

Antioxidant and hypoglycemic effects of Sulphurous water on alloxan-induced diabetic rats: a preliminary study

Efeitos antioxidantes e hipoglicemiantes da água sulfurosa em ratos com diabetes induzido pela aloxana: um estudo preliminar

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Recibido: 25/junio/2014. Aceptado: 24/octubre/2014.

ABSTRACT

Objective: the aim of this work was to evaluate possible anti-diabetic effects of drinking sulphurous water on rats.

Materials and Methods: rats were separated in control group, sulphurous water group, alloxan-induced diabetes mellitus with or without sulphurous water drinking. Glycemia and blood superoxide dismutase (SOD) values were determined.

Results: alloxan-induced diabetic rats had increased glycemic values and massive oxidative stress evidenced by decreased SOD values. Sulphurous water drinking reversed the diabetic effects of alloxan.

Conclusion: sulphurous water reversed the hyperglycemic state of rats and improved SOD synthesis, offering beneficial effects for diabetics.

KEY-WORDS

Sulphurous water; diabetes.

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RESUMO

Objetivo: o objetivo deste trabalho foi avaliar os possíveis efeitos anti-diabéticos de ingerir água sulfurosa em ratos.

Material e Métodos: ratos foram divididos em grupo controle, grupo de águas sulfurosas e diabetes induzido por aloxana com ou sem água sulfurosa. Foram determinados os valores sanguíneos de glicemia e da enzima superóxido dismutase (SOD).

Resultados: no grupo de diabetes induzido pela aloxana houve aumento dos valores de glicemia e de estresse oxidativo pela diminuição dos valores de SOD. A ingestão de água sulfurosa reverteu os efeitos diabéticos da aloxana.

Conclusão: a água sulfurosa inverteu o estado hiperglicêmico de ratos e melhorou a síntese de SOD, oferecendo efeitos benéficos aos diabéticos.

PALAVRAS-CHAVE

Água sulfurosa; diabetes.

List of abbreviations.

DM = diabetes mellitus.

EDTA = diaminoethanetetraacetic acid.

GPX = glutathion peroxidase.

GSH = glutathion.

NBT = nitroblue tetrazolium.

PBS = phosphate buffered saline.

SOD = superoxide dismutase.

T1DM = type 1 diabetes mellitus.

T2DM = type 2 diabetes mellitus.

INTRODUCTION

When oxygen, nitrogen or chlorine reactive species are excessively formed in cells and tissues they are implicated in many body disorders, including the type I and type II diabetes mellitus (DM)^{1,2}. Oxidative and nitrosative stresses occur when the production of free radicals is far higher the values of antioxidant defense substances, resulting in many peroxidative and nitrosative damaging reactions which in turn cause since DNA injury and mutations until cell death by necrosis or apoptosis². DM is characterized by impairment on pancreatic secretion of insulin and consequently the accumulation of carbohydrates on blood which is known as hyperglycemia.

In DM patients it is well established that oxidative and nitrosative stress decreases cell antioxidant enzyme defenses, especially the glutathione (GSH), and increase the blood and plasma superoxide dismutase (SOD) activity in order to detoxify the superoxide anion mitochondrial overload³⁻⁶. In macrophages of alloxan-induced diabetes, there were higher levels of SOD activity which were further stimulated by melatonin activity, but decreased by insulin action⁷. Then, the SOD enzyme activity suffers a dual role in DM pathogenesis.

The highly toxic and reactive hydroxyl free radicals are released as a consequence of the pro-inflammatory immunoglobulin-mediated beta cell attack in type I diabetes mellitus (T1DM) patients, whereas they are also produced in type 2 DM (T2DM) patients as a consequence of the blood glucotoxicity and lipotoxicity⁸.

Some studies had been addressed the potential benefits potential sulphurous water drinking on antioxidant systems and cell protection. Sulphurous water contains higher levels of hydrogen sulfide (H₂S) which modulates mitochondrial function, and acts as an electron donor to the respiratory chain⁹. Beyond antioxidant effects, H₂S is transformed to thiosulphate and sulphite which acts restoring glutathione levels⁹.

In this manner, the hydrogen sulfide from thermal mineral water decreased oxidative-induced DNA damage¹⁰. Another study reported that drinking of sulphurous water protected testicular cells against oxidative-induced apoptotic cell death¹¹. Drinking of sulphurous thermal water restored serum insulin levels, protected pancreatic beta cells from oxidative damage and improved pancreatic SOD values¹².

The objective of this work was to evaluate possible anti-diabetic effects of sulphurous water drinking on rats.

METHODS

Animals and operation procedures

Male Wistar rats (n=24) with 250g of weight, housed in constant conditions of light (photoperiod L:D 12:12) and temperature (20-22°C) with *ad libitum* access to water and food were maintained by one week for adaptation.

After the adaptation period, the animals were separated in four experimental groups: G1-control (n=6), G2-non-sensitized by alloxan but treated by sulphurous water (n=6), G3-alloxan-sensitized not treated by sulphurous water (n=6); and G4-alloxan-sensitized and treated by sulphurous water (n=6). G1 and G2 groups received an equivalent volume of saline to 0.9%, following the same experimental protocol, whereas G3 and G4 received intravenous injection of alloxan (42mg/Kg of body weight) in caudal region¹³.

After four days of alloxan sensibilization glycemia was checked using an "accu-chek advantage" (Roche) glucometer and this was considered the first experimental day. Then the glycemia was also evaluated after 7, 14, and 21 days.

The sulphurous water was daily collected from the "Andrade junior" source at the Barreiro's hydromineral ranch. The sulfur mud from Andrade junior source contains sulphurous mineral water as well as aluminum, iron, copper, boron, zinc, manganese, chromium, lead, and sulphur¹⁴. The mineral water from Andrade junior is alkaline, and rich in carbonatites, carbonates, calcium, magnesium, and silicon dioxide¹⁵.

To G3 and G4 groups sulphurous water was administered *ad libitum* as the unique drinking water resource.

In the morning of the 21th day, animals were anesthetized with pentobarbital (50mg/Kg body weight, in-

traperineal) and submitted to surgical procedure, which removed the organs for determination of body weight. The spleens were also removed and stored in phosphate buffered saline (PBS) for further superoxide determination. The blood samples were collected for superoxide dismutase (SOD) determination^{16,17}. All procedures were submitted to ethical evaluation and obtained institutional approval.

Obtaining plasma

The blood samples were collected within heparinized tubules, centrifuged for 15 minutes at 160G, and the plasma separated and stored at -20°C for later determination of the superoxide dismutase (SOD).

Cu,Zn-Superoxide Dismutase Determination (CuZn-SOD)

Analysis of the CuZn-SOD enzyme was performed using the nitroblue tetrazolium (NBT) reduction method (Sigma). Spectrophotometric reading was performed at 560 nm¹³. The individual plasma samples were placed in glass tubes, with another tube containing a standard solution. Each tube contained 0.5 mL of the sample, and the standard tube contained 0.5 mL of hydro-alcoholic solution. Next, 0.5 mL of chloroform-ethanol solution (1:1 ratio) and 0.5 mL of reactive mixture (NBT increased by diaminoethanetetraacetic acid - EDTA) was added to the tubes. The experimental and standard solutions received 2.0 mL of buffer carbonate, and the pH was increased to 10.2 after the addition of hydroxylamine. The tubes remained still at room temperature for 15 min and were subsequently read at 560 nm^{16,18}. Superoxide dismutase was calculated as follows:

$$\text{CuZn-SOD} = (\text{Ab standard} - \text{Ab sample} / \text{Ab standard}) \times 100$$

$$= \% \text{ reduction of NBT} / \text{CuZn-SOD.}$$

The results were expressed in international units (IU) of Cu,Zn-SOD (12,13).

Statistical analysis

For comparison of the glycemia and body weight values of the four experimental groups the non-parametric Kruskal-Wallis test was used. For analysis of the superoxide dismutase enzyme the t-student test was performed. A p value small than 0.05 was used to characterize a significant statistical difference among groups ($p < 0.05$).

RESULTS

After 21 days of follow up, animals treated by sulphurous water only (G2-group) which did not receive alloxan, had lower body weight (230g) in relation to those non-treated, non-sensitized G1-group animals (256g) and compared to alloxan-induced diabetes, treated or not with sulphurous water (243g e 241g, respectively).

Figure 1 represents the glycemic mean of experimental animals. It can be observed that alloxan had induced diabetes mellitus increasing the glycemic values to $247,3 \pm 12,8 \text{ mg/dL}$ (G3). This mean value was statistically different from those occurred with G1 and G2 groups ($p < 0.05$). Interestingly, sulphurous water drinking had lowered glycemic values in alloxan-induced DM (G4) compared to alloxan-treated only (G3) ($p < 0.05$).

Alloxan-treated animals had lower SOD values compared to sulphurous water. Diabetic rats who drunk sulphurous water had decreased glycemic values and increased SOD concentration (**Figure 2**).

DISCUSSION

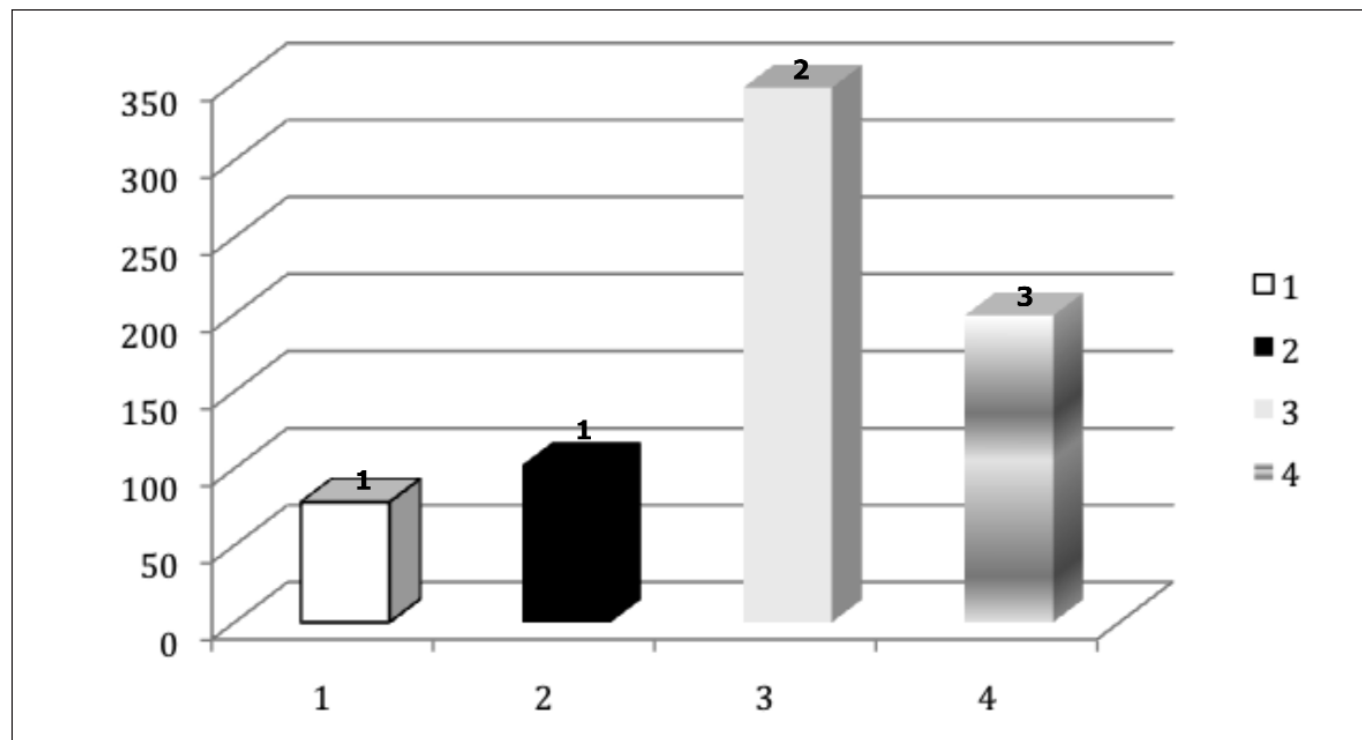
Although clinically controlled DM patients frequently have higher SOD levels^{5, 19,20} as a vivid expression of the defensive activity of the antioxidant system, uncontrolled DM causes impaired antioxidant defenses resulting in lower levels of SOD²¹. This is due to the fact that glycemic fluctuations have been associated with triggering of oxidative stress in DM patients²².

Chronic maintenance of hyperglycemia is suggested to be the main cause of long-term damage to the cardiovascular system, eyes, kidney, and nerves^{23,24}.

Free radical overload in diabetic patients induces both massive lipid peroxidation and hemoglobin glycation due to glucotoxicity which has been associated with decreased levels of erythrocyte glutathion peroxidase (GPX), GSH and increased levels of erythrocyte SOD²⁵. Although this study²⁵ had found increased levels of SOD in DM, other studies in diabetic brain, diabetic retinopathy, and other diabetic patients SOD levels were found to be decreased²⁶⁻³⁰. Diminishing levels of SOD values were also observed in the current work.

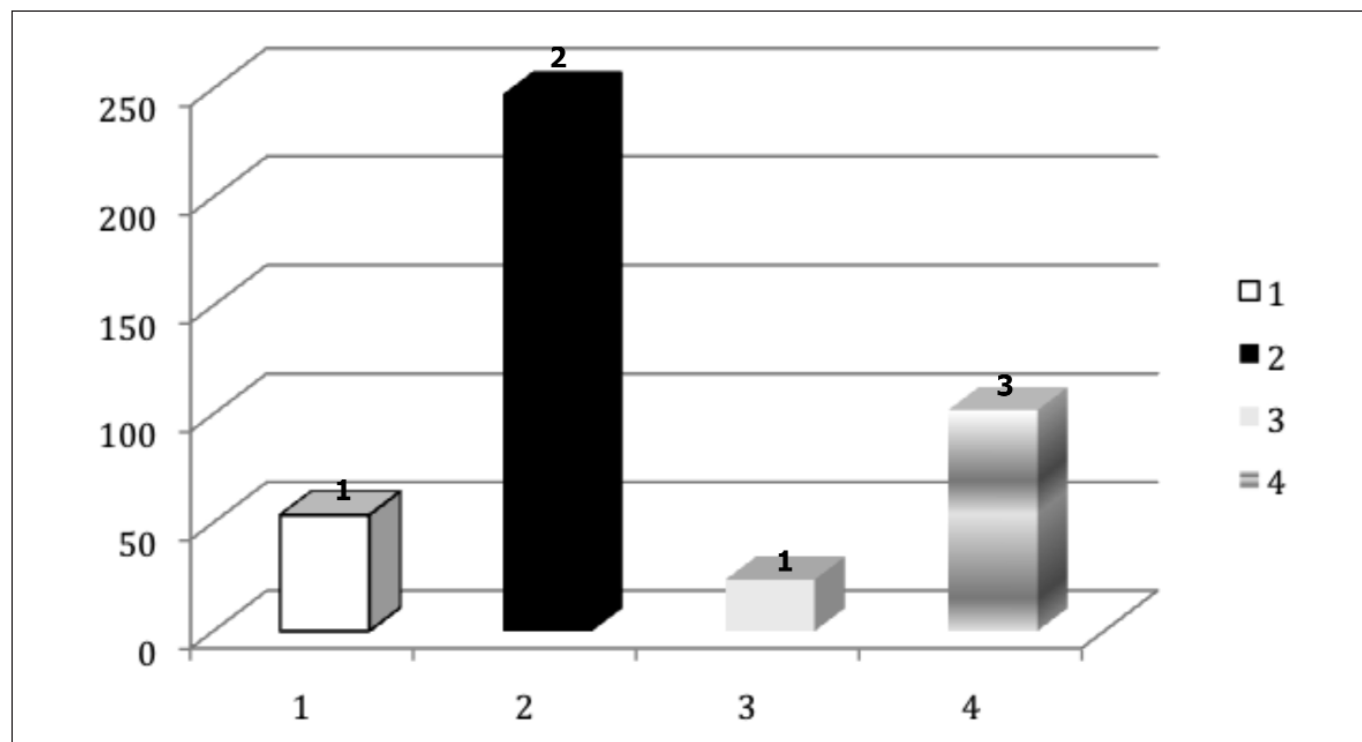
In the present study, experimental induction of diabetes had been associated with decreased SOD levels indicating that oxidative stress had depleted the antioxi-

Figure 1. Glycemic value of alloxan-induced diabetes treated or non-treated by sulphurous water.



1(G1): control (saline); 2(G2): sulphurous water; 3(G3): alloxan-induced DM; 4(G4): alloxan + sulphurous water. Different numbers mean significant statistical differences among groups ($p < 0.05$).

Figure 2. Blood superoxide dismutase (SOD) values of alloxan-induced diabetes animals treated or non-treated by sulphurous water.



1(G1): control (saline); 2(G2): sulphurous water; 3(G3): alloxan-induced DM; 4(G4): alloxan + sulphurous water. Different numbers mean significant statistical differences among groups ($p < 0.05$).

dant stores. Interestingly sulphurous water drinking partially reversed both the hyperglycemic state and SOD levels. As should be noted H₂S from sulphur water has important antioxidant activity including glutathione repletion and protection of cell DNA from oxidative damage^{9,10}. Possibly due to its cellular protective effects^{10,11}, sulphur water drinking had increased insulin secretion, restored pancreatic SOD values, and protected beta cells from oxidative-induced damage¹².

It should be noted conservation of the antioxidant systems (SOD and other antioxidant enzymes) and the turnover of the oxidative stress in diabetes mellitus is essential for better adequacy of the phagocytic function and destruction of intracellular and tissue pathogens^{30,31}.

Moreover, the positive antioxidant effect of sulphurous water is in accordance with previous studies which demonstrated that taking a shower with sulphurous water improved body antioxidant defenses and decreased oxidative stress³². Sulphurous water had also antioxidant effects on cultured neutrophils³³. Beyond, sulphurous water displayed protective effects on testicular function of diabetic rats, avoiding apoptotic cell death³⁴.

In DM patients, improvement of SOD levels is also essential to increase endothelial progenitor cells improving vascular repairing capacity as well as fundamental to preserve cardiac morphology and avoid cardiomyopathy³⁵.

Among the important limitations of the present study can be reported lower number of animals in the experimental groups, lack of a temporal oxidant-antioxidant analysis, and lacking of determination of other oxidant and antioxidant biomarkers.

CONCLUSION

Diabetes mellitus induced hyperglycemia and oxidative stress evidenced by decreased SOD values. Sulphurous water reversed the hyperglycemic state of rats and improved SOD synthesis, which suggest beneficial effects for diabetics.

ACKNOWLEDGE

Financial support: Edital IMEA/PRPPG n.3 – 2014 (Propesq-Unila 2014-2015). Authors wish to thank the reviewers for their important work.

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