Original Article

Evaluation of antihyperglycaemic activity of *Calotropis procera* leaves extract on streptozotocin-induced diabetes in Wistar rats


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**ABSTRACT**

*Calotropis procera* (Aiton) W. T Aiton, Apocynaceae, popularly known as “algodão-de-seda”, is a wild African bush, rich in bioactive substances that determine the medicinal potential of this species. Diabetes mellitus is a disease that affects about 10% of the population. This study aimed to evaluate the antihyperglycaemic activity of the hydroalcoholic extract of the leaves of *C. procera* of occurrence in coast of Pernambuco, Brazil. The hydroalcoholic extract of the leaves of *C. procera* (300 and 600 mg/kg/day), vehicle, insulin (6U, s.c.) or metformin (500 mg/kg/day) were administered orally to streptozotocin-induced diabetic rats (*n* = 7/group) for four weeks. Changes in body weight, food and water intake, biochemical markers, fasting glucose levels and oral glucose tolerance test were evaluated. The results showed that the *C. procera* dried extract (300 and 600 mg/kg) reduced significantly the level of blood glucose throughout the evaluation period and improved metabolic status of the animals and ameliorate the oral tolerance glucose test. The phytochemical screening revealed and quantified the presence of phenolic compounds and flavonoids in a percentage of 29.1 and 2.9%, respectively. Thus, we conclude that the extract of the leaves of *C. procera* has antihyperglycemic activity.

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**Introduction**

Diabetes mellitus is a disorder characterized by increased levels of blood glucose, consequence of impaired insulin production, insulin resistance or both. It is associated to long-term damage of eyes, liver, kidneys, nerves, blood vessels and it may cause degenerative diseases in central nervous system (Bhutada et al., 2011).

Plant compounds such as triterpenes (Wang et al., 2010), xanthines (Muruganandan et al., 2005), flavonoids, tannins...
(Roy et al., 2005), proteins (Ahmad and Beg, 2001), lignans, flavonol (Singhal and Kumar, 2009), among others, have been related to the improvement of hyperglycemia in diabetes.

Calotropis procera (Aiton) W. T. Aiton, Apocynaceae, is a wild bush originated from Africa, India and Persia (Gomes et al., 2006; Singhal and Kumar, 2009). In Brazil, it was introduced as an ornamental plant and in northeastern Brazil it is popularly known as cotton silk, silk flower, and “queimadeira” (Lima et al., 2011). In addition, C. procera has biologically active substances such as flavonoids, cardioactive glycosides, triterpenoids, alkaloids, resins, anthocyanins, tannins, saponins and proteolytic enzymes (Shaker et al., 2010). The latex of C. procera has been widely studied due to its antihyperglycaemic (Roy et al., 2005), anti-inflammatory, gastroprotective (Tour and Talele, 2011), anticoagulant and selective cytotoxic effects (Teixeira et al., 2011).

However, there are few information in the literature about the potential antidiabetic activity of secondary metabolites present in the leaves this species. Studies of Etuk and Mohammed (2009) using aqueous extract of C. procera demonstrated acute hypoglycemic activity in alloxan-diabetic rats. However, Rahmatullah et al. (2010) evaluated acute hypoglycemic activity of methanolic extract of C. procera leaves in diabetic mice and observed no significant effects. In this way, the present study was designed to investigate the effects of prolonged treatment with hydroalcoholic extract of C. procera leaves on biochemical blood parameters, oral glucose tolerance test and other diabetic disorders of streptozotocin-diabetic rats.

**Material and methods**

**Plant material**

The plant materials (Calotropis procera (Aiton) W. T. Aiton, Apocynaceae, was collected in Paulista, Pernambuco, Brazil (S 07°50`32" W 34°50`22"). The voucher specimen of the plant was identified by botanist Marlene Barbosa and deposited at the Geraldo Mariz herbarium from Federal University of Pernambuco, under the number 63707/2010.

**Preparation of hydroalcoholic extract of leaves of Calotropis procera**

The collected leaves of C. procera were subjected to the exclusion of screening for removal of damaged leaves and midribs from leaves. Then, they were dried in circulating air oven (45 ± 2°C), crushed and macerated until exhaustion in 50% ethanol solution at ratio of 1:10 (w/v) which was replaced every 72 h (Chechinel-Filho and Yunes, 1998). The final extract was concentrated in late rotary evaporator under reduced pressure at temperature of 60°C and subsequently lyophilized and kept at 4°C until use.

**Chromatographic analysis**

The methods described by Wagner and Bladt (1996) and Harborne (1998) were used to screen the leaves extract for the reducing sugars, alkaloids, coumarins, cinnamic derivatives, flavonoids, cardenolide glycosides, tannins, triterpenes, the total phenols, flavonoids and saponins. The phytochemical profile was drawn up using thin layer chromatography (TLC) on aluminum plates 20x20 cm (Fluka®) using the appropriate mobile phase, reagents and standards (Sigma-Aldrich®). The determinations of phenols and flavonoids were performed according to Peixoto Sobrinho et al. (2010).

**Animals**

Male Wistar rats (220-260 g) obtained from the Federal University of Pernambuco, Department of Physiology and Pharmacology, and Swiss mice (35-40 g) obtained from the Aggeu Magalhães Research Center, Pernambuco, Brazil, were used. They were kept under standard environmental conditions (12 h dark/light cycle) and temperature (22 ± 2°C). Water and industrialized dry food (Labina®, Purina, Brazil) were available ad libitum. All the experimental protocols were submitted to and approved by the Animal Experimentation Ethics Committee of the Federal University of Pernambuco, under license 051 739 in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Acute toxicity**

"Up and down" acute toxicity studies were performed on male Swiss mice as described by OECD 420 (2001), with slight modifications. The animals were randomly divided into four groups (n = 5/group) and deprived of feed for 12 h with access to water ad libitum. The treated groups received hydroalcoholic extract of the leaves of C. procera in a single oral dose of 5 g/kg and the control groups received water (0.1 ml/g). The observations were performed at 30, 60, 120, 180 and 240 min after the oral treatments and daily for fourteen days. Behavioral changes (piloerection, tremors, sedation, loss of corneal reflex, motor activity), weight, food and water consumption, clinical signs of toxicity and mortality were recorded daily (Malone, 1977).

**Induction of experimental diabetes**

Diabetes was induced using streptozotocin (STZ) from Sigma-Aldrich®, St. Louis, MO, USA. The animals were fasted overnight and diabetes was induced by way of a single intra peritoneal injection of a freshly prepared solution of STZ (50 mg/kg b.w.) in a 0.1 M citrate buffer (pH 4.5). On the third day of STZ-injection, the animals with fasting glycemia higher than 200 mg/dl and with signs of polyuria and polydipsia were considered to be diabetic and included in the study (Vasconcelos et al., 2011).

**Diabetic animals**

**Treatment**

In the experiment, the animals were randomly divided into six groups (n = 6/group): Group 1 (NDC: non-diabetic control) and group 2 (DC: diabetic control) consisted of rats treated with vehicle (water); group 3 (MTD: diabetic rats treated with metformin 500 mg/kg/day b.w.), groups 4 and 5 (diabetic rats treated with hydroalcoholic extract of the leaves of C. procera treated with hydroalcoholic extract of the leaves of C. procera...
Treatment was administered orally on a daily basis in a single dose for 28 consecutive days. Fasting glucose and body weight were recorded weekly, while food and water intake were monitored daily.

**Oral glucose tolerance test (OGTT) in STZ-diabetic rats**

On the 25th day of treatment, the animals from groups 1-5 were fasted for 12 h. Fasting glycemia was measured and defined as zero time. After this procedure, animals received their treatment orally and after 30 min, all groups received an oral load of \( \alpha \)-glucose (2 g/kg b.w.). Blood glucose levels were measured 5 min before and 30, 60, 120 and 150 min after glucose administration (Lima et al., 2012). Blood samples were obtained from the tail vein and glucose concentration determined using a blood glucose device monitor (Accu-Chek® active, Roche diagnostics Gmbh-Germain).

**Biochemical parameters**

At the end of treatment, blood samples were collected and centrifuged at 1500 × g for 10 min to obtain the serum, which was stored at -20°C (Silva et al., 2009) until the following parameters had been determined: glucose, blood urea nitrogen (BUN), creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triacylglycerides and the total cholesterol (TC). Dosages were made using Architect (Abbott®) automation with Boehringer Ingelheim® biochemical kits.

**Liver and tissue mass analysis**

Once blood had been collected, the animals were euthanized with an excess of Nembutal® (140 mg/kg, i.p.). Liver, epididymal adipose tissue, soleus and extensor digitorium longus muscles were carefully removed and individually weighed and the date was expressed in absolute and relative terms (g and g/100g b.w., respectively) in accordance to Vasconcelos et al. (2011).

**Statistical analysis**

The results were expressed as mean ± SEM. Statistical analysis was performed using Graph Pad Prism 5.0® software. The difference between groups was by analysis of variance (ANOVA), followed, when necessary, by Newman-Keuls test. The significance level for rejection of the null hypothesis was always ≥ 5% (p < 0.05).

**Results**

**Phytochemical analysis**

The phytochemical analysis of the hydroalcoholic extract of *C. procera* in TLC showed the presence of reducing sugars, flavonoids identified as luteolin and kaempferol, and cardenolide glycosides. The content of phenols and flavonoids were 29.3 and 3.0%, respectively.

**Acute toxicity**

It was observed that the hydroalcoholic extract of the leaves of *C. procera* (5 g/kg, p.o.) did not induce changes in the behavior of male mice during the first 30 min and for a period of up to 4 h after administration. No death was recorded during the fourteen days of observation. No significant changes in intake of food and water or in body weight were observed throughout the period (data not shown). The LD\(_{50}\) could not therefore be estimated and is possibly higher than 5 g/kg.

**STZ-Diabetic animals**

**The effect of *Calotropis procera* on fasting blood glucose**

Oral administration of *C. procera* 300 and 600 mg/kg/day b.w. in diabetic rats showed significant reductions in fasting glucose levels of more than 60% already in the first week of treatment when compared to DC and more than 45% in relation to MTD group. At the end of treatment, this reduction was of 68 and 51%, respectively, only in relation to DC (Fig. 1).

**Figure 1** - Effect of hydroalcoholic extract of *Calotropis procera* (Cp) on fasting blood glucose levels (mg/dl) of diabetic rats. NDC, non-diabetic control; DC, diabetic control, MTD, diabetic rats treated with metformin 500 mg/kg; Cp, diabetic rats treated with hydroalcoholic extract of the leaves of *Calotropis procera* 300 and 600 mg/kg; ITD, diabetic rats treated with insulin 6 U. The results are expressed as mean ± S.E.M. (n = 6/group).

\(^a\) Statistically different from DC and MTD.

\(^b\) Statistically different from DC (ANOVA followed by Newman-Keuls, p < 0.05).

**The effect of *Calotropis procera* on body mass gain, food and water intake**

Figs. 2, 3 and 4 show the evolution of body mass gain, food and water intake in the experimental groups during the 28-day treatment, respectively. During the period there was no significant difference in body mass gain in groups Cp (300 and 600 mg/kg) when compared to DC and MTD (Fig. 2).

The results shown in Fig. 3 reveal a significant reduction in food intake in the group receiving *C. procera* 300 mg/kg/day from the first week of the study until the end of the treatment when compared with DC. Statistical reduction in relation to MTD occurred from the third week. The group treated with *C. procera* 600 mg/kg/day showed reductions in food intake only in the first and third week of the study when compared with DC.
Polydipsia was significantly reduced in the group receiving *C. procera* 300 mg/kg/day already in first week of treatment compared to DC and, in the following weeks, this reduction was also significant for the DC and MTD. The administration of 600 mg/kg/day of *C. procera* did not show statistical differences for this parameter when compared to the DC (Fig. 4).

The effect of *Calotropis procera* on oral glucose tolerance test

Fig. 5 shows the blood glucose levels of the NDC, DC, ITD, MTD and *C. procera* 300 and 600 mg/kg b.w groups after oral administration of D-glucose (2 g/kg b.w). *C. procera* 300 and 600 mg/kg showed significant decrease in glycemia from 30 min after glucose administration when compared to diabetic control. Reductions of 35.6, 36.7, 43.7 and 39.1% and 43.9, 37.2, 40.9 and 40.9% were observed in glycemic levels of this group of 30, 60, 120 and 150 min, respectively, when compared to diabetic control.
The effect of Calotropis procera on biochemical parameters

Rats treated with *C. procera* 300 and 600 mg/kg showed statistically significant reductions in uric acid, AST and ALT levels when compared to DC and significant increases in creatinine, the total cholesterol and triacylglycerides in relation to same group (Table 1).

### Effect of Calotropis procera on the mass of organs and tissues

The effects of *C. procera* 300 and 600 mg/kg on masses of liver, kidney, epididymal adipose tissue (EAT), soleus and extensor digitorium longus (EDL) muscles are given in Table 2. *C. procera* 300 mg/kg induced a significant increase in EAT and soleus muscle relative mass, but reduced the relative mass of the kidneys in relation to DC group. While *C. procera* 600 mg/kg produced statistical increase in mass of the EAT when compared to DC group. Both doses of *C. procera* increased the mass of the EDL.

### Table 1

Biochemical parameters of normoglycemic and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NDC</th>
<th>DC</th>
<th>ITD</th>
<th>MTD</th>
<th>Cp 300 mg/kg</th>
<th>Cp 600 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>28.80 ± 1.25</td>
<td>114.50 ± 10.80</td>
<td>36.89 ± 3.60</td>
<td>70.75 ± 7.46</td>
<td>80.17 ± 19.62</td>
<td>77.00 ± 18.80</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.57 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>--</td>
<td>0.72 ± 0.044c</td>
<td>0.66 ± 0.044c</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.10 ± 0.07</td>
<td>3.05 ± 0.18</td>
<td>1.27 ± 0.08</td>
<td>0.97 ± 0.02</td>
<td>1.03 ± 0.22</td>
<td>1.13 ± 0.23</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>153.70 ± 8.00</td>
<td>596.20 ± 9.70</td>
<td>115.00 ± 3.84</td>
<td>212.30 ± 10.40</td>
<td>280.50 ± 92.79</td>
<td>222.67 ± 52.30</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>52.57 ± 2.30</td>
<td>406.70 ± 9.50</td>
<td>75.86 ± 7.46</td>
<td>139.80 ± 9.44</td>
<td>151.00 ± 54.22</td>
<td>115.17 ± 29.90</td>
</tr>
<tr>
<td>Triacylglycerides (mg/dl)</td>
<td>47.07 ± 3.94</td>
<td>35.77 ± 3.28</td>
<td>83.24 ± 4.83</td>
<td>139.30 ± 7.49</td>
<td>54.00 ± 4.27</td>
<td>63.17 ± 6.10</td>
</tr>
<tr>
<td>T. Cholesterol (mg/dl)</td>
<td>77.58 ± 5.15</td>
<td>35.77 ± 3.28</td>
<td>83.24 ± 4.83</td>
<td>70.75 ± 5.63</td>
<td>76.50 ± 6.75</td>
<td>62.00 ± 4.70</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; NDC, non-diabetic control; DC, diabetic control; ITD, Diabetic rats treated with insulin (6 U, s.c.); MTD, diabetic rats treated with metformin 500 mg/kg; Cp, diabetic rats treated with hydroalcoholic extract of the leaves of *Calotropis procera*. The values are expressed as mean ± S.E.M (n = 6-7/group).  

\*Statistically different from NDC.  
\*Statistically different from DC and MTD.  
\*Statistically different from DC (ANOVA followed by Newman-Keuls, p < 0.05).

### Table 2

Effect of the hydroalcoholic extract of the leaves of *Calotropis procera* on tissues masses of diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NDC</th>
<th>DC</th>
<th>ITD</th>
<th>MTD</th>
<th>Cp 300 mg/kg</th>
<th>Cp 600 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g)</td>
<td>13.56 ± 0.750</td>
<td>8.440 ± 0.440a</td>
<td>10.090 ± 0.660</td>
<td>8.360 ± 0.180b &lt; 0.05</td>
<td>8.140 ± 0.350b,c</td>
<td>8.230 ± 0.620b,c</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td>4.010 ± 0.110</td>
<td>3.770 ± 0.220</td>
<td>3.590 ± 0.150</td>
<td>3.830 ± 0.060 &lt; 0.05</td>
<td>2.860 ± 0.430a</td>
<td>3.650 ± 0.430</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>1.280 ± 0.020</td>
<td>1.190 ± 0.080</td>
<td>1.290 ± 0.050</td>
<td>--</td>
<td>1.141 ± 0.350</td>
<td>1.130 ± 0.720</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td>0.350 ± 0.010</td>
<td>0.520 ± 0.010a</td>
<td>0.380 ± 0.010a</td>
<td>--</td>
<td>0.390 ± 0.043b,c</td>
<td>0.500 ± 0.051a</td>
</tr>
<tr>
<td>EAT(g)</td>
<td>2.250 ± 0.010</td>
<td>0.570 ± 0.090a</td>
<td>4.290 ± 0.290</td>
<td>0.012</td>
<td>2.570 ± 0.03</td>
<td>1.380 ± 0.515b,c</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td>0.900 ± 0.020</td>
<td>0.240 ± 0.060</td>
<td>1.290 ± 0.011</td>
<td>0.05</td>
<td>0.950 ± 0.270d</td>
<td>0.570 ± 0.230d</td>
</tr>
<tr>
<td>Soleus Muscle</td>
<td>0.158 ± 0.002a</td>
<td>0.112 ± 0.007a</td>
<td>0.151 ± 0.030</td>
<td>0.101a</td>
<td>0.150 ± 0.006a</td>
<td>0.110 ± 0.016a</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td>0.052 ± 0.002</td>
<td>0.078 ± 0.034</td>
<td>0.078 ± 0.046</td>
<td>0.046</td>
<td>0.050 ± 0.010</td>
<td>0.050 ± 0.007</td>
</tr>
<tr>
<td>EDL (g)</td>
<td>0.162 ± 0.003</td>
<td>0.089 ± 0.008</td>
<td>0.149 ± 0.005</td>
<td>0.0793</td>
<td>0.150 ± 0.002</td>
<td>0.120 ± 0.012</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td>0.060 ± 0.004</td>
<td>0.040 ± 0.003</td>
<td>0.045 ± 0.006</td>
<td>0.036</td>
<td>0.050 ± 0.002</td>
<td>0.050 ± 0.009</td>
</tr>
</tbody>
</table>

EAT, Epididymal adipose tissue; Soleus: soleus muscle, EDL, extensor digitorium longus muscle. (NDC, non-diabetic control; DC, diabetic control; ITD, Diabetic rats treated with insulin (6 U, s.c.); MTD, Diabetic rats treated with metformin 500 mg/kg; Cp, diabetic rats treated with hydroalcoholic extract of the leaves of *Calotropis procera*. The values are expressed as means±S.E.M (n = 6-7/group).  

\*Statistically different from NDC.  
\*Statistically different from DC and MTD.  
\*Statistically different from DC (ANOVA followed by Newman-Keuls, p < 0.05).  
\*Statistically different from MTD (ANOVA followed by Newman-Keuls, p < 0.05).

### Discussion

Considering the wide use of this herb in folk therapeutics for the treatment of diabetes, the present study was conducted to investigate the antihyperglycaemic activity of *C. procera* in streptozotocin-induced diabetic rats. Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications due to their efficacy, low incidence of side effects and low cost (Valiathan, 1998).

This is the first study to show that the treatment with the hydroalcoholic extract of the leaves of *C. procera* for four weeks exhibited significant antihyperglycaemic effect in streptozotocin-induced diabetic rats.

The results of the acute toxicity test indicated that hydroalcoholic extract of leaves of *C. procera* when administered orally at a dose of 5 g/kg did not produce any sign of toxicity.
or death in the treated animals, suggesting an LD₅₀ of above 5 g/kg. Kennedy et al. (1986) reported that the substances present LD₅₀ higher than 5 g/kg after oral administration can be considered practically non-toxic. Therefore, it can be suggested that acute toxicity for C. procera is practically nil when administered in this way.

The phytochemical analysis of the hydroalcoholic extract of the leaves of C. procera showed the presence of redutors sugars, phenols and flavonoids. The latex has been shown to contain cardinolides, lignans and flavanol glycosides that have been considered to contribute to its antioxidant properties (Mueen Ahmed et al., 2003). In the same way, Roy et al. (2005) reported that the dry latex (100 and 400 mg/kg) has anti-hyperglycemic and antioxidant effects against alloxan-induced diabetes in rats. Indeed the role of oxidative stress and altered antioxidant level in the pathogenesis of diabetic complications is well established (Maxwell et al., 1997).

Persistent hyperglycemia leads to increased production of free radicals through glucose autooxidation and protein glycation (Zhang and Tan, 2000).

The streptozotocin destroys pancreatic β cells, giving rise to severe diabetes (Szkudelki, 2001). In our study, the induction of diabetes was confirmed by high levels of fasting glucose, and as expected, the diabetic rats had polyphagia, polydipsia and polyuria.

C. procera induced a decrease in blood glucose that was similar to the standard anti-diabetic drug metformin and this effect was also reflected by the decrease of daily water and food intake. In addition, the oral glucose tolerance test performed at the end of treatment that showed clearly the animals treated daily with C. procera not only had lower fasting glucose levels than the DC group, but also improved their metabolic state through increased glucose tolerance in a manner similar to the metformin-treated group. The group ITD presented an intense hypoglycemia after 60 min and, therefore, the administration was stopped to prevent animal death. In a similar way, Lima et al. (2012) have shown that the hydroalcoholic extract from the leaves from Persea americana, which is also rich in phenolic compounds, also presented anti-hyperglycemic effect in STZ-induced diabetes.

The effect of C. procera on the biochemical parameters and tissue mass were broads. In general, the extract improved the metabolic status of animals in relation to the DC group. In diabetic rats, there was an increase in urea and uric acid levels in blood. The values of uric acid were diminished in the treated groups. In general, the extract improved tissue mass were broads. In general, the extract improved adipose tissue and soleus and extensor longus digitorium muscles. These results also indicate also the decrease in protein catabolism through increasing glucose uptake. According to Umesh et al. (2005), during uncompensated diabetes, there is a decrease in body mass due to energy deficit and the cellular catabolism process characterized by glycogenolysis, lipolysis and proteolysis.

In conclusion, our results show that the hydroalcoholic extract of the leaves of C. procera has anti-hyperglycemic activity in streptozocin-induced diabetic rats. It may be suggested that this action have the contribution of phenols and flavonoids. However, the mechanism of action remains to be established. Some hypotheses include an antioxidant action, interference with insulin levels and the enzymatic pathways of protein kinase B and AMP-activated protein kinase.

Authors’ contributions

MCLN and VNT (Master students), CFBV and GFRC contributed in running the laboratory work, analysis of the date and drafted the paper. AVA, ELA and JHCS contributed in the laboratory work. FF, AFMO and AGW designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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REFERENCES


