Ex vivo Antispasmodic Activity of Aqueous Extract of Flowers of Muntingia calabura Linn. on Excised Rabbit’s Jejunum

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INTRODUCTION

Medicinal plants are sources of important therapeutic aid for alleviating human ailments. Approximately 80% of the people in the developing countries worldwide depend on traditional medicine for their primary healthcare. Muntingia calabura L. (family Muntingiaceae) is known throughout the world as Jamaican cherry. M. calabura is one of the most common roadside trees widely cultivated in warm areas of the Asian region and Southeast Asia such as Malaysia, Indonesia, and the Philippines. Several medicinal uses have been documented on various parts of this tree. M. calabura leaves, barks, flowers, and roots have been used as a folk remedy. In Peruvian folklore medicine, the flowers and barks are used as antiseptic and anti-inflammatory. In Colombia, the infusion of the flowers is used as a tranquilizer and tonic. The boiled barks can be used as a wash to reduce swelling in the lower extremities. In Mexico, the plant is used to treat measles, mouth pimplies, and stomach ache. In the Philippines, the flowers are also used to treat headache and incipient cold or as tranquilizers, antispasmodics, and antidiuretics. Other than that, the roots of M. calabura have been used as an emmenagogue in Vietnam and as an abortifacient in Malaysia.

MATERIALS AND METHODS

Drug and solution used were verapamil (Nicholas Piramal India Ltd.) and Tyrode’s solution.

Preparation of physiological solutions

Tyrode’s solution was prepared carefully using analytical grade reagents and distilled water. pH of the final solution was adjusted to 7.4. Composition of Tyrode’s solution is given in mmol/L: NaCl, 137; KCl, 2.7; MgCl₂•6H₂O, 1.8; NaH₂PO₄, 0.5; CaCl₂•2H₂O, 1.8; NaHCO₃, 0.4; NaClO₃, 12; glucose, 5.5.

Experimental animal

Either sex of New Zealand White Rabbits weighing 1.5–2.0 kg were used in the experiments. The animals were housed at a constant room temperature (25°C ± 2°C) with 12:12 h light and dark cycle and they were maintained on a standard pellet diet and water ad libitum.

Preparation of extract

The fresh flowers of M. calabura were collected from Guntur district of Andhra Pradesh and authenticated by Dr. S. M. Khasim M.Sc., SIMS: Southern institute of medical sciences, h: Hour, kg: Kilogram, μg: Microgram, mL: Milliliter, mM: Millimol, °C: Degree Celsius.

Abbreviations Used: IC100: Maximal inhibitory concentration, IAEC: Institutional animal ethics committee, SIMS: Southern institute of medical sciences, h: Hour, kg: Kilogram, μg: Microgram, mL: Milliliter, mM: Millimol, °C: Degree Celsius.

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Ph.D., Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh. The washed flowers were dried at room temperature (25°C–35°C), the dried flowers were powdered and passed through sieve number 80, the dried powdered flowers were defatted with petroleum ether and then extracted with water using Soxhlet extractor and dried at 45°C in an oven, and it was used for further phytochemical and pharmacological studies.\[4‑6\]

Qualitative phytochemical screening
Crude extract was qualitatively tested for the presence of chemical constituents by performing various tests such as Mayer's test for alkaloid, steroids with Salkowski's test, glycosides with Keller–Killiani test, carbohydrates with Molisch's test, flavonoids with aluminum chloride test, phenolic/tannins with ferric chloride solution, and saponins with water. These were identified by characteristic color change using standard tests.\[7,8\]

Experimental design
The experimental protocol was approved by the Institutional Animal Ethics Committee which follows the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The protocol approval number is IAEC/SIMS/2013/006 dated 07/May/2013.

Rabbit jejunum preparation
The spasmolytic activity of the plant materials was studied using isolated rabbit jejunum.\[9\] Rabbits were starved for 24 h before the experiment and they were stunned by a blow on the head and then pithed. The abdomens of pithed animals were opened, and the jejunum portion of about 2.0 cm was removed. Each preparation was cleared off the mesentery so that it could freely give spontaneous contractions. Each segment of about 2 cm lengths was suspended in a 10 mL tissue bath containing Tyrode's solution maintained at 37°C and aerated. The tissues were allowed to equilibrate for at least 30 min at preload of 1 g. Tension changes in the tissue were recorded on kymograph. The smooth muscles relaxant action of test material was observed by administration of flower extract of *M. calabura* in a cumulative fashion directly without the use of an agonist.

**RESULTS**

**Phytochemical screening**
Qualitative phytochemical screening on the *M. calabura* flower extract shows the presence of flavonoids, carbohydrates, glycosides, saponins, and phenolic compounds.

**Effects on rabbit jejunum**
In isolated rabbit's jejunum preparation, the flower extract of *M. calabura* inhibited the spontaneous contractions in a concentration-dependent manner with IC\(_{100}\) value of 36 ± 3.02 µg/mL [Figure 1], similar to that of verapamil with IC\(_{100}\) value of 40 ± 1.02 µg/mL on the rabbit's jejunum [Figure 2].

**DISCUSSION**
The current study was carried out to validate the traditional use of flowers of *M. calabura* as antispasmodic. Contractile effects of the intestine are due to the cytosolic-free calcium levels. Moreover, intracellular and extracellular calcium stores exchange with each other. Periodic depolarization and repolarization of the tissues are due to the influx of calcium into sarcoplasmic reticulum through voltage-dependent calcium channel. These events are responsible for spontaneous intestinal responses.\[10\] The aqueous extract of *M. calabura* flowers caused a concentration-dependent inhibition of spontaneous contractions in isolated rabbit jejunum preparations, thus showing an antispasmodic action which is equipotent to that of verapamil.

**CONCLUSION**
The present study on preliminary phytochemical evaluation of aqueous flower extract of *M. calabura* produced positive results for alkaloids, glycosides, carbohydrates, saponins, and phenols. The aqueous extract of flowers of *M. calabura* exhibited a significant dose-dependent relaxation of spontaneous contractions in isolated rabbit's jejunum preparations. In summary, the findings suggest that *M. calabura* has antispasmodic activity. These findings may explain the medicinal use of *M. calabura* in abdominal colic, diarrhea, and hypertension. However, more detailed studies are in progress to establish the possible mechanism of action, safety, efficacy, and isolation of active constituents responsible for this activity.

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Conflicts of interest
There are no conflicts of interest.

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