedro.barbanera@gmail.com
Contribution: Conduction of the experiment.

Priscilla Manfio Queiroz
Email: p.manq@hotmail.com
Contribution: Conduction of the experiment.

Nágilla Orleanne Lima do Carmo
Email: nagillaorleanne@hotmail.com
Contribution: Conduction of the experiment.

Ana Angélica Henrique Fernandes.
Professional Address: Department of Chemistry and Biochemistry, São Paulo State
Email: angelica@ibb.unesp.br
Contribution: Experimental design, conduction of the experiment, data analysis and write-up of the paper.

All authors have approved the final article.
Abstract

Diabetes is one of the leading diseases worldwide and, thus, finding new therapeutic alternatives is essential. The development of Non-alcoholic Fatty Liver Disease is a notable diabetic complication. Therefore, antioxidant therapy became a leading topic in the world of diabetes research. The objective of this present study was to evaluate the effects of antioxidant N-Acetyl-cysteine (NAC) administration on serum biochemical parameters and oxidative stress parameters in hepatic tissue of the diabetic rats. 32 animals were divided in 4 groups (n=8), G1: Normal rats, G2: Normal rats + NAC, G3: Diabetic rats, and G4: Diabetic rats + NAC. Diabetes was induced in diabetic groups through streptozotocin. NAC administration was effective in improving hyperglycemia and hypoinsulinemia, as well as reducing serum alanine-aminotransferase and urea, hepatic triglycerides accumulation and oxidative stress biomarkers in the diabetic liver, as well as improving hepatic antioxidant enzymes’ activities. This effect was likely due to NAC’s ability of restoring intracellular glutathione, an important compound for the antioxidant defense, as well as due to NAC’s direct antioxidant properties. Thus, NAC administration was useful for reducing hepatic oxidative stress and decreased the deposit of triacylglycerols, minimizing diabetic hepatic damage, making it a promising therapeutic adjuvant in the future.

Keywords: diabetes; antioxidant; n-acetyl-cysteine; oxidative stress; hepatic damage; streptozotocin; non-alcoholic fatty liver disease

Introduction

Considering insulin’s important metabolic role as a suppressor of lipolysis in adipose tissue, hypoinsulinemia in diabetes mellitus type 1 (DM1) leads to an increased release of free fatty acids (FFA) in the bloodstream (Adiels et al. 2008) and influx of acids to the liver. This contributes to increased β-oxidation, producing more acetyl-CoA, NADH and FADH$_2$ comparatively to glycolytic process (Leverve 2007), leading to
increased oxidative phosphorylation, which favors electron leakage and mitochondrial reactive oxygen species (ROS) generation (Kowaltowski et al. 2009).

Intrahepatic triglycerides (TG) accumulation happens when the influx of lipids to the liver exceeds the hepatic capacity to export TG to the bloodstream (Adiels et al. 2008). These conditions are ideal for the development of hepatic steatosis that includes the initial phase of Non-Alcoholic Fatty Liver Disease (NAFLD), one of the most relevant diabetic complications, with prevalence around 19% to 46%, and approximately of 50% in patients with type 1 diabetes (Cusi et al. 2017).

Studies show the importance of oxidative stress (imbalance between production and neutralization of reactive oxygen species (ROS)) as one of the mechanisms that aggravate NAFLD in the diabetes (Rolo et al. 2012; Ucar et al. 2013). TG accumulated in liver and phospholipids of cellular membranes can be targeted by ROS, leading to lipid peroxidation, alteration in the structure and permeability of membranes and consequently mitochondrial dysfunction (Vamecq et al. 2012), favoring ROS production further (Sarkhail et al. 2007). Diabetic hyperglycemia can also increase ROS production, by formation of Advanced glycation end products (AGEs), glucose auto-oxidation, increased activity of polyol pathway and suppression of antioxidants system (Fiorentino et al. 2013).

Another finding in the relation between diabetes and oxidative stress is the decrease of antioxidant enzyme activity, decreasing cellular ability to neutralize ROS, exacerbating oxidative stress (Sarkhail et al. 2007; Trivedi et al. 2014). There are indications that the glutathione (GSH) enzymatic system (glutathione peroxidase (GSH-Px), reductase (GSR) and transferase (GST)) is also impaired in the DM, as well as diminished
availability of reduced GSH, which is a substrate for GSH-Px (Ruffmann and Wendel 1991; Santos et al. 2014).

In this context, therapeutic strategies with the use of natural products with antioxidant and hypoglycemic properties have been the focus of several studies (Bajaj and Khan 2012; Abdali, Samson and Grover 2015; Öztürk et al 2017). N-acetyl-cysteine (NAC) is an antioxidant commonly found in *Allium* plants like garlic and onion, and contains cysteine in its composition, an essential precursor during the synthesis of reduced GSH, which has a key role in antioxidant defense as the substrate for GSH-Px (Ruffmann and Wendel 1991). Hence, NAC has the capacity of restoring intracellular reduced GSH levels (Suzuki 2009). There is evidence that NAC neutralize ROS by reducing them directly through its thiol group (Samuni et al. 2013).

Studies have reported positive effects of NAC administration on kidney and liver of diabetic rats (Hsu et al. 2004; Ribeiro et al. 2011), but some still show conflicting results (de Bairros et al. 2013; Patriarca et al. 2005). Although the literature reports improvement in both hypoinsulinemia and hyperglycemia with the administration of NAC (Hsu et al. 2004; Kamboj et al. 2010), there are still few studies addressing the influence of NAC on hepatic steatosis caused by DM1.

Thus, the objective of this study was to evaluate the effects of NAC administration on serum and hepatic biochemical parameters, TG accumulation and oxidative stress through the activity of antioxidant enzymes and important biomarkers of oxidative stress in the liver of diabetic rats.

**Material & Methods**

**Experimental groups and maintenance**
The animal experiment was conducted with approval of the Ethical Committee on the Use of Animals (CEUA) at the Institute of Biosciences of Botucatu, São Paulo State University (UNESP), under protocol number 706, and followed the guidelines highlighted by the Guide for the Care and Use of Laboratory Animals (8th edition). A total of 32 male Wistar rats (± 250g b.w.) with 60 days of age were housed in polypropylene cages in an environment with temperature (23 ± 2ºC) and relative humidity (55 ± 5 %) controlled, and 12 hours light/dark cycle. Animals were randomly divided into four groups of 8 animals in each: G1, control normal rats; G2, normal rats treated with NAC; G3, diabetic rats and G4, diabetic rats treated with NAC.

The experimental diabetes was induced with streptozotocin (STZ) administration at 60 mg kg⁻¹ b.w (single dose, ip.), diluted in 0.1 M sodium citrate buffer, pH 4.5. After 28 hours of administration of STZ, diabetes was confirmed in animals with glycemia above 250 mg dL⁻¹ according to Santos et al. (2014). NAC (sigma® A7250) was administered at the concentration of 25 mg kg⁻¹ b.w. day⁻¹, orally via gavage, for 37 days (Ribeiro et al., 2011).

The animals received water and standard rodent chow (Purina, Campinas, Brazil), which contained 22.0 % protein, 3.8 % fat, 44.5 % carbohydrate and 3.0 kcal/g of metabolizable energy. The control of intake water and food was daily between 9:00-10:00h am. Body weight was measured at interval of 7 days.

**Serum and hepatic biochemical analysis**

After 37 days of experimental period, animals were fasted overnight (12-14 h), anesthetized with ketamine chloride and xylazine chloride (solution 2:1) and euthanized by decapitation. The blood was centrifuged at 1,400 g/10 min to obtain the serum.
Glycemia was determined by colorimetric method in the presence glucose oxidase and peroxidase (test kit diagnostic LABTEST, Minas Gerais, Brasil. Serum concentration of insulin was determination by enzyme immune assay kit (EIA kit, Cayman Chemical, USA). Serum FFA were extracted, in medium containing chloroform, heptane and methanol, and determined according to the method described by Regouw et al. (1971). The activity of alanine aminotransferase (ALT) was measured by the rate of oxidation of NADH, which was proportional to activity of enzyme (Wilkinson 1965). Serum urea level was determined in the presence of urease and phenol-hypocloride with formation of indophenol-blue (Fawcett and Scott 1960).

Two samples of 100 mg of hepatic tissue were separated for biochemistry determinations. One of the samples was homogenized in sodium phosphate buffer 0.1 M; pH 7.4 and centrifuged (10,000 xg/ 15 min). In the obtained supernatant was parameters of oxidative stress.

The concentration of lipid hydroperoxide (LH) was determined in the presence of FeSO4, H2SO4, xylenol orange and butylated hydroxytoluene diluted in methanol (90 %; v/v) (Jiang et al. 1991).

The analysis of protein carbonyl (PC) was performed in the medium incubated with dinitrophenylhydrazine (DNPH), in the dark at room temperature, and precipitated in presence trichloroacetic acid (50 %, wt/v). The pellets, obtained after centrifugation (10 000g for 10 minutes), were washed with ethanol-ethyl acetate (1:1; v/v) and resuspended using guanidine hydrochloride. Through of extinction coefficient 22 000 M⁻¹.cm⁻¹ the concentration of PC was quantified at 360 nm according to method based in formation the of Schiff base described by Reznick and Packer (1994).
Glutathione reductase (GSR) and reduced glutathione (GSH) were assayed in sodium phosphate buffer 100mM; pH 7.4 with DTNB (5,5’–dithiobis-2-nitrobenzoic acid), NADPH, GSR and EDTA (ethylenediaminetetraacetic acid). Total GSH was measured in medium containing Tris-HCl buffer (0.1 M, pH 8.0), DTNB, GSR and EDTA (Tietze 1969). Through of nitro blue tetrazolium (NBT) reduction analyzed the superoxide dismutase (SOD) activity in the presence 50 mM sodium phosphate buffer pH 7.4, EDTA phenazine methosulfate and NADH according to the method described by Ewing and Janero (1995). Catalase (cat) activity was determined using sodium and potassium phosphate buffer (50 mM, pH 7.0) and hydrogen peroxide (Aebi 1974). GSH-Px activity was measured by NADPH oxidation in sodium phosphate buffer 0.15 M, pH 7.0 containing NADPH, GSR, reduced GSH, sodium azide and EDTA (Nakamura et al. 1974).

Glutathione transferase (GST) activity was assayed utilizing 1,2 – dichloro-4-nitrobenzene (CDNB) as substrate in reaction mixture with 0.1 M potassium phosphate pH 6.5 and reduced GSH (Habig et al. 1974).

Another sample of hepatic tissue (approximately 100 mg) was used for determination of concentration of TG. The extraction of total lipid took place through homogenization in mixture containing chloroform:methanol (2:1; v/v) (Bligh and Dyer 1959). In the chloroform layer, separated after 24 hours, quantified the level of TGs, using the enzymatic method with formation of the quinoneimine, which is directly proportional to the TG present in the sample (Moura 1982).
The values of absorbance as well as enzymatic activity were obtained in ELISA reader (µQuant-MQX/Bio-tech Instruments, Inc., USA) with monitored readings by Gen5 2.0 software to computer system.

**Statistical analysis**

Data was evaluated by analysis of variance (ANOVA) and Tukey test for comparison between the averages of the experimental groups. Statistically significant differences were considered when \( P \) values <0.05 (Zar 1996).

**Results**

*Hyperglycemia, hypoinsulinemia*

Table 1. Glycemia, serum insulin, serum urea, FFA and TG concentration in hepatic tissue.

Glycemia significantly increased in untreated diabetic rats (G3) when compared to other groups (G1, G2 and G4), while insulin levels decreased in G3. Diabetic rats treated with NAC (G4) improved these parameters, with lower glycemia and higher insulin levels, but not returning to the same levels of the non-diabetic (G1 and G2) rats. The levels of serum urea were increased by the diabetic condition in G3, and decreased by the treatment with NAC in G4. The level of serum FFAs was increased in G3 group and reduced in G4 group (Table 1).

*Liver Injury hepatotoxicity*

Hepatic TG accumulation was higher in G3, and significantly reduced by NAC administration in STZ-induced diabetic rats (G4) (Table 1).
Untreated diabetic rats (G3) had higher serum concentration of urea and ALT activity than the others groups (Table 2). Treatment with NAC was capable of reducing the concentration and ALT activity, but not enough to return them to normality. (Table 2)

**Hepatic oxidative stress biomarkers**

Figure 1 shows the data for hepatic oxidative stress biomarkers. LH and PC level was highest in G3 group, but was normalized by NAC administration, returning to non-diabetic levels.

G3 group had lower total and reduced GSH concentration. Note that there were no significant alterations in total GSH among the groups G1, G2 and G4.

GSR and GST activities were decreased in STZ-induced diabetic rats (G3), whereas NAC administration enhanced GR activity and normalized GT activity in G4 group.

**Hepatic antioxidant enzymes activity**

Table 3 shows that catalase activity decreased in the diabetic rats (G3 group) in comparison to the non-diabetic (G1 and G2), whereas no significant difference was found with NAC treatment in STZ-induced diabetic rats (G4 group).

Treatment in G4 group, compared with untreated G3 group, normalized SOD and GSH-Px activities. NAC was effective, increasing the antioxidant activity that was depressed by diabetes in G3, restoring it to activity levels of the non-diabetic groups (G1 and G2).

**Discussion**
There are evidences of an association between DM1 and nonalcoholic fatty liver disease (NAFLD) due poor glycemic control and dysfunction of lipid metabolism, elevating the delivery of fatty acids to the liver and consequently causing abnormal accumulation of triacylglycerol and oxidative stress. Considering the role of hyperglycemia-mediated oxidative stress, the development of novel pharmacotherapies, with antioxidant and antidiabetogenic properties, may be of great clinical interest. Thus, this study evaluated NAC’s effect on ectopic fat and oxidative stress, mainly characterized by increased lipid hydroperoxide production, protein oxidation and alteration of the antioxidant system in liver under diabetic conditions, and found an improvement in these parameters thanks to NAC’s administration, which is an indicative of NAC’s therapeutic potential.

Streptozotocin is considered a classic model of DM1 induction because provoke toxicity to pancreatic β-cells and cellular death (Lenzen 2007), resulting hypoinsulinemia and hyperglycemia.

Since insulin is responsible for the entry of glucose into the cell, low insulin leads to hyperglycemia in STZ-diabetic rats (G3). There is an association between poor glycemic and increase circulating FFA in diabetic state (Oliveira et al. 2013)

The increase in serum FFA level accompanied by TG accumulation in liver was another abnormality in response to hypoinsulinemia observed in G3 group. These observations are consistent with inhibitory action of insulin on lipolysis (degradation of stored TG in adipose tissue), therefore the absence of this hormone increase the availability and influx of FFA to the liver. Excess of FFA that are not β-oxidized are re-esterified in TG and accumulated, leading to hepatic steatosis, the first stage of NAFLD (Novelli et al.
In addition Donelly et al (2005) reported that the mobilization of FFA from adipose tissue is responsible for the approximately 60% of TG in the liver.

Others studies showed that hepatic steatosis is a prevalent pathological condition among DM1 patients (Regnell and Lernmark (2011) and in experimental diabetes (Tahara et al. 2014).

In present study, this pathological accumulation of TG may have caused a certain degree of liver injury, as observed by marked increase in the activities of ALT and GST in STZ-diabetic rats (G3). These observations are in agreement with Donelly et al. (2005) that demonstrated hepatic injury during the ectopic accumulation of TG in hepatocyte. Serum increase of both ALT and GST activity was utilized as biomarkers of liver injury status in experimental models (Cachón et al. 2016; Liu et al. 2014).

In addition, the high serum concentration of urea detected in G3 can be attributed to the special metabolism in diabetic condition, where there is excessive oxidative degradation of amino acid whose carbon skeletons are utilized as precursors for gluconeogenesis (Postic et al. 2004).

The administration of NAC in diabetic rats (G4) restored the level insulin and controlled the hyperglycemia. These results can be attributed to greater glucose uptake and utilization by peripheral and hepatic tissues. The increase of insulin levels by NAC-treated rats (G4) suggests the involvement of NAC in an insulin-dependent mechanism, possibly through its protective antioxidant effect on β-pancreatic cells in the DM animals, promoting pancreatic β-cell recovery, improving release of insulin and glycemic homeostasis. Hsu et al. (2004), Kamboj et al. (2010) and Malik et al (2016).
also described antidabetogenic effect of NAC on pancreatic β-cells with increase in the
insulin secretion preventing the hyperglycemia and diabetics complications.

The improvement of insulinemia reflected in lower concentration of circulating FFA
and consequently less TG content in the liver, after administration of NAC in diabetics
rats (G4), probably due antilipolitic effect exerted by insulin on adipose tissue.

Whereas other possible mechanisms may include the triacylglycerol-lowering effect in
liver, such as inhibitory action of NAC on lipogenesis with decrease in activity of malic
enzyme, contributing to suppress the TG biosynthesis (Lin et al. 2004).

The beneficial effect of NAC on intracellular TG deposition caused lower hepatic
lipotoxicity and minimized the degree of damage, reflecting a decrease in both ALT and
GST serum activity.

Similar findings were reported by Ali et al. (2015), where NAC presented a
hepatoprotective effect on non-diabetic NAFLD, reducing ALT activity in serum. In
addition, NAC treatment also improved the activity of this enzyme in hepatic toxicity
model by mercury chloride (Joshi et al. 2014) and patients with NALFD improved liver
function (Khoshbaten et al. 2010). In addition, Malik et al (2016) demonstrated that
NAC supplementation decreased degree of hepatic steatosis, a manifestation of the
metabolic syndrome characterized by the excessive accumulation of TG.

Under the pathological aspect, the NAFLD presents “two hits”. The first hit involves
TG accumulation which contributes to oxidative stress in liver (second hit) (Day, 2011).
Excessive formation of ROS in detriment to system endogenous antioxidant system
results in oxidative stress (Baydas et al. 2002).
The higher hepatic level of both LH and PC in diabetic rats (G3) indicates oxidative stress, since ROS-mediated lipid peroxidation and protein carbonylation. These results were corroborated by previous studies that observed increase of biomarkers of oxidative stress in hepatic tissue under diabetic state (Fidan and Dündar 2008; Naziroğlu and Butterworth 2005).

In the present study, the TG deposition may have caused elevation of the biomarkers in the hepatic tissue of G3, since lipotoxicity is a contributing factor to oxidative stress. Exacerbated accumulation of TG in the liver, mainly ceramide, makes the cell more susceptible to oxidative stress by promoting ROS generation, as described previously (Adiels et al. 2008; Fabbrini and Magkos 2015; Kowaltowski et al. 2009; Leverve 2007; Sarkhail et al. 2007; Vamecq et al. 2012).

Studies have reported the ROS oxidizing action on the structure of macromolecules, causing lipid peroxidation in plasma membranes (Toescu et al. 2004), provoking extravasation of both ALT and GST from intracellular medium into the bloodstream, as observed in G3.

NAC normalized hepatic LH and PC in diabetic demonstrating that oxidative stress was attenuated in steatosis induced by DM1. Previous studies have pointed towards NAC’s ability to reduce hepatic LH in sucrose-rich diet rats (Diniz et al. 2006) and protein carbonylation in a model of ischemia-reperfusion liver injury (Fernández et al. 2013). This evidences the direct antioxidant action of NAC through a thiol group present in its structure and improves the cellular redox status by ROS scavenger such as superoxide anion (O$_2^-$_{}), hydroxyl radical (•OH), hydrogen peroxide (H$_2$O$_2$) (Benrahmouse et al. 2000; Aruoma et al. 1989; Lasram et al. 2015; Pastor et al. 1997; Samuni et al. 2013).
The hepatic concentrations of total and reduced GSH as well as GSR and GST activity were diminished in STZ-induced diabetic rats (G3). These results can be explained by the polyol pathway.

Under the diabetic state, reduced GSH is commonly depleted (Ruffmann and Wendel 1991), which leads to low activity of GSH-Px (catalyzes the reduction of hydrogen peroxide to water), impairing the ability the cell to neutralize ROS and thus promoting oxidative stress (Jurkovic et al. 2008). This depletion occur during the stimulation of the polyol pathway, which is one of the mechanisms whereby the hyperglycemia can lead to oxidative stress – the accumulated glucose in blood activates aldose reductase, which converts glucose into sorbitol and oxidizes NADPH (Antony and Vijayan 2015). Since glutathione reductase requires NADPH as coenzyme for reducing the oxidized form of glutathione (GSSG) into reduced form, during polyol pathway stimulation, the concentration of GSH decreases, hence compromising the activity of both GSH-Px and GST.

The treatment with NAC was able to correct the alterations observed in the GSH system and improved antioxidant status in liver of STZ-diabetic rats through regeneration in GST, GSR and GSH-Px activities, as well as the levels of both total and reduced GSH. Therefore, the antioxidant property of NAC resides in its capacity to restore the intracellular content of GSH in hepatic tissue (Ribeiro et al. 2011). It is well known fact that GSH plays an important role in antioxidant defense because it acts as substrate of the GSH-Px and glutathione transferase, which are involved in the endogenous antioxidant system, maintaining redox homeostasis in the cell (Suzuki. 2009; Hayes et al. 2005; Ruffmann and Wendel 1991).
The activity of SOD, catalase and GSH-Px constitute the first line of antioxidant defense against oxidative stress. The low activity of these enzymes, observed in this study, confirms the establishment of oxidative stress in STZ-induced diabetic rats (G3 group). A possible explanation for this low activity includes accumulation of both superoxide anion and LH that cause deterioration of enzymes, impairing enzymatic catalysis (Punitha et al. 2005). The depression of hepatic antioxidant enzymes in streptozotocin-induced diabetes is supported by several studies (Ahmed et al. 2015; Bhakkiyalakshmi et al. 2016).

The supplementation of NAC increased SOD, GSH-Px and catalase activities under diabetic conditions (G4 group), suggesting the protective effect of NAC on hepatic tissue of the STZ-induced diabetic rats. The decrease LH, PC and TG concentrations accompanied concomitant increase in the activities of these enzymes, which modulates oxidative stress. NAC treatment attenuated oxidative stress in liver mediated by lipoperoxidation in STZ-induced diabetic rats and increased antioxidant enzymes activities. Nouri et al (2017) and Cai et al (2015) report that NAC increase SOD activity under hepatotoxic condition and hence decreased of superoxide anion ($O_2^-$) generation and greater control of oxidative stress in liver.

Furthermore, increased GSH-Px activity can be attributed to greater synthesis and consequently restoration of GSH levels, substrate of the enzyme, in hepatic tissue of diabetic rats treated with NAC (G4 group).

The relevance of this study was to demonstrate the importance of NAC supplementation in the control of oxidative stress caused by hepatic steatosis, one of the pathogenesis of DM1.
Conclusions

In conclusion, DM1 was associated with oxidative stress due accumulation of TGs in the liver. NAC intake promoted the release of insulin, ameliorated hyperglycemia, attenuated the hepatic exacerbated deposit of TG and consequently the oxidative stress, as shown by diminished biomarkers and increased activities of antioxidants enzymes. Considering the important role of oxidative stress in the development of diabetic complications and in the progression of the disease, the beneficial effect of NAC on liver is likely as a result of its antidiabetogenic and antioxidant properties.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

References


Bhakkiyalakshmi, E., Sireesh, D., Sakthivadivel, M., Sivasubramanian, S., Gunasekaran, P., and Ramkumar, K.M. 2016. Anti-hyperlipidemic and anti-


Oliveira, G.O.; Barga, C.P.; Fernandes, A.A.H. 2013. Improvement of biochemical parameters in type 1 diabetic rats after the roots aqueous extract of yacon [Samallanthus sonchifolius (Pepp.&Endl.)] treatment. Food and chem Toxicol. 59:256-260


Postic, C., Dentin, R., and Girard, J. 2004. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes Metab. 30: 398-408


Figure 1 - G1: control; G2: NAC 25mg-1bw.day-1; G3: diabetic; G4: diabetic + NAC 25mg-1bw.day-1. Values presented as means ± SD (n=8). Means not sharing a common superscripta,b,c,d shows significant difference in the ANOVA.
Table 1. Glycemia, serum insulin, serum urea, FFA and TG concentration in hepatic tissue.

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<td>Urea (mg mL⁻¹)</td>
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<td>FFA (mEq L⁻¹)</td>
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<td>TG (mg g⁻¹ tissue)</td>
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FFA: free fatty acids; TG: triacylglycerols. G1: control; G2: NAC; G3: diabetic; G4: diabetic+NAC. Values presented as means ± SD (n=8). Means not sharing a common superscript Shows significant difference by ANOVA. G1: control; G2: NAC 25mg⁻¹ bw.day⁻¹; G3: diabetics; G4 diabetics+NAC 25mg⁻¹ bw.day⁻¹.
Table 2. ALT and GST activities and level urea in hepatic serum.

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<td>ALT (UL⁻¹)</td>
<td>67.58 ± 8.36b</td>
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<td>GST (UL⁻¹)</td>
<td>26.78 ± 3.90a</td>
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<td>37.54 ± 5.00b</td>
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ALT: alanine aminotransferase. GST: glutathione transferase. G1: control; G2: NAC; G3: diabetic; G4: diabetic+NAC. Values presented as means ± SD (n=8). Means not sharing a common superscript shows significant difference by ANOVA. G1: control; G2: NAC25mg⁻¹bw.day⁻¹; G3: diabetics; G4: diabetics+NAC25mg⁻¹bw.day⁻¹
Table 3. Antioxidant enzymes activities in hepatic tissue

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<tr>
<td>Cat (µmol/g tissue)</td>
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<td>SOD (nmol/mg pt)</td>
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<td>GSH-Px (nmol/mg tissue)</td>
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Cat: catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase. G1: control; G2: NAC 25mg 1bw.day⁻¹; G3: diabetic; G4: diabetic+NAC 25mg 1bw.day⁻¹. Values presented as means ± SD (n=8). Means not sharing a common subscript shows significant difference by ANOVA.
Figure 1. Biomarkers of oxidative stress in hepatic tissue