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Original Article

A *Taliparitielatus* Sw. Fryxell flowers extract inhibits histamine-induced edema in mice

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Abstract

Introduction: *Taliparitielatus* Sw. Fryxell (Malvaceae) is a wooden botanical species that is endemic to Jamaica and Cuba. The main flavonoids identified in the flowers of this species have several biological activities, including the inhibition of vascular hyperpermeability induced by different stimuli. This work was aimed to assess whether a hydroalcoholic extract of *T. elatus* flowers (TFE) inhibits the edemagenic effect of histamine.

Methods: The flowers were collected from *T. elatus* trees at Cerro Municipality in Havana. TFE was obtained with 70% ethanol and was characterized by a phytochemical screening. Acute oral toxicity was determined in both sex SD rats. TFE (67; 200 and 600 mg/kg) or ketotifen (3 mg/kg) were orally administered to male Balb/c mice 1 hour before an intraplantar injection of histamine (50 µg) and the edemagenic reaction determined as the paw weight increment.

Results: Saponins, phenols- such as: tannins and flavonoids, anthocyanins, alkaloids, quinones, and coumarins were the major bioactive compounds in the extract. There were neither deaths nor any sign of toxicity among the rats treated with TFE. There was a significant inhibition of histamine-induced edema that was independent of TFE dose that was comparable with ketotifen.

Conclusion: This was the first evidence of the anti-edemagenic effect of the 70% ethanol extract of *T. elatus* flower petals against histamine-induced edema as a result of the early stage of a research project that deals with the pharmacological study of this endemic species.

Keywords: *Taliparitielatus*, Medicinal plant, Anti-edemagenic action, Histamine, Chemical composition, Acute toxicity

Introduction

Taliparitielatus Sw. Fryxell (majagua) fam. Malvaceae is a wooden botanical species that is endemic to Jamaica and Cuba (Figure 1).[1,2] However, there is little information about the biological properties of this species.[3]

Nonclinical studies have provided evidence on some biological activities of quercetin, rutin, and gossypitrin, the major flavonoids of *T. elatus* flowers.[4] For instance, quercetin and ruitn inhibited histamine release from mast cells,[5,6] reduced phospholipase A2 activity and recruitment of neutrophils and eosinophils into the lung.[5] Furthermore, rutin is a known protector from capillary permeability by histamine and other compounds.[7,8] Moreover, quercetin has induced the relaxation of airway smooth muscle, potentiated the β-agonist-induced effect on this tissue in vitro[9] and inhibited the transcriptional up-regulation of histamine H1 receptor.[10] Antioxidant capacity of the three flavonoids has been demonstrated.[11,12] These biological properties could be transferred to a given product derived from this part of the plant. Therefore, a research strategy for the characterization of its therapeutic potential should include the evaluation of its possible inhibitory effect on the release of chemical mediators, the contraction of airways smooth muscle and the increase of vascular permeability stimulated by histamine and other inflammatory agents. Accordingly, this study was aimed to assess the presence of one of these biological properties

Figure 1. *Taliparitielatus* Sw. Fryxell

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in a hydro-alcohol extract of *T. elatus* flower petals; this is the ability to inhibit histamine-induced increase of capillary permeability. It is the first stage of a research project that deals with the pharmacological study of this endemic species.

Materials and Methods

Plant Material

Flowers were collected from *T. elatus* Sw. Fryxell trees planted in parterres at Cerro Municipality of Havana, in the morning during July and August 2015. The taxonomic identification of this botanic species was performed by the Central Laboratory of Pharmacology from Salvador Allende Faculty of Medicine, University of Medical Sciences of Havana, Cuba. Its identity was demonstrated by comparison with the sample registered at Havana Botanic Garden (Voucher number HAJB 82587). The flower petals were separated and cut into pieces, dried in an oven at 40ºC for 12 hours (Figure 2), and reduced to small particles of about 3 mm by using a mesh battery.

Preparation of Plant Extract

An extract of *T. elatus* flower (TFE) petals was prepared by the method Miranda and Cuellar.[13] It was obtained by percolation of dried plant material with 70% ethanol. Afterward, rotoevaporation (at 40°C and 27 mm Hg reduced pressure) of the homogeneous liquid obtained was performed for ethanol elimination from it in order to avoid the possible bias of the results of the pharmacological evaluations. The remaining liquid was stored in closed dark bottles at 4 to 8°C until used. The total soluble solids (TSS) content of the extract was gravimetrically determined in dried 1 mL aliquots (three replicates) by using a Sartorius MA40 analytical balance. A mean value of 400 mg/mL was obtained.

Phytochemical Screening

A phytochemical screening of TFE was performed by the method described earlier,[13] that consisted of the formation of colored and/or insoluble chemical complexes from the secondary metabolites contained in the plant extract.

Animals

Sprague Dawley rats and Balb/c mice were purchased from the National Center for the Production of Laboratory Animals (Havana, Cuba). The animals were placed into polyurethane cages and housed at controlled environment (23°C and 50-60% relative humidity) with free access to food (AL- YCO® CMO 1000) and water.

Acute Oral Toxicity

TFE acute oral toxicity was evaluated in SD rats (both sexes, 200 ± 20 g b.w.) according to 423 OECD guideline.[14] A single intragastric dose of the extract (2000 mg TSS/kg b.w.) was administered to rats. They were observed for a 14-day period in order to detect the occurrence of deaths or any sign of toxicity. In addition, their body weights were determined before and after 7 and 14 days of treatment.

Figure 2. Drying of *T. elatus* flowers.

Increase of Histamine-Induced Vascular Hyper Permeability in the Mouse Paw

This assay was performed as described by Leal et al[15] with modifications. Ten male Balb/c mice (20-25 g b.w.) were randomly assigned to each treatment group: negative control (saline), positive control (ketotifen 3 mg/kg b.w.) and TFE (67; 200 and 600 mg TSS /kg b.w.). Food was retired 12 hours before the experiment. Saline, ketotifen and TFE solutions (0.1 mL/30 g b.w.) respectively were intragastrically administered to the animals 1 hour before a subplantar injection of 50 µL of saline containing 50 µg of histamine into the right hind paw and the same volume of saline solution alone in the left hind paw. The animals were sacrificed by cervical dislocation 1 hour later and their paws were cut and weighted in Sartorius MA40 analytical balance. Results are expressed as the difference between right and left paw weight.

Statistical Analysis

The experimental results were expressed as the mean ± standard deviation (SD) of each group of treatment. Normal distribution of data and homogeneity of variance were assessed by Shapiro-Wilk's and Bartlett´s tests, respectively. The statistical comparison between groups was performed by one-way ANOVA or Kruskal-Wallis test, according to the results of the previous tests. The means were compared by Dunn's and Bonferroni's tests. Differences lower than 5% were considered significant. The GraphPad Prism 5 statistical program was used for this purpose.

Ethics

All procedures described were carried out using a protocol approved by the Institutional Research Ethics Committee of Salvador Allende Faculty of Medicine. The guidelines for good practices for the management of laboratory animals[16,17] were followed.

Results and Discussion

Phytochemical Screening of TFE

As can be observed in Table 1, saponins and phenols –

such as: tannins and flavonoids, anthocyanins, alkaloids, quinones, and coumarins – were the major secondary metabolites found in TFE, as previously reported for the flowers and ethanol extracts of *T. elatus* plants grown in Cuba.[4,18,19] This qualitative similarity endorses the contribution of the results of this research for the chemical, pharmacological and toxicological characterization of this botanical species.

Oral Acute Toxicity

The demonstration that a research product has a low probability to be toxic for humans is pivotal for the strategy of new drug development.[20] This concept is valid for a plant extract, though it is obtained from parts of those called medicinal plants, since they have complex compositions, as demonstrated for TFE (Table 1), with the combination of different biological properties that not only could support their medicinal use, but also could result in undesirable bio- logical effects.

The evaluation of acute toxicity in experimental animals is a primary stage of non-clinical estimation of a product's safety. It consists of the evaluation of quantitative and qualitative changes, associated with the administration a single or repeated doses of the substance in a 24-hour period of time. This information offers a basis to calculate the doses of a product for its pharmacological characterization.

In agreement with a previous toxicological study of an ethanol extract of *T. elatus* flowers,[21] the acute intragastric administration of TFE 2000 mgTSS/kg b.w to rats (a maximal allowable dose[20]) caused neither deaths nor signs of toxicity among these animals during the 14-day period of observation following the treatment. Also, body weight gain was within the expected limits for this species (Figure 3), suggesting that this extract could be safe after a single dose treatment. However, other toxicological studies are necessary to complete the knowledge about this extract safety.

Table 1. Phytochemical Screening of TFE

Assay	Identified Metabolites	Results
Resins	Resinous products	–
Baljet	Lactonics compounds	++
Foam	Saponins	+++
Ferric chloride	Tannins and phenols	+++
Ninhidrine	Amino acids	–
Shinoda	Flavonoids	++
Kedde	Glycosides	–
Fheling	Reducing sugars	+++
Lieberman-Buchard	Triterpenes and steroids	+
Bortranger	Quinones	+++
Anthocyanidins	Anthocyanidins	+++
Drangendorff/Mayer /Wagner	Alkaloids	+++

+++ Strong reaction; ++ Intermediate reaction; + Weak reaction; - Absence of reaction.

Inhibition of the Increase of Histamine-Induced Capillary Permeability in the Mouse Paw

Preliminary experiments for standardization of this experimental model allowed the selection of the doses of histamine and the H1 antihistamine drug ketotifen, to be used in the experiments for the edemagenic stimulus and the positive control respectively. Fifty micrograms of histamine/paw showed to be a proper stimulus level because it produced a measurable, submaximal and reproducible effect. On the other hand, ketotifen 3 mg/kg b.w. had a submaximal inhibitory activity.

Since a single oral dose of TFE 2000 mg/kg showed no toxicity to animals a range of lower doses, being one 1/3 of the other (600; 200 and 67 mg/kg), was selected to assess the extract effect on histamine edemagenic activity in the mouse paw. These numbers approximately correspond to 1/3; 1/9 and 1/27 x 2000 mg/kg respectively.

Figure 4 shows that TFE significantly inhibited histamine induced edema at all dose levels tested but not in a dose-independent manner. Consequently, 67 mg/kg could be the highest effective dose of this extract and its ED 50 could be below it, thus suggesting that it has a favorable security index, higher than 27 after a single oral dose.

Histamine-induced paw edema was significantly lower in the group of mice treated with ketotifen with respect to the negative control group (Figure 4). The lack of statistical difference between the groups of animals treated with the extract and the one treated with the reference drug (Figure 4) is a demonstration of similar effectiveness in these experimental conditions that suggests a therapeutic potential of this plant preparation.

The flavonoids contained in TFE could be responsible for the inhibition of histamine effect but it has been

said that they do not have antihistamine activity.[22] Consequently, it seems that an unspecific rather than a specific mechanism of action could explain the results. Nevertheless, a contribution of other chemical

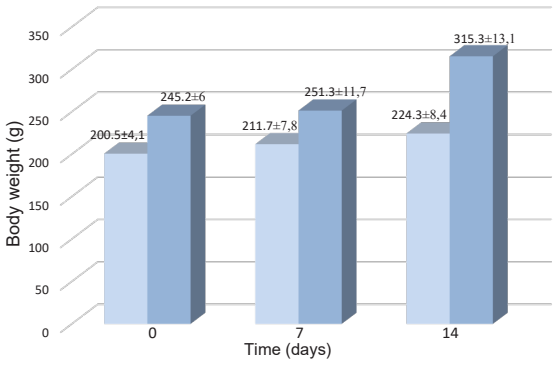

Figure 3. Increase of body weight in Wistar rats (female: gray and male: black) (N = 3 animals/group) after a single oral administration of TFE 2000 mg/kg b.w. One-way ANOVA with Bonferroni's post-test.

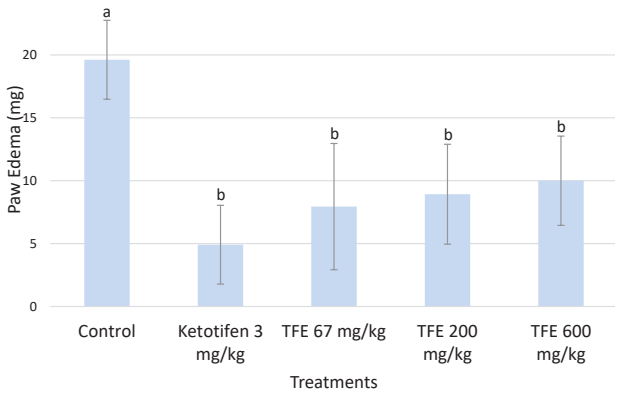

Figure 4. Antihistaminic effect of *Taliparitielatus* dried flowers fluid extract p.o. in Balb/c male mice (N = 10 animals/group). Kruskal–Wallis with Dunns´ post-test. Different letters means significant statistical differences for $P<0.05$.

components to the biological activity of this extract should not be discarded. Additional studies should be aimed to clarify it, and its influence on vascular hyper permeability induced by other stimuli.

The results presented here support the hypothesis of this research that an extract of *T. elatus* flowers could inhibit the edemagenic effect of histamine. Nevertheless, its probable inhibitory effects on the release of histamine, leukotrienes and other inflammatory mediators, as well as on airway contraction and other biological activities associated with the main secondary metabolites present in *T. elatus* flowers should be evaluated.

Somechronicdiseasesinvolvingedemaandinflammation induced by histamine could also be influenced by this extract. For instance, rutin pharmaceutical products and the micronized purified flavonoid fraction have a demonstrated activity as venotonic agents and are used for the treatment of chronic venous insufficiency.[23-25]

Accordingly, this plant preparation could have a wide [i] field of therapeutic use that deserves further investigation.

Conclusion

The present study has given the first qualitative experimental evidence that a 70% ethanol extract of the dried flower petals of *T. elatus* plants grown in Cuba contains saponins and phenols such as tannins and flavonoids, anthocyanins, alkaloids, quinones, and coumarins. Also, it did not show acute toxicity in rats. Furthermore, it was able to inhibit histamine-induced paw edema in mice. It was a result of the early stage of a research project that deals with the pharmacological study of this endemic species.

Competing Interests

None.

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