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EVALUATION OF BRONCHODILATOR ACTIVITY OF AQUEOUS EXTRACT OF FLOWER BUD OF SYZYGIUM AROMATICUM L. IN HISTAMINE INDUCED BRONCHOSPASM IN GUINEA PIGS

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Abstract:

Introduction: *Syzygium Aromaticum* L. (Clove) is most widely used across the globe especially in asian countries as spice and flavoring agent for its taste and aroma. Many patients with asthma use herbal therapies in addition to beta-agonists or steroids, often without proven efficacy or unknown mechanisms of action or drug interaction. Evaluation of both therapeutic and possible effects of isolated components of herbal therapies gain it's importance in the research field. *Syzygium Aromaticum* L. (clove) is used in traditional herbal medicine as antimicrobial, antispasmodic and carminative action but bronchodilator activity remained unexplored. Guinea pigs are most suitable experimental animal for respiratory system highly sensitive to histamine, a potent bronchoconstrictor. So, we designed the study to evaluate the bronchodilator activity of flower bud of *Syzygium Aromaticum* L. (clove) in histamine induced bronchospasm model in guinea pigs.

Method: After taking Institutional Animal Ethics Committee approval, 36 male guinea pigs weighing 300 -400 g were randomly divided into six groups. Group 1 received distilled water, while Group 2 was given formoterol (1.55 µg/kg) + budesonide (0.02 mg/kg). Guinea pigs in Groups 3 and 5 were given clove (150 and 300 mg/kg, respectively), while Groups 4 and 6 received clove (150 and 300 mg/kg, respectively) in addition to formoterol (1.55 µg/kg) and budesonide (0.02 mg/kg). After administration of 0.5 % w/v histamine inhalation on day 0 and day 14 pre-convulsion time was noted. Mean pre- convulsion time difference ($T_2 - T_1$) and percentage protection in each group was calculated using formulae:

Percentage protection = $\frac{T_2 - T_1}{T_2} \times 100$ (% Protection offered by the extract)

T_1 is the mean of PCT of group; (pre-convulsion time) before administration of test drugs;

T_2 is the mean of PCT of group; (pre-convulsion time) after the administration of test drugs.

Results: Statistically significant improvement in pre-convulsion time in group 2 Active Control Group ($p < 0.001$), in group 3 Low Dose extract Group ($p < 0.01$), group 4 Low Dose Extract with 1.55 µg/kg Formoterol and 0.04 mg/kg Budesonide inhalation ($p < 0.01$) group 5 High Dose Extract Group ($p < 0.001$), group 6 High Dose Extract with 1.55 µg/kg formoterol and 0.04 mg/kg budesonide inhalation ($p < 0.001$) was noted between day 0 to day 14. Statistically significant percentage protection with value 42.95 ± 3.13, 26.20± 4.44, 23.39 ± 7.06, 34.15± 3.61, 34.01± 6.36 were obtained respectively in group 2, 3, 4 and 5 and 6 as compared to group 1 disease control. **Conclusion:** Aqueous extract of flower bud of *Syzygium Aromaticum* L. improves pre-convulsion time and percentage protection with promising results as a potential bronchodilator. No add on benefit of *Syzygium Aromaticum* L. aqueous extract has been demonstrated by percentage protection or pre-convulsion time when combined with formoterol and budesonide. Rather there was slight reduction in efficacy of formoterol and budesonide standard combination trending towards antagonism which could not catch up with the statistical significance.

Keywords: *Syzygium Aromaticum* L. (Clove), Histamine, Bronchospasm, Pre-convulsion time, Percentage protection, Formoterol, Budesonide.

Introduction:

Bronchospasm is the dominant event of asthma associated with respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and intensity. About 300 millions of all ages are affected by asthma world-wide with increased prevalence in the developing countries for which ß-agonists and inhaled corticosteroids (ICS) are most commonly in use [1]. Many patients with asthma or C.O.P.D. use herbal therapies in addition to conventional asthma therapies to manage asthma symptoms, often without proven efficacy or known mechanisms of action [2]. Evaluation of both therapeutic and possible effects of unexplored components of herbal treatments on the airway gain its importance. The drugs used in the treatment of asthma mainly acts as bronchodilator, anti-inflammatory, mast cell stabilizers, leukotriene antagonists, anti IgE antibody [3]. *Syzygium Aromaticum* L. (Clove) is one of the most common spice used world-wide that bears an enormous number of pharmacological activities such as anti-inflammatory activity, antimicrobial activity, anti-oxidant property, anti-spasmodic, carminative action [4,5]. Clove (flower bud) contain essential oil (tannins, bicornin, flavonoids, eugenin, eugenitin, kaempferol, rhamnetin) [4]. Numerous studies have disclosed that, eugenol as the active ingredient of clove responsible for most of its pharmacological action. Acute oral toxicity study of aqueous extract of clove recorded
(Median Lethal dose) LD50 of *Syzygium Aromaticum* L. as 2500 mg/kg which showed it's relative safety as classified by (Organisation for Economic Co-operation and Development) OECD. However, on prolonged use of *Syzygium Aromaticum* L. (SYZ) for 90days, at 300-700mg/kg dose orally manifested hepato-nephrotoxicity and can predispose to hyper-coagulation state in rodents, so to be ingested with caution[6]. Eugenol is an active principle from flower bud of cloves that inhibits 5-lipoxygenase activity and leukotriene- C4 in human PMNL cells [7]. Guinea pig respiratory system is highly sensitive to histamine, so it is most suitable experimental animal for respiratory system, proposed model of bronchial asthma, C.O.P.D etc. [8,9]. So we designed this study, to evaluate the bronchodilator activity of flower bud of *Syzygium Aromaticum* L. (clove) in histamine induced bronchospasm model in guinea pigs by means of pre-convulsion time and percentage protection.

Materials and Method:

After taking approval from IAEC (Institutional Animal Ethics Committee) IAEC approval no: 61/2018, this study was conducted from June 2018 to June 2019 in the department of Pharmacology, Government Medical College, Bhavnagar [577/GO/Re/S/02/CPCSEA]. Histamine was procured from

Molychem, (Batch no. MCR 17723). Formoterol and budesonide (Foracort) Respule (Batch no SA00004 Cipla Limited), was purchased. 0.58 % w/w essential oil as active ingredient based aqueous extract of *Syzygium Aromaticum* L. (clove) was made by Kuber Impex Ltd, Indore, Madhya Pradesh, India (Batch no. DSHE125/B/CL01/04). The guinea pigs were handled with care as per internationally accepted guidelines and norms for handling and care of animals, as provided by CPCSEA, India [10,11] . Guinea pigs were housed in standard transparent polypropylene cages with wheat husk bedding, and kept under controlled room temperature and humidity (26 ± 3 °C; 40 ± 5 %) in a 12-h light/12-h dark cycle and were given standard laboratory diet and water *ad libitum*. Thirty-six male albino guinea pigs weighing 300 -400 g were randomly divided into six groups. Group 1 received distilled water, while group 2 was given formoterol (1.55 µg/kg) + budesonide (0.02 mg/kg).Guinea pigs in groups 3 and 5 were given clove (150 and 300 mg/kg, respectively), while groups 4 and 6 received clove (150 and 300 mg/kg, respectively) in addition to formoterol (1.55 µg/kg) and budesonide (0.02 mg/kg). After an overnight fast, the guinea pigs were exposed to inhalation of 0.5 % histamine in a histamine chamber through a nebulizer. The pre-convulsion time (PCT), i.e. the time from inhalation exposure to the start of dyspnea leading to the appearance of convulsion, was recorded in seconds (T1). As soon as the asphyctic convulsion occurred, the guinea pigs were removed from the chamber and resuscitated with humidified oxygen given through a face mask. After 14 days of treatment with extract/standard drug, the guinea pigs were again exposed to 0.5 % histamine inhalation, and pre-convulsion time was recorded (T2). Percentage protection in each group was calculated using formulae. [12].

$$\text{Percentage protection} = \frac{T_2 - T_1}{T_2} \times 100 \text{ (\% Protection offered by the extract)}$$

● T_1 is the mean of PCT of group; (pre-convulsion time) before administration of test drugs ● T_2 is the mean of PCT of group; (pre-convulsion time) after the administration of test drugs and PCT

Result The results obtained from each group were expressed as Mean ± SEM. The percentage of protection between mean PCTs on day 0 and day 14 in the individual group were compared using paired *t*-test. The values of percentage protection in the disease control group and active control group were compared with other groups using One-way ANOVA. All statistical analyses were performed using Graph Pad Instat trial version 3.06 Software. Values of $p < 0.01$ were considered significant.

Effect of Clove on Pre-convulsion Time:

Intragroup Comparison

On applying 'Paired t test' for comparing mean Pre-convulsion time within the individual group on day 0 (before treatment) and day 14 (after treatment), in (Group 1) Vehicle or Disease control group (p-value > 0.01) there was insignificant decrease in pre-convulsion time between day 0 PCT and day 14 PCT. Whereas, in other groups (Group 2) Active Control Group (Formoterol and Budesonide) (<0.001**), (Group 3) Low dose Clove Extract (<0.01*), (Group 4) Low Dose Clove Extract with Formoterol and Budesonide (<0.01*), (Group 5) High Dose Extract of Clove (<0.001**) and (Group 6) High Dose Clove Extract with Formoterol and Budesonide (<0.001**) revealed significant increase in the pre-convulsion time.

Intergroup comparison:

Comparison with Disease control : On applying one way ANOVA test, for comparing mean Preconvulsion time difference (Group 2) Active Control Formoterol and Budesonide(<0.001**), (Group 3) Low Dose Clove extract(<0.01**), (Group 4) Low Dose Clove extract with Formoterol and Budesonide (<0.01**), (Group 5) High Dose Clove Extract (<0.001**), (Group 6) High Dose Clove

Extract with Formoterol and Budesonide (<0.001**) displayed significant difference in comparison to Disease Control group.

Comparison with Active control: On applying one way ANOVA test, for comparing mean preconvulsion time difference statistically significant difference was revealed between (Group 3) Low Dose *Syzygium Aromaticum* L. group (*p* value < 0.01*) and (Group 4) Low Dose *Syzygium Aromaticum* L. + Active Control group group (*p* value < 0.01*) respectively in comparison to Active Control group. While, statistically insignificant difference was exhibited between (Group 5) High Dose *Syzygium Aromaticum* L. (*p* value > 0.01) and (Group 6) High Dose *Syzygium Aromaticum* L. + Active Control group (*p* value > 0.01) in comparison to (Group 2) Active Control group.

Comparison between Group 3 Vs Group 4: On applying Unpaired t test for comparing mean preconvulsion time difference among (Group 3) Low dose *Syzygium Aromaticum* L and (Group 4) Low dose *Syzygium Aromaticum* L. + formoterol and budesonide control group insignificant (p>0.01) mean Pre-convulsion time difference was noted.

Comparison between Group 5 Vs Group 6: On applying Unpaired t test for comparing mean Preconvulsion time difference between (Group 5) High dose *Syzygium Aromaticum* L and (Group 6) High dose *Syzygium Aromaticum* L + formoterol and budesonide control group insignificant (p>0.01) mean Pre-convulsion time difference was revealed. This observation implied that there is no add on benefit of adding *Syzygium Aromaticum* L. extract with formoterol and budesonide.

Effect of Clove on Percentage Protection:

Comparison with Disease Control group: On applying one way ANOVA test, for comparing percentage protection (Group 2) Active Control Formoterol and Budesonide(<0.001**), (Group 3) Low Dose Clove extract(<0.01*), (Group 4) Low Dose Clove extract with Formoterol and Budesonide (<0.01*), (Group 5) High Dose Clove Extract (<0.001**), (Group 6) High Dose Clove Extract with Formoterol and Budesonide (<0.001**) displayed significant difference in comparison to Disease Control group.

Comparison with Active Control group: On applying one way ANOVA test, statistically significant difference was revealed between (Group 3) Low Dose *Syzygium Aromaticum* L. group (*p* value < 0.01) and (Group 4) Low Dose *Syzygium Aromaticum* L. + Active Control group group (*p* value < 0.01) respectively in comparison to Active Control group. While, statistically insignificant difference was exhibited between (Group 5) High Dose *Syzygium Aromaticum* L. (*p* value > 0.01) and (Group 6) High Dose *Syzygium Aromaticum* L. + Active Control group (*p* value > 0.01) in comparison to (Group 2) Active Control group.

Table 1: Comparison between Pre- convulsion time on day 0 and day 14 in each groups.

Groups	**Pre-convulsion Time (seconds)**	**Mean Difference**	**Percentage of Protection (%)**	**P value**

	(PCT) 0 Day (Mean ± SEM)	**(PCT) 14th Day (Mean ± SEM)**			
Group-1 (Disease control) Distilled Water	73.33 ± 11.63	71.5 ± 6.83	1.8 ± 3	-2.37 ± 3.96	>0.01 NS#
Group-2 (Active-standard control) Formoterol and Budesonide	63.83± 4.401	113.33 ± 14.85	49.5 ± 6.6	42.95 ± 3.13	<0.001**
Group-3 (Low Dose *Syzygium Aromaticum* L. Extract group)	65 ± 13.84	89 ± 20.90	24 ± 4.6	26.20 ± 4.44	<0.01*
Group-4 (Low dose *Syzygium Aromaticum* L. Extract +Active control group)	72.166 ± 14.81	111.33 ± 26.43	18.6 ± 5.3	23.39 ± 7.06	<0.01*
Group-5 (High dose *Syzygium Aromaticum* L. Extract group)	61.5 ± 15.92	80 ± 5.77	39.16 ± 7.0	34.15 ± 3.61	<0.001**
Group-6 (High dose *Syzygium Aromaticum* L. Extract +Active control group)	52.33 ± 9.83	80.83 ± 13.68	28.5 ± 6.6	34.015 ± 6.36	<0.001**

NS # not significant * significant ** Very significant

Data are expressed as mean ± SEM) (n = 6); *$p < 0.01$ considered significant. (Intra-group comparison between days 0 and 14 PCT, using paired *t*-test), #$p < 0.01$ considered nonsignificant (Inter-group comparison amongst all groups with respect to PCT values for day 0 and day 14, using one-way ANOVA, followed by Tukey Kramer method)

Figure 1. Figure Showing Effect of Distilled Water, Formoterol and Budesonide, *Syzygium Aromaticum L.* in Low and High Dose and its Combination with Formoterol and Budesonide Group

on Pre-convulsion Time (PCT) on Histamine Induced Bronchospasm in Guinea Pigs in Mean ± SEM (Seconds) (n=6)

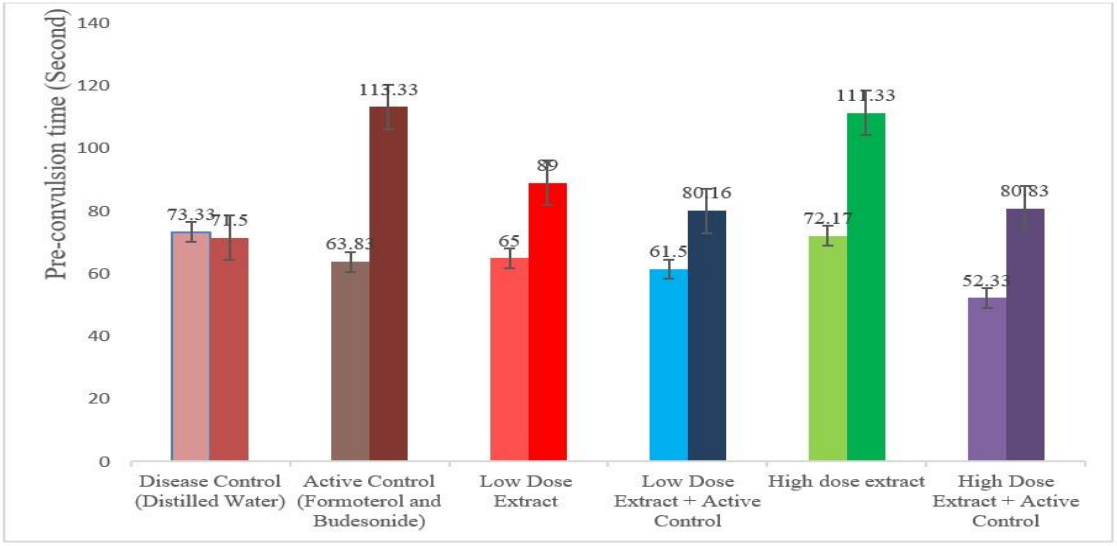
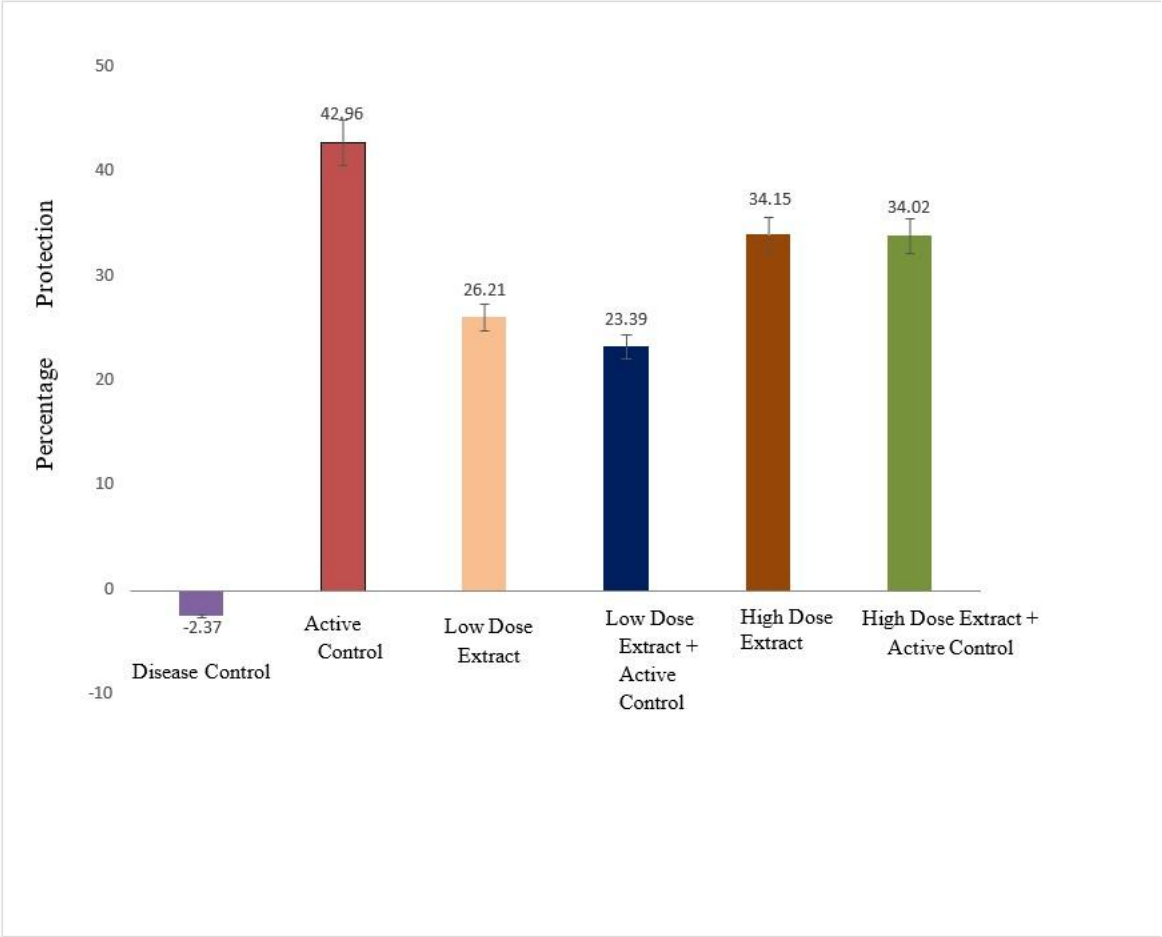


Figure 2- Figure Showing Percentage Protection obtained by Distilled Water, Formoterol and Budesonide, *Syzygium Aromaticum L.* in Low and High Dose and its Combination with Formoterol and Budesonide Group (Mean ± SEM) (n=6)



Discussion:

Histamine when inhaled causes contraction of bronchial smooth muscle leading to dyspnea and hypoxia in Guinea pigs resulting in convulsion. Bronchodilators can delay the occurrence of these symptoms. The increase in onset time of pre-convulsion dyspnea in guinea pig by test extract may be due to *in vivo* bronchodilator property [12]. Our study showed that oral administration of aqueous extract of (SYZ) *Sygyzium Aromaticum* L. in High dose 300 mg/kg and Low dose 150 mg/kg body weight for 14 days causes very significant increase in onset time of pre-convulsion time as compared with Disease control group. However, no add on benefit of SYZ aqueous extract has been demonstrated when combined with standard active control. Rather there was slight reduction in efficacy of formoterol and budesonide standard control trending towards antagonism which could not catch up with the statistical significance. Eugenol derived from clove extract exhibits inducing effect on Phase II liver enzymes[13] that might be the probable cause of reduction in the efficacy of active standard formoterol and budesonide [14,15]. The exact cause of this drug interaction remained unexplored. The bronchodilator activity of test extract may be due relaxation of bronchial smooth muscle or inhibition of mucus production in bronchioles or may be inhibition of histamine H receptors or stimulation of β adrenergic receptors [12,16]. The exact mechanistic action of Clove on airway smooth muscle remain unclear. Recent study by J.Haung et al, reported eugenol derived from the clove essential oil causes relaxation of isolated airway smooth muscle cells via calcium signaling in mice and human lung donor [17]. Though much more studies are required to explore and conclude it's exact mechanism. In-vivo and in vitro study of Kim HM et al, showed that aqueous extract of *Syzygium Aromaticum* L. when administered intraperitoneally inhibited compound 48:80induced systemic anaphylaxis in wister rats by inhibition of histamine released from mast cells [18].

Raghavenra et al, conducted a study that showed that eugenol—the active principle from cloves inhibited 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells [7]. An *in vivo* study by Rodrigues and coworkers on the effect of an hydroalcoholic extract of clove on pro-inflammatory cytokines production by macrophages of mice showed that clove oil caused cytokine inhibition due to the presence of eugenol, which imparts an anti-inflammatory activity [19]. All these studies validate our invivo study findings and it's probable mechanism behind potential bronchodilator Thus, eugenol an active compound of clove can be taken as a lead molecule for the development of bronchodilator.

Conclusion:

Our study parameters pre-convulsion time and percentage protection displayed that aqueous extract of *Syzygium Aromaticum* L., in high dose alone was found to be as effective as that of combination of formoterol and budesonide. This promising results of Aqueous extract of flower bud of *Syzygium Aromaticum* L. disclosed it's character as a potential bronchodilator. No add on benefit of *Syzygium Aromaticum* L. aqueous extract has been demonstrated by percentage protection or preconvulsion time when combined with formoterol and budesonide. Rather there was slight reduction in efficacy of formoterol and budesonide standard combination trending towards antagonism which could not catch up with the statistical significance.

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