ANTIHYPERTENSIVE ACTIVITY OF *Ruta chalepensis L.* LEAVES

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Summary

R. chalepensis is claimed in folk medicine and used in Mexico for treatment of hypertension and other cardiovascular disorders. The antihypertensive effect of an aqueous extract of the leaves from *R. chalepensis* (RCAE) and its methanol fraction (RCMF) was investigated in two models of hypertension. Acute administration of RCAE and RCME caused a significant fall in blood pressure in normotensive anaesthetized rats. RCAE (25 mg/Kg) produced displacement to the right in a dose-response curve to phenylephrine. In other experiments using 5 groups of rats, $N^{(\omega)}$-nitro-L-arginine methyl ester (L-NAME) was administered orally (70 mg/Kg) concomitantly with RCAE (25 and 50 mg/Kg), enalapril (5 mg/Kg), vehicle (control group) for ten days. Systolic blood pressure (SBP) was measured noninvasively before and after the treatment; the increment of SBP was less in the groups treated with RCAE than in the L-NAME-treated group. Enalapril also showed antihypertensive effect. Intravenous administration of RCAE and/or its methanol fraction (RCMF) after hypertension was established tended to normalize blood pressure. These data suggest that the aqueous extract of *R. chalepensis* leaves has antihypertensive effect, and support its use as an antihypertensive agent in traditional medicine.

Key words: *Ruta chalepensis*, hypotensive, N^{ω}-nitro-L-arginine methyl ester (L-NAME), arterial pressure, hypertension

Introduction

Nitric oxide (NO) is a very important vasodilator substance released by the vascular endothelium. It has a main role in maintaining vascular smooth muscle tone and in regulating blood pressure. In human and experimental hypertension impairment of endothelium-dependent vascular relaxation has been observed, and the ability of NO to maintain vascular tone has been shown to be deficient in this condition (1). In hypertensive patients, many studies suggest that endothelial dysfunction resulting from a diminished NO production may participate in the development and maintenance of hypertension (1). Hypertension is one of the most prevalent and powerful risk factors for cardiovascular disease and it is one of the most important preventable causes of premature morbidity and mortality in developed and developing countries (2). In Mexico, 36.9% of population between 20 and 65 years old is affected by hypertension; in other words, there are 16 million persons affected by this condition in Mexico (3).

It is well known that the study of medicinal plant species had allowed the isolation of several agents, used as leads for the development of new therapeutic drugs (4). Although there is availability of low-cost therapy for hypertension, the Mexican folk medicine has used a wide variety of plants to treat many diseases, and some herbal remedies such as *R. Chalepensis* are widely used as antihypertensive treatment (5).

Ruta chalepensis (Rutaceae), commonly called "ruda" in Mexico, is a native herb to the Mediterranean region, though at the present time it grows in many parts of the world, including Latin America (6). *R. chalepensis* was introduced to Mexico by the Spaniards, and it is used in decoction as a medicinal remedy to treat several cultural diseases and for "spiritual cleansings". The flowers are cymes with 4–5 sepals, 4–5 petals, 8–10 stamens and a superior ovary. Oil glands are principally present in leaves, having a strong odor. The leaves and young stems have been reported to contain alkaloids, flavonoids, phenols, amino acids, furanocoumarins, tannins, volatile oil and saponins (7-9). R. Chalepensis is known to be used as laxative, anti-inflammatory, analgesic, antipyretic, abortifacient, anti-epileptic, emmenagogue, anti-helmintic, spasmolytic and for dermatopathy treatment (9-13). It also has been reported in folk medicine to have an antihypertensive effect (5).

Therefore the aim of the present study was to investigate the antihypertensive effect and to explore the mechanism of action of aqueous extract from *R. chalepensis* and its methanol fraction.

Methods

Animals

Male Wistar rats were maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted in accordance with *Federal Regulations for Animal Experimentation and Care* (SAGARPA, NOM-062-ZOO-1999, México). The protocol was approved by the Institutional Animal Care and Use Committee.

Drugs

Acetylcholine chloride, N^{ω}-nitro-L-arginine methyl ester (L-NAME), enalapril maleate and L-phenylephrine chloride were purchased from Sigma Chemical Co., St. Louis, MO, USA and Sodium pentobarbital (Barbital) from Holland de México S.A. de C.V. All drugs were dissolved in distilled water to a 10 mM concentration. Subsequent dilutions were done with saline solution (0.9%) immediately before use.

Preparation of the plant aqueous extract and its methanol fraction

The leaves of *R. chalepensis* were bought in the Sonora's market, a place in Mexico City where medicinal plants are sold. The plant's botanical identity was verified at the Izta Herbarium of the Botanical Department, Facultad de Estudios Superiores Iztacala. The specimen deposited with the voucher number MPF 149 was authenticated by Edith López Villafranco, biologist in charge of the Herbarium. *R. chalepensis* leaves were dried at room temperature and then powdered with a mechanical grinder and stored in airtight containers, 5 g of powdered leaves were mixed with 100 mL distilled water for 15 min at 70°C, filtered and vacuum dried. The aqueous extract was adjusted to 15 mg/mL and pH 7.4 for biological experiments. The aqueous extract of *R. chalepensis* dry powder was extracted with methanol at room temperature; the methanol fraction was filtered and concentrated on a rotary evaporator at reduced pressure until dryness. Before using the methanol fraction of *R. chalepensis*, it was dissolved in saline solution (0.9%) to a 15 mg/mL concentration and pH was adjusted to 7.4.

L-NAME-induced hypertension

Animals were randomly allocated into five groups of six animals each. All the experiments were followed-up for ten days. Group 1 (control) received vehicle, group 2 received L-NAME (70 mg/kg body weight), groups 3 and 4 received L-NAME plus aqueous extract of *R. chalepensis* (RCAE) leaves (25 and 50 mg/kg/day, respectively), and group 5 received enalapril (5 mg/kg body weight). All the drugs were administered in the drinking water and the actual doses of each group were calculated from the daily water intake. At the end of the treatment, systolic blood pressure (SBP) was measured noninvasively using a tail cuff computer-aided monitoring device (Automatic Blood Pressure Computer, Model LE 5007, LSI Letica Scientific Instruments, Barcelona Spain).

Dose-response curves to *R. chalepensis* and phenylephrine in normotensive and hypertensive rats.

Normotensive and L-NAME induced hypertensive rats were anesthetized with sodium pentobarbital (45 mg/kg by i.p. injection). The femoral vein and carotid artery were isolated and cannulated for drug administration and blood pressure recording, respectively. The cannulae were filled with heparinized saline to prevent clotting. Blood pressure was recorded with a pressure transducer (Narco Bio-Systems Mod. P1000B) connected to the arterial cannula. The signal from the transducer was electronically dampened and inscribed on a physiograph (Narco Bio-Systems, Mod. DMP-4B). Once the blood pressure was stabilized, dose-response curves to aqueous extract of *R. chalepensis* leaves and its methanol fraction (1-50 mg/kg) were done. In other experiments acute hypertension was induced by phenylephrine. Dose–response curves using from 1 to 50 μg/kg were recorded, then when blood pressure returned to baseline, RCAE was administered (25 mg/kg), and again the dose-response curves to phenylephrine were registered.

Phytochemical analysis

Chemical tests were carried out on the RCAE leaves and its methanol fraction for detection of alkaloids, cardiac glycosides, flavonoids, anthraquinones, saponins, sterols and triterpenes (14, 15).

Statistical analysis

The experimental data are expressed as mean and standard error mean (SEM) of at least five experiments. Differences among groups were analyzed by two-way ANOVA and Bonferroni post-test for the concentration-response curves and Student's t-test. Differences were considered significantly different when $p < 0.05$.

Results

Effect of RCAE and RCMF on normotensive animals

Acute intravenous administrations of RCAE to anaesthetized normotensive rats produced transient, dose-related, significant reductions in systolic blood pressure (SBP). Maximal decrease of SBP obtained with RCAE (50 mg/kg) was 40.9 ± 8.4 mmHg (Fig.1).

Similarly RCMF produced SBP reduction in anaesthetized normotensive rats, maximal blood pressure decrease obtained with 50 mg/kg dose was 46 ± 5 mmHg (Fig.2).

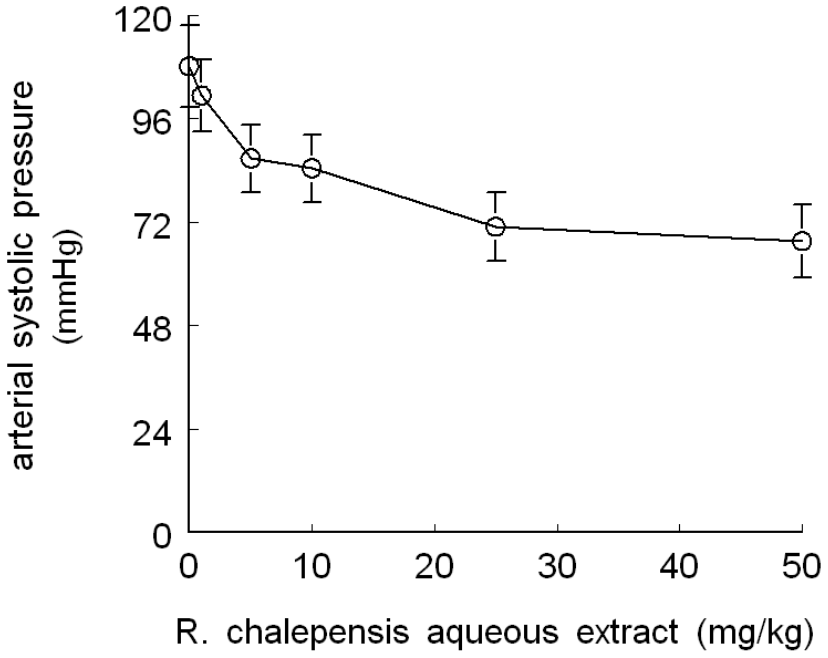

Figure 1. Effect of RCAE leaves on SBP of anesthetized normotensive rats. Values presented are means ± SEM (*n* = 5 in each group).

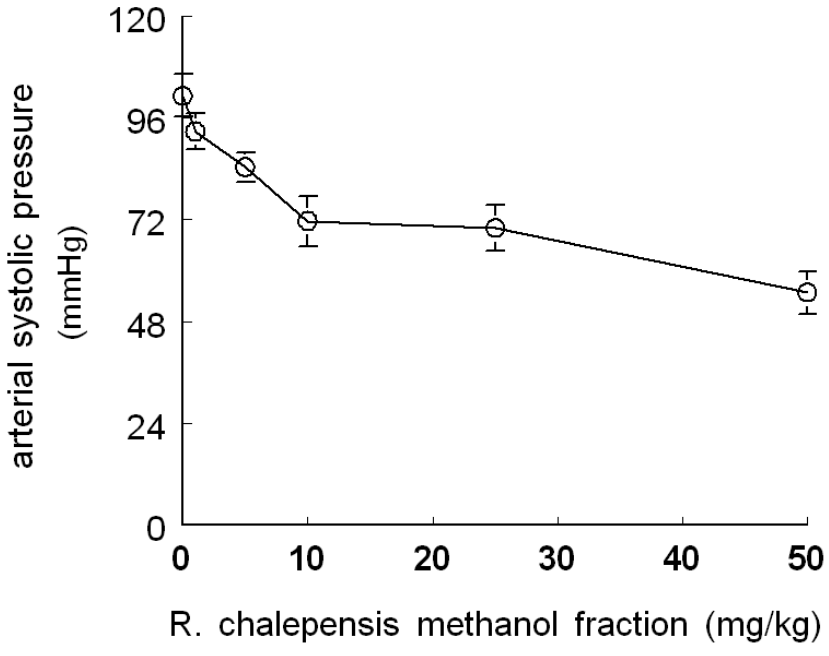

Figure 2. Effect of RCMF on SBP of anesthetized normotensive rats. Values presented are means ± SEM (*n* = 5 in each group).

Antihypertensive effect of aqueous extract in anesthetized rats

The administration of phenylephrine, a α-adrenergic receptor agonist, increased SBP in normotensive rats in a dose dependent manner. RCAE (25 mg/kg) reduced by 81.8% the increment of SBP induced by phenylphrine (25 μg/kg, Fig. 3).

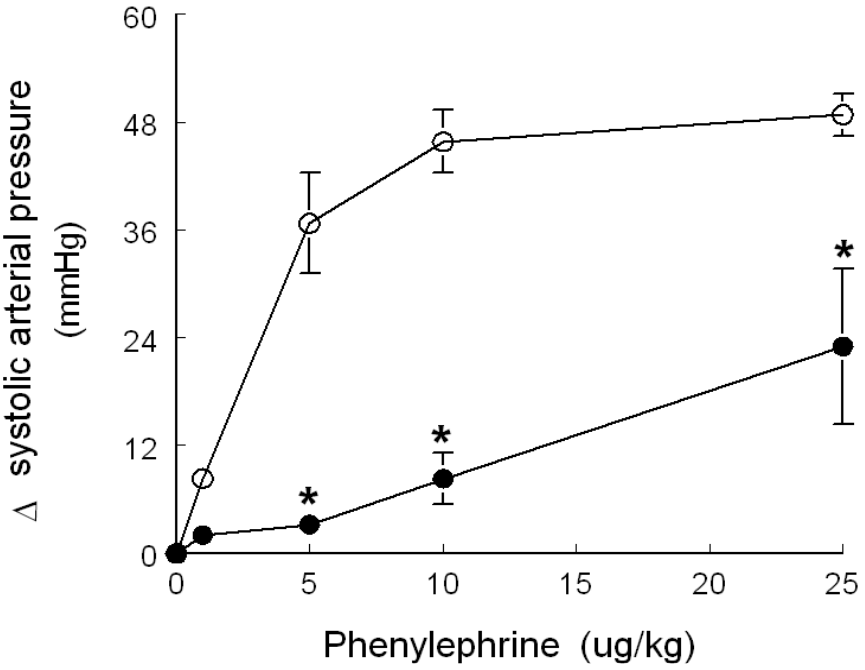

Figure 3. Effect of phenylephrine on SBP in normotensive rats with (●) or without (o) previous administration of the RCAE (25 mg/kg). Phenylephrine dose was from 1 to 25 μg/Kg body weight. (n=5) mean ± S.E.M, *p < 0.05.

Effect of RCAE on the development of L-NAME-induced hypertension

The administration of L-NAME orally for 10 days elevated significantly SBP by 36.3%, from a mean of 122.8 ± 4 mmHg in control animals to 152.3 ± 11 mmHg. Treatment with RCAE (25 and 50 mg/kg), administered simultaneously with L-NAME prevented the rise in blood pressure induced by L-NAME (123 ± 9 and 112.4 ± 5 mmHg, respectively). Enalapril administration also prevented the increase in blood pressure (130 ± 3 mmHg, Fig. 4).

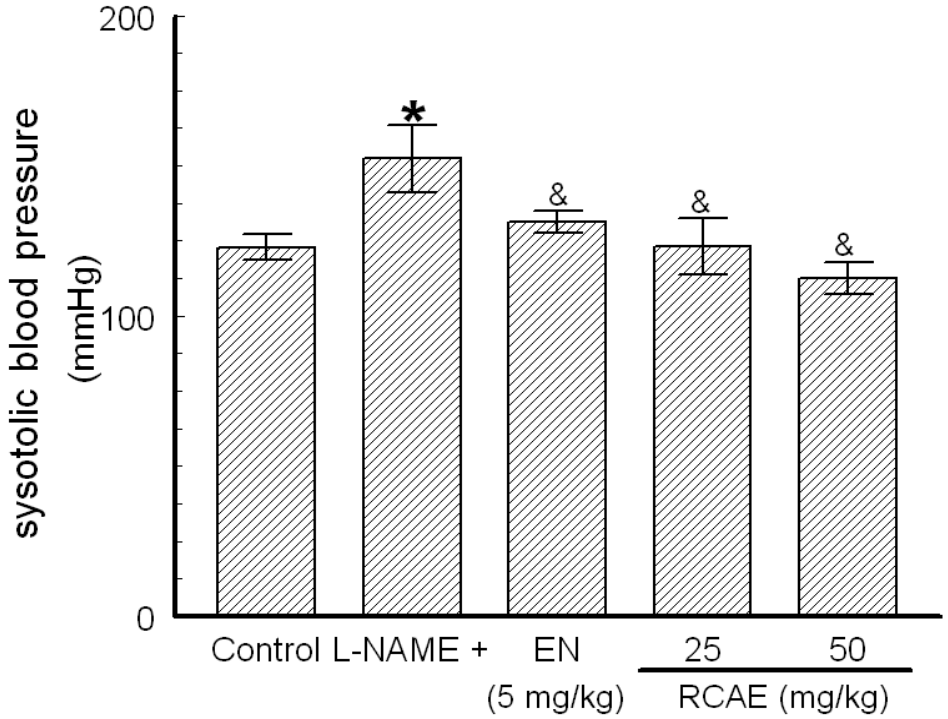

Figure 4. Effect of RCAE and enalapril (EN) treatment on SBP of L-NAME-induced hypertensive rats. Each bar represents the mean ± SEM of 5 experiments. *$p < 0.05$ from corresponding normotensive control values; $^{\&}p < 0.05$ from corresponding hypertensive control values.

Effect of RCAE and RCMF on hypertensive animals

Acute intravenous administrations of RCAE to anaesthetized rats treated with L-NAME produced transient, dose-related, significant reductions in SBP. Maximal decrease of SBP obtained with RCAE (50 mg/kg) was 75.5 ± 14.6 mmHg (Fig.5). Similarly, intravenous administration of RCMF (50 mg/kg) showed antihypertensive action. The maximal decrease in SBP was 74.8 ± 9.3 mmHg (Fig. 6).

Phytochemical analysis

Phytochemical analyses of the aqueous and methanol extracts of *R. chalepensis* leaves revealed the presence of flavonoids, coumarins, sterols, and alkaloids.

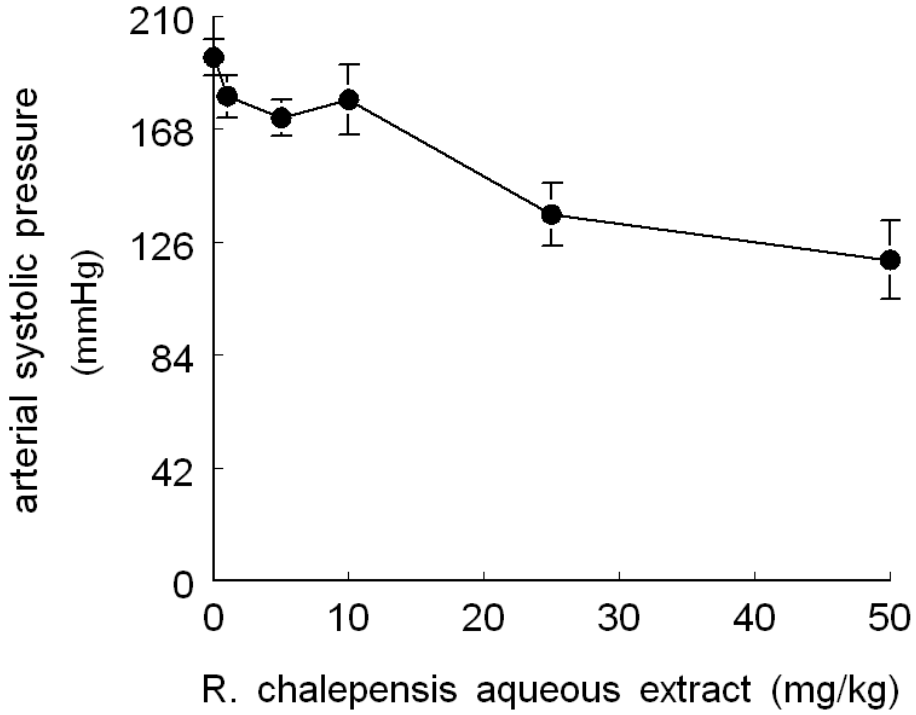

Figure 5. Effect of the oral administration of RCAE on blood pressure in anesthetized L-NAME-treated rats. Values represent mean ± SEM (n = 5 in each group).

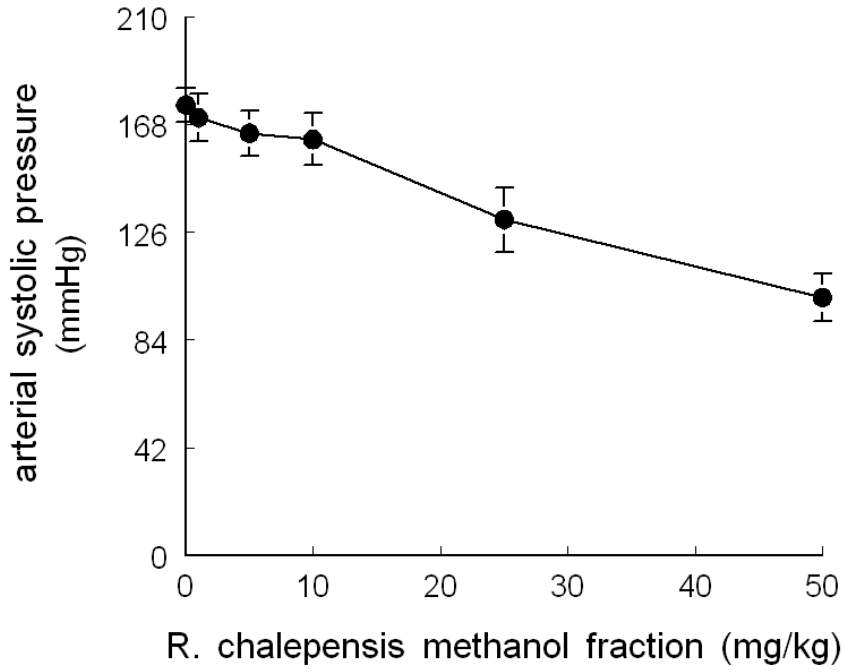

Figure 6. Effect of RCMF on blood pressure in anesthetized L-NAME-treated rats. Values represent mean ± SEM (n = 5 in each group).

Discussion

In this study we show that aqueous extract of *R. chalepensis* and its methanol fraction, when tested on blood pressure of normotensive rats and L-NAME-treated rats induced a dose-dependent reduction the blood pressure, which is in line with its traditional use in hypertension (5). The hypotensive effect of the RCAE was brief in normotensive rats. We have observed in our earlier studies that the duration of action of the plant extracts or pure chemicals even with other action mechanism such as Ca^{+2} channel blockade, is usually brief when tested in normotensive animals (16, 17) mainly because of intact physiological compensatory mechanisms in these animals. RCMF showed a greater hypotensive effect that RCAE, suggesting the enrichment of the active compounds. The observation that RCAE reduced the hypertension produced by the acute administration of phenylephrine suggest that the hypotensive effect of RCAE could, partially at least, be mediated through α-adrenergic mechanisms.

To test the antihypertensive activity of the *R. chalepensis* extract, we used the experimental model of hypertension induced by oral administration of L-NAME, an inhibitor of nitric oxide synthase (NOS) (18). Its administration for ten days causes marked increase in systolic blood pressure, confirming the main role of NO in maintaining the vascular smooth muscle tone and in regulating blood pressure (19, 20). The co-administration of RCAE (25 and 50 mg/Kg) diminished such increase, compared to the group of L-NAME-treated rats which only received vehicle. The angiotensin-converting enzyme inhibitor enalapril also decreased blood pressure, confirming the participation of angiotensin II in this hypertension model (21-23).
The mechanisms participating in the L-NAME-induced hypertension rat model have not been fully characterized, but it is now established that the renin-angiotensin system, and the sympathetic nervous system are involved, and interference with calcium channels and arachidonic acid derivatives has also been demonstrated (20, 24-27). This model of hypertension may also be associated with free oxygen species generation (26). Oxidative stress induced by glutathione depletion can cause severe hypertension in normotensive rats, associated with disturbances in the NO system (27). In this model, hypertension can be neutralized by the administration of free radical scavengers. It is thus clear that agents that decrease L-NAME-induced hypertension, such as the investigated extract, may do so in more than one way.

Sub-chronic RCAE treatment of hypertensive rats prevented the development of hypertension, presumably by alkaloids, flavonoids, coumarins, and sterols detected by the qualitative phytochemical analysis. These compounds have a large variety of pharmacological actions which include stimulation of endothelial nitric oxide (NO) synthesis and inhibition of angiotensin converting enzyme (ACE). *Ruta chalepensis* is a rich source of important secondary metabolites such as furanocoumarins and alkaloids (9).The furanocoumarin imperatorin is responsible for the vasodilatory activity of *Angelica dahurica var. formosana* regulated by NO in an endothelium-dependent manner (27). Another compound present in *R. chalepensis* is rutin flavonoid (28, 29), which is a diglycoside of quercetin that can be hydrolyzed to quercetin in the gastrointestinal tract (30). It has been demonstrated that quercetin possess antihypertensive activity in several models of hypertension (31). So rutin may be the antihypertensive active compound of *R. chalepensis*.
In conclusion, this study shows that *R. chalepensis* reduces the elevation of blood pressure of hypertensive rats treated with L-NAME, which supports the use this plant in ethnomedical practices as antihypertensive agent. Further studies are in progress to identify the active constituents from extracts of this plant to allow understanding its effects better.

Acknowledgements

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