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Hypoglycemic Effect and Histopathological Changes of the Ethanolic Extract of *Witheringia Solanaceae* L´Hér Fruits

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ABSTRACT

Introduction: the *Witheringia solanaceae* is a short weak shrub belonging to the Solanaceae family and widely used in folk medicine of Central American countries. In Costa Rican traditional medicine it is employed as an antidiabetic agent. However, there is no scientific evidence demonstrating the biological effects of the fruits of this plant. The aim of this work was to evaluate the hypoglycemic effect and histopathological changes in the target organs of mice exposed to the ethanolic extract of *W. solanaceae* fruits.

Material and Methods: a ethanolic extract of *W. solanaceae* fruits was prepared with ethanol (95%). To evaluate the hypoglycemic activity, a single oral dose of 200 and 400 mg/kg of the extract was administered in an oral glucose tolerance test in normal mice. The blood glucose level was determined at different times by the glucose oxidase method. Then, it was evaluated the histopathological changes in liver, kidneys, heart and lungs.

Results: The administration of the ethanolic extract of *W. solanaceae* fruits at 200 and 400 mg/kg was not able to reduce the blood glucose levels of mice. In addition, this extract led to some histological changes, such as microsteatosis and congestion in liver. The treated groups showed slight changes in the glomeruli and in the proximal convoluted tubules. No relevant changes were found in the lungs and the heart.

Conclusion: the ethanolic extract of *Witheringia solanaceae* fruits showed no hypoglycemic activity in mice at doses of 200 and 400 mg/kg. Besides, it was able to cause some histopathological changes, especially in liver.

Keywords: *Witheringia solanaceae*; diabetes; hypoglycemic activity; histopathology; herbal medicine.

Introduction

Diabetes mellitus (DM) is undoubtedly one of the most serious health problems of the 21st century. DM is a chronic metabolic disorder characterized by increased blood glucose levels (hyperglycemia) due to a disorder in the secretion or action of insulin. The main symptoms of diabetes are polyuria, polydipsia, polyphagia, blurred vision and weight loss. In its most severe form, it can lead to coma and death[1]. DM is associated with the development of microvascular complications such as retinopathy, nephropathy and neuropathy, as well as macrovascular complications, including accelerated cardiovascular disease and cerebrovascular disease in the form of cerebrovascular accident[2,3].

Researchers around the world have agreed that there is a need to find drugs that provide stable, durable and safe glycemic control through proper control of glucose metabolism[4,5]. Several studies have demonstrated the effect of medicinal plants that have been shown to be promising in glycemic monitoring. Plants of the Solanaceae family present hypoglycemic, antihyperglycemic or antidiabetic effects, especially those belonging to the genus Physalis[6–10]. Besides, Herrera *et al*. (2011) showed that the aqueous extract of *W. solanaceae* leaves have hypoglycemic and antihyperglycemic effects in normal and alloxan-induced hyperglycemic rats[11]. However, there are no studies demonstrating the hypoglycemic effect of fruits of *W. solanaceae*.

In this context, the aim of this study was to evaluate the hypoglycemic effect and histopathological changes in mice exposed to ethanolic extract of *Witheringia solanaceae* fruits.

Material and methods

Plant Material

The plants of *W. solanaceae* were obtained from Santa Clara in San Carlos (Alajuela, Costa Rica) in the month of November and authenticated by Dr. Luis Poveda (Universidad Nacional, Costa Rica). The voucher specimen (No. JVR14890) was deposited at the Herbarium "Juvenal Valerio Rodríguez" of the Universidad Nacional, Costa Rica. The authorization for accessing and studying samples from Costa Rican genetic heritage was obtained from the Costa Rican government through National Commission for the Management of Biodiversity (CONAGEBIO)

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Preparation of extract

The fruits were selected and dried in an oven with air circulation at 50ºC for 7 days and then grounded until obtaining a powder of fine texture. To prepare the ethanolic extract, ethanol (95%) was added to the powder for extraction until exhaustion, then the extract was filtered and concentrated in rotatory evaporator at 35 ± 5ºC under reduced pressure, to obtain a semisolid material, which was then lyophilized to get a powder.

Animals

Male Swiss mice (*Mus musculus*) of 25 and 30 g were obtained from the Oswaldo Cruz Institute, Pernambuco (FIOCRUZ-PE). The animals were accustomed with laboratory conditions for one week and housed in polypropylene cages at room temperature between 22 ± 3°C, in light-dark cycles of 12/12 h, with 50 to 60% humidity. All animals received water and food *ad libitum*. All experimental procedures were developed according to ethical precepts of animal research and previously approved by the Ethical Committee on Animal Research of the Universidade Federal Rural de Pernambuco (CEUA/UFRPE No. 93/2018).

Hypoglycemic activity

For the determination of hypoglycemic activity, the animals were randomly distributed in three groups of six mice, which were fasted for 6 h prior to Oral Glucose Tolerance Test (OGTT). The ethanolic extract of *W. solanaceae* fruits were administered orally, by gavage, in single and fixed doses at 200 and 400 mg/kg. The saline group received a saline solution. At 10 min after the administration of the treatments, 2 g/kg of glucose was administered to each mouse. The blood glucose level was measured before the treatment and at 30, 60, 90 and 120 min after the treatment. The blood samples were drawn from the coccygeal vein and the blood glucose level was determined by the glucose oxidase method with the Kit Accu-Chek Activ® glucose analyzer, which was validated. At the end of the experiment, the animals were euthanized, and target organs were collected for histopathological analysis.

Histopathological changes

After euthanasia, liver, kidneys, heart and lung samples were removed, fixed in 10% buffered formalin were embedded in paraffin, sliced at 5mm, and stained with hematoxylin and eosin (H&E) according to standard protocols. Then, the samples were blindly analyzed by an experienced pathologist.

Statistical Analyses

Data are reported as means±SD. Statistical significance of differences between groups was assessed using one-way ANOVA, followed by the Tukey test. $P<0.05$ was considered to be significant.

Results and Discussion

Hypoglycemic activity

OGTT is one of the tests of choice to evaluate the hypoglycemic effect of drugs on glucose metabolism. It is a fast and inexpensive technique usually used in scientific research with animals. In normoglycemic mice, post-prandial glycemia elevation after glucose overload, and consequent normalization of onset, after about 2 h, characterize a normal glucose metabolism function[12]. Some oral hypoglycemic agents, such as metformin, are capable of altering glucose metabolism inhibiting the elevation of postprandial glycemia in about 25 to 30%[13].

Our results showed that the ethanolic extract of the fruits of *W. solanaceae* at 200 and 400 mg/kg was not enough to decrease the glycemia of normal mice submitted to the OTTG test (Fig. 1). In contrast, a study with fruits of *Physalis peruviana* L., a plant belonging to the Solanaceae family, showed hypoglycemic effects when young adults intook fruits of *P. peruviana*, which were able to reduce the glucose levels at 90 and 120 minutes postprandial[14].

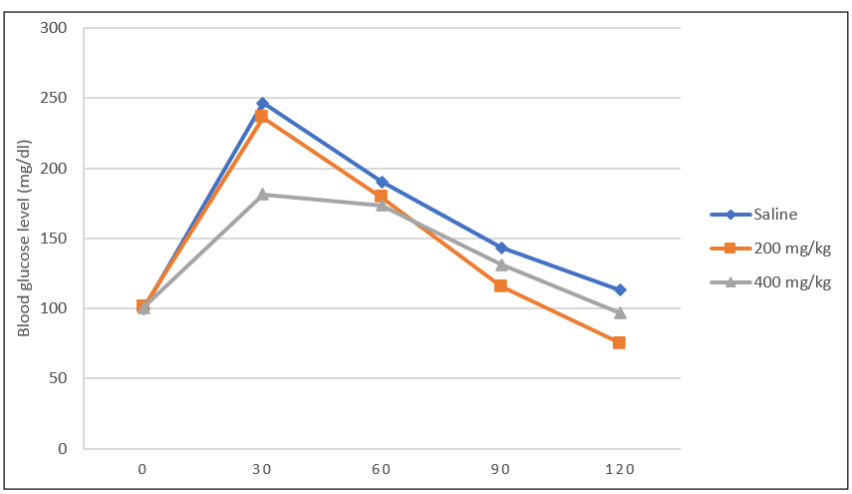

Figure 1. Hypoglycemic activity of ethanolic extract of *W. solanaceae* fruits in mice. Reduction of blood glucose produced by ethanolic extract of *W. solanaceae* fruits at different doses after oral administration in glucose load mice (n = 6).

Besides, the effects of the ethanol extract of the fruits of W. *solanaceae* do not coincide with those reported by Herrera *et al*. (2011), who demonstrated that the aqueous extract of the leaves of W. *solanaceae* presented a hypoglycemic and antihyperglycemic effect both in normoglycemic rats as in diabetic induced rats by alloxan[11]. It is important to consider that although the study by Herrera *et al*. was made with the same plant, they used leaves and not fruits. In addition, the experiment by Herrera *et al*. was performed in rats whose glucose metabolism is different when compared to mice.

Histopathological changes

According to the histopathological evaluation, the liver of the control group showed normal histological characteristics with preserved hepatic architecture. The normal liver was devoid of necrosis, fibrosis, hemorrhage or fatty changes (Fig. 2A). The portal triad contained a bile duct branch, a portal vein branch, and a hepatic artery branch. In treated groups with ethanolic extract of fruits, the morphology of portal tracts was preserved, and the findings most frequently found in the liver were a microsteatosis diffuse and congestion (Fig. 2B and 2C). Although both *P. peruviana* and W. *solanaceae* belong to the family Solanaceae and possess physalins in their composition, our results do not agree with those reported by Dkhil *et al*. (2014) and Chang *et al*. (2008), who showed a potential hepatoprotect effect of the *P. peruviana* against cadmium-induced hepatic and renal toxicity in Wistar rats[15] and a protect effect against hepatotoxicity caused by acetoaminophen in rats[16].

Regarding the kidneys, the control group showed a normal structure of glomeruli and renal tubules (Fig. 3A). The 200 mg/kg group had slight glomerular atrophy and hydropic cells in the proximal convoluted tubule (Fig. 3B), whereas the 400 mg/kg group, it was observed loss of microvilli at the apical pole. Both treated groups had cell debris at the tubular lumen (Fig. 3C). Despite the reported lesions, the renal architecture was not modified. However, our results do not agree with those reported by Dkhil *et al*., (2014), who revealed the potential protective role of *Physalis peruviana* L. fruit in cadmium-induced hepatotoxicity and nephrotoxicity[15]. Besides, Ahmed *et al*. (2012) showed that the *P. peruviana* confers an appealing nephroprotective effect which might be explained partially via diminishing the generation of malondialdehyde and nitric oxide by induction of antioxidant systems[17]. No relevant changes were found in the lungs and the heart.

Conclusions

In conclusion, this study revealed that the ethanolic extract of *Witheringia solanaceae* fruits does not present a significant hypoglycemic effect in

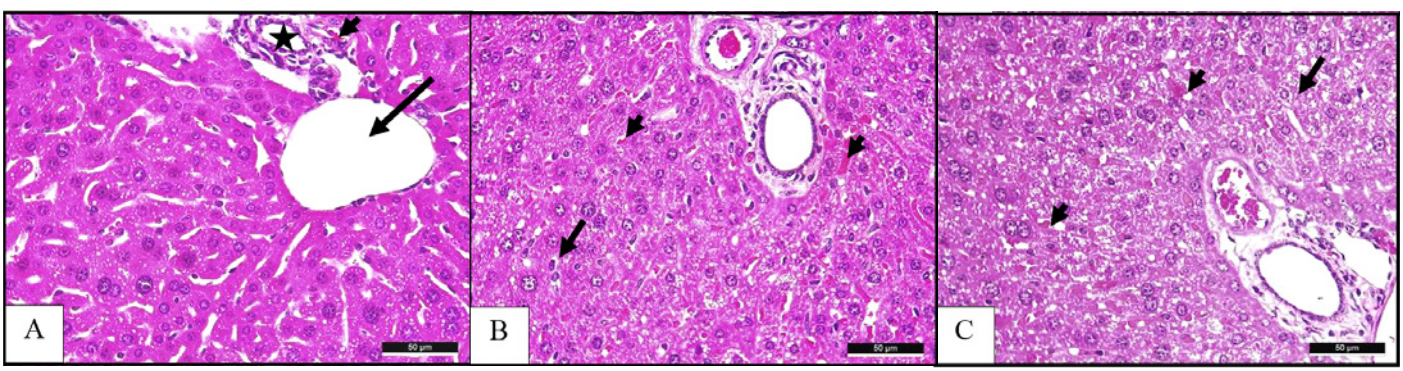

Figure 2. Histopathological analysis of the liver (H&E: 40X). (A) Control group with normal hepatic triads: portal vein branch (long arrow), hepatic artery branch (short arrow) and bile duct branch (star). (B) 200 mg/Kg group and (C) 400 mg/Kg group with diffuse microsteatosis (long arrow) and congestion (short arrow).

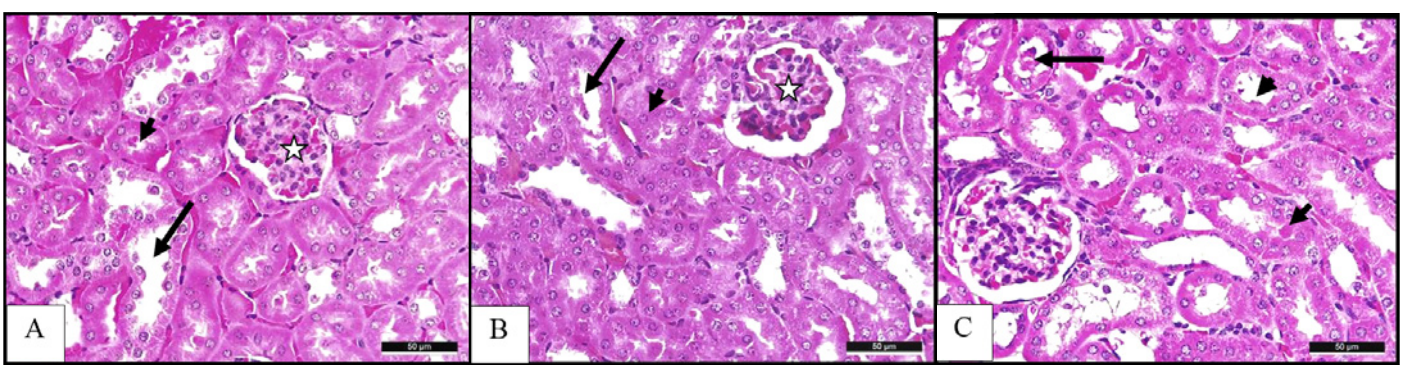

Figure 3. Histopathological analysis of the kidney (H&E: 40X). (A) Control group with normal structure of glomeruli (star) and proximal tube (short arrow) and distal tube (long arrow). (B) 200 mg/Kg group with discrete glomerular atrophy (star), hydropsy in lining cells (short arrow), cell debris in the tubular lumen (long arrow). (C) 400 mg/Kg group with degenerating cells (short arrow), loss of microvilli at the apical pole (arrowhead) and cell debris in the tubular lumen (long arrow).

normoglycemic mice at doses of 200 and 400 mg/kg. Besides, this extract in these doses led to some histopathological changes, especially microsteatosis and congestion in liver.

Acknowledgments

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