Calcium Channel Blocking Activity of Fruits of *Callistemon citrinus*

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Summary: *Callistemon citrinus* is a plant of family myrtaceae that has a great medicinal importance. Traditional uses of the aerial parts of *Callistemon citrinus* in ethnic tribal communities are in practice, and very little are known about its importance on scientific grounds. Therefore, the crude methanolic extract of fruits of *Callistemon citrinus* (C.c) was screened for possible spasmolytic activity on isolated rabbit's jejunum preparations. The extract produced a relaxing effect on spontaneous contraction of rabbit's jejunum. Explaining the mode of action, the extract produced a dose dependent relaxant effect and shifted the calcium response curves to the rightward (EC_{50} ± SEM = -2.05 ± 0.05 vs. control EC_{50} ± SEM = -2.5 ± 0.05). The effect of extract was comparable with the effect of verapamil, a standard calcium channel blocker and therefore, the plant specie could be a potential target for isolation of calcium antagonist(s).

Introduction

Callistemon citrinus belongs to a family myrtaceae that has a great medicinal importance [1]. Nitisinone has been isolated from the plant that exhibit a herbicidal activity. Nitisinone has also been successfully used in the management of human tyrosinaemia Type-I [2]. The alpha-amylase inhibitory activity of *Callistemon rigidus* in mouse's gastrointestinal tract has been documented that has blood glucose lowering effect at postprandial state [3, 4]. Similarly, antibacterial and anthelmentic activity of *Callistemon viminalis* have also been documented [5, 6]. The 1, 8-cineole and alpha-terpineol have been isolated as major compounds from the leaves and flowers of *Callistemon citrinus* (syn. *Callistemon lanceolatus*) that have anthelmentic activity [7]. Moreover, antistaphylococcal, nematicidal, larvicidal, pupicidal, antithrombotic activities of the genus callistemon, and antioxidant activities have been documented [8, 9]. Moreover, traditional uses of the aerial parts of *Callistemon citrinus* in ethnic tribal communities are in practice, and very little are known about its importance on scientific grounds [10]. Keeping in view the literature and traditional uses of *Callistemon citrinus*, we screened the plant extract for possible pharmacological activities on isolated rabbit's jejunum preparations.

Results and discussion

Preliminary Phytochemical Screening / Chemical Composition

The tested plant material contains triterpenes, triterpenoids and saponin glycosides.

Spasmolytic and Calcium Channel Blocking Activity

The extract of C.c inhibited the spontaneous contractions of isolated rabbit's jejunum produced by KCl (80 mM) as shown in Fig. 1.

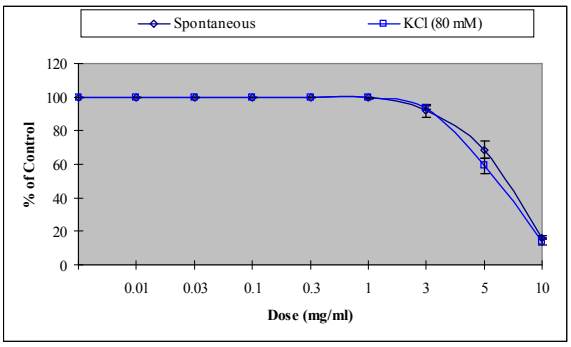

Fig. 1: Dose response curves of the crude extract of *Callistemon citrinus* on spontaneous contractions of isolated rabbit's jejunum preparations. The effect of extract on KCl induced contractions are also shown. All values are Mean ± SEM (n = 6).

The relaxant effect of the extract was in a dose dependent manner starting from 3.0 mg. The maximum response was obtained at 10.0 mg/mL (EC_{50} ± SEM = 5.2 ± 0.8).

To explain its possible mode of action, sustained contractions were produced in the tissues by KCl (80 mM). The contractions were inhibited by the extract in similar fashion (Fig. 1). The

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contractility of the smooth muscles of the intestines is due to the cytosolic free calcium levels and there is an exchange of calcium between extra cellular and intra cellular calcium stores. The voltage dependent calcium channels (VDCs) are responsible for the influx of calcium into the sarcoplasmic reticulum [11-14]. This leads to periodic depolarization and repolarization of the intestinal tissues that account for its spontaneous responses. As positive relaxing effect on the KCl induced contractions doesn't always imply for calcium channel blockade [15], hence, the results were confirmed by plotting the calcium chloride curves vs. verapamil, a standard calcium channel blocker, as shown in our previous study [16, 17].

The results are summarized in the Fig. 2. According to Fig. 2a, it is evident that the extract supposed to inhibit the calcium channels and even a maximum dose of calcium (Log10 [Ca^{++}] M= -1.6) could produce only 63.0 % response vs. control maximum *i.e.* 100 %. Thus, there is a right shift in the calcium curves (Fig. 2a) that resemble the curves of verapamil Fig. 2b. According to Fig. 2a, a right shift at concentration of 0.03 mg/mL of extract treated tissues (EC_{50} ± SEM = -2.05 ± 0.05 (Log10 [Ca^{++}] M vs. control EC_{50} ± SEM = -2.5 ± 0.05 (Log10 [Ca^{++}] M) for calcium response resembles the effects of verapamil (EC_{50} ± SEM = -1.7 ± 0.1 vs. control Max.) at concentration 0.1 µ M (Fig. 2b). This right shift of the response confirmed the calcium channel blocking activity [18] of the fruit of *Callistemon citrinus*.

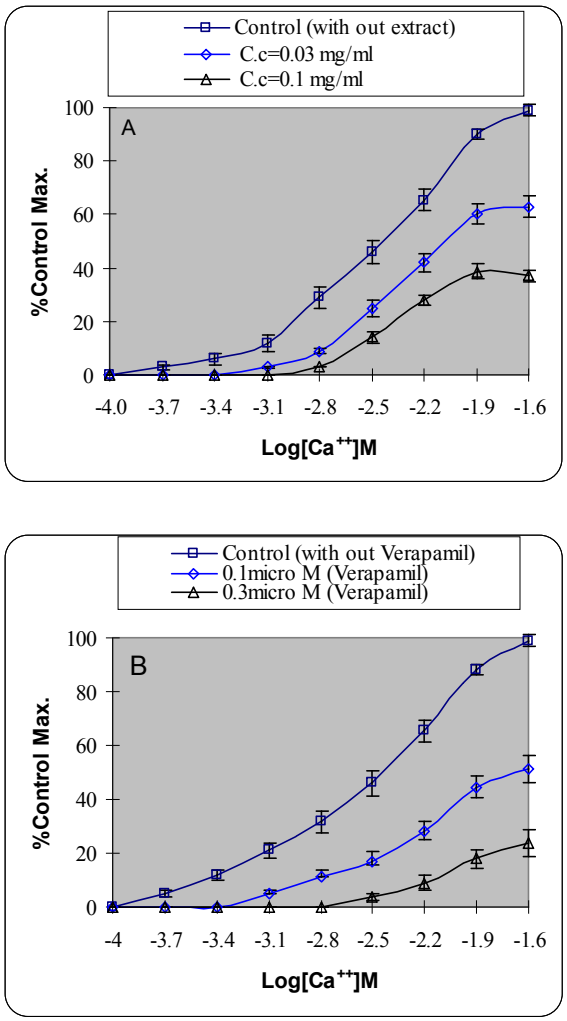

Fig. 2: Concentration response curves of Ca^{++} in absence and presence of the increasing concentration of (A) Extract of *Callistemon citrinus* and (B) verapamil in isolated rabbit's jejunum preparations. All values are shown as Mean ± SEM (n = 6).

Experimental

Collection and Extraction of Plant Materials

The dried ripe fruits of *Callistemon citrinus* were collected from the University of Malakand campus-I. The plant was identified by Professor Dr. Jehandar Shah, vice chancellor Shaheed Benazir Bhutto University, Sheringal Dir, Upper. A voucher specimen (CC-01-2009) has been deposited to the herbarium of University of Malakand. The plant materials were subjected to shade drying and the fruits were grinded to get a fine powder. The powdered materials were soaked in commercial grade methanol available. After ten days, the material was filtered through ordinary filter paper. The process was repeated thrice. The filtrate was combined and concentrated to dark brownish crude extract at temperature of 40 °C using a rotary evaporator.

Preliminary Phytochemical Screening

Preliminary Phytochemical screening of the crude extract of fruits and powdered materials of the plant was carried out to determine the presence of chemical constituents like alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones and cardiac glycosides [19, 20].

Drugs

All the chemicals used in this study were of analytical grade. The drugs were dissolved in distilled water, and all solutions were prepared on the

same day of experiments. Acetylcholine was purchased from BDH Chemicals, Poole, England. Rests of the chemicals were purchased from E. Merck Germany.

Animals

Local breed rabbits (1.0-1.4 kg) of either sex were used in the experiments. The animals were housed at the "Animal House of the University of Malakand" complying with standards mentioned in the "Animals Bye-Laws 2008 of the University of Malakand (Scientific Procedures Issue- 1)". Standard diet and tap water was provided to the animals. The animals were kept without food, for 24 h prior to the start of the experiments. They were given only water.

Data Recording

Data related to intestinal responses were recorded using Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) connected with Power lab (Model No: 4/25 T) AD Instruments, Australia through Bridge Pod Amplifier. Data were recorded at the range of 20 mv, Low pass 5Hz X 10 gain using input 1, rate 40/S.

Statistical Analysis and Interpretation of Data

Chart 5 for Windows supplied with Power lab was used for the interpretation of graph tracings. Microsoft-XL Sheet was used to calculate mean and standard error mean (SEM) of the data (not shown). Student's *t* test was used to compare the data and *P* values less or equal to 0.05 were considered as statistically significant.

Rabbit's Jejunum Preparations

The animals were subjected to cervical dislocation and their abdomens were opened and a portion(s) of the (2 Cm long) jejunum were isolated and kept in the tyrode's solution. The solution was maintained at 37 °C and aerated with carbogen gas (5 % Carbon dioxide and Oxygen mixture) to keep the tissues alive and ready for use [21, 22]. The tissues were then mounted in 10 mL tissue bath containing tyrode's solution, and were stabilized for about 25-30 minutes. After a reproducible response, the tissues were treated with crude methanolic extract at dose(s) of 0.01, 0.03, 0.1, 0.3, 1.0, 5.0 and 10.0 mg/mL (final bath solution) in cumulative manner [22]. Earlier, a pressure of one gram was applied to the tissues. The composition of tyrode's solution in mM was: KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8, and glucose 5.55.

Spasmolytic and Calcium Channel Blocking Activity

Because of the dose dependent fall in the spontaneous activity of the rabbit's jejunum preparations, we tried to explain the possible mode of action of the extract. In another series of experiments, tissues were pre-treated with high concentration of KCl (80 mM in final bath volume) to depolarize and get them in sustained contractions [23]. The sustained contractions produced by KCl were relaxed by the methanolic extract tried in cumulative manner. Their relaxation was expressed as % of the KCl induced contractions [22, 24].

For the confirmation of calcium channel blocking activity, the tissues were exposed to calcium free tyrode's solution having EDTA (0.1 mM). The tissues were then exposed to potassium rich tyrode's solution for 30 minutes having following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Earlier, all the tissues were stabilized in normal tyrode's solution for at least 30 minutes. Calcium response curves were constructed in the decalcified tissues with cumulative addition of Ca^{++} at concentration range 1×10^{-4} - 256×10^{-4} M.

The extract at different concentration were added to the decalcified tissues and left for on hour interval. Calcium response curves were constructed in the extract treated tissues. The extract treated calcium response curves were plotted vs. control maximum calcium curves. In other series of experiments, the decalcified tissues were exposed to different concentrations of verapamil (3×10^{-9} - 1×10^{-6}M). The curves were then compared with that of verapamil for confirmation of possible calcium channel blocking activities.

Conclusion

The fruit of *Callistemon citrinus* has a spasmolytic effect on rabbit's jejunum through the calcium channel blocking mechanism; and the plant could be a potential target for activity guided isolation of the active constituent(s).

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