Full Length Research Paper

Administration of the aqueous extract of the stem bark of Hancornia speciosa Gomes (Apocynaceae) does not alter obesity induced by high-fat diet in mice

Luana, M. Cercato¹, Pollyanna, A. S. White¹, Vanessa, S. Batista¹, Luciana, C. Brito², Charles dos Santos Estevam¹, Márcio R. V. dos Santos¹ and Enilton A. Camargo¹*

¹Department of Physiology, Federal University of Sergipe, (UFS), São Cristóvão, 49100-000, SE, Brazil.
²Department of Physical Education, Federal University of Ceará (UFC), Fortaleza, CE, Brazil.

Received 11 November, 2015; Accepted 17 February, 2016

Ethnobotanical surveys have shown that the stem bark of Hancornia speciosa Gomes (Apocynaceae) is popularly used to treat obesity and diabetes. However, there is no experimental evidence that confirms such use. The present study investigated the effects of the aqueous extract of the stem bark of H. speciosa (AEHS) on the glycemic and adipogenic profiles of obese mice. Mice were divided into four groups that received standard diet (SD), standard diet plus AEHS (SDE), high-fat diet (HD) and high-fat diet plus AEHS (HDE). The administration of AEHS (in a concentration of 0.3 mg.mL⁻¹ ad libitum in the drinking water) was performed for the last 8 weeks totaling a period of 18 weeks, in which the animals received the diets. Whole body weight, liquid intake and food consumption were measured during the entire experiment. Blood glucose levels, insulin sensitivity, glucose tolerance and adipose pads weight were evaluated. Animals from the HD group presented higher body weight in comparison to animals from the SD group. That was associated with insulin resistance and glucose intolerance, as well as increased blood glucose levels (p < 0.05) and weight of adipose tissue pad (p < 0.05), when compared to the SD group. The treatment with AEHS did not alter obesity induced by high-fat diet, because no significant difference was observed between the HD and the HDE groups in all of the parameters evaluated. These findings allowed the conclusion that AEHS does not reverse the alterations caused by high-fat diet in mice, what goes against the popular use.

Key words: Hancornia speciosa, obesity, high-fat diet, adipose pads, glucose intolerance, insulin resistance

INTRODUCTION

Obesity can be defined as a multifactorial syndrome consisting of biochemical, metabolic and anatomical alterations, such as increased adipose tissue and body weight (Go et al., 2013). Nowadays, obesity is an important risk factor for several types of diseases that lead to poor quality of life, considerable morbidity and premature death (Flegal et al., 2012). Obesity is increasing at an alarming rate and is considered as

*Corresponding author. E-mail: enilton.camargo@pq.cn.pq.br

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
a worldwide epidemic condition that affects all age groups. It is a chronic and multifactorial disease and may be a result of endogenous and/or exogenous factors. It is important to mention that the exogenous factors hold the majority of cases related to environmental factors, especially the lack of physical activity and negligible eating habits (Yach et al., 2006).

Disappointing results after lifestyle modification or pharmacotherapy have indicated the need of other treatment modalities to produce better results in terms of weight loss (Abdollahi and Afshar-Imani, 2003). Nevertheless, herbal supplements and diet-based therapies for weight loss are among the most common treatments in complementary and alternative medicine. Equally, a wide variety of these natural and herbal products, including crude extracts and compounds isolated from plants, can be used to induce weight loss and prevent diet-induced obesity. Furthermore, in recent decades, medicinal plant preparations have been widely used in the treatment of obesity (Barnes et al., 2004; Han et al., 2005; Cercato et al., 2015). These plants contain a variety of components that may interfere with the metabolism and oxidation of fatty acids, possibly increasing their lipolysis, thus presenting anti-obesity and antioxidant properties. Some herbs have been investigated and discovered as being useful in the treatment of obesity, diabetes and other chronic diseases (Hasani-Ranjbar et al., 2009, 2010); however various plants used by population have not been considered for the scientific evaluation.

Hancornia speciosa Gomes (Apocynaceae) is a tree naturally found in Brazil, where it is distributed throughout the Midwestern, Southeastern, Northern and Northeastern regions, with higher abundance in the areas of coastal plains and plateaus of the Northeast. In popular medicine, H. speciosa, or “mangabeira”, is used in various ways: the bark is used to treat dermatoses, liver diseases and diabetes, and is also used as an anti-inflammatory and for weight loss; the roots are used in the treatment of dislocations, rheumatism, and also as stomatic and antihypertensive; the latex and leaves are used as astringent, in the treatment of menstrual cramps, dermatitis, tuberculosis, ulcers, herpes and warts, and in the treatment of diseases affecting the liver; the fruits are used as a food source (Grandi et al., 1989; Rodrigues and Carvalho, 2001; Macedo and Ferreira, 2005; Souza and Felfili, 2006; Conceição et al., 2011; Pasa, 2011; Ribeiro et al., 2012).

It is important to highlight that scientific information related to the popular use of this plant for the treatment of metabolic syndromes, such as obesity and hyperlipidaemia, would be of a great clinical importance. This popular use has been extensively mentioned in ethnobotanical surveys in different regions of Brazil. However, few biological activities have been evaluated (Grandi et al., 1989; Rodrigues and Carvalho, 2001; Macedo and Ferreira, 2005; Silva et al., 2010a, b; Cercato et al., 2015).

Therefore, an intense popular use of H. speciosa for several conditions, including body weight loss in obese or overweight people, is observed. Yet, there is lack of research analyzing its therapeutic potential to treat obesity. In this way, this study aimed to verify the beneficial effect of the aqueous extract of the stem bark of H. speciosa on the glycemic and adipogenic profiles of obese mice in the high-fat diet model.

MATERIALS AND METHODS

Plant material and preparation of the stem bark aqueous extract

For this study, the stem of H. speciosa Gomes (Apocynaceae Juss.) was collected in the town of Pirambu-SE, Brazil, in March 2012. The identification of the plant was confirmed by Dr. Ana Paula Prata, from the Federal University of Sergipe, and a voucher specimen was deposited in the Herbarium of the Federal University of Sergipe (ASE30170). For the preparation of the aqueous extract of the stem bark H. speciosa (AEHS), 500 g of the stem bark of H. speciosa was dried, ground and subjected to extraction by infusion in 5 L of distilled water at 100°C for 30 min. The solution obtained was kept and cooled to room temperature (25°C). It was then filtered with a filter paper of 125 mm to obtain 3.4 L of the final solution. Hence, this solution was lyophilized and 52.5 g was obtained and stored at -20°C for later use. The yield of this extraction was 10.5%.

Animals for experimentation and experimental conditions

Male Swiss mice (21 to 23 days, 10 to 14 g) were obtained from the Central Animal Facility of the Federal University of Sergipe. After one week of adaptation in the laboratory, animals were randomly divided into 4 groups of 8 animals that received:

1) Standard diet for 18 weeks (SD).
2) Standard diet for 18 weeks and the aqueous extract of the stem bark H. speciosa (AEHS) in the last 8 weeks (SDE).
3) High-fat diet for 18 weeks (HD).
4) High-fat diet for 18 weeks and AEHS in the last 8 weeks (HDE).

These animals were maintained on identified polypropylene cages with 4 animals each, with diet and water ad libitum. In the groups supplemented with AEHS, the administration was carried ad libitum in the drinking water. The temperature remained at the 22±2°C range, with light / dark cycle of 12 h. The Ethics Committee on Animal Research of the Federal University of Sergipe approved the experimental protocol of this study, under the reference number 81/12. During all experimental procedures, the ethical principles for animal testing were adopted, following the National Council for Animal Experiment Control (CONCEA).

Induction of obesity

For the induction of obesity in mice, a high-fat diet was offered ad libitum to animals during 18 weeks (HD and HDE groups), according to White et al. (2013). Control groups received a standard diet (normal lipid content) for the same period (SD and SDE groups). The diets were commercially obtained from...
PragSoluções (São Paulo, Brazil) and their compositions are specified in Table 1.

Supplementation with the aqueous extract

The aqueous extract of the stem bark of *H. speciosa* was offered *ad libitum* to mice of groups HDE and SDE, at room temperature, during the 8 weeks of the experiment at a concentration of 0.3 mg.mL\(^{-1}\), which resulted in an estimated dose of 200 mg.kg\(^{-1}\) based on the daily water consumption of the animals.

Evaluation of water intake, food intake and weight gain of animals

The evaluation of both water intake and food consumption was performed daily for each box of animals during the entire period of the experiment. Body weight, in turn, was measured once a week.

Evaluation of glycemic profile

**Insulin tolerance test (ITT)**

The blood glucose was measured after 5 h of fasting at the end of the 18 weeks, 3 days before euthanasia. The blood supply obtained from the animal’s tail vein was used, using Accu-check® (Roche) glucometer, according to the manufacturer’s specifications. The insulin was intraperitoneally injected in the proportion of 0.7 U.kg\(^{-1}\) and blood glucose levels were measured after 20, 40 and 60 min post-injection (Ali et al., 2011). The total area under the curve was calculated from 0 to 60 min.

**Glucose tolerance test (GTT)**

At the end of 18 weeks, with 2 days before ITT, D-glucose (1 g.kg\(^{-1}\), prepared in saline solution) was administered intraperitoneally to animals submitted to 12 h of fasting and blood glucose levels were measured before and after 5, 15, 30, 45, 60 and 120 min post-injection (Faulhaber-Walter et al., 2011). The total area under the curve was calculated from 0 to 120 min. The blood supply was obtained from the tail vein of the animals and glucose levels were measured using the blood Accu-check® glucometer, according to the manufacturer's specifications.

Blood glucose

Blood glucose was measured with the animals fasting for 5 h. For this determination, the blood of the animal’s tail vein was collected and glucose levels were measured by using the Accu-check® glucometer, according to the manufacturer’s specifications.

Removal of adipose tissue and determination of adiposity index

After anaesthesia and euthanasia of animals by using inhaled isoflurane (3-5%) and blood collection, a longitudinal incision in the abdomen was performed to remove the periepididymal, perirenal and retroperitoneal adipose pads. Then, adipose tissues were immersed in saline solution, the excess solution was taken up with gauze and tissues were immediately weighted. The adiposity index was obtained by dividing the sum of the animal’s pads by the total animal body mass (White et al., 2013).

Statistical analysis

The results were presented as Means±Standard Error of Means (SEM) and the comparison between them was performed with one- or two-way analysis of variance (ANOVA) followed by Bonferroni’s post-test, as specified in the legends of each figure. Values of \(p < 0.05\) were considered significant.

---

**Table 1.** Composition of diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Standard diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U (g/kg) kcal</td>
<td>U (g/kg) kcal</td>
</tr>
<tr>
<td>Corn starch</td>
<td>415.0 1 660</td>
<td>14.3 57.2</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>305.0 1 281</td>
<td>410.0 1 722</td>
</tr>
<tr>
<td>Sucrose</td>
<td>80.0 320</td>
<td>80.0 320</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>70.0 280</td>
<td>70.0 280</td>
</tr>
<tr>
<td>Lard</td>
<td>0.0 0</td>
<td>302.0 2 718</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0.0 0</td>
<td>0.0 0</td>
</tr>
<tr>
<td>Soybean fatty acid</td>
<td>50.0 350</td>
<td>50.0 350</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>31.7 0</td>
<td>25.4 0</td>
</tr>
<tr>
<td>L-cystine</td>
<td>1.8 7.2</td>
<td>1.8 7.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.5 0</td>
<td>1.5 0</td>
</tr>
<tr>
<td>Buty-hydroxytoluene</td>
<td>0.014 0</td>
<td>0.028 0</td>
</tr>
<tr>
<td>Mix min. mod 50 gps</td>
<td>35.0 0</td>
<td>35.0 0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10.0 40</td>
<td>10.0 40</td>
</tr>
<tr>
<td>Total</td>
<td>1 000.0 3 938</td>
<td>1 000.0 5 494</td>
</tr>
</tbody>
</table>

Standard diet (SD): 73.9% of carbohydrate, 14.8% of protein and 9.8% of lipid. High-fat diet (HD): 26.3% of carbohydrate, 14.4% of protein and 57.6% of lipid.
RESULTS

Figure 1 shows that at the beginning of the experiment, there was no significant difference in body weight among the groups (10.7±1.2, 11.5±0.5, 11.6±0.2 and 11.5±0.4 g respectively for the SD, SDE, HD and HDE groups). On the 10th week of the experiment, the body weight of the animals did not differ statistically, but a clear tendency for higher values for animals of the HD and HDE groups (41.4±0.9 and 39.4±0.6 g, respectively) was observed in comparison with the animals of the SD and SDE groups (36.3±1.0 and 37.8±0.7 g, respectively). After 14 weeks of treatment with high-fat diet, a significant difference between the HD and SD groups was found (p < 0.05, Figure 1). No difference was observed for groups treated with AEHS (SDE or HDE) when compared with their respective control for diets (respectively SD or HD). Both the diet consumption and the liquid intake were measured during the 18 weeks of the experiment. These parameters were not altered in the groups evaluated (data not shown), both before and after the animals that received the standard or high-fat diet were exposed to AEHS (in the last 8 weeks).

Figure 2 shows the weight of adipose pads and adiposity index. The animals of the HD group had significantly higher adipose retroperitoneal (p < 0.05; Figure 2A), perirenal (p < 0.001; Figure 2B) and periepididymal (p < 0.05; Figure 2C) pad weight when compared to the SD group, which resulted in higher adiposity index in the HD group (p > 0.05; Figure 2D), when compared to the SD group. The treatment with AEHS lessened the weight pad of the perirenal pad (p < 0.05; Figure 2B), without affecting epididymal or retroperitoneal pads (Figure 2A and C). However, this difference did not reflect on the alteration in the adiposity index (Figure 2D), thus indicating no influence of AEHS upon the total fat mass.

At the end of the 18 weeks of the experiment, ITT and GTT were also carried out. Figure 3A shows that glucose levels of mice from the HD group were significantly increased when compared to the animals of the SD group, at 0, 20 or 60 min post-injection of insulin, which was also confirmed by higher values of AUC in the HD group than in the SD group (p < 0.01, Figure 3B). The treatment with AEHS, in the last 8 weeks, did not significantly modify the glucose levels or AUC after intraperitoneal injection of insulin, when compared to the respective control for diet.

After a challenge with intraperitoneal injection of glucose, animals from the HD group showed significantly higher levels of blood glucose from 15 to 60 min post-injection in comparison to the SD group (Figure 4A). That
Figure 2. Weight of adipose tissue (g) for peripididymal (A), perirenal (B) and retroperitoneal (C) pads and adiposity index (D) of the groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus the aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. * p < 0.05 for SD vs. HD groups, # p < 0.05 for HD vs. HDE groups. One-way ANOVA followed by Bonferroni’s post test.

Figure 3. Insulin tolerance test (ITT, Panel A) for groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus the aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. Glucose levels (mg.dL⁻¹) were measured at 0 (baseline) and 20, 40 and 60 minutes post-intraperitoneal insulin injection. * p < 0.05 or ** p < 0.001 for HD vs. SD groups. Two-way ANOVA followed by Bonferroni’s post-test. (B) Panel B shows the values of area under the curve (AUC) of the same groups. ** p < 0.01 for HD vs. SD groups. One-way ANOVA followed by Bonferroni’s post-test.
resulted in higher AUC ($p < 0.001$) in the HD group than in the SD group (Figure 4B), indicating a glucose intolerance in mice treated with high-fat diet for 18 weeks. However, the treatment with AEHS in the last 8 weeks did not significantly alter the effect of high-fat diet over the glucose intolerance.

Glucose levels were increased in mice from the HD group after a 5 h period of fasting ($p < 0.05$), when compared to the SD group (Figure 5). However, the treatment with AEHS caused no significant change in basal blood glucose levels both in animals submitted to standard or high-fat diet.
DISCUSSION

Data presented in this study showed that the aqueous extract of the stem bark of *H. speciosa* (AEHS) did not alter the body weight gain, adiposity index, blood glucose levels, sensitivity to insulin and tolerance to glucose in mice fed with high-fat diet, which is consistent with the lack of anti-obesity or favorable glycemic effects of AEHS in the conditions used in the present study. In addition, animals fed with standard diet did not present any change in these parameters.

The hypothesis that the aqueous extract from the stem bark of *H. speciosa* could present such activity was based on the ethnobotanical surveys describing that the population in Brazil uses this medicinal plant to treat obesity or to promote body weight loss (Cercato et al., 2015). That is the case of the study published by Conceição et al. (2011), which described that people from Nova Xantina (MT), Brazil, indicated the use of the infusion or decoction of the bark of *H. speciosa* as anorectic, representing an alternative to appetite control thus reducing food consumption. Other ethnobotanical studies have also demonstrated that people use the bark of *H. speciosa* to lose weight in different regions of Brazil (Grandi et al., 1989; Silva et al., 2010a, b; Santos et al., 2013; Cercato et al., 2015).

In spite of these descriptions, data from the present study failed to show any effect that could corroborate the ethnobotanical description that the bark of *H. speciosa* can be useful both to treat obesity and to produce weight loss. Thus, mice treated with high-fat diet plus AEHS did not show important alteration of the parameters evaluated, and no change was observed in mice treated with standard diet plus AEHS. The model of high-fat diet used in this study was previously standardized (White et al., 2013). This previous study demonstrated a difference in body weight of Swiss mice after ten weeks of exposition to the same high-fat diet utilized in the present study. In fact, a clear tendency for higher values of body weight was found in animals from the HD group on the 10th week, but even eight weeks of treatment with AEHS (~ 200 mg.kg⁻¹.day⁻¹) in the drinking water did not modify the body weight and other parameters of mice.

The amount of adipose tissue, measured as the mass of peripididymal, retroperitoneal and perirenal adipose pads, was increased in animals treated with high-fat diet, along with the augmented whole body mass. These results are consistent with the previous study from this study group (White et al., 2013). The treatment with AEHS did not affect the mass of peripididymal or retroperitoneal pads, but interestingly, reduced the weight of perirenal adipose pad. Unfortunately, that was the lower adipose pad and it did not cause a significant effect of AEHS over the adiposity index, which allowed us to conclude that AEHS, in the conditions used in this study, was not effective to promote body weight loss in mice.

It is worthwhile noting that the via of administration chosen in the present study was the drinking water, in order to avoid gavage for eight weeks, which could cause some damage related to the administration that could interfere in the swallowing of mice. One could suggest that the treatment with AEHS in the drinking water could change the liquid intake or the consumption of food, but AEHS promoted alteration of neither the liquid intake (which demonstrates that it was well tolerated by the animals) nor the food intake. Animals continued to consume the same amount of liquid and food that they used to before the AEHS had been introduced. Therefore, there was no significant difference in consumption in grams and absolute consumption in kcal among the groups treated and their respective controls.

Another possibility of bias of the present study could be the dose of AEHS used. The estimated dose was 200 mg.kg⁻¹.day⁻¹, which was considered as a dose high enough to cause any possible effect that AEHS could induce, and that could offer biological relevance to the treatment of obesity. Unfortunately, there is no description of how much bark of *H. speciosa* is used by the population in the preparation of decoction or infusion. Besides, other studies have shown that treatment with similar doses of extracts of plants can alter the induction of obesity or other associated conditions. For example, the study from Song et al. (2014) demonstrated that the methanol extract from the stem of *Sasa borealis* (150 mg/kg) reduced the body weight and hepatic steatosis in rats made obese by a high-fat diet consumption. Kim et al. (2014) showed that the treatment with the ethanol extract from the rhizomes of *Boesenbergia pandurata* (200 mg/kg) decreased the whole body and adipose pad weight of C57BL/6J mice submitted to a high-fat diet through activation of AMP-activated protein kinase and regulation of lipid metabolism. However, differences in species of animals, composition of extracts or via of administration do not permit a direct comparison between the studies.

Concerning the effects of AEHS over the glycemic profile, it was observed that AEHS did not reverse glucose intolerance and insulin resistance, nor did it normalize the basal blood glucose levels in the HDE group. A study carried out in fifteen traditional communities (non-indigenous) in the Upper Paraguay River Basin and two in the Guaporé Valley collected data about hypoglycemic plants through qualitative approach and with the aid of semi-structured and opened interviews. Among the seventeen identified species, the bark of *H. speciosa* was cited as medicinal and used by community leaders, traditional healers, midwives and other plant users for the treatment of diabetes (Macedo and Ferreira, 2004). Many plants that have been used to reduce blood glucose and that were pharmacologically evaluated have their hypoglycemiant activity confirmed. Among their constituents, the steroid and triterpenoid glycosides are bioactive substances present in many of them (Rao and Gurfinkel, 2000). Some saponins derived...
from triterpenoid have hypoglycemic action and their possible effect involves the stimulation of pancreatic β-cells with subsequent secretion of insulin (Ojewole, 2002; Connolly and Hill, 2001).

Studies by Rodrigues et al. (2007), Costa et al. (2008) and Santos et al. (2013) have indicated the presence of organic acids and derivatives, xanthones, proanthocyanidins, volatile compounds, flavonoids, triterpenes and cyclitols in parts of H. speciosa. Also, rutin and cyclitol L-(−)-bornesitol were identified in the bark of this plant (Pereira et al., 2012), which are considered primary bioactive compounds. In spite of the presence of triterpenes and other components that could possess a hypoglycemic activity, this effect was not observed in animals receiving AEHS from groups treated with both standard and high-fat diets, probably due to the difference in the solvents used to extract (ethanol vs. water). Interestingly, it was demonstrated that the ethanol extract of the leaves of H. speciosa or its dichloromethane fraction reduced the in vitro activity of α-glucosidase, and it also enhanced the uptake of glucose in freshly dissociated adipocytes (Pereira et al., 2015). In this study, the treatment of mice with 300 mg/kg of this extract or its dichloromethane fraction reduced glycemia in starch or glucose tolerance tests, which suggests that the ethanol extract of the leaves of this plant may also present some potential to induce a hypoglycemic activity.

Conclusions

Altogether, these results demonstrate that the aqueous extract of the stem bark of H. speciosa (AEHS) administered to obese mice did not cause alteration in weight gain, insulin resistance, glucose intolerance or hyperglycemia. Data obtained in the present study do not exclude the possibility that preparations from other parts of H. speciosa could affect obesity. However, these results from experimental animals disagree with the popular uses demonstrated in the ethnobotanical surveys about the bark of this plant and claim for attention for this use.

Conflicts of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors sincerely thank “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES; Social Demand Program of Scholarships) and “Conselho Nacional de Pesquisa e Desenvolvimento Científico” (CNPq) for their financial support. EAC and MRVS are beneficiaries of CNPq productivity grants.

REFERENCES


888:133-137.