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Aqueous extracts of *Sphagneticola trilobata* attenuates streptozotocin-induced hyperglycaemia in rat models by modulating oxidative stress parameters

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Abstract
Male albino rats with diabetes induced by the administration of streptozotocin (45 mg/kg, i.v.) were treated with oral administration of *Sphagneticola trilobata (S. tri)*. *S.tri* reduced blood glucose levels and improved weight gained which was accompanied by a marked restoration of decreased vitamin C and reduced glutathione in liver and kidney tissues) of STZ-treated rats. *In vitro* data revealed that *S.tri* caused an inhibition of lipid peroxidation under Fe^{2+} or sodium nitroprusside assaults. Conversely, *S. tri* also caused a reduction in the high levels of thiobarbituric acid reactive substances (TBARS) observed in the liver, kidney, and testes as well as high serum triglyceride, ALT and AST of diabetic rats. Finally, the inhibition of catalase, delta aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase accompanied by hyperglycemia were relieved by *S. tri* in all tissues examined. Hence, the empirical use of *S. tri* in folkloric medicine may have some scientific justification.

Keywords: *Sphagneticola trilobata*; diabetes; antioxidant; vitamin C; glutathione; TBARS; lactate dehydrogenase; delta aminolevulinic acid dehydratase.

Introduction

Diabetes is a chronic disease that cannot be completely cured and may develop complications if not properly regulated. A serious metabolic disorder with micro- and macrovascular complications results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics worldwide. In fact, despite the implementation of dietary intervention strategies and the presence of numerous educational programs, the prevalence of diabetes continues to increase (Boston *et al*., 1997) Prolonged hyperglycemia is a primary cause of most long-term complications of diabetes. It causes increased protein glycation, which has been shown to be a source of free radicals (Ceriello, 1999). Reactive oxygen species (ROS) and the products of advanced glycosylation are significant in the onset and development of complications in chronic diabetes (Jakus *et al*., 1998). In addition, it has been equally suggested that cell membrane lipid peroxidation is involved in the etiology of neurodegenerative disorder associated with diabetes (Jenner, 1994); lipid peroxides may cause oxidative damage to the myelin sheath surrounding the nerve. Therefore, oxidative stress may predispose diabetic patients to the development of neuropathy by a mechanism involving increased lipid peroxidation (Dickinson *et al*., 2002).

The current treatment of diabetes although provide good glycemic control but do a little in preventing complications. Besides, these drugs are associated with side effects (Rang *et al*., 1991). Moreover, providing modern medical healthcare across the world (especially in developing countries) is still a far-reaching goal due to economic constraints. Consequently, there is a growing need to develop novel approaches towards the management and prevention of diabetes. In this context, it has been shown that despite access to conventional medical facilities, the use of and dependence upon complementary and alternative medicine continues to thrive (Johns *et al*., 1990; Berman *et al*., 1999). Thus, it is not surprising that numerous publications over the last 2 decades have underlined the importance of exploring

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traditional medicine as a potential source of effective diabetic remedies (WHO, 1980; Bailey and Day, 1989; Ryan et al., 2000). A wide variety of the traditional herbal remedies are used by diabetic patients, especially in the third world countries (Day, 1998; Gray and Flatt, 1998; Shimada *et al*., 1998) and may therefore, represent new avenues in the search for alternative hypoglycemic drugs. In fact, in recent years, traditional or complementary medicine has seen an upsurge and reports have indicated both in developing and developed countries, people had used at least one form of unconventional therapy including herbal medicine in the treatment of diabetes (Eisenberg *et al*., 1993; Maclennan *et al*., 1996). Therefore, medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system.

Indeed, according to the previous investigations there are some medicinal plants and herbs which exert hypoglycemic effect such as *Balanites aegyptiaca* fruits (Kamel *et al*., 1991; Eskander, 1994), *Trigonella foenum* (*Hulba* seeds) (Ali *et al*., 1995) and two Acacia species (*Acacia nilotica* and *Acacia farnesiana L. wild*) (Wassel *et al*., 1992). Additionally, there are reports indicating that worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes (Marles and Farnsworth, 1995). Although most of these species have not undergone rigorous scientific evaluation, over 80% of those that have been tested show antidiabetic activity (Marles and Farnsworth, 1995). It is also worth noting that a number of drugs currently used to treat diabetes are historically derived from plants. These include metformin (derived from *Galega officinalis*) (Oubre *et al*., 1997) and 4-hydroxyisoleucine that is derived from *Trigonella feonum-feacum*) (Broca *et al*., 2004). Despite this convincing evidence, and the presence of several documents which confirm the wealth of ethnomedicinal no work has been done to examine the antidiabetic potential of *Sphagneticola trilobata*.

Sphagneticola trilobata is a creeping herb, native to the tropics of Central America and has naturalised in many wet tropical areas of the world. It forms a dense ground cover, crowding out or preventing regeneration of other species. Cultivated as an ornamental, it is tolerant to inundation and high levels of salinity (Wagner *et al*., 1990). Unpublished reports indicate that aqueous infusion of *Sphagneticola trilobata* has been employed locally and empirically in southern part of Brazil in the management of diabetes. In fact, it is popularly referred to as *insulina* due to its observed antidiabetic properties. However, there has not been a scientific appraisal of this plant for antidiabetic effect. The objective of this study was to evaluate *Sphagneticola trilobata* as a medicinal plant that demonstrate an antidiabetic potential so that it may be integrated into alternative and/or complementary intervention strategies in the management of type 2-induced hyperglycemia.

Materials and Methods

Chemicals

ð-Aminolevulinic acid (ð-ALA), *p*-dimethylaminobenzaldehyde, streptozotocin (STZ), reduced glutathione (GSH), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Plant material

Leaves of *S. trilobata* were gathered from different places around the university campus of the Federal University of Santa Maria, RS, Brazil. The plants were identified at the Biology/Botany Department of the same University. The leaves were air-dried for 14 days (temperature range 30±4^{0}C). After drying, the leaves were grounded into powder using a blender. The powdered leaves were stored in polythene bags and placed in a freezer (temperature range 0–4^{0}C) until they were used.

Preparation of plant extracts

Aqueous extracts infusion of *S. trilobata* was prepared by weighing ten grams of powdered leaves in a beaker and two hundred milliliters of distilled water was added to the beaker containing the powdered leaves, placed on a heating element, and allowed to boil for ten minutes for extraction. Thereafter, the solution was filtered separately using a Whatman filter paper. The extracts were evaporated using an oven preset at 45^{0}C. The extract was reconstituted in distilled water prior to usage.

Animals

Adult male Wistar rats weighing 180–200 g were used for the experiments. All rats received food (Guabi, Ribeirao Preto, SP, Brazil) and water *ad libitum* and were kept on a 12 h ligth/12 h dark

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cycle, in a room with the temperature regulated to 21–25°C and humidity at roughly 56%. The animals were used accordingly to guidelines of the Committee on Care and use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil.

Diabetes induction

Diabetes was induced by a single intravenous injection of streptozotocin (STZ) 45 mg/kg), diluted in 0.1M citrate-buffer (pH 4.5). Control rats received an equivalent amount of the buffer. Diabetic state was checked 72 h after induction with STZ. Blood samples were taken from the tail vein of rats to determine glucose levels by an automatic autoanalyzer (GLUCOTREND®). Animals were considered diabetic when blood glucose levels exceeded above 250 mg/dl.

Treatment

The animals were randomly divided into the following groups: (1) control; (2) *S. trilobata* (3) streptozotocin (STZ); and (4) STZ + *S. trilobata.* Groups 2 and 4 were administered with aqueous infusion of *S. trilobata* by gavage at the dose of 50 mg/kg (once a day) for 30 days after the administration of STZ. Control rats were orally administered with water. At the end of the experimental period, diabetic rats and the corresponding control animals were anesthetized with ether and euthanized by decapitation. Rats were fasted 12 h prior to the euthanasia.

Tissue preparation

Tissue samples (liver, kidney, spleen, and testes) were quickly removed, placed on ice and homogenized in cold 50mM Tris–HCl pH 7.4. The homogenate was centrifuged at 4000×*g* for 10 min to yield the low-speed supernatant (S1) fraction that was used for biochemical assays. For all analysis, protein content was determined by the method of Lowry *et al*., (1951), using bovine serum albumin as the standard.

In vitro TBARS assay

Hepatic tissues from untreated rats were prepared for *in vitro* TBARS assay as described earlier. An aliquot of 100 µl of S1 was incubated for 1 h at 37^{0}C in the presence of *S. trilobata* with and without the pro-oxidants; iron (final concentration (10µM) and sodium nitroprusside (final concentration 3 µM). This was then used for lipid peroxidation determination. One rat was used per experiment. Production of TBARS were determined as described by Ohkawa *et al.* (1979) excepting that the buffer of color reaction have a pH of 3.4. The color reaction was developed by adding 8.1% sodium dodecyl sulphate (SDS) to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95^{0}C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

In vivo TBARS assay

Production of TBARS was determined as described by Ohkawa *et al*. (1979) except that the buffer for the color reaction was pH 3.4. The color reaction was developed by adding 300 µl 8.1% SDS to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% TBA. This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532 nm, and the absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

Antioxidant defense systems

Vitamin C content

Vitamin C levels were determined colorimetrically as described by Jacques-Silva, *et al.,* (2001). Briefly, proteins were precipitated in 10 volumes of cold 4% trichloroacetic acid solution. An aliquot of the sample (1 ml) was incubated for 3 h at 38°C then 1 ml H_2SO_4 65% (v/v) was added. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and CuSO4 (0.075 mg/ml), and the absorbance of the colored product was measured at 520 nm. The content of ascorbic acid is related to tissue amount (µmol ascorbic acid/g wet tissue).

GSH levels

Reduced glutathione (GSH) content was estimated using Ellman's reagent after deproteinization with TCA (5% in 1 mmol/EDTA) as described by Ellman, 1959.

Enzymes activity

δ-ALA-D

ð-ALA-D activity was assayed according to the method of Sassa, (1982) by measuring the rate of product porphobilinogen (PBG) formation except that 84mM potassium phosphate buffer, pH 6.4 and 2.4mM ALA were used. The reaction was started 10 min after the addition of enzyme

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by adding the substrate (ð-ALA). Incubations were carried out 1 h (liver), 2 h (kidney) and 2 h (spleen) and 2 h (testes) at 37^0C. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of 6.1×104M^{-1} for the Ehrlich-PBG salt.

LDH Activity

LDH activity was monitored spectrophotometrically by the rate of increase in absorbance at 340 nm at 37°C resulting from formation of NADH. The assay medium contained 21 mM of glycine-KOH buffer (pH 10), 50 mM of lactate (pH 6.8), 30µL of liver (150 to 200µg of protein) or spleen (150 to 200 µg of protein) or kidney (120 to 180 µg of protein), testes (120 to 150 µg of protein) and the reaction was started by adding neutralized NAD^+ to provide a final concentration of 0.25 mM.

Catalase

Catalase activity was assayed spectrophotometrically by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of the homogenate at 240 nm. The enzymatic reaction was initiated by adding an aliquot of the homogenized tissue and the substrate (H_2O_2) to a final concentration of 0.3 mM in a medium containing 50 mM phosphate buffer, pH 7.0. The enzymatic activity was expressed in units (1 U decomposes 1 µmol H_2O_2 min^{-1} at pH 7 at 25°C).

Biochemical analyses

Plasma AST (aspartate aminotransferase), ALT (alanine aminotransferase) and urea, creatinine, cholesterol, triglyceride and uric acid levels were determined using commercial Kits (Labtest, Minas Gerais, Brazil).

Statistical analysis

All values obtained are expressed as Mean ± SEM. The data were analyzed by ANOVA and MANOVA analyses of variance followed by Duncan's multiple range tests when appropriate. Differences between groups were considered to be significant when $p < 0.05$.

Results

Body weight of animals

Two-way ANOVA [(with or without STZ) × (with or without *S. trilobata*)] revealed that from the third week, there was a marked reduction in body weight gain in all STZ-treated rats when compared to the control group (p<0.05) (Fig. 1). It is noteworthy that *S. trilobata* treatment did not affect the marked reduction in body weight observed in diabetic rats (groups 2 and 4).

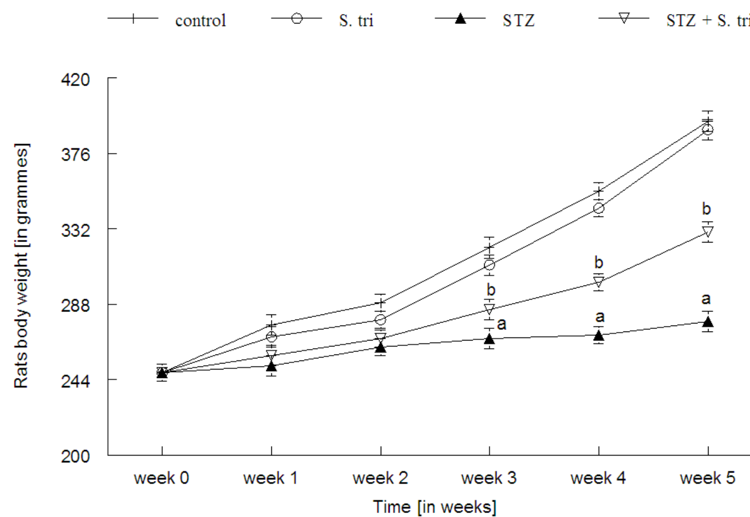

Figure 1: Body weight (mean ± SEM, n=8) of streptozotocin (STZ)-induced diabetic rats treated with *S. trilobata*. Lower case letters ([a]) and ([b]) indicate significant difference from the control group at p < 0.05.

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Organ to body weight ratio

Table 1 shows that STZ caused an increase in the organ to body weight ratio for all the tissues analyzed. In fact, two-way ANOVA shows that treatment of animals with *S. trilobata* significantly restored the organ to body weight ratio to control level (p < 0.05).

Table 1: Organ-to-body weight ratio (mg/g) in STZ-induced diabetic rats treated with *S. trilobata*.

Groups	Liver	Kidney	Spleen	Testes
Control	34.2 ± 11.2	8.5 ± 0.6	6.7±1.2	15.4±3.6
S. tri.	33.5 ± 10.3	8.2 ± 0.5	6.8±1.4	15.8±2.9
STZ	47.7 ± 11.3*	15.7 ± 1.3*	7.19±1.2	15.2±3.1
STZ+*S. Tri.*	34.2 ± 11.1	8.3 ± 0.7	7.0±1.5	16.1±2.7

Data are expressed as Mean ± S.E.M. of seven animals.
Denoted $p < 0.05$ as compared to the STZ group (ANOVA/Duncan).
* Denoted $p < 0.05$ as compared to the control group (ANOVA/Duncan).

Plasma glucose levels

Blood glucose levels were significantly (P < 0.05) higher in diabetic rats than in control groups. *S. trilobata* caused a significant decrease in the glucose levels of STZ diabetic. This anti-hyperglycemic effect of *S. trilobata* was evident in the fifth week after STZ administration (Figure 2).

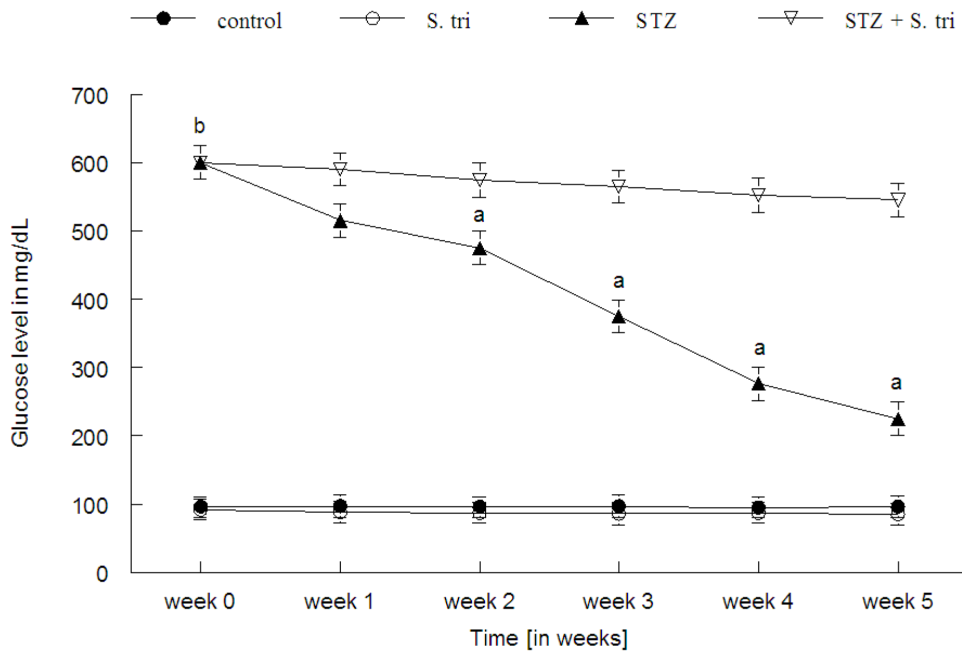

Figure 2: Glucose level (mean ± SEM, n=8) of streptozotocin (STZ)-induced diabetic rats treated with *S. trilobata*. Lower case letter ([a]) indicates significant difference from the control group ([b]) at p < 0.05.

Biochemical analysis
Plasma AST, ALT and triglyceride was increased in diabetic rats when compared to control group (Table 2A). *S. trilobata* treatments restored the activities of the enzymes as well as triglyceride levels in STZ treated rats. Conversely, the activities of hepatic ALT and AST were significantly decreased whereas treatment with *S. trilobata* prevented the leakage of these enzymes into the plasma (table 2B). However, as observed in table 2A, urea and creatinine were not significantly modified by STZ and/or *S. trilobata* treatments.

Table 2: Biochemical parameters of STZ-induced diabetic rats treated with *S. trilobata* Data are expressed as means ± SEM of eight animals. ALT and AST denote alanine aminotransferase and aspartate aminotransferase.

Parameters	Control	*S.tri*	STZ	STZ + *S. tri*
A) Plasma				
ALT*	121.5±10.5	155±28	229.7±34.6[a]	165.7±62.4
AST*	133.8±17.3	141.2±5.4	232.4±56.2[a]	162.3±45.3
Triglyceride#	193.7±33.2	187.5±31.43	377.1±37.3[a]	283.5±56.3[b]
Urea#	71.2±5.12	67.32±3.4	66.3±5.3	71.8±4.3
Creatinine#	0.593±0.09	0.672±0.21	0.621±0.34	0.611±0.29
B) Liver				
ALT*	342.5±15.3	355±35	121.7±21[a]	275.7±47
AST*	354.8±67	341.2±34	133.4±56[a]	283.3±56

Data are expressed as means ± S.E.M. eight animals.
Data of renal markers, lipid and uric acid levels are presented as mg/dl.
* Data of enzyme activities are presented as U/l.
[a]Significantly different from the control group (ANOVA/Duncan, $p < 0.05$).
[b]Significantly different from the diabetic group (ANOVA/Duncan, $p < 0.05$).

Ex vivo thiobarbituric acid reactive substances
Levels of TBARS in both liver and kidney and spleen were markedly elevated (P<0.05) in STZ -treated animals in comparison with control rats (Table 3). However, there was no marked difference in the TBARS level of testes tissue of either STZ- or *S. trilobata*-treated rats. It was also observed that, in relation to group 1 animals, *S. trilobata* caused a decrease in the levels of TBARS in both liver, kidney and spleen tissues of group 4 animals. Nevertheless, the levels of hepatic and renal TBARS in group 4 were still significant (P<0.05) in the fifth week in relation to the control group.

Effect of S. trilobata on in vitro Fe (II) and SNP-induced hepatic TBARS production
Fig. 3 shows that *S. trilobata* caused a significant concentration dependent inhibitory effect on Fe (II) and sodium nitroprusside induced lipid peroxidation in the liver homogenate ($p < 0.05$). Apparently, the inhibition pattern of *S. trilobata* on the two prooxidant-induced TBARS production is similar. In contrast, *S. trilobata* has no effect on basal production of TBARS in the hepatic tissues (P < 0.05).

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Table 3: Levels of thiobarbituric acid reactive substances (TBARS) and non-enzymatic antioxidant defenses in STZ-induced diabetic rats treated with *S. trilobata*. Data are expressed as means±SEM of eight animals.

Parameters	Control	*S.tri*	STZ	STZ + *S. tri*
TBARS[A]				
Liver	521.6±28.2	586±43.11	952±128[a]	692.1±35.3[b]
Kidney	332.1±37.22	367.7±23.2	653.2±43.01[a]	435.8±23.02[b]
Spleen	432.2±36.11	456.1±135.3	682.2±135[a]	498.9±126.1[b]
Testes	197±21.02	201.1±27.12	210.2±23	189.9±21
Vitamin C[B]				
Liver	519.45±20.43	545.44±47.34	287.21±29.10[a]	514.22±89.7[b]
Kidney	373.1±41.18	367.8±32.19	228.7±21.9[a]	365.5±37.8[b]
Spleen	547.71±44.24	537.11±41.6	532.4±39.8	521.8±29.7
Testes	293.3±27.37	289.7±28.28	281.7±28.5	284.2±25.6
GSH[D]				
Liver	31.3±4.11	28.73±3.43[b]	14.37±1.37[a]	20.87±4.54[b]
Kidney	15.76±3.12	14.89±2.35[b]	9.73±0.73[a]	11.37±1.48[b]
Spleen	11.31±1.76	11.28±1.39	10.99±1.87	11.01±1.57
Testes	8.47±0.97	8.27±1.78	8.88±1.85	9.01±1.98

Data are expressed as means±S.E.M. of eight animals. [A]Unit of TBARS is μM MDA/hr/g tissue; [B]Unit of Vit C is μg AA/g tissue; [D]GSH levels are presented as μmol/g of tissues. [a]Different from the control group (ANOVA/Duncan, $p < 0.05$). [b]Different from the diabetic group (ANOVA/Duncan, $p < 0.05$).

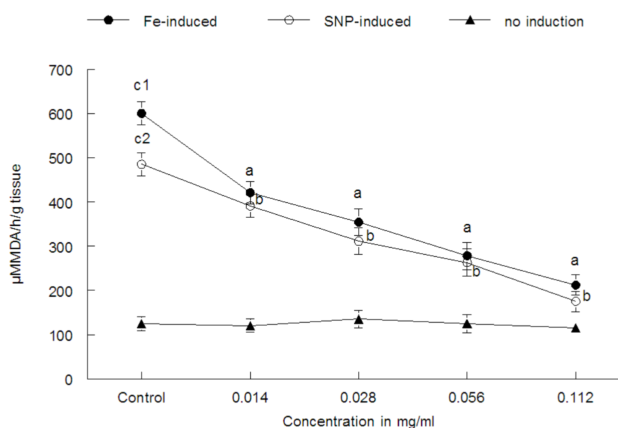

Figure 3: *In vitro* effect of different concentrations of *S. trilobata* on iron (II) (10 μM) and sodium nitroprusside (3μM) –induced TBARS production in liver homogenates. Data shows mean ± SEM values average from three independent experiments performed in duplicate in different days. ([a]) and ([b]) indicate significant difference from the control groups (c1) and (c2) respectively.

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Antioxidant defenses

Vitamin C levels

Hepatic, renal and testes levels of vitamin C were decreased in STZ-treated rats. *S. trilobata* treatment normalized Vitamin C levels in all tissues of STZ treated rats (Table 3). It is noteworthy that the level of vitamin C in the spleen was not modified either by STZ or *S. trilobata* ($p < 0.05$).

GSH levels

Hepatic and renal GSH levels were significantly diminished by STZ treatment. *S. trilobata* treatment was able to restore the diminished level of GSH in both organs. On the other hand, neither STZ nor *S.trilobata* alter the levels of GSH in the spleen and testes of the animals. (Table 3).

Enzyme activity

δ-ALA-D

Generally, ð-ALA-D activities were significantly reduced in the liver ($P<0.0001$), kidney ($P<0.0001$), spleen and testes ($P<0.05$) of STZ-treated rats when compared to the control group (Table 4). However, a comparison of group 3 and 4 rats revealed that *S. trilobata* significantly relieved the inhibition of ð-ALA-D imposed by STZ in all tissues ($P<0.05$). A two-way ANOVA revealed a significant interaction for the enzyme activity in all tissues ($P<0.00001$) (Table 4).

Lactate dehydrogenase

Table 4 shows that STZ treatment caused a significant decrease ($P<0.001$ for liver; $P<0.0001$ for kidney, spleen and testes) in all isoforms of LDH evaluated as compared to the control group. However, treatment of diabetic rats with *S. tri.* significantly improved LDH activities, returning them to control levels. Two-way ANOVA of the activities of all isoforms of LDH yielded a significant interaction ($P<0.00001$). The inhibition is significant because, as we observed, STZ caused a marked decrease in the activities of LDH in the different tissues analyzed, and *S.tri.* treatment was able to markedly relieve the observed inhibition. In addition, for all isoforms, the increase in LDH activity in group 4 rats was significant ($P<0.0001$) when compared to that in groups 3 rats.

Catalase

Catalase activity was significantly elevated in all the tissues analyzed in the diabetic rats when compared to the control group. *S. tri.* caused a decrease in the activity of catalase in the kidney and spleen and this decrease was significant ($P < 0.05$) in the liver (Table 4). Two-way ANOVA yielded a significant ($P<0.05$) interaction only for the renal and hepatic enzyme.

Table 4: Activity of catalase, lactate dehydrogenase and ð-Aminolevulinic acid dehydratase in STZ-induced diabetic rats treated with *S. trilobata*.

Parameters	Control	*S. tri*	STZ	STZ + *S. tri*
Catalase[A]				
Liver	73212±8398	73739±9898	97893±15478[a]	69898±17564
Kidney	54387±17675	52897±16398	58878±17899	50827±17867
Spleen	56721±13219	53765±13456	59342±11234	55643±11658
Testes	43756±12343	44878±13786	48787±14356	47175±12343
Lactate dehydrogenase[B]				
Liver	3954±43	3787±56	1673±48[a]	3546±61
Kidney	1743±39	1878±27	1237±44[a]	1897±49
Spleen	1742±30	1702±37	1323±41[a]	1687±29

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Testes	996±37	787±38	503±34[a]	998±39
ð-aminolevulinic acid dehydratase[D]				
Liver	0.721±0.08	0.779±0.09	0.299±0.07[a]	0.728±0.08
Kidney	0.575±0.062	0.583±0.074	0.373±0.073[a]	0.545±0.092
Spleen	0.251±0.06	0.244±0.09	0.109±0.05[a]	0.221±0.014
Testes	0.150±0.042	0.148±0.024	0.132±0.03[a]	0.148±0.018

Data are expressed as means ± S.E.M. of eight animals. [A]Unit of Catalase is μmol H_2O_2 /mg protein/min; [B]Values are expressed as change in absorbance/mg protein/minute and [D]Unit of ALAD is nmol of PBG/μg protein/hr. [a]Different from the control group (ANOVA/Duncan, $p < 0.05$).

Discussion

Although no literature data have given any insight into the possible toxicological potentials of *S. trilobata,* the dose of 50mg/kg body weight chosen for treating animals did not cause obvious signals of toxicity. In fact, administration of aqueous infusion of *S. trilobata* 50 mg/kg did not affect the body weight gain of animals and did not cause evident signs of liver injury as indicated by the activities of plasma AST and ALT. In STZ-diabetic rats the activities of plasma AST and ALT were significantly (P<0.05) increased relative to their normal levels. The increment of the activities of AST and ALT in the plasma may be associated to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993), which gives an indication on the hepatotoxic effect of STZ. On the other hand, treatment of the diabetic rats with aqueous extracts of *S. trilobata* caused reduction in the activity of these enzymes in plasma compared to the mean values of the diabetic group. In contrast, the activity of AST and ALT were significantly (P<0.05) decreased in the liver tissue of STZ-diabetic rats relative to the control values (Table 2). Reduction of such activities is mainly due to leakage of these enzymes from the liver into the blood stream as a result of STZ toxicity which leads to the liver damage. However, treatment of STZ-diabetic groups with the aqueous suspension of *S trilobata* for five consecutive weeks restored the activities of the above enzymes to their normal levels possibly by inhibiting the liver damage induced by STZ.

It is noteworthy that diabetic hyperglycemia did not alter the plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). In contrast, there was a significant increase in the level of triglyceride in the diabetic groups and treatment with *S. trilobata* normalized this observed effect. Although the precise mechanism(s) of most antidiabetic agent is yet to be fully understood, authors have postulated that these agents possibly reduce blood glucose by stimulating glucose uptake in insulin-responsive tissues resulting in phosphorylation reactions involving the β-subunit of the insulin receptor and other downstream components of insulin signaling pathways (Stapleton *et al*. 1997; Ezaki 1990). Such phosphorylation also positively affects the expression and activities of enzymes associated with both carbohydrate and fatty acid metabolism *in vitro* (Berg *et al*. 1995; Stapleton *et al*. 1997). Therefore, we could partly explain the reason for the observed increase in the level of triglycerides in STZ-treated rats which was significantly modified by S trilobata treatment (Table 2).

The effect of oral administration of aqueous suspension of *S. tri.* on plasma glucose is presented in Figure 1. The experimentally induced diabetes significantly (P<0.05) increased the level of plasma glucose in relation to the control. However, treatment of the STZ-diabetic rats with the aqueous suspension of *S. trilobata* significantly reduced their plasma glucose levels by compared with the diabetic group. Several postulations may be advanced for the observed antidiabetic effect of this plant. On one hand, the hypoglycemic effect of these herbs may be due to the increased level of serum insulin and on the other hand, it may be due to the enhancement of peripheral metabolism of glucose (Eskander and Won Jun, 1995). In addition, significant fall in diabetic rats suggests that the active hypoglycaemic compound present in the aqueous extract of *S*

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trilobata does not necessarily require the presence of functioning β-cells for its favourable action. It means that it can act in a variety of diabetic conditions with or without functioning pancreatic β-cells.

STZ-induced diabetic animals tend to show renal hypertrophy. The entry of glucose in renal tissue is not dependent on action of insulin and, therefore, in the event of hyperglycemia there is an increase in the entry of glucose (Belfiore *et al*., 1986). This has been postulated to cause increased intra-renal glycogen deposition, which leads to glycosylation of basement membrane collagen in the kidney (Anderson and Stowring, 1973). The present result showed that the diabetic rats showed a significant increase in the two-kidney versus body weight ratio in comparison to controls. Rise in renal weight has been reported previously with varying degree of renal hypertrophy in comparison with the present study (Nielsen *et al*., 1999; Barbosa *et al*., 2006). The reason for this disparity in the present and the past results can be attributed to the difference in the duration of the studies. Interestingly, *S. trilobata* significantly reduced renal hypertrophy to normal level. The literature regarding the effect of diabetes on liver weight is contradictory, as some workers have shown an increase in hepatic weight in animals (Chen and Ianuzzo, 1982; Sadique *et al*., 1987; Murphy and Anderson, 1974) as well as humans (Van Lancker, 1976) while others have reported no change (Gupta *et al*., 1999). In the present case diabetic rats showed significantly higher liver/body weight ratio which was normalized by *S. tri*. Although the exact reasons for the observed hepatic hypertrophy are not known, however fat deposition has been proposed to be the cause (Sadique *et al*., 1987). In contrast to both kidney and liver, there was no increase in both the spleenic and testes to body weight ratio respectively, either in animals treated with only STZ, *S. tri.* or both.

Since tissue damage associated with hyperglycaemia has been related to oxidative stress, we evaluated the enzymic and non-enzymic antioxidant status of liver, kidney spleen and testes, since these organs have been object of targets in diabetic complications (Ratner 2001). With respect to non-enzymic antioxidants such as GSH and vitamin C, levels were observed to be significantly decreased in the liver and kidney but not in the spleen and testes and were restored by *S. tri.* treatment, indicating that *S. tri.* besides acting as an antioxidant, may promote an increase in antioxidant systems. Similarly, administration of *S. tri.* to diabetic rats, exhibited antioxidant effect by reducing the level of hepatic, renal and spleenic TBARS which were elevated in STZ treated rats. This is in agreement with our *in vitro* result that shows that *S. tri.* significantly diminishes the production of TBARS under two prooxidant-induced assaults on hepatic lipids (Figure 3).

It has been observed that reduced lipid peroxidation and improved antioxidant status may be one mechanism by which dietary treatment contributes to the prevention of diabetic complications (Armstrong *et al*. 1996). Improvement in antioxidant status in diabetes is likely to have a number of benefits. Evidence is accumulating to support the idea that there is a close relationship between the processes of oxidation and glycation, and that antioxidants may specifically inhibit glycation of proteins (Davie *et al*. 1992; Ceriello *et al*. 1991). Clinical trials of antioxidant therapy to prevent ischemic heart disease in non-diabetic subjects are currently beginning. It has been speculated that diabetic patients may represent a group who would achieve particular benefit in terms of reduced atherosclerosis from antioxidant supplementation. In fact, Morel and Chisolm (1989) have shown that antioxidant treatment can inhibit both lipoprotein oxidation and cytotoxicity in diabetes.

Furthermore, catalase, an antioxidant enzyme that reduces the level of hydrogen peroxides formed by cellular metabolism, was significantly elevated only in the hepatic tissues of STZ-treated rats, and this elevation was modified by *S. tri.* It is noteworthy that discrepancies in the levels of liver antioxidant enzymes have been reported in diabetic rats (Wohaieb and Godin 1987; Ozkaya *et al*. 2002).

ð-ALA-D is a sulfhydryl-containing enzyme that is extremely sensitive to oxidizing agents (Farina *et al*. 2003), and plays a fundamental role in most living aerobic organisms by participating in heme biosynthesis. We have previously observed that the activity of ð-ALA-D is inhibited in cases of diabetes (Folmer et al. 2002; Kade *et al*., 2009, 2009a). In the present study, we observed that STZ caused a significant inhibition in the activity of ð-ALA-D in both liver and kidney, and that *S. tri.* was able to significantly relieve this inhibition.

Diabetes is generally characterized by a loss of insulin activity, culminating in profound changes in glucose metabolism (in glycolysis)

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(Yechoor *et al.,* 2002), and these changes alters the dynamics between the reduced and oxidized forms of the LDH coenzyme, nicotinamide adenine dinucleotide (Wahlberg *et al*., 2000). Table 4 shows that STZ caused a marked decrease in LDH activity in all tissues (liver, kidney, spleen and testes) studied. However, administration of *S. tri.* significantly relieved this inhibition in the different isoforms of LDH studied. We earlier proposed that the apparent increase in the levels of the activity of LDH in diabetic rats may be consequent from the need to regulate the extent of cellular acidosis and respiration, which are markedly increased in diabetes (Kade *et al*., 2009).

Conclusively, it would therefore be observed that the treatment of diabetic rats with *S. tri.* exerted a considerable hypoglycemic effect with concomitant improvement on the antioxidant status. In addition, *S. tri.* could ameliorate the impaired dysfunction in hepatic, renal, spleenic and testes tissues that are associated with diabetes, suggesting that *S. tri.* is a potential candidate in the management of pathological conditions in human models of diabetes. However, it is noteworthy that extensive pharmacological and toxicological work may be required to validate the safe dose of extract of *S. tri.* before its final incorporation into alternative medicine practices.

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