Lack of antidiabetic activity of the methanol extract of *Costus spicatus* in rats

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(Recebido em 30 de junho de 2010; aceito em 20 de dezembro de 2010)

Costus spicatus (Costaceae), conhecida popularmente como "cana-do-brejo", é utilizada na medicina popular como diurética, nas infecções urinárias e como agente antidiabético. O objetivo deste estudo foi investigar a possível atividade antidiabética do extrato metanólico de *Costus spicatus* (EMCS) em ratos. O efeito anti-hiperglicemiante foi avaliado em ratos Wistar machos (150-200 g) normoglicêmicos. Estes animais foram divididos em cinco grupos com cinco animais cada e foram tratados pela via oral com solução salina (0,9%), metformina (500 mg/kg) ou EMCS nas doses de 100, 200 e 400 mg/kg. Após os tratamentos, os animais receberam pela via oral 3 g/kg de glicose e suas glicemias foram mensuradas durante 120 min. A glicemia média dos animais antes dos tratamentos foi de 82 ± 2 mg/dL. A metformina foi capaz de inibir significantemente a hiperglicemia após a administração da glicose. No entanto esse efeito não foi observado com o EMCS. A fim de avaliar a atividade hipoglicemiante, o EMCS foi testado em ratos diabéticos induzidos pela aloxana. Estes animais apresentaram uma glicemia média no jejum de 453 ± 14 mg/dL. A metformina foi capaz de reduzir em 51 ± 11% a glicemia dos ratos diabéticos, entretanto, o EMCS não apresentou efeito significativo. Em conclusão, estes resultados sugerem que o extrato metanólico obtido das folhas de *Costus spicatus* não apresenta atividade antidiabética, ao menos nos modelos experimentais testados neste estudo. Outros experimentos são necessários para confirmar estes resultados.

Palavras-chaves: atividade antidiabética, *Costus spicatus*, aloxana, extrato metanólico

Costus spicatus (Costaceae), popularly known as "cana-do-brejo" in folk medicine is used as a diuretic, for urinary tract infections and as antidiabetic agent. The aim of the present work was to evaluate the antidiabetic activity potential of the methanol extract os *Costus spicatus* (MECS) in rats. The antihyperglycemic effect was evaluated in normoglycemic male Wistar rats (150-200 g). These animals were divided into five groups of five animals each and were treated orally with saline solution (0.9%), metformin 500 mg/kg (reference drug) or MECS at the doses of 100, 200, and 400 mg/kg. After treatments, animals received orally glucose (3g/Kg) and their blood glucose levels were measured during 120 min. The average blood glucose before treatment was 82 ± 2 mg/dL. Metformin was able to inhibit significantly the hyperglycemia after glucose administration. However, these effects were not observed with MECS. In order to evaluate the hypoglycemic activity MECS was tested in alloxan-induced diabetic rats. These animals showed average fasting blood glucose of 453 ± 14 mg/dL. Metformin was able to reduce 51 ± 11% glucose levels in diabetic rats, however, MECS not showed significantly effect. In conclusion, these results suggest that methanol extract obtained from *Costus spicatus* leaves not presents antidiabetic activity, at least in the experimental model tested in this study. Other experiments are required to confirm these results.

Keywords: antidiabetic avtivity, *Costus spicatus*, aloxan, methanol extract

1. INTRODUCTION

The use of medicinal plants is based on family tradition and became widespread in folk medicine of several developed or developing countries. These plants have been used as an alternative treatment for various diseases, including diabetes mellitus (DM) [1].

DM is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia resultant or deficiency in insulin secretion by pancreatic beta cells, as occurs in type DM 1, or insulin action or both, as occurs in type 2 DM [2].

Costus spicatus Swartz (Costaceae), popularly known in Brazil as "cana-do-brejo" is a native species found in wet places in Latin America [3]. In folk medicine, it is used as a diuretic, for urinary tract infection and as antidiabetic agent [4]. Some studies have shown that other species of the genus *Costus* have hypoglycemic effect in rats such as, *Costus afer* [5] and *Costus speciosus* [6]. However, there are no studies to prove efficacy and safety of using this plant for therapeutic purpose [7].

The aim of this work was to evaluate the antidiabetic activity potential of the methanol extract of *C. spicatus* (MECS) in rats.

2. MATERIAL AND METHODS

2.2- Plant material

C. spicatus leaves were collect from the campus of the Federal University of Sergipe (Latitude: 10º 55' 47" S and Longitude: 37º 06' 04 " W), Sergipe State, Brazil, in November 2008 and were identified by Dr. Ana Paula Prata. A voucher specimen (ASE 11453) is deposited at the Herbarium of the Federal University of Sergipe.

2.3- Methanol extract preparation

The methanol extract was obtained from *C. spicatus* leaves harvested of the campus of the Federal University of Sergipe. After harvesting the leaves, they were adequately selected and then submitted to drying in a sterilizer with hot air circulation and renewal (Model Marconi, MA – 035/5, Brazil) at 40 ºC during 72 h. Subsequently, the leaves were triturated in an electric mill until a dust of fine granulation was obtained. This powder was subjected to extraction with 90% methanol, by exhaustive maceration for five days at room temperature. After, the methanol extract was concentrated under reduced pressure, obtaining 260 g of concentrated extract. In each experiment, the methanol extract was diluted in saline, with the addition of two drops of cremophor, until minutes before the desired concentrations of the treatments.

2.4- Animals

We used fifty healthy adult male Wistar rats (*Rattus norvegicus*), aged approximately 2 months and weighing between 150-180 g, obtained of Central Biotery from Federal University of Sergipe. Experimental protocols were approved by the Committee on Animal Research at the Federal University of Sergipe (nº 58/07). The animals were kept under controlled conditions of temperature, 12 h light and 12 h dark cycle, minimum noise and housed in polypropylene cages, with food and water *ad libitum.*

2.5- Assessment of antihyperglycemic effect of MECS

The acute antihyperglycemic effect of MECS was evaluated by analysis of curves obtained from the oral glucose tolerance test (OGTT), according to the method described in Souza *et al.* (2009) [8]. To perform this test, they were used 25 normoglicemic animals divided into five groups of six animals each. The groups were defined as: negative control group, treated with saline; positive control group, metformin treated (500 mg/Kg), a reference drug [9] and three experimental groups treated with MECS at the dose of 100, 200, and 400 mg/kg.

Before experiments, all animals were placed in fasting with water *ad libitum* during 12h. After, the glucose levels was measured in fasting and defined as glucose level time 0 (zero). Then, animals received their respective treatments and after 30 minutes, glucose was administered at the dose of 3 g/kg. Glucose levels were then measured in 30, 60, 90 and 120

minutes after glucose administration. The glycemia was measured by reagent strips (ACCU-CHEK Advantage II, Roche) coupled to a portable glucometer in a blood drop collected from tail of the animals.

2.6- Assessment of acute hypoglycemic effect of MECS in diabetic rats

2.6.1- Induction of experimental diabetes

The experimental DM was induced by the method described by Prince *et al.*, 2004 [10]. After 12 hours of non-water fasting, the animals received 40 mg/kg body weight of alloxan monohydrate (INLAB) diluted in saline solution (0.9%) by a single intravenous injection. After 30 min of the injection, the animals were normally fed. To prevent a fatal hypoglycemia due to massive release of insulin that occurs after the destruction of pancreatic beta cells [11], it was made available to the animals a glucose solution 10% as single water source for 24 hours.

2.6.2- Experimental protocols

The acute hypoglycemic effect of MECS was assessed in diabetic rats by measures of glucose in according to the method described in Zanatta *et al.* (2006) [12]. To perform this test, they were used 25 animals divided into five groups of five animals each. The groups were defined as: negative diabetic control group, treated with saline (0,9%); positive diabetic control group, treated with metformin (500 mg/Kg), a reference drug [9], and three experimental groups treated with MECS in 100, 200, and 400 mg/kg. Before experiments, all animals were placed in fasting with water *ad libitum* during 12h. After, the glucose levels was measured in fasting and defined as glucose level time 0 (zero). Then, animals received their respective treatments and glucose levels were then measured in 1, 2, 3, 4, and 5h after treatments. The blood glucose values were measured as previously described and values compared between groups.

2.7- Statistical Analysis

Data were expressed as mean ± standard error mean (S.E.M.). Two-way analysis of variance (ANOVA) followed by Bonferroni post-test was used to identify significantly different groups. For these procedures was used the statistical software GRAPH PAD PRISM ™ 3.02 version.

3. RESULTS AND DISCUSSION

In normoglycemic animals, the average blood glucose after fasting for 12 hours was 82 ± 2 mg/dL (n = 25). In the negative control group, oral administration of 3 g/kg glucose was able to increase significantly ($p < 0.05$) glucose levels at 30, 60, and 90 (142 ± 15, 133 ± 7 and 109 ± 7 mg/dL, respectively). As shown in Table 1, administration of metformin (500 mg / kg) induced a significant antihyperglycemic effect after 60 min of administration; however the MECS at all doses tested, did not show the same effect. Both the fasting glucose and variations in blood glucose after glucose ingestion were similar to those found in other studies using the same method and same animal species [8, 9, 13].

Table 1: Variations in blood glucose (mg/dL) obtained from the curve of OGTT in normoglycemic rats after administration of saline, metformin (500 mg / kg) or methanol extract of C. spicatus (100, 200 and 400 mg / kg).

*Values represent the mean ± SEM, n = 6. * p <0.05 vs Saline. Data were analyzed using two-way ANOVA followed by Bonferroni post-test.*

As reported in the literature, metformin is an oral hypoglycemic of the biguanide class [14].

Treatment	Time (min)				
	Dose (mg/kg)	30	60	90	120
Saline	--	54.8 ± 18.3	17.3 ± 8.6	19.9 ± 9.9	5.6 ± 2.8
Metformin	500	25.5 ± 7.8	24.8 ± 6.7*	14.8 ± 6.2	13.5± 6.7
MECS	100	56.5 ± 4.0	43.3 ± 1.2	18.8 ± 5.9	9.5 ± 3.7
MECS	200	61.0 ± 7.1	47.8 ± 4.3	19.2 ± 9.6	10.0 ± 6.6
MECS	400	64.8 ± 16.4	47.5 ± 5.5	25.3 ± 3.6	8.3 ± 5.2

It is able to alter glucose metabolism by inhibiting the elevation of postprandial glycemia in approximately 25-30% [15]. Its mechanism of action involves the inhibition of glucose absorption from the gastrointestinal tract, reduction of hepatic glucose production and increase sensitivity of peripheral tissues to insulin [15]. We choose to use metformin as a drug reference, because it is administered by the same route of MECS and avoid creating variables that could affect the outcome.

In the next step of experiments, the hypoglycemic activity of MECS was assessed in alloxan induced diabetic rats. As described in the literature, alloxan is a diabetogenic agent widely used in science for the induction of experimental diabetes. This drug has specific cytotoxicity to beta cells, causing impaired insulin followed by the establishment of permanent diabetes [16]. This model has clinical similarities, such as glycosuria, polyphagia, polydipsia, hyperglycemia [17], laboratorial and histopathological features with human diabetes [16, 18, 19]. In this model of experimental diabetes, the animals had fasting blood glucose of 453 ± 14 mg/dL (n = 25). The oral administration of metformin (500 mg/kg) was able to produce a significant hypoglycemic effect, but the MECS, at all doses tested, did not show any significant effect (Figure 1).

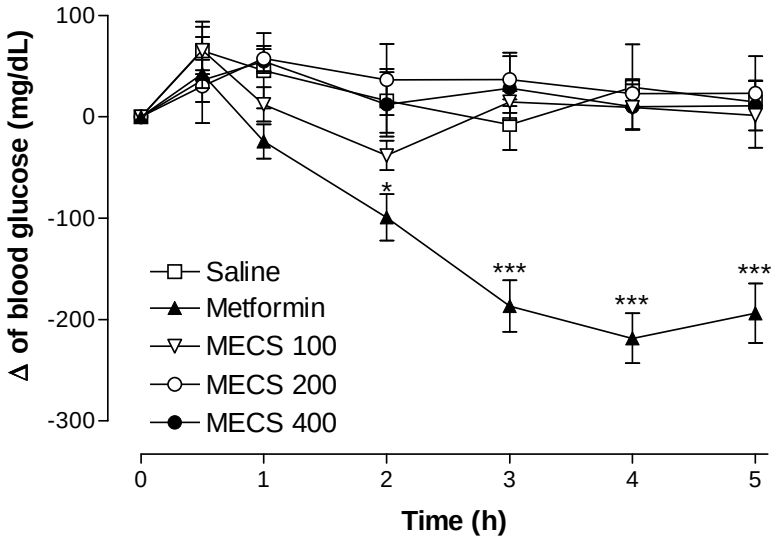

Figure 1: Effect saline, metformin (500

*mg/kg) and MECS (100, 200, and 400 mg/kg, po) on blood glucose of alloxan induced diabetic rats in the times of 1, 2, 3, 4, and 5h after administration. Values represent the mean ± S.E.M. of five experiments. * P <0.05 and *** p <0.001 vs. Saline. Data were analyzed by using two-way ANOVA followed by Bonferroni post-test.*

In earlier studies by Keller *et al.*, 2009 [20] and Souza *et al.*, 2009 [8], it was observed that the aqueous extract of *C. spicatus* was unable to promote improvement in glucose homeostasis in diabetic rats, confirming in part with our results.

One possible explanation for the absence of antidiabetic effect of MECS could be related to factors such as geographic location where the plant was collected, the season of collection, the concentration of the extract or the experimental model of diabetes used here. It is possible that the MECS can provide anti-diabetic activity in other experimental models, such as the type 2 DM, since the model used in this study is more related to type 1 DM.

4. CONCLUSION

These results suggest that methanol extract of *Costus spicatus* leaves appears no present antidiabetic activity in rats, at least in experimental models used in this study. Further experiments are necessary for confirm clearly these negative results.

5. ACKNOWLEDGEMENTS

This research was supported by CAPES, CNPq, FAPITEC/SE, Ministério da Saude and SES/SE, Brazil.

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