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CHRONIC TREATMENT WITH *DUGUETIA FURFURACEA* IMPROVES HYPERGLYCEMIA AND METABOLIC PARAMETERS IN DIABETIC RATS

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Abstract: *Duguetia furfuracea* (St. HIL) Benth & Hook, is a Brazilian's cerrado plant that display, among their pharmacological actions, a considerable hypoglycemic effect in normoglycemics rats. However, it hadn't been reported in literature the use of *D. furfuracea* in diabetics rats. The aim of this study is to evaluates the hypoglycemic effect of the water extract of this plant in diabetic rats. We evaluated several metabolic and functional factors related to diabetes type 1. Wistar rats were divided in two groups: normoglycemic or diabetic rats, who were treated with vehicle (water) or with *D. furfuracea* treatment (4g of the plant for kg/rat, water extract), for 14 days. An additional group of diabetic rats were treated with insulin. In the end of treatment, rats were kept in metabolic cages, for 24 hours, to conduct evaluation of diuresis, ketonuria, water and food intake. Animal's blood samples were collected to analyze the glucose and albumin levels. The aorta was removed to evaluate vascular isometric force, using an organ bath system. *D. furfuracea* demonstrated a considerable hypoglycemic effect in diabetics animals, favoring an improvement in the dysfunction endotelial and in the kidney function. *D. furfuracea* did not change blood pressure, and water or food intake. Conversely, it favored the correction of the ketonuria, weight loss and hyperglycemia. The results suggest that *D. furfuracea* display relevant action in the treatment of the diabetes.

Keys word: Phytotherapy. Diabetes type 1. Wistar rats.

TRATAMENTO CRÔNICO COM *DUGUETIA FURFURACEA* MELHORA A HIPERGLICEMIA E OS PARÂMETROS METABÓLICOS EM RATOS DIABÉTICOS

Resumo: *Duguetia furfuracea* (St. HIL) A Benth & Hook, popularmente conhecida como sofre do rim quem quer, é uma planta brasileira de cerrado que apresenta, entre suas ações

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farmacológicas, um considerável efeito hipoglicemiante em ratos normoglicêmicos. No entanto, o uso de *D. furfuracea* em ratos diabéticos ainda não foi descrito. O presente estudo teve como objetivo avaliar o efeito hipoglicemiante do extrato aquoso dessa planta em um modelo diabético. Ratos Wistar foram divididos em dois grupos: ratos normoglicêmicos ou diabéticos, que foram tratados com veículo (água) ou com tratamento com *D. furfuracea* (4g da planta por kg/ rato, extrato aquoso), por 14 dias. Um grupo adicional de ratos diabéticos recebeu tratamento com insulina. No final do tratamento, os ratos foram mantidos em gaiolas metabólicas, por 24 horas, para avaliação da diurese, cetonúria, ingestão de água e alimentos. Amostras de sangue de animais foram coletadas para analisar os níveis de glicose e albumina. A aorta foi removida para avaliar a força isométrica vascular, utilizando um sistema de banho de órgãos. *D. furfuracea* demonstrou considerável efeito hipoglicemiante em animais diabéticos, favorecendo uma melhora na disfunção endotelial e na função renal. *D. furfuracea* não alterou a pressão arterial ou a ingestão de água ou alimento. Por outro lado, favoreceu a correção da cetonúria, perda de peso e hiperglicemia. Os resultados sugerem que *D. furfuracea* apresenta ação relevante no tratamento do diabetes.

Palavras-chave: Fitoterápicos. Diabetes tipo 1. Ratos Wistar.

Introduction

Diabetes mellitus is a common metabolic disease characterized by elevated blood glucose levels, resulting from absence or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action (Ortiz-Andrade et al., 2007). This illness is possibly the world's largest growing metabolic disorder and about 400 million people around the world suffer from this disorder, and the prevalence is predicted to continue rising if current trends prevail (Arulrayan et al., 2007; Subash-Babu et al., 2008; Sezik et al., 2005; Schoenfelder et al., 2006; Cetto et al., 2008).

The increase in the prevalence of diabetes is associated with several factors, such rapid urbanization, nutritional transition, sedentary lifestyle and overweight (Asmat et al., 2016; Dominguete et al., 2016). Delay in diagnosis and non-compliance favors the constant increase of levels of glucose in blood (hyperglycemia) that promotes the emergence of chronic irreversible complications. In fact, the hyperglycemia is the main cause of complications related to coronary artery disease, cerebrovascular disease, renal failure, limb amputation, neurological complications and endothelial dysfunction (Lopez-Candales, 2001).

In an ever-increasing population of patients with diabetes, morbidity and mortality due to the secondary complications require prompt identification of the underlying

mechanisms. Alterations in the vascular endothelial cell function may be a key element in the development and progression of the chronic diabetic complications (Khan et al., 2006).

Several species of plants have been described in the scientific and popular literature for their potential hypoglycemic property (Ali et al., 1993), one of which is *Duguetia furfuracea* (A. St.-Hil.) Benth. & Hook. However, further scientific evidence supporting the hypoglycemic effect should be studied. The aim of our study was to investigate the hypoglycemic effects of *D. furfuracea* in hyperglycemic experimental model, using comparative methodology between groups of normal and diabetic rats. Beyond, we evaluated some metabolic and functional factors that are changed in type I diabetic.

Methods

Plant material

The aerial part (leaves) of *D. furfuracea* (A. St.-Hil.) Benth. & Hook. f. Annonaceae, were collected on the UFMT Campus I, Pontal do Araguaia, MT, Brazil. The species was identified and authenticated by Dr. Maryland Sanchez. A voucher specimen (number 878) has been deposited in the Herbarium in the UFMT.

Preparation of the aqueous infusion

The leaves of *D. furfuracea* were dried at 40^0C for 7 days and then reduced to powder. The infusion obtained from botanic material was administered orally by gavage (4g for Kg body weight) for 14 days. Preliminary data from pilot studies showed that this was the dose that great improved glycemic levels (data not shown). Distilled water was used in the control animals during the treatment.

Animals

The present experimental protocols were approved and performed in accordance with the Ethics guide from Brazilian College of Animal Experimentation (COBEA) and the

University of Sao Paulo (authorization n°. 115/2000–107/2000). Animals Male Wistar rats (10–12 weeks) were obtained from our breeding colony at the institute. All animals were housed according to institutional guidelines (constant room temperature, 12/12- hour light/dark cycle, 60% humidity, standard rat chow and water ad libitum). Diabetes mellitus was induced with an injection of alloxan hydrate (40 mg/kg, i.v.) dissolved in physiological saline. Hyperglicemia (> 250 mg/dL) was confirmed by blood sample, extracted from the tail artery, and glycemia was recorded by a digital glycosimeter (Accu Check – Roche). Control rats were injected with physiological saline alone. The rats were divided into 5 groups of 6 rats each, as the following: Group 1 (C) - control rats treated with vehicle; Group 2 (C + P) - control rats treated with *D. furfuracea*; Group 3 (D) – diabetic rats treated with vehicle; Group 4 (D + In) – diabetic rats treated with vehicle and insulin (IP); and Group 5 (D + P) – diabetic rats treated with *D. furfuracea*. The Group 4 received subcutaneous injections of 1 IU of NPH insulin (Iolin; Biobras, Montes Carlos, Brazil). Control rats received injections of vehicle (physiological saline solution).

Determination of the arterial pressure

Systolic blood pressure was measured by the standard tail-cuff method (PowerLab 4/S, ADInstruments Pty Ltd., Castle Hill, Australia) in conscious restrained rats.

Determination of the blood albumin and ketonuria levels

Albumin levels were evaluated using colorimetric assay in blood samples obtained from the abdominal aorta in anesthetized animals. Ketonuria levels were measured by urine test.

Determination of the moist weight of the kidneys

After euthanasia, the kidneys were removed, cleaned and weighed separately. The weight was normalized by the body weight of the respective animal.

Vascular functional studies

After euthanasia, the thoracic aorta was removed and cleaned from fat tissue in an ice-cold physiological salt solution (PSS), containing (mM): NaCl, 130; $NaHCO_3$, 14.9; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$ 1.18; $CaCl_2 \cdot 2H_2O$, 1.56, EDTA, 0.026, glucose 5.5. Segments of thoracic aorta (4 mm in length) were carefully mounted as ring preparations on standard organ chambers for isometric tension recordings by a PowerLab 8/SP data acquisition system (ADInstruments Pty Ltd., Castle Hill, Australia). The tissue was continuously bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C, under a resting tension (30mN for rats; 5mN for mice). After a 45 min equilibration period aorta integrity was assessed first by stimulation of vessels with potassium chloride solution (KCl - 120 mM) and, after washing and a new stabilization period, by contracting the segments with phenylephrine (PE; 1μM) followed by stimulation with acetylcholine (ACh; 10 μM). Endothelium-dependent relaxation was assessed by measuring the relaxation response to ACh (0.1 nM to 30 □□) in PE-contracted vessels (1 □□). Relaxation is expressed as percent change from the PE contracted levels.

Statistical analyses

All the grouped data were statistically evaluated using Graph Pad Prism 3.02 program. The values were analyzed by One way analysis of variance (ANOVA) followed by Newman-Keuls Multiple comparison. Values of $p<0.05$ vs. vehicle were considered statistically significant.

Results

In order to explore the effects of *D. furfuracea* leaves water extract on the metabolic and endothelial factors compromised by type I diabetes in male rats, several parameters that are commonly changed during diabetes pathology were evaluated.

As shown in the figure 1, oral administration of *D. furfuracea* (4g/kg b.w.), for 14 days, showed significant plasma glucose lowering effect in diabetic rats (D+P), when

compared to the diabetic animals that did not receive any treatment (D). *D. furfuracea* did not change glucose levels in normotensive animals (C+P). As expected, treatment with insulin (D+In) restored the glucose levels in diabetic animals.

Table 1 contents the effects of oral *D. furfuracea* treatment on food and water intake in normal and diabetic rats. In diabetic rats (D) there was a significant increase in food and water uptake, compared to normal rats (C). Insulin treatment normalized food consumption and *D. furfuracea* treatment did not change food or water uptake. No changes in the systolic arterial pressure were observed among the groups.

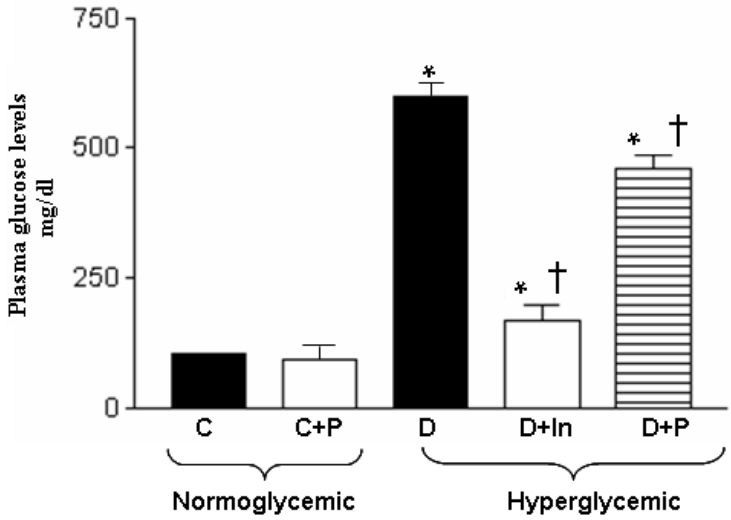

Figure 1: Effect of 14 days treatment with *Duguetia furfuracea* or vehicle in plasma glucose, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, $p<0.05$ by comparison with control rats; †, $p<0.05$ by comparison with diabetic rats.

Table 1: Effect of daily administration of *D. furfuracea* on food, water intake and systolic arterial pressure in normal and diabetic rats, after 14 days of treatment.

Groups	Control	Control + *D. furfuracea*	Diabetic	Diabetic + Insulin	Diabetic + *D. furfuracea*
Water intake (ml/day)	39±2.64	53±4.1 *†	129±6.17 *	111±2 *	135±5.3 *
Food intake (g/day)	27.26±2.3	26.1±1.74 †	35.96±1.0 *	30.2±1.5 †	37.7±1.16 *

Systolic arterial pressure (mmHg)	110.8±9	108.7±5	118.4±16	123.9±14	117.4±9

Results are presented as mean ± SEM for n=6 in each experimental group. * P < 0.05 vs. control; † P < 0.05 vs. diabetic rats.

Body weight gain was increased in controls animals, while the diabetic animals displayed lose of body weight during the period of experiment. *D. furfuracea*, as well as, insulin treatment prevented lost of body weight, compared to diabetic animals (Fig.2).

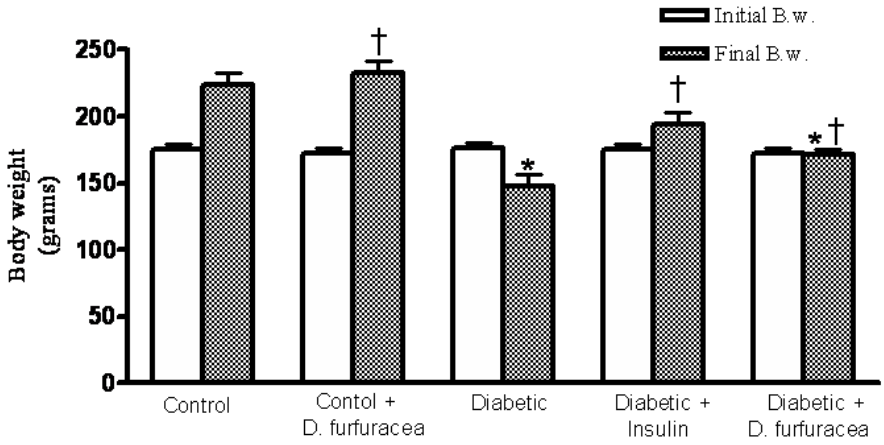

Figure 2: Effect of 14 days treatment with *Duguetia furfuracea* on the body weight gain, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, p<0.05 by comparison with normal; †, p<0.05 by comparison with diabetic rats.

Kidney weight was increased in diabetic rats, when compared to control rats. Both *D. furfuracea* and insulin treatment decreased kidney weight when compared to diabetic rats (Fig.3).

Diabetic animals showed decreased albumin plasma levels, compared to normal rats (Fig.4). Treatment of diabetic rats with *D. furfuracea* or insulin normalized albumin levels.

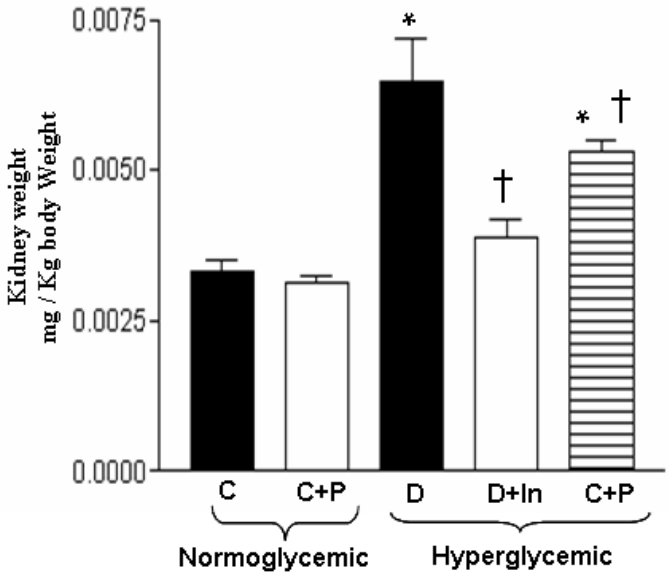

Figure 3: Effect of 14 days treatment with *Duguetia furfuracea* on kidney weight, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, $p<0.05$ by comparison with normal; †, $p<0.05$ by comparison with diabetic rats.

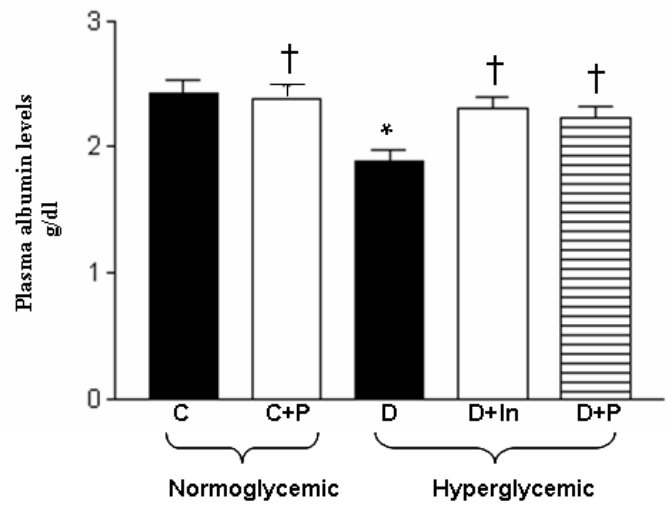

Figure 4: Effect of 14 days treatment with *Duguetia furfuracea* on plasma albumin, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, $p<0.05$ by comparison with normal; †, $p<0.05$ by comparison with diabetic rats.

Ketonuria levels were significantly increased in diabetic animals, as compared to normal animals (Fig.5). Oral administration of *D. furfuracea* decreased ketonuria levels and treatment with insulin restored ketonuria in diabetic animals.

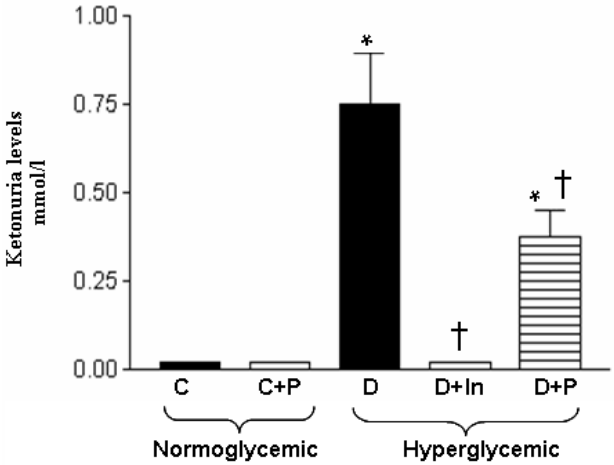

Figure 5: Effect of 14 days treatment with *Duguetia furfuracea* on ketonuria levels, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, p<0.05 by comparison with normal. †, p<0.05 by comparison with diabetic rats.

Endothelium-dependent relaxation was determined by assessing responses to ACh in aortas (Fig.6). Normal rats treated with *D. furfuracea* showed significantly enhancement in ACh-induced relaxation in aorta. As expected, diabetes impaired ACh-induced relaxation in rat aorta compared to normal rats. Diabetic rats treated with *D. furfuracea* showed improvement in the Ach-induced responses, when compared to untreated diabetic rats. Treatment with insulin abolished changes in ACh dilation between hyperglycemic and normoglycemic rats.

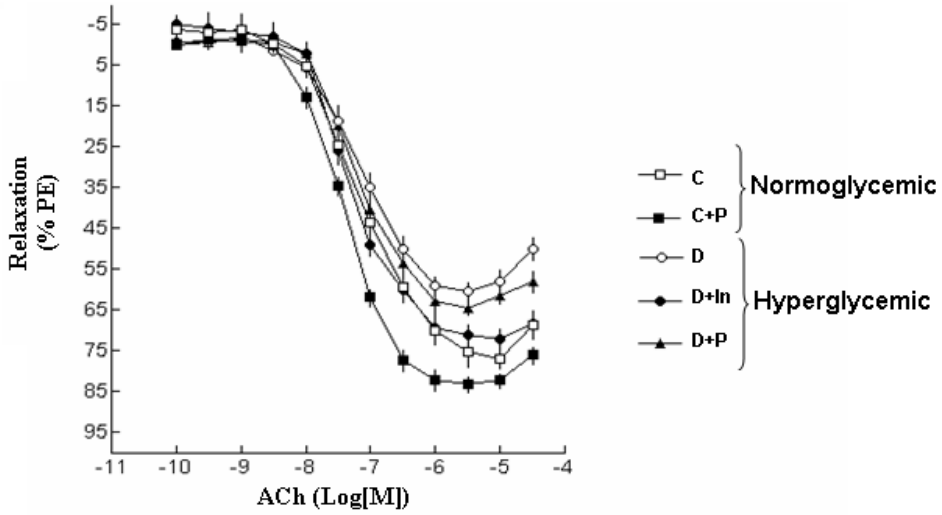

Figure 6: Effect of 14 days treatment with *Duguetia furfuracea* on ACh-induced relaxation, both in normoglycemic and hyperglycemic rats. Results are presented as mean ± SEM for n=6 in each experimental group. *, p<0.05 by comparison with normal. †, p<0.05 by comparison with alloxan-induced diabetic rats.

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Discussion

The discovery of insulin was a major breakthrough in medicine and initiated a new era in the understanding and treatment of diabetes (Subash-Babu et al., 2008; Silva et al., 2002). Even though diabetes mellitus has a high prevalence, morbidity and mortality worldwide, it is regarded as a uncurable, but controllable disease. In addition, different synthetic drugs, plants remedies and dietary traditions play an effective role in diminishing the suffering that it causes (Schoenfelder et al., 2006).

The potential role of medicinal plants as hypoglycemic agents has been reviewed by several authors and is supported by ethnobotanical surveys and the use of traditional medicines in numerous cultures (Jouad et al., 2001). In this study we show here for the first time that chronic oral administration of *D. furfuracea* significantly decreased blood glucose levels in diabetic rats and did not alter glucose levels in normal rats. This result suggests that the active hypoglycemic compound present in the plant does not necessarily require the presence of functioning beta cells for its favorable action, as suggested in studies with different medicinal plants (Subash-Babu et al., 2007 and 2008).

The hypoglycemic effect of *D. furfuracea* has been shown not to act directly on functioning pancreatic beta cells, a significant effect since Type I diabetes mellitus has a strong autoimmune destruction of these cells and consequently the production of insulin, the hormone responsible for reducing the levels of blood glucose. In fact, we observed that, like insulin, the administration of *D. furfuracea* was also able to promote a hypoglycemic effect compared to the diabetic group (Asmat et al., 2016; Dominguete et al., 2016).

The active constituents responsible for these hypoglycemic effects of some plants have been isolated and identified to include, among other substances, some diterpenes, flavonoids, alkaloids, amino acid derivatives and steroidal glycosides (Akah et al., 2002; Sezik et al, 2005). Some of these compounds are finding in *D. furfuracea* (Valter et al., 2008; Silva et al., 2007), and additional studies should be performed to elucidate witch constituents are related to the hypoglycemic properties of *D. furfuracea*.

In addition to the hypoglycemic property of *D. furfuracea*, we observed that other parameters, related to diabetes pathophysiological development, were ameliorated by the

plant treatment. Besides oral administration of *D. furfuracea* did not improve food consume, it did prevent the lose weight in the diabetic group. Decreased in body weight of diabetic rats is possible due to catabolism of fats and proteins, even though the food intake is more in diabetic rats than control. Due to deficiency protein content is decreased in muscular tissue by proteolysis (Subash-Babu et al., 2007).

It is well known that alloxan induces diabetes mellitus in rats by selective necrotic action on the beta cells of pancreas, leading to insulin deficiency (Sakurai et al., 2001). Insulin deficiency leads to various metabolic alterations in animals, such as increased blood glucose level, decreased protein content and increased fatty acid oxidations, resulting in increased ketonic formation (Sumana et al., 2001; Dhanabal et al.,2007). *D. furfuracea* treatment restored the levels of plasmatic proteins, as showed by albumin levels, in the diabetic group. The improvement was comparable as the insulin treated group. Furthermore, diabetic animals treated with *D. furfuracea* displayed decreased levels of ketonic formation.

Because diabetes is known to leads to endothelial dysfunction, resulting in impairment of vascular reactivity to agonists that lead to relaxation (Tabit el al., 2010), we decided to investigate if *D. furfuracea* could prevent this alteration. ACh-induced relaxation was impaired in thoracic aorta from diabetic rats and insulin treatment restored ACh-induced relaxation. In addition to the beneficial properties on metabolic parameters, *D. furfuracea* treatment improved endothelial-dependent vascular relaxation in diabetic animals and further relaxed arteries control animals.

Conclusion

Taken together, our findings suggest that treatment with *D. furfuracea* improves various parameters that are altered by diabetes. *D. furfuracea* has been shown to be a positive candidate for alternative and/or complementary medicine in the management of diabetes mellitus. Prospect studies are required to elucidate the constituents and mechanisms of action of the anti-diabetic effect of *D. furfuracea*.

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