

Assessment of the Hypoglycemic Effects of the Aqueous Extracts of *Bauhinia forficata* in Wistar Rats Fed with a High Fat Diet

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Abstract

This study aimed at analyzing both body and glycemic parameters resulting from the administration of high fat diet and *Bauhinia forficata* aqueous extract (AqE) in rats. Initially, sixty-day-old rats were divided into two groups. Group C was fed with normocaloric diet and group D was fed with a high fat diet composed of 20% proteins; 48% carbohydrate, 20% lipid, 4% cellulose, 5% vitamin and mineral salts. On the 120 day-old group D was subdivided with another D + I group, fed a hyperlipid diet plus administration of *Bauhinia forficata* AqE for gavage. During experimental period, body mass, food intake and glucose were evaluated. The animals were sacrificed at the age of 150 days. Group D, at 120 days, presented higher body mass compared to group C, but there were no changes in dietary intake. The glycaemia of group D increased compared to group C. At 150 days, the D + I group had a decrease in body mass and glycaemia, and group D continued to gain body mass without changing food intake. Therefore, the findings show that the two-month-diet-period increased body weight and blood glucose. AqE of plant *B. forficata* has pharmacological potential in reducing body mass and decreasing in blood glucose concentrations.

Keywords: *Bauhinia forficata*, aqueous extract, high fat diet, diabetes

1. Introduction

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder of various known etiologies. One of the DM etiologies, a world's health problem, is the ingestion of high fat diet, raising the risk of morbidity and mortality (Mohammad, 2014). Associated conditions such as increased hyperglycemia, elevated insulin level or alteration insulin action presents disorder of carbohydrate metabolism and may cause insulin resistance and DM (Christian et al., 2013; Marlon, 2013). Medicine has developed several methods treating diseases, many people constantly choose to use natural plants as treatment. In this sense, many plants with antidiabetical potential are used and some papers prove that diabetes mellitus control may bring new therapeutic possibility (Patel et al., 2012; Pinheiro et al., 2017).

Several species of the *Bauhinia* genus are used as folk medicines worldwide including Africa, Asia, South America and Central America (Trojan-Rodrigues et al., 2012). *B. forficata* (Figure 1) is the most studied species in folk Brazil (in a medicinal list) because of its wide geographic distribution, low cost and facility to get the samples, and it is known that this plant has an antidiabetical activity (Cunha et al., 2010; Batista et al., 2013). It is popularly known here as “cow paw” due to the bilobulated aspect of its leaves (Cechinel, 2009).

Considering the need to search for new resources for DM treatment, the present study aimed to evaluate the properties of an aqueous extract of *B. forficata* fresh leaves in Wistar male rats.



Figure 1. Habitus of *B. forficata*

Note: The representative image of *B. forficata* commonly known as “Pata de Vaca”.

2. Material and Methods

2.1 Botanical Samples

Plant Specimen and Extraction *B. forficata* Link leaves were obtained in Nova Iguaçu, Rio de Janeiro, Brazil (-22.9035, -43.2096 (latitude/ longitude)) in March 2016 and were identified by Botanical Ph.D. Vitor Tenório Rosa; a voucher specimen numbered 1052 was deposited in the Universidade Iguaçu (“Iguaçu University”) herbarium. The fresh leaves were infused into an extraction (Pepato et al., 2002). In this process, the infusion with the species leaves was performed at a ratio of 150 g of fresh leaves/liter of water. Subsequently, the extracts were filtered and concentrated under vacuum to obtain the Aqueous Extract (AqE) from *B. forficata* leaves. The extract was divided into aliquots that were kept in a freezer until the moment of consumption.

2.2 Biological Material

This study was carried out in compliance to the ethical principles of experimental animal adopted by Brazilian College of Animal Experimentation (COBEA) and it was approved by Ethic Committee in animals use from Universidade Iguaçu (“Iguaçu University”) docketed: PEBIO/UNIG/006/2016.

It was used in male rats of Wistar lineage coming from the vivarium colony of the Universidade Iguaçu (“Iguaçu University”). The rats was kept in cages measuring 34x41 cm with controlled room temperature (77 to 80.6°F), constant humidity and clear/dark cycle of 12 hours (6:00 to 18:00). From 21 day-old on, the animals were fed with regular food (Nuvilab®) and filtered water *ad libitum*.

During all experimental period, animals were weighty and food intake was evaluated. The protocol of offering high fat and normocaloric diet lasted for two months from 60 day-old. The normocaloric diet consisted in commercial food for rats (Nuvilab®) containing by weight: 19.0% protein, 56.0% carbohydrate, 3.5% lipids, 4.5% cellulose, 5.0% of vitamins and minerals, totalizing 17.03kJ/ and high fat (Estadela et al., 2004; Estadela et al., 2011) consisted of a mixture of foods with the following ratio: 15g of standard food (Nuvilab®), 10g of Roasted peanuts, 10g milk chocolate and 5g of mastic biscuit. These ingredients were ground, mixed and offered in the form of Pellets, containing by weight: 20% protein, 48.0% carbohydrate, 20.0% lipid, 4.0% of cellulose, 5.0% of vitamins and minerals. Energy content of the hyperlipidic diet was here 21.40kJ/g. The Experimental groups: (C) rats fed with normocaloric diet; (D): rats fed with high fat diet, (D+I): rats fed with high fat diet plus administration of AqE *B. forficata*.

The administration to AqE *B. forficata* was daily at 120 day-old by and was administered via oral gavage. The treatment was performed in the morning at 8:00 am with 10mg/kg for 30 days. At 150 days-old, the rats were

sacrificed by exsanguination. The glucose monitoring (60, 90, 120 and 150 day-old) was accomplished by means of blood collection attained from the rats' caudal vein and deposited at glicemic tape which was analyzed with a glucometer ADVANTAGE® (Boehringer Mannheim, USA).

2.3 Statistical Assessment

For analysis of food consumption, body mass and glucose levels to the 60 and 120 days it was used the t test of Student not paired. The significance level was set out at $p < 0.05$.

The outcomes of both food consumption and body mass regarding the 150 day-old were analyzed by means of the variance analysis method (ANOVA) bivariate, followed by Bonferroni post test. To further results, the ANOVA univariate method was used, followed by Newman Keuls post test of multiple comparisons. All outcomes were expressed as average \pm standard deviation, considering the significance level of $p < 0.05$. The software used was Prism (GraphPad Prism version 6.00 for Windows).

3. Results and Discussion

Obesity is a worldwide epidemic causing overconsumption of food, with change in eating patterns and often causing metabolic comorbidities. The increase of blood insulin and glycemc levels are associated to obesity and resistance insulin (Jianping, 2014; Kim et al., 2015).

Our findings showed that high fat diet for 60 calendar days was able to induce obesity. Group D showed significant increase in body mass gain 78 at 120 day-old compare to group C (Fig 2a). The increased energy gain presented is naturally expected from increased fat deposits, especially visceral fat, which is typically proven in resistance insulin and DM (Paniagua, 2016). The D+I group revealed AqE of *B. forficata* gradative normalize body mass (Figure 2b). The literature is controversial regarding acute use of *B. forficata* tea, stating no alterations on the body mass and water intake (Pepato et al., 2002).

The food intake assessment at 60 and 120 (table 1) day-old showed similarities between groups. The D+I (table 1) group showed food intake decrease at 150 with AqE of *B. forficata*. According to literature, *B. forficata* could lead to tissue toxicity by hydrolysable tannins and simple phenols in the leaves (Faria et al., 2004; Salgueiro et al., 2016).

Table 1. Food intake at 120 and 150 days of life

Food intake (g)	C	D	D+I
120 days	48.00 \pm 1.00	47.33 \pm 1.33	47.70 \pm 1.30
150 days	48.90 \pm 0.50	48.25 \pm 0.19	44.50 \pm 1.25*

Note. Food intake at 120 and 150 day-old. C (rats fed with normocaloric diet) and Wistar rats, D (rats fed with high fat diet), D+I (rats fed with high fat diet plus administration of AqE *B. forficata*). Values represent mean \pm SEM * $p < 0.05$, ANOVA univariate, followed by Bonferroni post test.

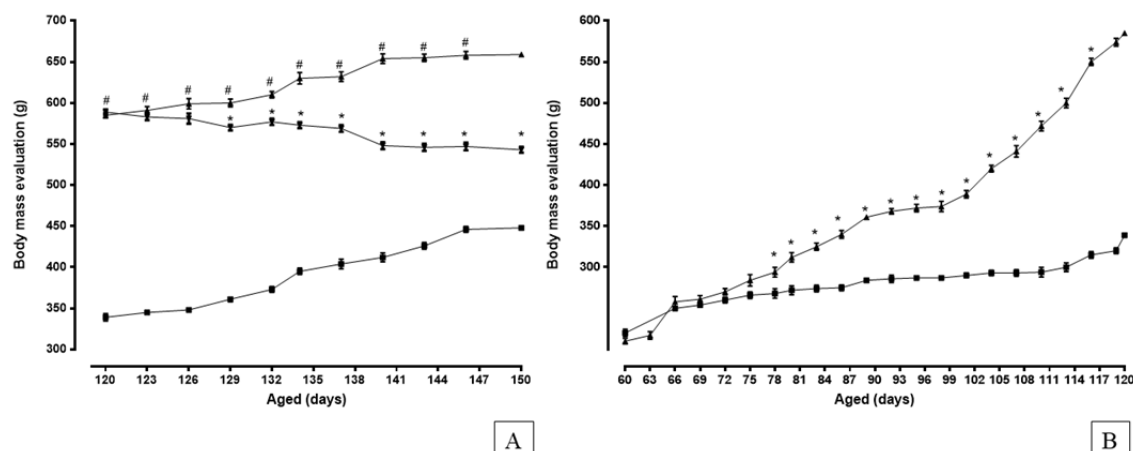


Figure 2. Body mass evaluation

Note. A) 60 at 120 days Wistar rats C (rats fed with normocaloric diet) and wistar rats, D* (rats fed with high fat diet) T test Student, not paired. B) 120 at 150 days wistar rats, D+I # (rats fed with high fat diet plus administration of AqE *B. forficata*). Values represent mean \pm SEM. ANOVA bivariate, followed post test Bonferroni, $p < 0.05$.

Group D showed higher significant glucose at 120 and 150 days compare to C and D+I groups. These results corroborated with Duarte et al. (2006) who observed the use of high fat and hypercaloric diets unleash the increase on the insulin plasma concentration. Insulin seems to exercise positive feedback on its secretion through the interaction with their receptors in pancreatic beta cells. Desensitization on the action mechanisms of the insulin signaling pathways show correlation between the insulin resistance and the decrease of insulin secretion and these considerations are homologous to the observed in type2-diabetic rats (Fante et al., 2016, Meng et al., 2017).

Group D+I (Figure 3c) that received AqE *B. forficata* significantly reduced glucose at 150 day-old compared to D group. The hypoglycemic properties of the flavonoids occur in *B. forficata* (Trojan-Rodrigues et al., 2012). Kaempferol is isolated from *B. forficata* enabling promoting metabolic homeostasis with the improved fasting blood glucose. Reports showed lower glucose level and increase glucose uptake in the rat soleus muscle when isolated from *B. forficata*. The author suggests that kaempferol stimulates glucose uptake in the rat soleus muscle via the PI3K and PKC pathways and, at least in part, regardless of MEK pathways and the synthesis of new glucose transporters (Zanata et al., 2008).

Future studies need to be accurate so that the results can provide understanding regarding underlying mechanisms.

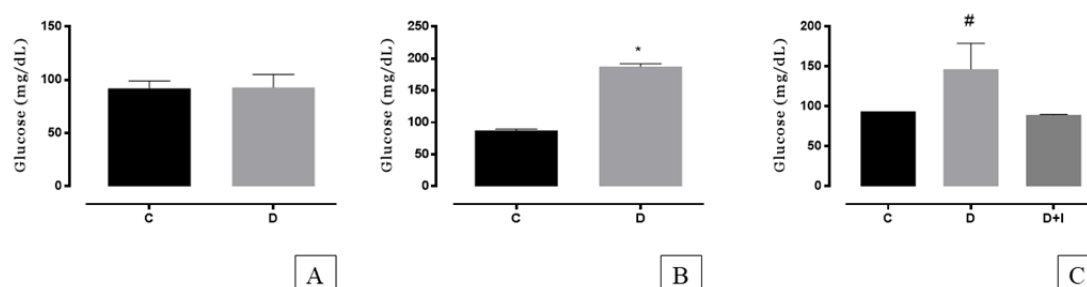


Figure 3. Concentration of blood glucose

Note. a) 60 day-old, b) 120 day-old, c) 150 day-old. C (rats fed with normocaloric diet) and Wistar rats, D (rats fed with high fat diet D+I (rats fed with high fat diet plus administration of AqE *B. forficata*)). Values represent mean \pm SEM * p <0.05, t test of Student, not paired; # p <0.05, ANOVA univariate, followed by Bonferroni post test.

4. Conclusion

The experimental high fat diet may represent obesity by its significance on the body mass gain and increased levels of glucose blood in differ times. These observations showed the efficiency of the applied diet here.

The AqE *B. forficata* was able to decrease blood glucose, body mass and food intake due to its antidiabetic capacity. Moreover, it has been studied and its use is very promising for the design of new pharmaceutical forms. The value of this plant in pharmacological therapy, it is very important to be studied and its use is very promising.

Conflicts of interests

The authors have declared no conflict of interests.

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