PHYTOCHEMICAL AND PHARMACOLOGICAL PRELIMINARY STUDY OF THE METHANOLIC EXTRACT FROM *STRUTHANTHUS VENETUS* *IN CARDIOVASCULAR SYSTEM OF ANESTETHIZED RAT.*

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Summary

Hypertension is the most common cardiovascular disease. It is among the first ten death causes all around the world. That is why it is very important to develop new antihypertensive agents. Studies about the methanolic extract taken from the *Struthanthus venetus* (Sv), better known as "injerto"- graft- (Voucher 33393, Herbario Nacional de Mexico, Instituto de Biología, UNAM), showed hypotensive and cardiotoxic effects over the anesthetized rats. It also produced a vasorelaxant effect over endothelium free rings precontracted with norepinephrine taken from guinea pig aorta. Due to the purpose of learning about these action mechanisms as well as isolate the responsible substance or substances, the methanolic extract separation from Sv leaves (SvME) was carried out. This SvME was dissolved in several solvents. The hypotensive fraction analytical chemical study through). This fraction produced into anesthetized rats hypotension dependent dose as well as a cardiotoxic effect characterized by rat electrocardiogram T wave flat. These results suggest that both the hypotensive as well as the cardiotoxic effects take place because of water soluble compounds like polyphenols. Besides, the hypotensive effect mechanism is related to beta adrenergic activity. The active compounds structural elucidation total is currently been studied.

Key words: Struthanthus venetus, cardiovascular activity, hypotensive effect, rat, polyphenols.

Introduction

The gender *Struthanthus* form a group hemi parasites plants.[1]. *Struthanthus venetus* is known as "injerto"-grafting- and belongs to: Plantae> Magnoliophyta> Magnoliopsida> Santalales> Loranthaceae>*Struthanthus* >specie *venetus*. This plant is adhered to different bushes with flowers stems such as tulips, peach and tangerine trees and "casuarinas".

This plant is used in traditional medicine to cough and as hypoglycemic agent [2, 3] and its biological activity has not been studied within experimental animals. Preliminary studies have been carried out in our laboratory with *Struthanthus venetus* methanolic extract (SvME) obtained from dry leaves in order to evaluate cardiovascular activity in anesthetized rat. The SvME produced significant decrease of the mean arterial pressure (MAP) and decreased of heart rate (HR).

In continuation of our pharmacological studies this paper deals about the pharmacodinamic analysis of hypotensive effect and on preliminary phytochemical study in order to characterize the compounds which could be responsible of cardiovascular effects.

Materials and Methods

Plant material

*Struthanthus venet*us was collected in Oaxtepec Morelos, Mexico City, during spring season 2004 and a specimen was deposited in the Mexican National Herbarium at the Institute of Biology, UNAM (Voucher 33393).

Extraction and isolation

Plant leaves were dried down shared and powdered. The powder was exhaustively extracted with methanol at 20^{o} C. The solvent of the liquid extract was removed completely by evaporation at room temperature (20^{o} C). The dried extract yield was 10/100 g of the starting crude material.
An extract part of SvME was dissolved into saline solution (sodium chloride 0.9 %) and centrifuge to 5000 rpm/10 min, with a final concentration of 50 mg/ mL for administration intravenous.

Other part of SvME was defatted with hexane and fractionated with dichloromethane. The soluble fraction (4.0 g) was redissolved in methanol and insoluble part was set aside. The evaporation to environmental temperature led to dark brown residue giving positive to polyphenols green color with $FeCl_3$. This material was separated with mixture of n-butanol-water (100 to 100 mL) and hydrosoluble fraction was employed for chemical analysis.

Chemical analysis

In order to identify the main chemical groups, HPLC was carried out in a system Hewlett Packard 1050 series with a Hypersil BDS-C18 (5µm, 250 x 4.6 mm) column, using water-methanol gradient at a flow rate of 0.7 mL min^{-1}. The detection was achieved at 260 and 280 nm. ^1H NMR was determined in methanol–d_4 using Varian Mercury VX 400 MHz spectrometer.
The IR analysis was recorder in a Perkin Elmer FT-IR Spectrometer Paragon 1000.

Anesthetized rats preparation.

Studies were carried out in male Wistar rats (Rattus norvegicus) weighting between 250 and 280 g. They were bred into the animal facilities of the School of Medicine, National Autonomous University of Mexico. They were subjected to a 12 h light/dark cycle and were maintained at 21-23 ^oC and 45 % humidity; tap water and food pellets (5001 Rodent Laboratory Chow; Agribrands Purina Canada, Woodstock, ON, Canada) were available freely. Animals were brought daily to the laboratory for the experiments, which were conducted according to the Guide for the Care and use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1996).
The rats were anesthetized with a mixture chloralose 50 mg/kg and urethane 800 mg/kg i.p. Cannulas were placed in three places: the trachea in order to facilitate spontaneous breathing, into the left femoral artery for blood pressure recording, and into the right femoral vein for drug administration. The arterial cannula was filled with heparin, 50 U/mL, in order to prevent clotting; no anticoagulant was administered systemically. The MAP was recorded on a Grass Model 79 polygraph with a Gould-Statham P23ID transducer (Grass Instrument Division, Astro-Med, Warwick West, R I). High frequencies of the transducer signal were electronically filtered in order to obtain a smooth tracing equivalent to MAP. The HR was simultaneously recorded through another polygraph channel with a Grass 7P4 cardiotachograph triggered by the pulse waves from the unfiltered transducer signal. After a stabilization period of at least 20 min (basal values), one dose (80 mg/kg) as well as growing SvME doses (20, 40, 60, 80 and 100-mg/kg in 0.25 mL) were injected by i.v. The doses were administered as bolus every 15 min and the MAP and HR recordings were continued for 90 min. In other sets of experiments the responses doses curves at SvME were obtained after and before the treatment with propranolol (1 mg/ Kg i. v.). The values were sent to a computer in order to be tabulated. All experiments groups consisted of 6 rats.

Drugs and Chemicals.

The propranolol hydrochloride, the heparin sodium and Methanol-d_4 to RMN were obtained from Sigma-Aldrich (St. Louis, MO). Methanol, petroleum ether, ethyl acetate, acetone, chloralose-urethane and NaCl were reagent grade of J.T. Baker S.A. of C.V. Methanol and Water grade HPLC was obtained of Mallinckrodt.

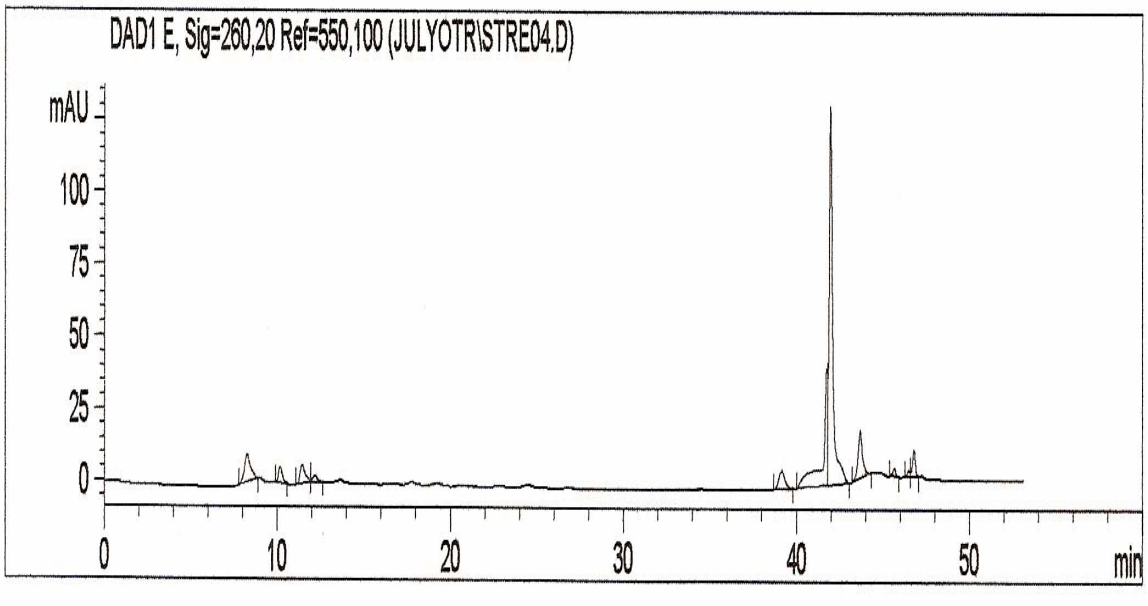
Statistical analysis.

The results are presented as means ± standard deviation (S.D.) of MAP and HR are depicted in mm Hg and beats/min, respectively. MAP and HR values at each 10 min tabulation period were compared with the corresponding controls by unpaired t tests or one way variance analysis. In all cases probability level of less than 0.05 was accepted as significant.

Results

The figure 1 is a picture of *Struthantus venetus* main stem. Observe that the branches and leaves are implanted on tulip typical leaf.

The figure 2 show the chromatographic profile of hydrosoluble fraction recorded at 260-280 nm. The main peak (49.6 % area) was recorded to 41.9 min retention time.



The present study was carried out in anesthetized rats using the MAP and HR responses to SvME as an index of the cardiovascular activity of the *S. venetus*. The initial MAP and HR were 115 ± 2 mmHg and 319 ± 4 beats/min, respectively. The SvME administration produced an immediate lessen of MAP and HR from 120 mm Hg to 50-70 mm of Hg and 350 to 100 beats/min, respectively. Some animals ended up with respiratory arrest (not shown).

The Fig. 3 shows the dose-dependent effects on MAP and HR of several doses of SvME in anesthetized rat.

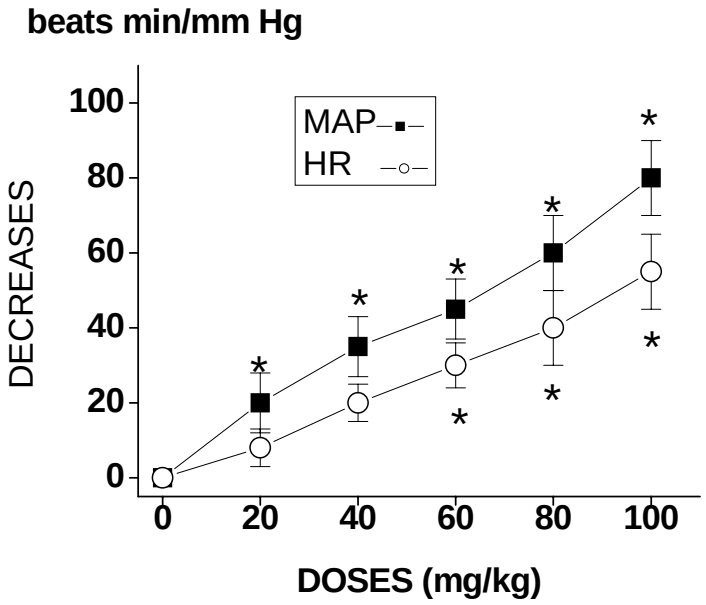
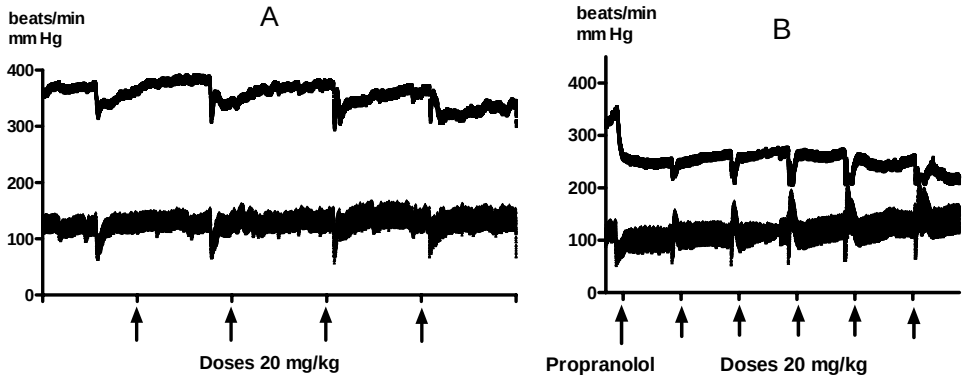


Figure 3. Symbols correspond to means six experiments; vertical lines are. S.D. Asterisks denote significant differences from values with respect at time 0.

The Figure 4A, shows that several doses of SvME (20 mg/kg iv) decreased the MAP and HR. Propranolol (antagonist of β adrenergic receptors [5])was administered (1 mg/kg iv) after 15 min. HR decreased significantly whereas MAP decreases moderately. Once the MAP and HR were normalized, the same SvME doses were administered again. Propranolol blockaded and inverted the hypotensor SvME response (Fig. 4B).



Discussion

The objective of the present work was study the cardiovascular activity of SvME, using anesthetized rats. Previous studies have been showed that SvME elicited significant relaxant effect in aortic rings from *Guinea pig* which could be attributed partially to calcium inhibition influx in vascular smooth muscle [4]. This relaxant vascular effect may be the responsible mechanism for cardiovascular activity produced by SvME.

These results suggest that the S. venetus hypotensive effect action mechanism could be mediated by β adrenergic receptors. However, the phytochemical preliminary analysis allowed identify functional groups like to polyphenols which have a broad spectrum the biological an pharmacological activities including antihypertensive and vasorelaxant effects make them good prospect for the effects observed in the present study. However further chemical and pharmacological experiments are required in order to find out its potential cardiovascular activity and to identify the responsible active principle(s). RMN studies are in progress.

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