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In vivo evaluation of the hypoglycemic effect of wolf-apple flour (*Solanum lycocarpum* A. St.-Hil)

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The prevalence of diabetes has increased at alarming rates worldwide, and has become a serious health problem in modern society, highlighting the need for adjuvants to assist in its treatment. The starch from wolf-apple is a product extracted from the pulp of the unripe wolf-apple (*Solanum lycocarpum* A. St.-Hil), which has been used empirically by the population due to various therapeutic effects, among them, its hypoglycemic action. The objective of this study was to evaluate the hypoglycemic effect of the administration of wolf-apple starch on diabetic Wistar rats, during five weeks. The animals were randomly divided into three groups: normal control, diabetic control and treated diabetic (received 100 mg/day of wolf-apple flour by gavage), and diabetes was induced with streptozotocin (40 mg/kg rat). The following parameters were evaluated: glycemia, animal weight, food intake, diuresis, water intake and histopathological analyses of liver and pancreas. The results show that the flour presented a hypoglycemic effect of 19.76%, and there was no significant difference in food consumption, water consumption and weight gain among the evaluated groups. On the other hand, the treated diabetic group showed a urine volume significantly higher than the other groups. The treated animals did not show toxicity in the liver and pancreas. It is concluded that the starch from wolf-apple has hypoglycemic potential.

Key words: Wolf-apple, flour, hypoglycemic effect, diabetes.

INTRODUCTION

Currently, diabetes represents a serious public health problem due to its high frequency in the population, complications, mortality, high financial and social costs involved in the treatment and a significant decrease in quality of life (Santo et al., 2012). Diabetes mellitus is a metabolic disease of multifactorial cause, characterized

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by the relative deficiency in insulin production or a decrease in its action, causing hyperglycemia (Gallego, 2005; American Dietetic Association (ADA), 2009). The disease has an increasing prevalence worldwide and could become a pandemic in the next decades, due to an increasingly aging population, but mainly due to the increasing prevalence of obesity and physical inactivity (Murussi et al., 2003; Rato, 2010). Facing that reality, the search of new alternatives in the treatment of diabetes becomes indispensable.

Although there is no cure for the disease, its control is viable by the adhesion of the diabetic patient to a series of care behaviors: diet, regular physical exercises, monitoring of glucose levels and appropriate use of hypoglycemics and/or insulin when necessary (Cruz, 2005). Studies on new hypoglycemic drugs have been conducted with special focus on the medicinal plants used in traditional medicine, because data in the literature shows that it is much more probable to find biological activity in plants oriented for use in traditional medicine than in plants chosen at random (Barbosa-Filho et al., 2006).

Solanum lycocarpum A. St. Hil is a plant species of the Solanaceae family that can be found throughout the entire Brazilian territory, mainly in savannas (Santos and Coelho, 2002). Its fruit is referred to as wolf-apple and presents a slightly flattened bulbous form (8 to 12 cm diameter) and could weigh up to 500 g (Rocha et al., 2012). Sedative, soothing, hypocholesterolemic and hypoglycemic properties have been attributed to wolf-apple (Dall and Von Poser, 2000; Vieira et al., 2003; Clerici et al., 2011). In the Southeast and Central-West regions of Brazil, the wolf-apple flour is widely used as an oral hypoglycemic. It is a powder extracted from the pulp of the unripe wolf-apple, which can be elaborated domestically or acquired in drugstores specialized in herbal medicines in the form of capsules (Clerici et al., 2011; Rocha et al., 2012). Given the above, the objective of this study was to evaluate the hypoglycemic effect of the wolf-apple flour on diabetic Wistar rats.

MATERIALS AND METHODS

Plant material

The fruits of the polant species *Solanum lycocarpum* A. St. - Hil were collected in the pasture area of Departamento de Zootecnia at Universidade Federal de Lavras. A voucher specimen is on deposit in Esal herbarium 00836. The wolf-apple flour was obtained according to Rocha et al. (2012). Sixty (60) wolf-apples were collected, weighing an average of 350 g each, and taking into account the degree of maturation, excluding mature and very unripe fruit.

The fruits were weighed, washed with distilled water and peeled. The seeds were removed and the pulp was chopped into pieces, weighed, crushed in an industrial blender with distilled water, at the ratio 2:1 (pulp/distilled water), for two minutes. The homogenized pulp was filtered on a cotton cloth, the filtrate was taken to a refrigerator (4°C) for 6 h, to decant. After decanting, the supernatant was discarded and the precipitate was washed with distilled water and put back in the refrigerator to decant for 12 h. On the next day, the sample was washed and decanted again. The supernatant was discarded and it was possible to observe the formation of a light fraction in the bottom of the beaker, and a dark fraction above that one, which was discarded. The light fraction was placed in a tray and brought into a ventilated oven, at 30°C, for three days. The dry light fraction was called starch, which was crushed, weighed and then stored in a hermetically sealed glass container protected from light, at room temperature.

Animals and experimental conditions

24 adult male Wistar rats were used, (*Rattus novergicus*), in growth phase and with an initial average weight of 193±25 g, from the Biotery of Departamento de Medicina Veterinária da Universidade Federal de Lavras. The animals were randomly divided into three groups: normal control (NC), diabetic control (DC) and treated diabetic (TD), with eight animals in each group. The animals were maintained in individual cages, at a room temperature of 25±3°C (12 h light/dark cycle) and with access to food (food suitable for this species, Nuvilab CR1®) and water *ad libitum* for five weeks. This study was examined and approved by the Ethics Committee on Animal Use of Universidade Federal de Lavras-UFLA (Ruling № 006/2009).

Diabetes induction

The animals of the diabetic control and treated diabetic groups, after anesthesia with 35 mg/Kg thiopental, received 40 mg/kg streptozotocin (Sigma®), diluted in a 0.01 mol/L citrate buffer pH 4.5 via intravenous administration, using the penile vein as access (Cunha et al., 2009).

A week after the application of the diabetogenic agent, blood glucose in the animals was measured. The rats with glycemic levels equal or superior to 120 mg/dL (Delfino et al., 2002) were considered as diabetic, and the remaining ones, which did not reach the levels, were discarded. Soon afterwards, these animals were randomly divided into the diabetic control and treated diabetic groups.

Determination of glycemia

Blood glucose was determined weekly, using the glucose oxidase method, with the use of glucose test strips, and reading was conducted in the Optium Xceed® model apparatus (Abbott). The blood samples were collected from the tail of the animals, after an 8 h fast.

Treatment with the flour

The group diabetic treated received 100 mg/day of wolf-apple flour in a single dose, which is equivalent to approximately 10 times the recommended dose for adult humans (1,500 mg/day). An orogastric tube was used (gavage) for delivery and the flour was dissolved in 1 mL of filtered water. In the control and diabetic control groups, 1 mL of water was administered by the same procedure.

Biological evaluation

Food consumption

The animals were placed in individual metabolic cages and fed with

Table 1. Weekly glucose levels (mg dL^{-1}), during the five weeks of experiment, for the groups normal control, diabetic control and treated diabetic.

Treatment/week	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Normal control	99.0±7.6C	91.12±1.8C	103.5±5.8C	113.3±5.3C	101.6±3.3C	108.2±7.7C
Diabetic control	121.0±6.8B	94.0±2.6B	121.5±1.4A	131.8±3.4A	129.1±10.4A	129.8±2.9A
Treated diabetic[1]	156.0±16.9A	114.3±3.7A	118.2±2.2B	124.0±3.3B	109.5±5.7B	125.3±1.3B

Data are the mean of eight replicates ± standard deviation. Averages followed by the same letter in the columns do not differ by the Tukey test (p≤0.05). [1]Treated diabetic: received 100 mg/day of wolf-apple flour.

25 g food/day. The consumption was recorded daily and calculated based on the remainder verified on the following day.

Water consumption

Water consumption was verified every two days, through the difference between the placed and the remaining volume.

Weight of animals

The animals were weighed weekly, on a Bel Engineering® digital scale.

Urine volume

The urine of the animals was collected in a graduated beaker and volume readings were taken every other day.

Euthanasia of animals

At the end of the experiment, the animals were anesthetized with 35 mg/Kg sodium thiopental, intraperitoneally. A median laparotomy was carried out in the pelvic-cranial direction of the abdominal and thoracic cavities. Soon afterwards, the exsanguination was performed to sacrifice the animals, and the liver and the pancreas of the animals were removed.

Histopathological analyzes

The liver and the pancreas of each animal were collected, weighed and fixed in a formalin solution. The organs were transversely sectioned, dehydrated and diaphanized at room temperature. 4 µm cuts were made in a rotary microtome, stained with hematoxylin-eosin, mounted on slides/coverslips and analyzed under light microscopy (100x magnification) for the qualitative analysis.

Statistical analysis

The data are the average of eight replicates ± standard deviation analyzed by Sanest program and when this analysis showed a significant difference, the Tukey test (P≤0.05) was used for the comparison of means. For the variance analysis, the design used was completely randomized, in a 3 x 5 (groups x weeks) factorial outline, with eight repetitions, for the parameters glycemia, food consumption, weight gain, water consumption, urine volume and liver weight.

RESULTS AND DISCUSSION

It was observed (Table 1) that there was a significant difference in blood glucose levels between the evaluated groups of animals. The group treated with wolf-apple starch showed a decrease of 19.76% in their glycemic levels over the five weeks of experiment, whereas in the diabetic group, there was an increase of 6.61% and, in the normal control group, an increase of 9.29%. At the beginning of the experiment (week 0), the treated diabetic group had glycemic levels higher than those of the normal control and diabetic control groups. However, throughout the experiment, the glycemic levels of the treated diabetic group decreased, while those of the normal control and diabetic control groups showed a small increase, demonstrating the hypoglycemic activity of wolf-apple starch.

Martha et al. (2000) demonstrated that a drug is considered effective when it reduces the glucose levels by at least 15% of the initial values. Therefore, it can be suggested that the wolf-apple flour is effective in the reduction of blood glucose levels in Wistar rats.

Studies show the presence of dietary fiber and alkaloids in wolf-apple starch (Dall-Agnol and Von-Poser, 2000; Rocha et al., 2012), and these substances may be responsible for the reduction in glycemia observed in this study. Dietary fiber delays gastric emptying, decrease in absorption of carbohydrates by the inclusion of sugars in the fiber matrix and modification in hormone secretion. On the other hand, alkaloids present in the Solanaceae family can stimulate the release of insulin by the beta cells of the pancreas (Dall-Agnol and Von-Poser, 2000), therefore reducing glycemia.

Table 2 shows the consumption of water, food, weight gain and urine volume of the experimental animals, with no statistical difference (p≤0.05) between treatments for the consumption of water, food and weight gain. The treated diabetic group presented a urine volume significantly higher than the other groups, showing that the reached glycemic levels were enough to lead to polyuria in those animals.

Regarding food consumption, water and weight gain, there was no significant difference, probably due to the fact that streptozotocin led to mild diabetes in the rats. If the disease were more severe, more severe differences would probably have been observed in those parameters.

There was no significant difference (p≤0.05) in the three studied groups, when evaluating the weights of the livers of the animals and the LW/BW ratio (Table 3), probably due to the fact that the animals did not present high glycemia.

Table 2 Average weekly consumption of water, food, weight gain and urine volume during the five weeks of experiment, for the groups normal control, diabetic control and treated diabetic.

Parameter	Treatment		
	Normal control	Diabetic control	Treated diabetic
Water consumption (mL)	180.6±73.0A	203.9±63.1A	208.1±61.0A
Food intake (g)	162.0±43.4A	151.7±26.3A	151.9±32.9A
Weight gain (g)	19.7±7.5A	17.5±23.2A	13.6±16.5A
Urine volume (mL)	10.4±3.3B	18.5±10.2B	57.3±24.1A

Data are the mean of eight replicates ± standard deviation. Averages followed by the same letter in the lines do not differ by the Tukey test (p≤0.05). [1]Treated diabetic: received 100 mg/day of wolf-apple flour.

Table 3. Average liver weight and liver weight/body weight (LW/BW) for the groups normal control, diabetic control and treated diabetic, after five weeks of experiment.

Treatment	Liver weight (g)	LW/BW (%)
Normal control	10.06±1.7^{A}	3.89±0.45^{A}
Diabetic control	11.00±2.05^{A}	4.13±0.34^{A}
Treated diabetic	10.52±2.04^{A}	4.50±0.52^{A}

Data are the mean of eight replicates ± standard deviation. Averages followed by the same letter in the columns do not differ by the Tukey test (p≤0.05). [1]Treated diabetic: received 100 mg/day of wolf-apple flour.

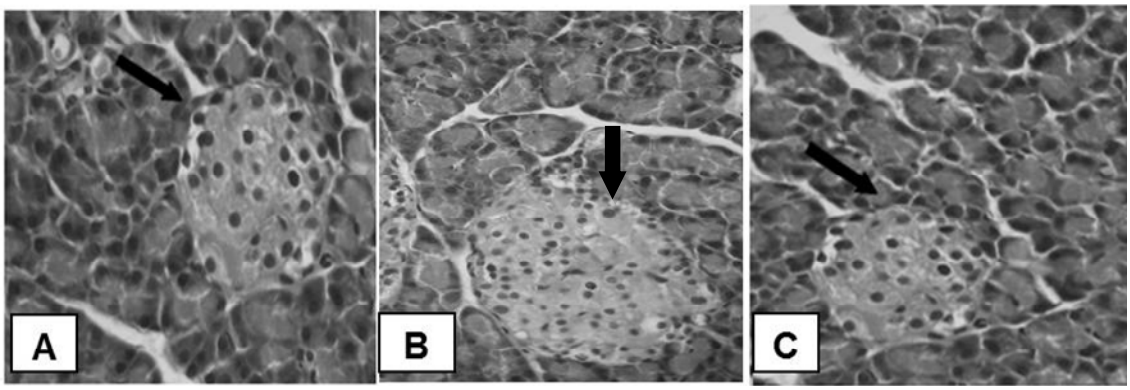

Figure 1. Histological section of the pancreas (islet of Langerhans is indicated by black arrow) stained with hematoxylin-eosin, for the groups normal control (A), diabetic control (B) and treated diabetic - received 100 mg/day of wolf-apple flour (C), for six weeks. 40 X magnification.

The histological sections of the pancreas and liver of the animals are presented in Figures 1 and 2, respectively. No macroscopic changes were observed in the organs analyzed, and neither were injuries, or significant microscopic changes.

Observing the pancreas and liver slides from all of the studied groups, it was noticed that there was no difference among the groups under optical microscopy analysis. This is probably due to the fact that the animals presented mild diabetes, not leading to lesions in those organs, observable by this technique.

The wolf-apple flour did not present any toxic effect to the liver, under the tested conditions. To affirm the non-hepatic toxicity of the wolf-apple flour, other analyses would be necessary, such as the plasmatic analyses of aminotransferases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Conclusions

Although the wolf-apple flour did not normalize the

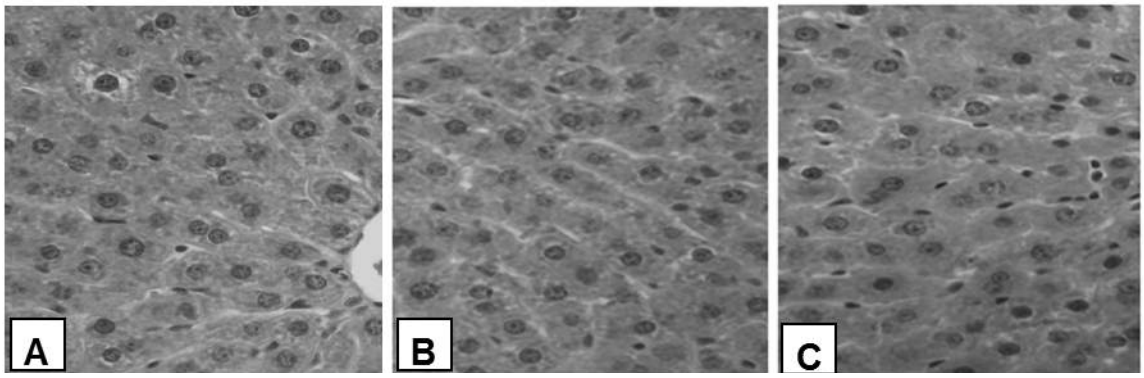

Figure 2. Histological section of liver stained with hematoxylin-eosin, for the groups normal control (A), diabetic control (B) and treated diabetic - received 100 mg/day of wolf-apple flour (C), for six weeks. 40 X magnification.

glycemic levels of the treated rats, there was a relevant reduction of 19.76% in glycemia. However, in the non-treated group, the glycemia practically did not change, and it could be inferred that the wolf-apple flour can aid in the glycemic control of diabetics.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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