Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats

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1. Introduction

Diabetes mellitus is a serious, complex chronic condition which is a major source of ill health all over the world [1]. This metabolic disorder is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occur secondary to an absolute or relative lack of insulin [1].

The precise cellular and molecular mechanism which underlies the etiology and progression of diabetes is still not fully understood. However, increasing evidence suggests that oxidative stress plays a crucial role in the pathogenesis of diabetes and its complications [2,3]. Elevated levels of blood glucose can induce non-enzymatic and auto-oxidative glycosylation, increase polyol and hexosamine pathway, promote protein kinase-C activation and lead to alterations of inflammatory mediators, as well as in the status of antioxidant defense [2–5]. These pathways are involved in the...
generation of reactive oxygen species (ROS) in diabetic state, which directly contribute to the increase of oxidative stress in various organs and tissues [2,5]. In addition to the pancreatic β-cells, supraphysiological glucose levels are notorious to provoke oxidative damage in hepatic and renal cells and consequently has been associated with functional and morphological alterations in liver and kidney of diabetic rats [6–8]. In fact, the liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus [8].

The most commonly recognized effect of oxidative stress is the oxidation and damage of macromolecules such as proteins, lipids, DNA, and enzymes involved in energy production, thereby contributing to cellular injury, energetic deficit, and the acceleration of cell death through apoptosis and necrosis [9,10]. In both human and animal diabetics, significant increases in lipoperoxidation products and/or decreases of some antioxidants have been reported, and the presence of oxidative stress has been judged by these indices [10,11].

The effect of ROS is balanced by the antioxidant action of non-enzymatic antioxidants as well as by antioxidant enzymes. The most efficient enzymatic antioxidants involve superoxide dismutase (SOD) and catalase (CAT) while non-enzymatic antioxidants include non-proteics thiol, vitamin C and vitamin E [2,9,12]. These antioxidant defenses are extremely important since they represent the direct removal of free radicals, providing maximal protection for biological sites [12].

Of particular interest, aminolevulinate dehydratase (δ-ALA-D), also known as porphobilinogen synthase, is the second enzyme of the heme pathway. This enzyme catalyzes the condensation of two molecules of δ-aminolevulinic acid (δ-ALA), yielding to the monopyrrole porphobilinogen [13]. It is a metalloenzyme, containing sulfhydryl (-SH) groups and zinc, which are essential for its activity [14]. Notably, its cysteinyl residues are highly sensitive to heavy metals [15], molecular oxygen, and other pro-oxidant conditions such as hyperglycemia [16] that induces disulfide bond formation and enzyme inhibition. Therefore, the sulfhydryl enzyme δ-ALA-D, used together with other parameters, can play an important role as a marker of oxidative stress and impairment of metabolic processes [15,16].

In addition, the use of compounds with antioxidant properties has demonstrated a series of beneficial effects in the prevention and treatment of diabetic complications caused by oxidative stress [17]. Resveratrol (3, 4′, 5′-trihydroxy-trans-stilbene), a natural polyphenol found mainly in grapes and red wine, has been reported to have a wide range of biological properties [18]. The potent antioxidant activity of resveratrol is well established in the literature [19], however, evidence has demonstrated that this compound also possesses anti-inflammatory [20], antiaggregant and neuroprotective properties [21,22]. Based on these findings, resveratrol has become attractive as a therapeutic agent in the treatment of a variety of pathologies including neurodegeneration, cancer, cardiovascular disease and diabetes mellitus [20,22,23]. The mechanisms underlying the beneficial effects of resveratrol are not totally elucidated, but have been related mainly to its antioxidant activity that has demonstrated to protect tissues, such as liver, kidney and brain against a variety of damage caused by oxidative stress [19,24].

Therefore, considering that resveratrol has important antioxidant actions and that the oxidative stress has been associated with the pathogenesis of different diabetic complications the aim of this study was to investigate the effects of resveratrol in the oxidative stress parameters in liver and kidney, and in the blood biochemical parameters in streptozotocin (STZ)-induced diabetic rats, a well-characterized animal model of type 1 diabetes, in order to verify the potential therapeutic this compound in hepatic and renal oxidative damage in diabetic state in rats with STZ-induced type 1 diabetes.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ), resveratrol (3, 5, 4′-trihydroxy-trans-stilbene, approximately 99% purity), δ- aminolevulinic acid (δ-ALA), reduced glutathione (GSH), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), tris (hydroxymethyl)-aminomethane GR, thiobarbituric acid (TBA) and Coomassie brilliant blue G were purchased from Sigma Chemical Co (St. Louis, MO, USA). All other reagents used in the experiments were of analytical grade and of the highest purity.

2.2. Animals

Adult male Wistar rats (70–90 days; 180–280 g) from the Central Animal House of the University Federal of Santa Maria (UFSM) were used in this experiment. The animals were maintained at a constant temperature (23 ± 1°C) on a 12 h light/dark cycle with free access to food and water. All animal procedures were approved by the Animal Ethics Committee from the Federal University of Santa Maria (protocol under number: 21/2007).

2.3. Experimental induction of diabetes

Type 1 diabetes was induced by a single intraperitoneal injection of 55 mg/kg streptozotocin (STZ), diluted in 0.1 M sodium citrate buffer (pH 4.5). The age-matched control rats received an equivalent amount of the sodium-citrate buffer. STZ-treated rats received 5% of glucose instead of water for 24 h after diabetes induction in order to reduce death due to hypoglycemic shock. Blood samples were taken from the tail vein 48 h after STZ or vehicle injection to measure glucose levels [25]. Glucose levels were measured with a portable glucometer (ADVANTAGE, Boehringer Mannheim, MO, USA). Only animals with fasting glycemia over 16 mmol/L were considered diabetic and used for the present study. During the experiment the levels of blood glucose was verified four times (2, 10, 20 and 30 days after the beginning of treatment). The animals that maintained fasting glycemia higher than 16 mmol/L were considered diabetic and selected for the assays.

2.4. Treatment with resveratrol

The animals were randomly divided into six groups (8 rats per group): Control/saline; Control/RV 10 mg/kg; Control/RV 20 mg/kg; Diabetic/saline; Diabetic/RV 10 mg/kg; and Diabetic/RV 20 mg/kg. Two week after diabetes induction, the animals belonging to group/control/RV10 and diabetic/RV10 received 10 mg/kg of resveratrol and the animals from control/RV20 and diabetic/RV20 groups received 20 mg/kg of resveratrol, while the animals from control/saline and diabetic/saline groups received saline solution. Resveratrol was freshly prepared in 25% ethanol and was administered intraperitoneally at between 10 and 11 a.m. once a day during 30 days, at a volume not exceeding 0.1 ml/100 g rat weight. The choice of the doses of 10 and 20 mg/kg of resveratrol was made based in previous works of our research group, in which diabetic rats were treated with resveratrol in the concentrations of 10 and 20 mg/Kg (i.p.) [25–27,53,54] and also in several studies that used theses same concentrations of resveratrol and obtained beneficial results [28–30]. Furthermore, the choice of these concentrations take into account that red wine is the main
dietary source of resveratrol [31]. Assuming that the average concentration of trans-resveratrol in wine is 5 mg/L [32] and moderate daily consumption of wine is 250 mL, the mean daily intake of resveratrol under these conditions is 0.02 mg/kg. Thus, we selected a dose that was 500 (10 mg/kg) and 1000 (20 mg/kg) times higher than this estimate to provide a sufficiently large safety margin [28].

2.5. Tissue preparation

Twenty-four hours after the treatment, the animals were previously anesthetized with halothane and submitted to euthanasia. The samples of liver and kidney were quickly removed, placed on ice and homogenized within 10 min in cold 50 mM Tris–HCl pH 7.4 (1/10, w/v). The homogenate was centrifuged at 2000 g, at 4 °C, for 10 min to yield the low speed supernatant (S1) that was used immediately for TBARS, vitamin C, non-protein thiol group (NPSH) and δ ALA-D assay. Furthermore, during all procedures the S1 was maintained on ice. It is important to note that the immediate use of sample can prevent a possible alteration in the sample caused by storage time and by freezing process. Moreover, it is important point out that if any oxidation occurs spontaneously in samples from diabetic and diabetic rats treated with resveratrol it will be deleted, since all the procedures for the test samples and control sample are carried out together. In order to perform SOD and CAT assay, liver and kidney were diluted and homogenized as described in the respective (Sections 2.7).

2.6. Determination of lipid peroxidation

Lipid peroxidation in liver and kidney was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the method described previously by Ohkawa et al. [33]. In short, the reaction mixture contained 200 µL of samples of S1 from liver and kidney or standard (MDA-malondialdehyde 0.03 mM), 200 µL of 8.1% sodium dodecylsulfate (SDS), 750 µL of acetic acid solution (2.5 M HCl, pH 3.5) and 750 µL of 0.8% TBA. The mixtures were heated at 95 °C for 90 min. TBARS tissue levels were expressed as nmol MDA/mg protein.

2.7. Catalase (CAT) and superoxide dismutase (SOD) activities

For the CAT assay, liver and kidney were homogenized in 50 mM potassium phosphate buffer, pH 7.5, at a proportion of 1:9 (w/v) and 1:5 (w/v), respectively. The homogenate was centrifuged at 2000 g for 10 min to yield a supernatant that was used for the enzyme assay. CAT activity was measured by the method of Nelson and Kiesow [34]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7), 10 mM H₂O₂ and 20 µL of the supernatant. The rate of H₂O₂ reaction was monitored at 240 nm for 2 min at room temperature. The enzymatic activity was expressed in units mg⁻¹ protein (One unit of the enzyme is considered as the amount of CAT which decomposes 1 µmol of H₂O₂ per min at pH 7 at 25 °C).

With the purpose of performing the SOD assay [35] kidney and liver was adequately diluted with Tris–HCl pH 7.4 at a proportion of 1:40 (w/v) and 1:60(w/v) respectively. Briefly, epinephrine undergoes auto-oxidation at pH 10.2 to produce adrenochrome, a colored product that was detected at 480 nm. The addition of samples (10, 20, 30 µL) containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored during 180 s. The amount of enzyme required to produce 50% inhibition was defined as 1 unit of enzyme activity.

2.8. Vitamin C and non-protein thiol group (NPSH) content

Hepatic and renal vitamin C levels were determined by the method of Jacques-Silva et al. [36]. Proteins of liver and kidney were precipitated in a cold 10% trichloroacetic acid (TCA) solution at a proportion of 1:1 (v/v) and submitted to centrifugation again. This supernatant was then used for analysis. A 300 µL aliquot of sample in a final volume of 575 µL of solution was incubated for 3 h at 37 °C then 500 µL H₂SO₄ 65% (v/v) was added to the medium. The reaction product was determined using a color reagent containing 4.5 mg/mL dinitrophenyl hydrazine (DNPH) and CuSO₄ (0.075 mg/mL). Vitamin C levels are expressed as µg ascorbic acid/g tissue.

NPSH was measured spectrophotometrically with Ellman’s reagent [37]. An aliquot of 100 µL for liver and 200 µL for kidney in a final volume of 900 µL of solution was used for the reaction. The reaction product was measured at 412 nm after the addition of 10 mM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (0.05 mL). A standard curve using cysteine was added to calculate the content of thiol groups in samples, and was expressed as µmol SH/g tissue.

2.9. δ-Aminolevulinic acid dehydratase activity (δ-ALA-D)

Hepatic and renal δ-ALA-D activity was assayed according to the method of Sassa [38] by measuring the rate of porphobilinogen (PBG) formation, except that in all enzyme assays the final concentration of ALA was 2.2 mM. An aliquot of 200 µL of sample S1 was incubated for 0.5 h (liver) and 1 h (kidney) at 37 °C. The reaction was stopped by addition of 250 µL of trichloroacetic acid (TCA). The reaction product was determined using modified Ehrlich’s reagent at 555 nm. ALA-D activity was expressed as nmol porphobilinogen (PBG) mg⁻¹ protein h⁻¹.

2.10. Biochemical analysis

The activities of the enzymes of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl-transferase (γ-GT) and the levels of urea and creatinine were determined using standard methods on Cobas MIRA™ (Roche Diagnostics, Basel, Switzerland) automated analyzer. In addition, serum total cholesterol and triglycerides concentrations were measured using standard enzymatic methods using Ortho-Clinical Diagnostics™ reagents on the fully automated analyzer (Vitros 950® dry chemistry system; Johnson & Johnson, Rochester, NY, USA).

2.11. Protein determination

Protein was measured by the method of Bradford [39] using bovine serum albumin as standard.

2.12. Statistical analysis

Statistical analysis was done by the commercial SPSS package for Windows®. All data were expressed as mean ± S.D. Data were analyzed statistically by one-way ANOVA followed by the Duncan’s multiple tests. Differences were considered significant when the probability was P < 0.05.

3. Results

3.1. Blood glucose and body weight

The body weight and blood glucose levels determined at the onset and at the end of the experiment are presented in Table 1. As can be observed, the blood glucose levels and body weight at the onset of the study showed no significant differences among the
groups. In the end of experiment, the blood glucose levels were significantly increased, while the body weight was significantly decreased in diabetic/saline, diabetic/RV 10 and diabetic/RV20 groups \((P < 0.05)\) when compared to the control/saline group. However, the treatment with resveratrol had no effects on glucose levels or on body weight in the diabetic/RV10 and diabetic/RV20 groups, when compared to the control/saline group. Similarly, no significant differences in glucose levels and body weight were observed when resveratrol was administered per se in the control/RV10 and control/RV20 groups at the end of the study when compared to the control/saline group.

### 3.2. Lipid peroxidation

Hepatic and renal lipid peroxidation estimated by TBARS levels is shown in Fig. 1A and B, respectively. As can be observed, TBARS levels in liver and kidney were significantly increased in the diabetic/saline group when compared to the control/saline group \((P < 0.05)\). However, treatment with resveratrol prevented an increase of lipid peroxidation in both tissues in the diabetic/RV10 and diabetic/RV20 groups, when compared to the diabetic/saline group \((P < 0.05)\). No significant difference on TBARS level in kidney was observed in the control/RV10 and control/RV20 groups when compared to the control/saline group.

### 3.3. Catalase (CAT) and superoxide dismutase (SOD) activities

The effect of resveratrol on the activities of enzymatic antioxidants CAT and SOD in hepatic and renal tissues are presented in Figs. 2 and 3, respectively. Liver (Fig. 2A) and kidney (Fig. 2B) CAT activity was significantly decreased in the diabetic/saline group when compared to the control/saline group \((P < 0.05)\). The treatment with resveratrol prevented this decrease in the enzyme activity in liver and kidney in the diabetic/RV10 and diabetic/RV20 groups when compared to the control/saline group \((P < 0.05)\). In

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**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose levels (mmol/L)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset</td>
<td>End</td>
</tr>
<tr>
<td>Control/saline</td>
<td>6.30 ± 1.10</td>
<td>6.20 ± 1.19</td>
</tr>
<tr>
<td>Control/RV 10 mg/kg</td>
<td>5.60 ± 0.86</td>
<td>6.10 ± 2.87</td>
</tr>
<tr>
<td>Control/RV 20 mg/kg</td>
<td>6.20 ± 0.90</td>
<td>5.80 ± 1.27</td>
</tr>
<tr>
<td>Diabetic/saline</td>
<td>6.10 ± 1.28</td>
<td>25.10 ± 1.26*</td>
</tr>
<tr>
<td>Diabetic/RV10 mg/kg</td>
<td>5.80 ± 2.13</td>
<td>25.60 ± 2.24*</td>
</tr>
<tr>
<td>Diabetic/RV20 mg/kg</td>
<td>5.90 ± 1.19</td>
<td>23.20 ± 1.52*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. *Significant difference when compared to the control/saline group \((P < 0.05; n = 6–8)\). ANOVA-Duncan’s Test.

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**Fig. 1.** Levels of thiobarbituric acid reactive substances (TBARS) in liver (A) and kidney (B) from STZ-induced diabetic rats and those treated with resveratrol. Bars represent means ± S.D. Groups with different letters are statistically different \((P < 0.05; n = 8)\). ANOVA-Duncan’s Test.

**Fig. 2.** CAT activity in liver (A) and kidney (B) from STZ-induced diabetic rats and those treated with resveratrol. Bars represent means ± S.D. Groups with different letters are statistically different \((P < 0.05; n = 8)\). ANOVA-Duncan’s Test.
addition, treatment with resveratrol per se did not alter significantly the CAT activity in both types of tissues, in the control/RV10 and control/RV20 groups when compared to the control/saline group.

Results found for SOD activity were similar to those obtained for CAT activity. A significant decrease in liver (Fig. 3A) and kidney (Fig. 3B) SOD was observed activity in the diabetic/saline group when compared to the control/saline group ($P < 0.05$). Treatment with resveratrol prevented this decrease in SOD activity in both tissues, in the diabetic/RV10 and diabetic/RV20 groups reaching similar values to the control/saline group ($P < 0.05$). The administration of resveratrol per se caused no significant effect on SOD activity in the control/RV10 or control/RV20 groups when compared to control/saline group.

3.4. Vitamin C and NPSH levels

Table 2 shows the hepatic and renal vitamin C and NPSH content. As can be seen, in both liver and kidney vitamin C and NPSH content was significantly reduced in the diabetic/saline group when compared to the control/saline group ($P < 0.05$). However, the treatment with resveratrol was able to prevent the reduction in the hepatic and renal content of these non enzymatic antioxidants in the diabetic/RV10 and diabetic/RV20 group when compared to diabetic/saline group ($P < 0.05$). When resveratrol was given per se, no significant difference in liver and kidney was observed in the in the control/RV10 or control/RV20 groups when compared to control/saline group.

3.5. 3-ALA-D activity

Liver and kidney 3-ALA-D (Fig. 4A and B, respectively) activity presented a significant decrease in the diabetic/saline group when compared to the control/saline group ($P < 0.05$). The treatment

Fig. 4. 3-ALA-D activity in liver (A) and kidney (B) from STZ-induced diabetic rats and those treated with resveratrol. Bars represent means ± S.D. Groups with different letters are statistically different ($P < 0.05$; $n = 8$). ANOVA-Duncan’s Test.
with resveratrol prevented this decrease in δ-ALA-D activity in both types of tissues in the diabetic/RV10 and diabetic/RV20 groups when compared to the diabetic/saline group (P < 0.05). Administration of resveratrol per se did not modify significantly hepatic and renal δ-ALA-D activity in the control/RV10 or control/RV20 groups when compared to the control/saline group.

3.6. Biochemical analysis

The activities of serum enzymes AST, ALT and γ-GT were significantly higher in the diabetic/saline group compared to the control/saline group (P < 0.05) (Table 3). The treatment with resveratrol prevented significantly the rise of AST, ALT and γ-GT activities in the diabetic/RV10 and diabetic/RV20 groups (P < 0.05). When resveratrol was given per se, no significant differences were observed in the control/RV10 or control/RV20 groups when compared to the control/saline group.

Levels of renal function markers, urea and creatinine also presented a significant increase in diabetic/saline group when compared to control/saline group (P < 0.05) (Table 3). However, the administration of resveratrol prevented this increase of urea and creatinine levels in the diabetic/RV10 and diabetic/RV20 groups (P < 0.05). The treatment with resveratrol per se caused no effect in urea or creatinine levels in the control/RV10 or control/RV20 groups when compared to the control/saline group.

Cholesterol and triglycerides levels were significantly increased in the diabetic/saline group when compared to the control/saline group (P < 0.05) (Table 4). Treatment with resveratrol prevented the increase of cholesterol and triglycerides levels in the diabetic/RV10 and diabetic/RV20 groups (P < 0.05). The administration of resveratrol per se did not alter significantly the cholesterol or triglycerides levels in the control/RV10 and control/RV20 groups when compared to the control/saline group.

4. Discussion

Extensive evidence has shown that chronic hyperglycemia leads to a series of biochemical events resulting in the production of high levels of ROS and eventual oxidative stress [2–5, 28–30]. STZ-induced diabetes is a well-characterized experimental model for type 1 diabetes due to its ability to selectively destroy pancreatic islet β-cells leading insulin deficiency and hyperglycemia [40]. Diabetogenic effect of STZ is mainly attributed to the excess production of ROS leading to toxicity in pancreatic cells which reduces the synthesis and the release of insulin, while affecting organs such as liver, kidney, and hematopoietic system [40]. In view of this, several experimental and clinical studies have indicated the potential usefulness of polyphenolic compounds as antioxidants therapeutic agents for the prevention and/or reduction of oxidative damage in diabetes [41–43]. Thus, the effects of resveratrol on oxidative stress in liver and kidney, as well as on blood biochemical parameters of STZ-induced diabetic rats were investigated in this study.

Lipid peroxidation is considered a hallmark of oxidative stress, in which ROS interact with polyunsaturated fatty acids, and leads to the formation of lipid products such as MDA and 4-HNE (4-hydroxynonenal), which then causing damages to the membrane components of the cell, cell necrosis and inflammation [44]. Extensive evidence has demonstrated that the increase of lipid peroxidation plays an important role in the progression of diabetes by altering the transbilayer fluidity gradient, which could hamper the activities of membrane-bound enzymes and receptors [3, 45]. In our study, there were significant increases in lipid peroxidation in liver and kidney of diabetic rats, as measured by TBARS formation (Fig. 1A and B). These results are in agreement with several studies that have reported an increase in TBARS levels in kidney, liver, serum and erythrocytes of animal with experimental diabetes [11, 43, 46, 47]. Taken together these findings suggest that the exposure to high glucose levels may elevate the generation of ROS through the non-enzymatic glycation of protein and glucose auto-oxidation. As a consequence, it provokes damage in structural and functional integrity of hepatic and renal tissues, as evidenced by an increase in the oxidative deterioration of the lipids of cellular membrane in diabetic state.

In addition, the increased lipid peroxidation under diabetic conditions could be due to increased oxidative stress in the cell as a result of the depletion of antioxidant defense systems [48]. Antioxidant enzymes form the first line of defense against ROS in the organism include the enzymes SOD, CAT and GSH-Px, which play an important role in scavenging the toxic intermediate of incomplete oxidation. CAT and SOD are the two major enzymes that remove ROS in vivo. SOD catalyzes the dismutation of superoxide anion (O2−) into hydrogen peroxide (H2O2), which is then degraded to H2O by CAT or by glutathione peroxidase. A decrease in the activity of these antioxidants may lead to an excess of availability of O2− and H2O2, which in turn generates hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation [2, 9, 48, 49]. In line with this, in our study, it was observed a decrease in the activities of antioxidants enzymes SOD and CAT in liver and kidney of diabetic rats associated with a concomitant increase in lipid peroxidation in these tissues (Figs. 2A, B and 3A, B). Corroborating with these results, other studies have demonstrated

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (mU/mL)</th>
<th>AST (mU/mL)</th>
<th>γ-GT (mU/mL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/saline</td>
<td>197.1 ± 39.7</td>
<td>64.0 ± 13.5</td>
<td>7.7 ± 0.6</td>
<td>52.1 ± 3.8</td>
<td>0.41 ± 0.05</td>
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<tr>
<td>Control/RV10</td>
<td>192.1 ± 45.2</td>
<td>55.1 ± 6.5</td>
<td>9.1 ± 1.0</td>
<td>44.1 ± 1.9</td>
<td>0.38 ± 0.08</td>
</tr>
<tr>
<td>Control/RV20</td>
<td>176.2 ± 13.2</td>
<td>57.8 ± 8.9</td>
<td>9.5 ± 1.3</td>
<td>54.2 ± 4.5</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>Diabetic/saline</td>
<td>223.4 ± 77.3</td>
<td>94.4 ± 19.0</td>
<td>12.3 ± 1.7</td>
<td>86.4 ± 9.5</td>
<td>0.60 ± 0.09</td>
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<tr>
<td>Diabetic/RV10</td>
<td>192.1 ± 24.8</td>
<td>68.2 ± 9.4ab</td>
<td>8.6 ± 3.8</td>
<td>62.2 ± 7.2</td>
<td>0.48 ± 0.06</td>
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<tr>
<td>Diabetic/RV20</td>
<td>197.8 ± 64.5</td>
<td>61.3 ± 11.2</td>
<td>8.4 ± 0.92</td>
<td>61.5 ± 6.9</td>
<td>0.35 ± 0.08</td>
</tr>
</tbody>
</table>

Data of enzyme activities ALT, AST and γ-GT are presented as U/L. Data of renal markers (urea and creatinine) are represented as mg/dL.

Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/saline</td>
<td>1.56 ± 0.12</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Control/RV10</td>
<td>1.27 ± 0.09</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Control/RV20</td>
<td>1.64 ± 0.17</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Diabetic/saline</td>
<td>2.17 ± 0.06</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Diabetic/RV10</td>
<td>1.57 ± 0.20</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>Diabetic/RV20</td>
<td>1.75 ± 0.21</td>
<td>0.74 ± 0.10</td>
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that the activity of the antioxidant enzymes, such as SOD and CAT are reduced in tissues of diabetic rats. This may result in a number of deleterious effects due to the accumulation of ROS [4]. One possible mechanism for this reduction in SOD and CAT activities may be due to the inactivation caused by the excess of free radicals and/or by non-enzymatic glycation due to the persistent hyperglycemia, which has been extensively reported to occur in diabetes [2–5,50].

On the other hand, our study demonstrated that the treatment with resveratrol (10 and 20 mg/kg) prevented the increase in TBARS levels and the reduction in SOD and CAT activity in liver and kidney of STZ-induced diabetic rats, indicating a possible role of this polyphenol in free radical inactivation and in the antioxidant defense (Figs. 1A, B–3A, B). These results are consistent with reductions in oxidative stress found in other studies, where the resveratrol treatment greatly ameliorated antioxidants enzyme activities and prevented the rise in lipid peroxides in tissue and blood cells of diabetic animals [24,51–54]. In addition, Cao and Li [55] and Leonard et al. [56] reported that resveratrol is able to up-regulate mRNA expression for antioxidant enzymes and decrease the activation of nuclear factor NF-kB (ROS-sensitive transcription factor). Based on these findings, we can suggest that resveratrol is able to modulate SOD and CAT activities, which may be very important since it increases the scavenging capacity of ROS of liver and kidney providing higher protection against oxidative damage induced by diabetes in these tissues. In fact, the reactivation in SOD activity promoted by resveratrol may accelerate the dismutation of \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \), which is quickly removed by CAT protecting the hepatic and renal tissues of diabetic rats against highly reactive and toxic hydroxyl radicals and consequently preventing the lipid peroxidation [20,56].

Numerous investigations have reported that resveratrol inhibits effectively the lipid peroxidation of cellular membranes, the protein oxidation as well as the DNA damage due its ability to directly scavenge various free radicals, including superoxide radicals and peroxyl and hydroxyl radicals [20,56,57]. Moreover, it showed to reduce \( \alpha \)-tocopherol radical to regenerate the endogenous tocopherol, which further strengthens the antioxidant defense mechanism [58]. The capacity of resveratrol to scavenge ROS may be attributed to a hydrogen-electron donation from its hydroxyl groups [59]. It has been reported that due to hydroxylated structure of resveratrol, it can form a radical derivative stabilized by the delocalization of two electrons between the two aromatic cycles and the methylene bridge joining these two cycles [60]. It is important to observe that due to its highly lipophilic character resveratrol is able to bind the lipoprotein particles suggesting that this event improved its antioxidant activity [61].

Simultaneously with the decrease in the activity of the antioxidants enzymes, ruined antioxidant competence of non-enzymatic antioxidants such as non-protein thiols and vitamin C have been reported in diabetes [62–64]. In our study, a marked decrease in NPSH and vitamin C content was observed in liver and kidney of diabetic rats, as reported by other studies [52,63,64] (Table 2). The decrease in hepatic and renal NPSH levels in diabetic rats may represent a depletion of this antioxidant due to its increased utilization in removing \( \text{H}_2\text{O}_2 \) and other peroxides produced in excess due to oxidative stress [65]. On the other hand, the reduction in vitamin C content in the diabetes may be attributed to elevated utilization of this antioxidant in trapping the oxiradicals and/or to a decrease in the GSH level, since GSH is required for the recycling of ascorbic acid [66]. This deficiency in NPSH levels, mainly GSH, and in the vitamin C content found in our study can increase the susceptibility to oxidative damage in hepatic and renal cells and may contribute to the pathogenesis of complications associated with the chronic diabetic state [67].

In our study, the treatment with resveratrol restored NPSH and vitamin C content in liver and kidney of diabetic rats close proximity to normal levels, which in turn reveals the antioxidant potential of resveratrol (Table 2). Similarly, Palsamy et al. [43,51] also observed that resveratrol was able to prevent the decrease in GSH levels in liver and pancreas of diabetic rats. In fact, polyphenols are reported to enhance the activity of \( \gamma \)-glutamylcysteine synthetase and demonstrated simultaneous escalation in the intracellular GSH level [41]. In addition, data of literature demonstrated that elevated levels of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies ROS [67]. In this line, we can suggest that a prevention of a decrease in NPSH content in liver and kidney of diabetic rats found in our study could be in part responsible for the decrease in ROS formation and in the lipid peroxidation levels and the resultant of low oxidative stress obtained in vivo in the animals treated with resveratrol.

Persuasive evidence has indicated that \( \delta \)-ALA-D enzyme is highly sensitive to the presence of a variety of pro-oxidant elements, which oxidize -SH groups of this enzyme impairing its activity [15,68]. The inhibition of \( \delta \)-ALA-D activity may prejudice heme biosynthesis and can result in the accumulation of aminolevulinic acid which under physiologically conditions, can have pro-oxidant effects contributing to the oxidative stress [69]. In line with this, in the present study, the activity of \( \delta \)-ALA-D was significantly decreased in hepatic and renal tissues of diabetic rats (Fig. 4A and B). Our results are in accordance with data found in human and experimental diabetes, where a significant inhibition of \( \delta \)-ALA-D is described and has been related mainly to high glucose levels and overproduction of ROS [16,64,70]. Thus, we can suggest that the inhibition of \( \delta \)-ALA-D activity in liver and kidney of diabetic rats may be a consequence of either the glycation in the lysine residue from the active site of \( \delta \)-ALA-D, which is involved in the formation of the Schiff basis with the first molecule of ALA [71], or by oxidation of essential reduce cysteinyl residues of the enzyme by ROS [64,68,72]. Furthermore, the decrease in \( \delta \)-ALA-D activity found in our study may be linked to the significant reduction in the antioxidant defenses in hepatic and renal tissues of diabetic rats, especially in NPSH content, which is responsible for preventing the oxidation of the sulphhydril groups necessary for the activity of this enzyme [15].

The treatment with resveratrol at doses of 10 and 20 mg/kg was able to prevent the inhibition in \( \delta \)-ALA-D activity in liver and kidney of diabetic rats suggesting that this compound can prevent the oxidation of essential -SH groups located at its active site of \( \delta \)-ALA-D and consequently its inhibition (Fig. 4A and B). Indeed, in our study resveratrol prevented the reduction of NPSH levels in hepatic and renal tissues in STZ- induced diabetic rats; hence, it could be expected to protect other endogenous thiols such as those found in \( \delta \)-ALA-D enzyme. Consequently, we can suggest that the prevention of a decrease of NPSH content as well as a decrease of oxidative stress in diabetic rats by resveratrol could be associated with a prevention of a decrease of \( \delta \)-ALA-D activity. Furthermore, this study clarifies that the inhibition of \( \delta \)-ALA-D enzyme is not closely related to the development of hyperglycemia in rats, pointing out the importance of antioxidants as resveratrol to minimize deleterious effects of diabetes in activity of this biologically important enzyme.

Another important aspect to be discussed in this study is serum aminotransferases activities, which have long been considered as sensitive indicators of hepatic damage [73]. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [73,74]. Therefore, the marked release of AST and ALT from liver cytosol into circulation and an increase of \( \gamma \)-GT, a membrane enzyme, indicate severe damage to
hepatic tissue membranes during the diabetes [74]. Thus, increased activities of AST, ALT, and γ-GT found in this study may be interpreted as a result of the liver cell destruction or changes in the membrane permeability indicating severe hepatocellular damage is induced by diabetes, which is in accordance with other reports [64,74,75] (Table 3). The administration of resveratrol in doses of 10 and 20 mg/Kg was able to protect against the increase in the activity of these enzymes in diabetic rats, demonstrating the protective effect of this polyphenol against hepatic damage induced by diabetic state (Table 3). These results are in agreement with those found in studies using resveratrol [51,76] and other antioxidants as rutin [76], caffeic acid and quercetin [77] and can be attributed to the capability of resveratrol to conserve the membrane integrity of cellular organelles in the diabetic state. Indeed, in our study resveratrol was able to prevent the lipid peroxidation in hepatic tissue.

In addition to the hepatic damage, in the current study, diabetic rats also presented renal damages that were evidenced by the elevation in serum urea and creatinine levels, which are considered as significant markers of renal dysfunction [78] (Table 3). Of great importance, resveratrol prevented the increase in the levels of urea and creatinine in diabetic rats. These findings suggest that resveratrol possesses the potential to attenuate renal injury caused by hyperglycemic state and this can be associated directly with the antioxidant capacity of this polyphenol, which protects the kidneys against oxidative damage, as evidenced in this and other studies [79,80].

Another important aspect to be discussed is that, in contrast with our results, Hassan-Khabbar et al. [80] related that the dose of 20 mg/kg of trans-resveratrol became pro-oxidant with an aggravation of liver injury associated with a depletion of total and reduced glutathione levels and a decrease of antioxidant enzyme activities. In addition, Gadacha et al. [81] related a possible pro-oxidant effect of resveratrol at 0.8, 2, and 5 mg/kg, depending on day/night rhythm. In fact, studies has related that depending of the concentration of the phytoalexin and on the cell type, the resveratrol can exhibit pro-oxidant properties, leading to oxidative breakage of cellular DNA in the presence of transition metal ions such as copper. Recently, it has been proposed that such a pro-oxidant action could be a common mechanism for anticancer and chemopreventive properties of plant polyphenols [82]. Furthermore, it has been suggested that resveratrol can work as a pro-oxidant under low oxidative conditions, while it becomes antioxidant under strong oxidative conditions [81]. Therefore, studies have shown that resveratrol possess biphasic function [83]. However, its pro-oxidant or antioxidant action appears to be depending on the dose, environmental factors, interactions with other compounds, medications, cell types and different pathological conditions [81,84]. For example, in our study the dose of 20 mg/kg had an important antioxidant effect in diabetic rats, while in other studies, in different pathologic conditions, the same dose showed pro-antioxidant effects [80]. Thus, more research is needed to understand the action of resveratrol on all cell types and conditions, and the optimum therapeutic concentration that applies to each condition in order to gain a complete understanding of the biphasic response of resveratrol.

Literature data have showed that the diabetes is usually associated with abnormal high levels of serum lipids, which may contribute to the development and progression of micro- and macrovascular complications [85]. In our study, there is a significant rise in serum cholesterol and triglycerides levels in diabetic rats (Table 4). However, when diabetic rats received resveratrol the levels of serum triglycerides and cholesterol presented a significant decrease. These results are supported by other works that have reported that administration of resveratrol improved lipid profile including reduction of serum cholesterol levels [86]. In addition, resveratrol appears to be beneficially modulating the lipid and lipoprotein levels, inhibiting hepatic triglycerides synthesis and reducing cholesterol and triglycerides accumulation in liver [87]. Considering the cholesterol lowering property of resveratrol, it has been suggested that it may act as inhibitor for some enzymes such as hydroxy methyl glutaryl CoA reductase, which participates in the cholesterol synthesis [86,87]. Based on these results, we can suggest that resveratrol improves the dyslipidemia while inhibiting the progression of hepatic and renal dysfunction in STZ-induced diabetic rats.

An important datum of this study is that despite the absence of the effect of resveratrol in the reduction of hyperglycemia of the diabetic rats treated with this polyphenol, there was a reduction of death of these animals when compared with the animals of diabetic/saline group. Indeed, only about 50% of the animals of diabetic/saline group survived until the end of experiment, while in the groups of diabetic rats treated with resveratrol there was a survival of about 80% of the animals (data not shown). Literature data have demonstrated that resveratrol extends the lifespan of mice through the overexpression of sirtuin 1 in a yeast diet and is linked to longer life in humans [88,89]. In addition, Baur et al. [90] shows that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet and significantly increases their survival. Although our study indicates an increase in survival of diabetic rats treated with resveratrol, our protocol lasted 30 days, therefore, no full response can be obtained and a longer-lasting protocol could determine the effects of resveratrol on lifespan of STZ-induced diabetic rats. Nevertheless, the treatment with resveratrol produced changes associated with longer lifespan, including an increase in antioxidant defense system and consequently a decrease of the oxidative stress in liver and kidney of diabetic rats.

In conclusion, the findings of the present study demonstrated that resveratrol treatment may provide effective protection against oxidative damage in liver and kidney STZ- induced type 1 diabetic rats, since this compound was able to ameliorate enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues. Moreover, it is important to point out that this is the first study to investigate the effects of resveratrol on δ-ALA-D activity in diabetic rats demonstrating the important role of this enzyme as a marker of oxidative stress. Taken together, these results may contribute to a better understanding of the hepatoprotective and renoprotective role of resveratrol, emphasizing the influence of this polyphenol and other antioxidants in the diet for human health, possibly preventing hepatic and renal complications associated with diabetes mellitus.

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