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RESEARCH ARTICLE

Relaxant Effects of Syzygium Cumini Leaves on Guinea Pig Tracheal Chains and its Possible Mechanism(s)

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ABSTRACT

The *leaves* of *Syzygium cumini* is an attractive *leaves* of myrtaceae family . The purpose of this study was investigating experimentally the possible anti asthma activity of macerated and soxhlet extracts of leaves of this plant on tracheal chains of guinea pigs were evaluated. The relaxant effects of 4 cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 W/V) in comparison with saline as negative control and 4 cumulative concentrations of theophylline (0.25, 0.5, 0.75, and 1.0 mM) as positive control were examined on precontracted tracheal chains of two groups of 6 guinea pig by 60 mM KCl (group 1, N=6) and 10 µM methacholine the non-incubated tissues (group 2, N = 6) and tissues incubated with 1 µM propranolol (group 3, N = 4) [15]. Decrease in contractile tone of tracheal chains was considered as relaxant effect. The isolated guinea-pig trachea pre-contracted with KCl, methacholine and tissues incubated with propranolol were used to study the relaxation of macerated and soxhlet extracts of leaves *Syzygium cumini.* In group 1 experiments only the last one higher concentrations of theophylline and soxhlet extract showed significant relaxant effect compared to that of saline ($p<0.001$ for both concentrations), which were significantly greater than those of macerated extracts ($p<0.001$ for all cases).In group 2 experiments only the last two higher concentrations of theophylline and soxhlet extract showed significant relaxant effect compared to that of saline. The effects of two higher concentrations of theophylline in this group were significantly greater than those of macerated and soxhlet extracts ($P < 0.01$). And in group 2 and 3 experiments both macerated and soxhlet extracts showed concentration-dependent relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$ for both extracts).The relaxant effects of macerated and soxhlet extracts in group 1 were significantly lower than those of groups 2 and 3.In group 3 experiment potent relaxant effect was observed.

KEYWORDS: *Syzygium cumini,* Bronchodilatory Guinea pig. Trachea

INTRODUCTION:

Myrtaceae is a plant family widely used in folk medicine in different countries and *Syzygium cumini* is a evergreen plant is originally from Indonesia and India, belongs to the Myrtaceae family. All parts of the plant can be used medicinally and it has a long tradition in alternative medicines. The *leaf has* anti-flammatory activity [1], respiratory diseases [2], and allergic disorders [3] and used in India for anemia, antibioticactivity, asthma, bronchitis. The ethanolic *leaves* extract has been reported to have anti-inflammatory activity in carrageenan and formaldehyde paw edema [2]. The same extract was also shown to inhibit histamine-, serotonin (5-HT) and prostaglandin 2-induced paw edema [4]. The allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed. During this process, mast cells release several inflammatory me-diators including histamine, 5-HT, platelet-aggregating factor (PAF), leukotrienes, and a variety of cytokines which can elicit many events associated with allergic inflammation, such as edema formation and cellular infiltration [5].

Leaves juice for gingivitis and also used against diarrhea and fever. Leaves used for diabetes which reduce the blood sugar level quickly. It also used against diarrhea & abdominal pains, skin diseases [6] leucorrhoea & general debility. *Leaves*s give orally reduced blood sugar & glycosuria in patients. Powdered *leaves*s are used as a remedy in diabetes and in metrorrhagia. Extract of *leaves* of *S.cumini* were found to have antidiabetic, antiinflammatory, hepatoprotective, antihyperlipidemic, diuretic and antibacterial activity. These properties of *S.cumini leaves* have been attributed to its saponins, tannin and flavonoids. These medicines are free from side effects and cheap compared to allopathic medicines, but still common people/educated people do not use them as they are prepared & practiced by the traditional medical practitioners [6-9]. Flavonids prevent oxidative cell damage suggesting antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties. *Syzygium cumini* tree about 8 to 15 meters high with white branches and reddish young shoots. Leaves are opposite, shiny and leathery, oblong-ovate to elliptic or obovate-elliptic, 6 to 12 centimeters long, the tip being broad and shortly pointed. Panicles are borne mostly from the branchlets below the leaves, often being axillary or terminal, about 4 to 6 centimeters long. Flowers are small, numerous, scented, pink or nearly white, in clusters, without stalks, borne in crowded fascicles on the ends of

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the branchlets. Calyx is funnel-shaped, about 4 millimeters long, and 4-toothed. Petals cohere and fall all together as a small disk. Stamens are numerous and about as long as the calyx. *Leaves* are oval to elliptic, 1.5 to 3.5 centimeters long, dark purple or nearly black, luscious, fleshy and edible with a sweet astringent taste; containing a single large *leaves* [10]. In the present study, the relaxant effect of macerated and soxhlet extracts of the leaves of *Syzygium cumini* and its possible mechanism(s) on guinea pig tracheal chains were examined.

MATERIALS AND METHODS:

PLANT AND EXTRACTS:

Leaves of *Syzygium cumini* was collected from the chandaka jungle, Bhubaneswar. The leaves of the plant was authenticated by Prof P K Sahu, Taxonomist, Botany Dept, Utkal University, Bhubaneswar. The soxhlet extract was prepared as follows: Fifty grams of the chopped, dried plant leaves were extracted with 300 ml distilled water by suxhelat apparatus. For the preparation of the macerated extract, the same amount of plant was macerated with 300 ml distilled water (on a shaker) for 48 hr. The solvent of both extracts were then removed under reduced pressure at 50^{o}C and distilled water were added to residues in such a way that plant ingredient concentration in the final soxhlet extracts were 10% W/W.

TISSUE PREPARATIONS:

Male guinea pigs (400-700g) were killed by a blow on the neck and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). The cartilages of all rings were then cut open opposite to the trachealis muscle, and sutured together to form a tracheal chain [11]. Tissue was then suspended in a 10 ml organ bath (Pinnacle Biomedical Research Institute(PBRI) Syamala Hills Bhopal (M.P.) India) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11. The Krebs solution was kept at 37^{o}C under stream of 95% O_2 and 5% CO_2 gases. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min [13].

PROTOCOLS:

The relaxant effects of four cumulative concentrations of macerated and soxhlet extracts (0.25, 0.5, 0.75 and 1.0 g/100 ml), four cumulative concentrations of theophylline anhydrous (S.D Fine, Mumbai,India) (0.25, 0.5, 0.75, and 1.0 mM) as positive control, and saline as negative control were examined. For preparation of different concentrations in the case of macerated and soxhlet extracts, 0.25 ml of 10% W/V of the concentrated extracts and in the case of theophylline, 0.25 ml of 10 mM solution were added to the organ bath. The consecutive volumes were added to organ bath at 5 min intervals. In each experiment the effect of four cumulative volumes of each extract, four cumulative volumes of theophylline, or saline on contracted tracheal smooth muscle were determined after exposure of tissue to the solution for 5 min. A decrease in tone was considered as a relaxant (broncho-dilatory) effect and expressed as positive percentage change in proportion to the maximum contraction and an increase in tone was consi-dered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change [12] [14].
The relaxant effect of different solutions ware tested with two different experimental designs as follows.

1. On tracheal chains contracted by 60 mM KCl (group 1experiments N = 6).

2. On non-incubated tracheal chains contracted by 10 µM methacholine hydrochloride (S.D Fine, India) (group 2 experiments N = 6).

3. On incubated tracheal chains with 1 µM propranolol hydrochloride 30 min prior to beginning and during the testing relaxation of different solutions. In this series of experiments, tracheal chains were also contracted by 10 µM methacholine hydrochloride (Group 3 experiments, N = 4).
The relaxant effect of theophylline was examined only on groups 1 and 2. The relaxant effects in three groups of experiments were examined in three different series of tracheal chains. All of the experiments were performed randomly with a 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution [13]. In all experiments responses were recorded on a kymograph and were measured after fixation.

STATISTICAL ANALYSIS:

All data were expressed as mean ± SEM. Data of relaxant effects of different concentrations of extracts were compared with the results of negative and positive control using ANOVA. The data of relaxant effect obtained in three groups of experiments were also compared using ANOVA. The relaxant effect of two extracts and theophylline were related to the concentrations using least square regression. Significance was accepted at $P<0.05$.

RESULTS:

RELAXANT (BRONCHODILATORY) EFFECT:

In group 1 experiments only, the highest volumes of theophylline and soxhlet extract showed significant relaxant effects compared to those of saline ($P < 0.05$ to $P < 0.001$).The effects of the last three concentrations of both extracts were significantly lower than those of theophylline ($P < 0.01$ to $P < 0.001$).In addition the effects of the last one concentrations of soxhlet extract were significantly higher than those of macerated extract in this group (Table 1). In groups 2 soxhlet extracts from *Syzygium cumini* and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea pig. The relaxant effects of the most concentrations of extracts and theophylline were significantly higher than those of saline ($P < 0.01$ to $P < 0.001$). Only the four concentration of macerated extract and two lower concentrations of soxhlet extract and two lower concentrations of theophylline did not show significant relaxant effects (Table 2). Only the one lower concentration of macerated extract and one lower concentrations of soxhlet extract did not show significant relaxant effects (Table 3). The relaxant effects of most concentrations of both extracts in group 2 and 3 were statistically greater than those of group 1 experiments ($P < 0.05$ to $P < 0.001$). The relaxant effect of most concentrations of both extract in group 3 were higher than those of group 2, There were significant positive correlations between the relaxant effects of both extracts and theophylline with concentrations of the solutions in all three experimental groups except that for macerated extract in group1 ($P < 0.05$ to $P < 0.001$) (Table 4).In addition, the effects of the different concentrations of soxhlet extract in this group were greater than those of macerated extracts, which were statistically significant ($p<0.05$), (Table 1 and 2).

Different Concentration	Saline	Macerated extract	Soxhlet extract	Theophylline
0.25	0.0 ± 0.0	0.0 ± 0.0 NS, ns, nS	0.0 ± 0.0 NS, ns	0.70 ± 6.14 NS
0.5	0.0 ± 0.0	0.0 ± 0.0 NS, ns, nS	5.93 ± 4.93 NS, ns	21.64 ± 7.13 NS
0.75	0.0 ± 0.0	0.0 ± 0.0 NS, +++, nS	14.41 ± 2.24 NS, ++	45.00 ± 5.67 ***
1	0.10±0.24	5.54 ± 2.04 NS, +++, ¶	45.55 ± 13.70 *, +++	82.83 ± 6.41 ***

Table No. 1. Relaxant effect of two different extracts from *syzygium cumini* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (contracted tracheal chains with 60 mM KCl).

NOTE: Values are presented as mean ± SEM. Statistical differences between the effect of extracts and negative control (saline); NS: non-significant difference, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Statistical differences between the effect of extracts and positive control (theophylline); ns, non-significant difference, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$. Statistical differences between the effect of two extracts; nS, non-significant difference; $P<0.05$; and $P<0.01$. The unit of concentration for extracts was w/v and for theophylline was mM.

Different Concentration	Saline	Macerated extract	Soxhlet extract	Theophylline
0.25	0.0 ± 0.0	7.94 ± 3.76 NS, ns	12.31 ± 5.80 NS, ns, nS	10.86 ± 3.44 NS
0.5	0.1±0.26	21.41 ± 12.74 NS, ns	27.59 ± 11.40 *, ns, nS	21.97 ± 3.71 NS
0.75	0.21±0.24	29.31 ± 11.21 ***, ns	46.91±8.26 ***, ns, nS	52.56 ± 4.37 *
1	0.32±0.21	34.94 ± 13.32 ***, ns	67.83 ± 6.08 ***, ns, nS	80.99 ± 7.44 ***

Table No. 2. Relaxant effect of two dofferent extracts from *Syzygium cumini* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (contracted tracheal chains by 10 µM methacoline).

NOTE: For abbreviations See Table I.

Different Concentration	Saline	Macerated extract	Soxhlet extract
0.25	_	24.37 ± 12.04	26.26 ± 12.07
0.5	_	62.10 ± 14.64**	65.19 ± 10.33**
0.75	_	74.47 ± 11.66***	81.70 ± 13.55***
1	_	85.54 ± 12.21***	89.57 ± 4.60***

Table No. 3. Relaxant effect of two different extracts from *syzygium cumini* in comparison with negative control (saline) in group 3 experiments (incubated preparation with 1µM propranolol contracted tracheal chains by 10 µM methacholine).

NOTE: For abbreviations see Table I.

Different substances	Group 1	Group 2	Group 3
Soxhlet extract	0.611 ***	0.731 ***	0.812 ***
Macerated extract	0.311 NS	0.547 ***	0.689 ***
Theophy-lline	0.876 ***	0.912 ***	_

Table No. 4. Correlation(r) between the relaxant effects of two different extracts from *syzygium cumini* and theophylline with concentration in three groups of experiments.

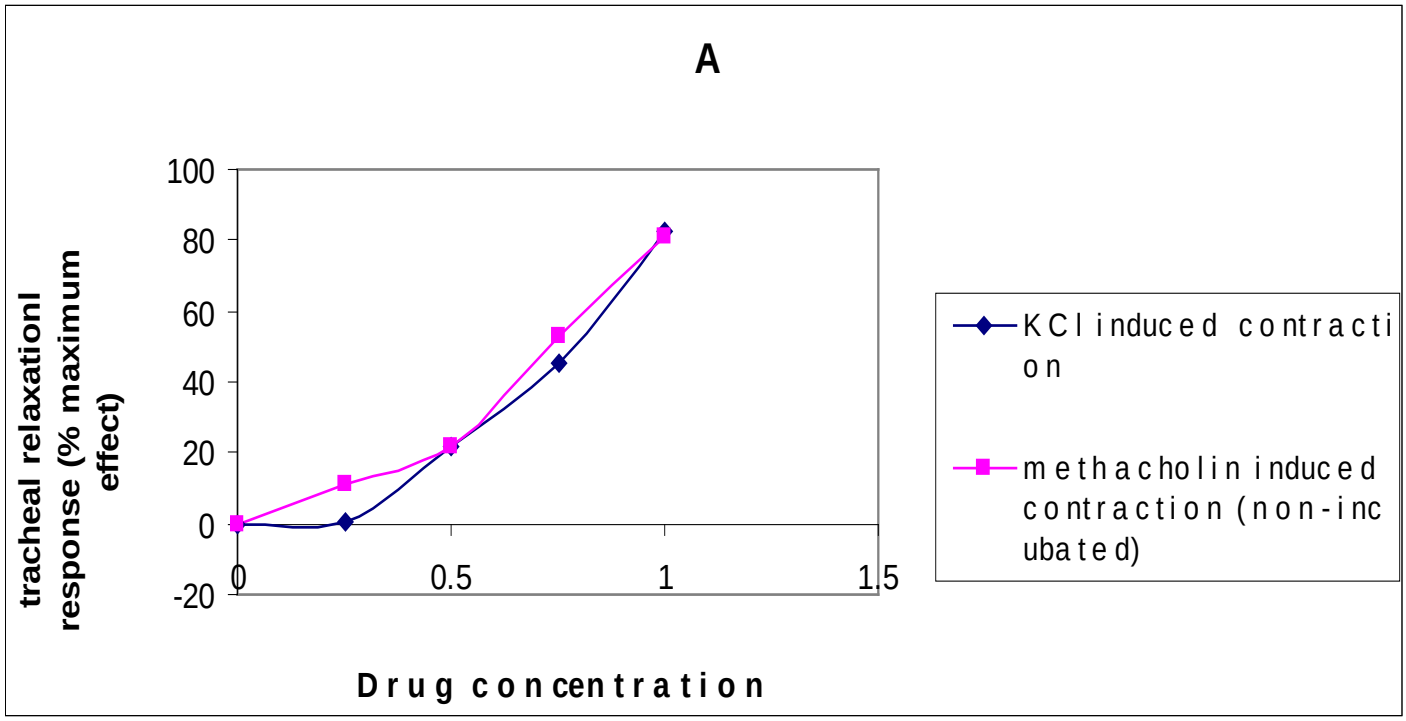

Figure No. 1. Concentration response curves of the relaxant effects of theophylline (a).

Three different groups of experiments were as follows: group 1, kcl induced contraction on non-incubated tracheal chains (o n = 6); group 2, methacholine induced contraction on non-incubated tracheal chains (n = 6), and group 3, methacholine-induced contraction on incubated tracheal chains of guinea pig with propranolol(n = 4). Statistical differences in the relaxant effect of different substances between group 1 with those of group 2 and 3; ns, non-significant difference; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.002$. Statistical differences in the relaxant effect of different substances between groups 2 and 3; ns, non-significant difference; + $p < 0.05$; and ++ $p < 0.01$.

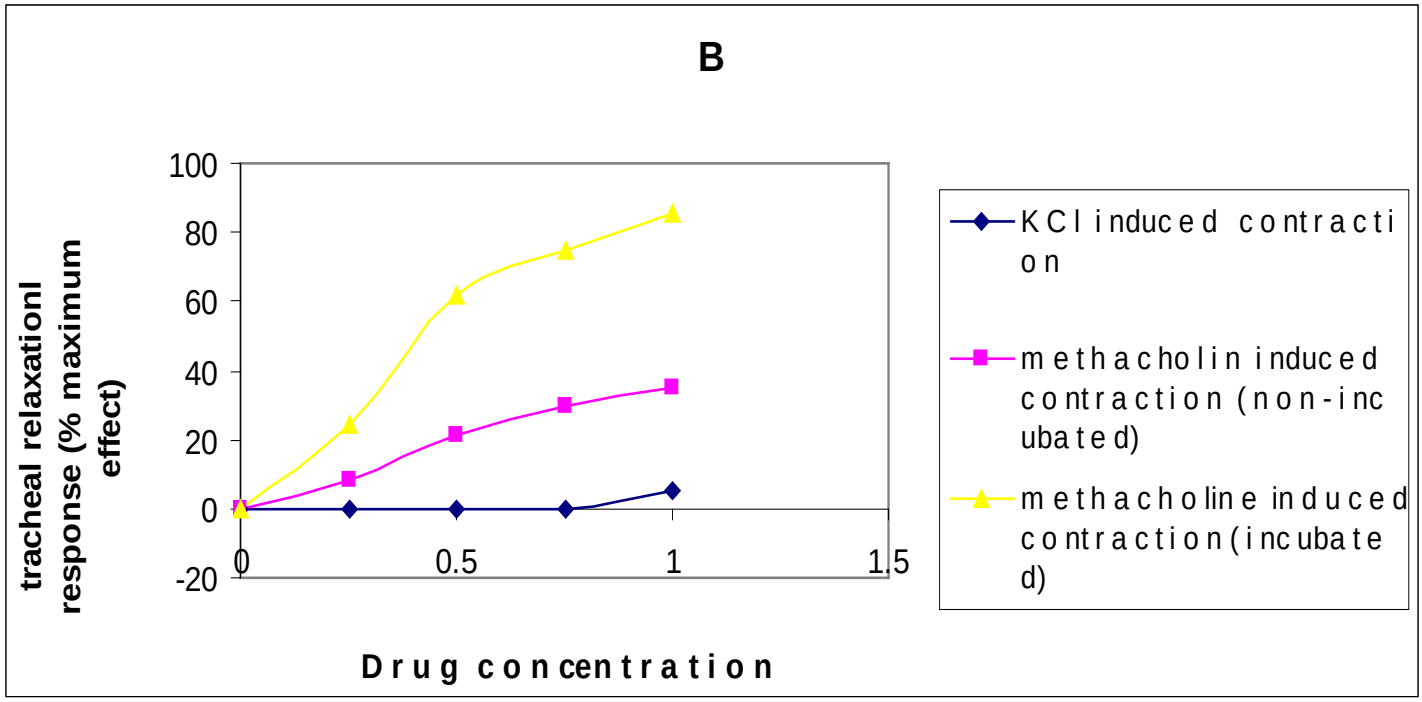

Figure No. 2. Concentration response curves of the relaxant effects of macerated extract (b).

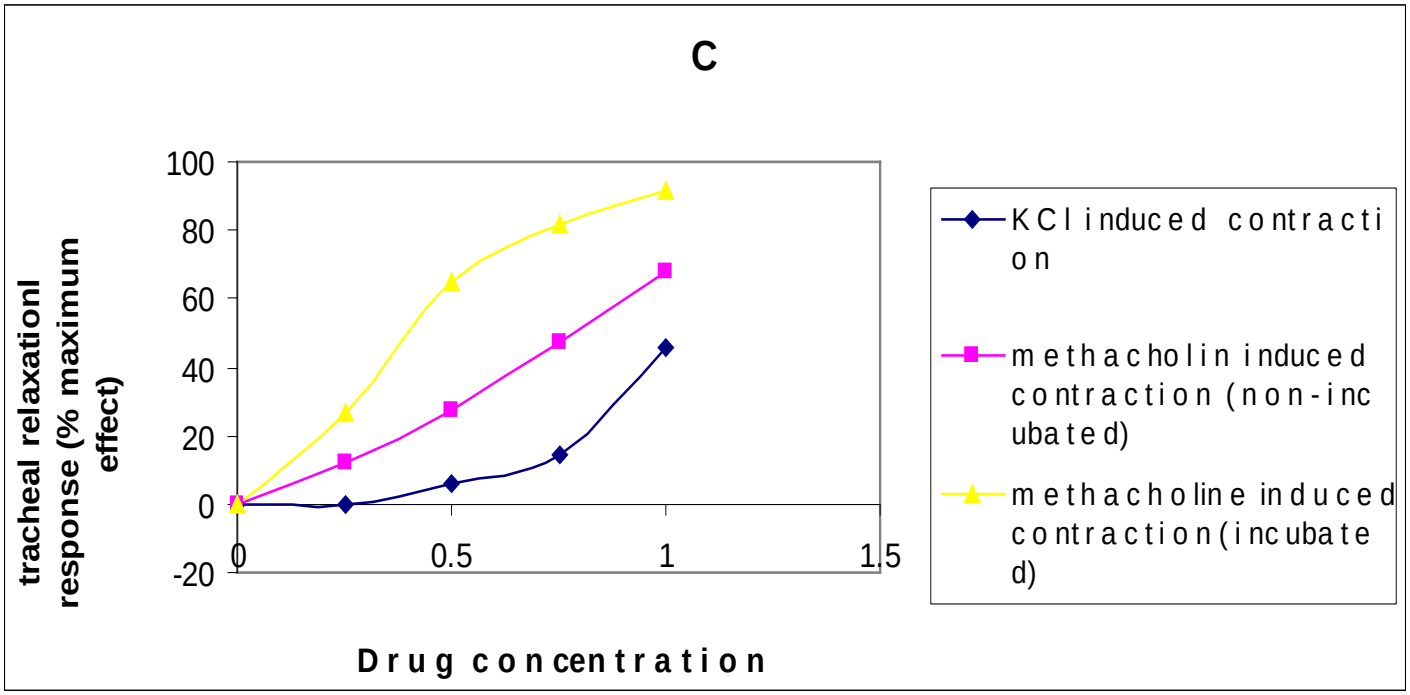

Figure No. 3. Concentration response curves of the relaxant effects of soxhlet extract (c).

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