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In vivo and ex vivo evaluation of the anti-asthmatic potential of *Pranacare*: A polyherbal formulation

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ABSTRACT

The anti-asthmatic efficacy of *Pranacare*, a polyherbal formulation, was previously evaluated using various *ex vivo* and *in vivo* animal models. Prior animal studies focused on histamine- and acetylcholine-induced hyperactivity as well as compound 48/80-induced mast cell degranulation activity. Here, *in vivo* dose-response studies were conducted to examine the effect of *Pranacare* on histamine- and acetylcholine-induced bronchospasm as well as histamine- and acetylcholine-induced contractions in the ileum tissue of guinea pigs. Moreover, the effects of *Pranacare* on mast cell degranulation using rat mesentery mast cells are evaluated *ex vivo*. In addition, the effects of *Pranacare* on 5-hydroxytryptamine (5HT)-induced contractions in the fundus tissue of Wistar rats are assessed *ex vivo*. Our *in vivo* findings reveal that oral administration of *Pranacare* (100, 200, and 400 mg/kg) leads to a significant reduction in histamine- and acetylcholine-induced bronchospasm in guinea pigs and mast cell degranulation in Wistar rats. Our *ex vivo* findings also reveal that *Pranacare* causes a significant, dose-dependent inhibition of histamine- and acetylcholine-induced smooth muscle contraction in guinea pig ileum and 5HT-induced smooth muscle contraction in rat fundus. Together, *Pranacare* exerts potent inhibitory effects against bronchospasm, mast cell degranulation, and smooth muscle contraction. Such anti-histaminic, anti-cholinergic, and anti-allergic properties of *Pranacare* present it a potential therapeutic agent that could be of great efficacy in the prevention and/or treatment of asthma.

Keywords: *Pranacare*, Anti-histaminic, Anti-cholenergic; Mast cell degranulation; Compound 48/80; Polyherbal formulation.

1. INTRODUCTION

Asthma is a chronic respiratory disease related to hypersensitivity[1]. Asthma, whose prevalence is alarmingly on the rise, is a disease that does not respect the boundaries of race, age, or gender[2]. Despite the availability of various medications that can ameliorate the symptoms associated with asthma, such medications do not withstand effectively to eradicate and cure the disease. Conventional or synthetic drugs used in the treatment of asthma are either inefficacious

Table –1: Exact composition of *Pranacare* capsules.

Botanical name	Source	Family	mg/Capsule
Adhatoda vasica	Leaves	Acantheceae	80 mg
Balsamoden dronmyrrha	Leaves	Burseraceae	20 mg
Coleus aromaticus	Leaves	Labiatae	100 mg
Ocimum sanctum	Leaves	Labiatae	30 mg
Piper betle	Leaves	Piperaceae	100 mg
Piper longum	Fruits	Piperaceae	25 mg
Tylophora asthmatica	Leaves	Asclepidaceae	75 mg

or have serious adverse effects. Hence, there is a worldwide tendency to go back to traditional medicinal plants as an alternative to treat/cure asthma. Indeed, a considerable number of medicinal plants have been reported in Ayurveda to offer natural and substantial protection against asthmatic attacks without any side effects, thus gradually leading to full recovery from asthma[3].

Phyto-pharmaceuticals derived from medicinal plants are widely used in many countries as part of folk medicine to treat different inflammatory conditions including contact dermatitis, arthritis, and asthma. A large portion of the world population, especially in the developing countries, depends on traditional medicine to treat a variety of infectious and non-infectious diseases. Several hundred genera are used medicinally, mainly as herbal preparations, in the indigenous systems of medicine in different countries, and they serve as invaluable sources of very potent and efficacious drugs that have stood the test of time and modern chemistry, leading to their perseverance and continuity [4].

Many successful polyherbal formulations are available in the market for the management of asthma. Efficacy and cost effectiveness are among the main advantages of anti-asthma polyherbal formulations. Even though the available polyherbal formulations are efficacious and cost effective, there is still a great need for more potent and effective formulations. A few studies have reported hepatoprotective, immunomodulatory, anti-inflammatory, anti-allergic, anti-tussive, anti-anaphylactic and anti-asthmatic effects of some of the individual ingredients of *Pranacare*[5-8]. *Pranacare* is one such polyherbal formulation that was, and still is, subject to well-designed preclinical evaluation. In the present study, the objective is to evaluate the potential effects of *Pranacare* on various mediators involved in histamine- and acetylcholine-induced bronchospasm and mast cell degranulation. The possible mechanisms of action by which *Pranacare* manifest such physiological effects are also examined using different *ex vivo* and *in vivo* animal models.

2. MATERIALS AND METHODS

2.1. Polyherbal formulation

The polyherbal formulation, *Pranacare*, was generously supplied by Universal Pharmaceuticals (Chennai, India). *Pranacare* consists of seven crude drugs (Table 1). *Pranacare* was suspended in 1% (w/v) solution of sodium carboxymethylcellulose (SCMC) before administration into animals.

2.2. Chemicals and Reagents

Compound 48/80, histamine, 5-hydroxytryptamine (5HT), and fine chemicals used in this study were purchased from Sigma-Aldrich (St. Louise, MO, USA). Acetylcholine and all other analytical grade chemicals were obtained from SD Fine-Chem Limited (Mumbai, India).

2.3. Animals

Colony inbred strains of Wistar rats (150-250 g) and guinea pigs (400-600 g) were used for the pharmacological experiments. The animals were kept in polypropylene cages under standard conditions (day/night cycle) at room temperature. The animals were fed on standard pelleted diet (Hindustan Lever Limited, Bangalore, India) and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee at C.L. Baid Metha College of Pharmacy, Chennai.

2.4. Effect of *Pranacare* on histamine-induced bronchospasm

Guinea pigs of either sex (400-600 g) were housed under uniform environmental conditions. They were divided into four groups, with six animals in each group. Animals in Groups I, II, and III received 100, 200, and 400 mg/kg (p.o.) of *Pranacare*, respectively. The animals in these three groups served as a test group in which

the eighteen animals were exposed to histamine aerosol. Animals in Group IV received 2 mg/kg (p.o.) of chlorpheniramine maleate. The six animals in this group served as a standard group in which they were exposed to histamine aerosol. Prior to drug treatment, the animals were exposed to micro-aerosol of histamine acid phosphate (1% w/v) using a nebulizer under constant pressure (1 kg/cm^2) in an aerosol chamber (24x14x24 cm) made of perplex glass. The animals exposed to the asthmatic agents showed progressive dyspnoea. The endpoint pre-convulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. As soon as the PCD was noted, the animals were removed from the chamber and placed in fresh air. 0-day values of PCD were taken before treatment. The animals were administered with the test formulation (*Pranacare*) and standard drugs as described above. On day 7, two hours after the last dose, the time for the onset of PCD was recorded as on day 0, as previously outlined[9]. Animals that withstood exposure to histamine aerosol for 10 min were considered to be completely protected[10].

The protection offered by the treatment was calculated according to the following formula:

$$\text{Percentage protection} = \left\{1 - \frac{T_1}{T_2}\right\} \times 100$$

Where, T_1 is time for PCD onset on day 0 and T_2 is the time for PCD onset on day 7.

2.5. Effect of *Pranacare* on acetylcholine-induced bronchospasm

Guinea pigs of either sex (400-600 g) were housed under uniform environmental conditions and were divided into four groups, with six animals in each group. Animals in Groups I, II, and III received 100, 200, and 400 mg/kg (p.o.) of *Pranacare*, respectively. The animals in these three groups served as a test group in which the eighteen animals were exposed to acetylcholine aerosol. Animals in Group IV received 2 mg/kg (p.o.)of atropine sulfate. The six animals in this group served as a standard group in which they were exposed to acetylcholine aerosol. Prior to drug treatment, the animals were placed in the aerosol chamber and exposed to micro-aerosol of acetylcholine chloride (5% w/v) using a nebulizer under constant pressure (1 kg/ cm^2). The procedure was continued in the same manner as described above for evaluating histamine-induced bronchospasm.

2.6. Effect of *Pranacare* on rat mesentery mast cell degranulation

Wistar rats of either sex (150-170 g) were housed under uniform environmental conditions. The animals were divided into five groups, with six animals in each group. Animals in Group I received a daily dose of 2ml/kg (p.o.) of 1% SCMC for 7 days and served as a control group. For 7 days, animals in Groups II, III, and IV received a daily dose of 100, 200, and 400 mg/kg (p.o.) of *Pranacare*, respectively, suspended in 1% SCMC, and served as test groups. Animals in Group V received a daily dose of 10 mg/kg (p.o.) of prednisolone suspended in 1% SCMC for 7 days and served as a standard group. Two hours after the last dose on day 7, all animals were sacrificed. Pieces of animal guts with intact mesentery tissue were isolated and kept in Locke-Ringer solution. The Locke-Ringer solution is composed of 154 mM NaCl, 6.0 mM $NaHCO_3$, 5.6 mM KCl, 2.2 mM $CaCl_2$, 5.55 mM D-glucose, and water. The mesentery tissue was incubated in 2.5 µg/ml of compound 48/80 solution for 10 min at 37°C. After incubation, the mesentery tissue was carefully spread on a clean dry microscopic glass slide and it was allowed to air-dry. The attached piece of intestine was gently removed from the mesentery tissue as previously outlined[11]. Subsequently, mast cells were stained with toluidine blue (1% w/v) for 2 min, and the mesentery tissue was washed with distilled water. Next, mast cells were stained with light green (0.1% w/v) for 30 sec, and the mesentery tissue was washed with distilled water and allowed to air-dry. The numbers of intact and ruptured, degranulated mast cells in five different fields were determined using a high power objective[12]. The percentage protection wascalculated as outlined above.

2.7. Ileum tissue preparation and assessment of histamine- and acetylcholine-induced smooth muscle contraction

Guinea pigs (300-500 g) of either sex were starved overnight with water *ad libitum*. The animals were killed by a sudden blow on the head and the neck was exsanguinated. The abdomen was cut open and a suitable length of the ileum (approximately 2 cm long) was placed in a Petri dish containing Tyrode's solution. Tyrode's solution is composed of 137 mM NaCl, 12 mM $NaHCO_3$, 0.3 mM NaH_2PO_4, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, 5.6 mM D-glucose, and water. Experiments were performed in a 30 ml organ bath containing Tyrode's solution maintained at 37^{o}C and gassed with air mixture (O_2 plus CO_2). Isometric contractions were recorded on a smoked kymograph paper with frontal writing lever adjusted in horizontal position and exerting 0.5 gm tension. After an equilibration period of 30 min during which the Tyrode's solution was changed at 10 min intervals, contractile responses were recorded for histamine (8 µg/ml) and

acetylcholine (8 µg/ml). The tissue response for contact time of 30 sec was recorded at 5 min time intervals. The *Pranacare*-tissue contact time was set at 1 min before the addition of histamine and acetylcholine. The percentage inhibition caused by *Pranacare* against histamine- and acetylcholine-induced muscle contractions was calculated as previously described[13].

2.8. Fundus tissue preparation and assessment of 5HT-induced smooth muscle contraction

Wistar rats of either sex (150-200 g) were starved overnight with water *ad libitum*. The animals were sacrificed by cervical decapitation. The abdomen was cut open and the stomach was exposed. The fundus of the stomach was identified, incised from the junction of pyloric part, and transferred to a Petri dish containing Tyrode's solution. The isolated fundus was incised from the lesser curvature, opened longitudinally, and cut into sheets, from where 2-3 cm long strips were prepared. The experiments were performed in a 30 ml organ bath containing Tyrode's solution maintained at 37°C and gassed with air mixture (O_2 plus CO_2). Isometric contractions were recorded on a smoked kymograph paper using a frontal writing lever adjusted in horizontal position and exerting 1.0 gm tension. After an equilibration period of 60 min, contractile responses were recorded for 5-hydroxytryptamine (5HT) (8 µg/ml). A90 sec contact time and 5 min time cycles were kept for proper recording of the contractile responses. *Pranacare*-tissue contact time was set at 1 min before the addition of 5HT. The effects of *Pranacare* on 5HT-induced muscle contractions were recorded. The percentage inhibition caused by *Pranacare* against 5HT-induced muscle contractions was calculated as previously described [14].

2.9. Statistical analysis

Statistical significance of dose-responses was determined by significant correlation structure and modeling the data on a linear regression equation. For multiple comparisons, statistical significance was determined using paired t-test, student t-test, and ANOVA coupled with two-sided Dunnett's post-hoc test. *p<0.01, and **p<0.001 are considered statistically significant.

3. RESULTS AND DISCUSSION

Asthma is a chronic, episodic disease of the airways that is manifested as recurrent episodes of variable respiratory symptoms with airflow obstruction that is often reversible[15, 16]. The major goals in the management of asthmatic attack include relaxation of the smooth muscle of the bronchial tree and inhibited release of pro-inflammatory mediators such as histamine, 5HT, leukotriene, etc., thereby relieving airway obstruction and hyper-responsiveness [15, 16].In this study, we evaluated the potential anti-asthmatic property of *Pranacare* using different *ex vivo* and *in vivo* animal models. Specifically, the effects of *Pranacare* on histamine-induced bronchospasm and acetylcholine-induced bronchospasm were assessed *in vivo* using guinea pigs. Moreover, the effect of *Pranacare* on mast cell degranulation was evaluated *in vivo* using Wistar rats. Additionally, the effects of *Pranacare* on histamine-induced smooth muscle contraction and acetylcholine-induced smooth muscle contraction were assessed *ex vivo* using ileum tissue of guinea pigs. Finally, the effect of *Pranacare* on 5HT-induced smooth muscle contraction was assessed *ex vivo* using fundus tissue of Wistar rats.

3.1. Effect of *Pranacare* on histamine-induced bronchospasm in guinea pigs

Guinea pigs are highly sensitive to histamine-induced bronchospasm. In histamine aerosol-induced challenge, the animals displayed PCD. However, as shown in Figure 1, pre-treatment with *Pranacare* (100, 200, and 400 mg/kg) led to a significant delay in the onset of PCD in a dose-dependent manner (Figure 1), indicating that *Pranacare* provides marked protection against the histamine aerosol-induced challenge. In comparison to the before treatment the percentage protection was found to be 52.73%, 66.05% and 73.64% for Group I, Group II, and Group III, respectively (Figure 1). The standard group (Group IV) also showed significant delay in the onset of PCD and the percentage protection was found to be 78.37% in comparison to the sample before treatment (Figure 1).

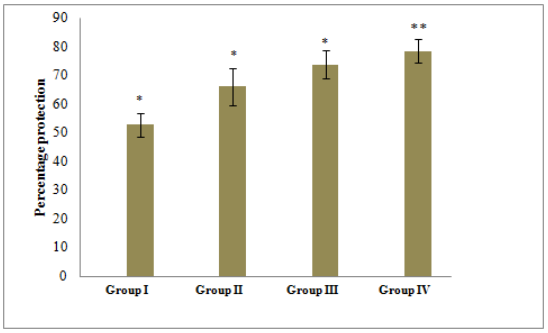

Figure – 1: Effect of *Pranacare* on histamine-induced bronchospasm in guinea pigs.

PCD was evaluated in guinea pigs that were subject to oral administration of 100, 200, and 400 mg/kg *Pranacare* (Group I, Group II, and Group III, respectively) or chlorpheniramine maleate (Group IV). Bronchospasm was induced

by histamine aerosol, and % protection was calculated as described above. Statistical significance (*p<0.01 and **p<0.001) was determined in comparison to the sample before treatment Values are expressed as mean ± SEM (n = 6).

3.2. Effect of *Pranacare* on acetylcholine -induced bronchospasm in guinea pigs

Guinea pigs are also highly sensitive to acetylcholine-induced bronchospasm. In acetylcholine aerosol-induced challenge, the animals displayed PCD. However, as shown in Figure 2, pre-treatment with *Pranacare* (100, 200, and 400 mg/kg) led to a significant delay in the onset of PCD in a dose-dependent manner (Figure 2), indicating that *Pranacare* provides marked protection against the acetylcholine aerosol-induced challenge. In comparison to the sample before treatment, the percentage protection was found to be 42.73%, 50.53% and 58.32% for Groups I, Group II, and Group III, respectively (Figure 2). The standard group (Group IV) also showed significant delay in the onset of PCD and the percentage protection was found to be 64.57% in comparison to the sample before treatment (Figure 2).

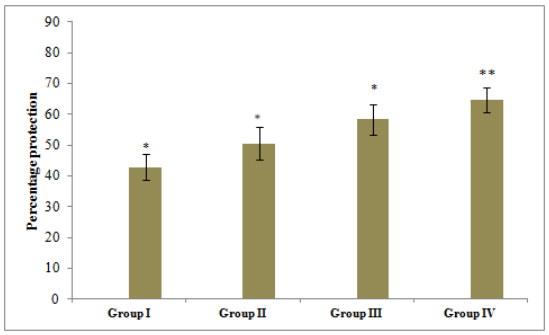

Figure – 2: Effect of *Pranacare* on acetylcholine-induced bronchospasm in guinea pigs.

PCD was evaluated in guinea pigs that were subject to oral administration of 100, 200, and 400 mg/kg *Pranacare* (Group I, Group II, and Group III, respectively) and atropine sulfate (Group IV). Bronchospasm was induced by acetylcholine aerosol, and % protection was calculated as described above. Statistical significance (*p<0.01 and **p<0.001) was determined in comparison to the sample before treatment. Values are expressed as mean ± SEM (n = 6).

3.3. Effect of *Pranacare* on rat mesentery mast cell degranulation

Mast cells have been thought to play a major role the development of many physiological changes during asthma[17, 18]. Mast cell activation, both by IgE-dependent and IgE-independent stimuli, triggers the process of degranulation that results in the fusion of the cytoplasmic granular membrane with the plasma membrane, leading to the release of the granular content outside the activated mast cell[17, 18]. Mast cell degranulation can be elicited by a number of positively-charged substances collectively known as the basic secretagogues of mast cells[19]. The most potent secretagogues include compound 48/80, a synthetic polymer of basic amino acids[19]. Figure 3 clearly shows that treatment with *Pranacare* (100, 200, and 400 mg/kg) resulted in a significant dose-dependent protection against compound 48/80-induced rat mesentery mast cell degranulation. In comparison to the control group (Group I), the percentage protection against mast cell degranulation was found to be 38.46%, 54.53%and 65.78% for Group II, Group III, and Group IV, respectively (Figure 3). The standard group (Group V) also showed significant protection (78.49%) against mast cell degranulation when compared with the control group (Group I- 23.12%) (Figure 3). These findings suggest that *Pranacare* can potently suppress mast cell degranulation, a mechanism of action that, at least partially, explains its anti-asthmatic effects. It is possible that *Pranacare* protects against mast cell degranulation by directly inhibiting IgE production. However, this likely possibility needs to be examined by future

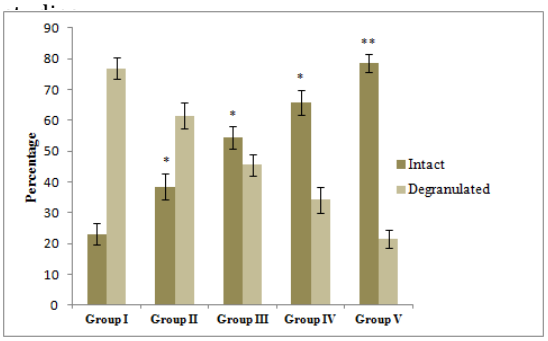

Figure – 3: Effect of *Pranacare* on rat mesentery mast cell degranulation.

Mast cell degranulation was evaluated in Wistar rats that were subject to oral administration of 2 ml/kg 1% SCMC (Group I - control), 100, 200, and 400 mg/kg *Pranacare* (Group II, Group III, and Group IV, respectively), or 10 mg/kg prednisolone (Group V - standard). Mast cell degranulation was induced by compound 48/80 (2.5 µg/ml), and % protection was calculated as described above. Statistical significance (*p<0.01 and **p<0.001) was determined in comparison to the Group I (control). Values are expressed as mean ± SEM (n = 6).

Table - 2: Effect of *Pranacare* on histamine-induced contractions of guinea pig ileum.

Treatment		Mean contraction (mm)	% Inhibition
Histamine (µg/ml)	*Pranacare* (mg/ml)		
8.0	-	49.00 ± 2.51	0
	1	35.33 ± 6.48	27.90*
	2	16.00 ± 4.04	67.35*
	4	8.00 ± 1.52	83.67*
	8	3.30 ± 0.33	93.27*
	16	0	100*

Values are mean ± SEM (n = 3). *p<0.01 when compared with control (49.00 mm is taken as 100% contraction).

3.4. Effect of *Pranacare* on histamine and acetylcholine-induced smooth muscle contraction in guinea pig ileum

Mediators like histamine and acetylcholine are spasmogens that are critically implicated in various ways in the pathogenesis of asthma[20]. Histamine is the most implicated mediator in bronchoconstriction that accompanies asthma[20]. Acetylcholine has also been shown to cause bronchoconstriction[21]. We wanted to assess the potential effects of *Pranacare* on smooth muscle contraction. To this end, the effects of *Pranacare* on histamine-induced contraction of guinea pig ileum were examined *ex vivo*. Isolated ileum tissue was treated with 1, 2, 4, 8, and 16 mg/ml *Pranacare* in absence and presence of histamine (8 µg/ml) and responses were recorded. *Pranacare* treatment did not exhibit any changes in the contractile activity of isolated guinea pig ileum in absence of histamine. However, *Pranacare* significantly inhibited histamine-induced contraction of guinea pig ileum in a dose-dependent manner, with the 16 mg/ml concentration leading to complete inhibition of histamine-induced contraction (Table 2).

Similarly, the effects of *Pranacare* on acetylcholine-induced contraction of guinea pig ileum were examined *ex vivo*. Isolated ileum tissue was treated with 1, 2, 4, 8, 16, 32, and 64 mg/ml *Pranacare* in absence and presence of acetylcholine (8 µg/ml), and contractions were recorded. *Pranacare* treatment did not exhibit any changes in the contractile activity of isolated guinea pig ileum in absence of acetylcholine. To a lesser extent than histamine, acetylcholine (8 µg/ml) caused marked contractile responses in guinea pig ileum tissue (Table 3). Acetylcholine-induced contractions were significantly inhibited by *Pranacare* in a dose-dependent manner, with the 64 mg/ml concentration leading to complete inhibition of acetylcholine-induced contraction (Table 3).

Collectively, these findings clearly indicate that *Pranacare* is a potent inhibitor of histamine- and acetylcholine-induced contraction of guinea pig ileum. Thus, the protection offered by *Pranacare* against histamine- and acetylcholine-induced bronchospasm (Figure 1 and Figure 2) may be attributed to its ability to inhibit the contraction of bronchial smooth muscle that is caused by histamine and acetylcholine. The anti-asthmatic effects of *Pranacare* may likely be due to its anti-histaminic/anti-cholinergic activity on histamine/acetylcholine receptors expressed on the surface of bronchial smooth muscle cells.

3.5. Effect of *Pranacare* on 5HT-induced smooth muscle contraction in rat fundus

We further evaluated the potential effects of *Pranacare* on 5HT-induced smooth muscle contraction in rat fundus *ex vivo*. To this end, isolated rat fundus tissue was treated with 1, 2, 4, 8, 16, 32, 64, and 128 mg/ml *Pranacare* in presence and absence of 5HT (8 µg/ml), and contractions were recorded. *Pranacare* treatment caused no significant changes in the contractile activity of isolated rat fundus in absence of 5HT. However, 5HT-induced contractions were subject to a significant, dose-dependent inhibition by *Pranacare*, with the 128 mg/ml concentration leading to complete inhibition of 5HT-induced contraction (Table 4). These findings indicate that *Pranacare* is not only a potent inhibitor of histamine- and acetylcholine-induced contraction of guinea pig ileum (Table 2 and Table 3), but it is also an effective inhibitor of 5HT-induced contraction of rat fundus (Table 4). Hence, *Pranacare* can exert potent inhibitory effects against smooth muscle contraction, making it a potential therapeutic agent that could hamper bronchospasm, and thus, prevent/treat asthmatic attacks.

Table – 3: Effect of P*ranacare* on acetylcholine-induced contractions of guinea pig ileum.

Treatment		Mean contraction (mm)	% Inhibition
Acetylcholine (µg/ml)	*Pranacare* (mg/ml)		
8.0	-	35.33 ± 0.33	0
	1	33.67 ± 0.66	4.70*
	2	31.33 ± 0.88	11.32*
	4	27.33 ± 1.20	22.64*
	8	17.00 ± 1.15	51.88*
	16	10.33 ± 1.45	70.76*
	32	3.66 ± 0.33	89.64*
	64	0	100*

Values are mean ± SEM (n = 3). *p<0.01 when compared with control (35.33 mm is taken as 100% contraction).

Table – 4: Effect of *Pranacare* on 5HT-Induced contractions of rat fundus.

Treatment		Mean contraction (mm)	% Inhibition
5HT (µg/ml)	*Pranacare* (mg/ml)		
8.0	-	16.00 ± 3.05	0
	1	14.67 ± 2.33	8.31*
	2	14.00 ± 2.51	12.50*
	4	12.33 ± 1.76	22.94*
	8	9.33 ± 1.45	41.69*
	16	7.66 ± 1.20	52.13*
	32	5.33 ± 0.88	66.69*
	64	3.00 ± 0.55	81.25*
	128	0	100*

Values are mean ± SEM (n = 3). *p<0.01 when compared with control (16.00 mm is taken as 100% contraction)

4. CONCLUSION

Pranacare is a polyherbal formulation that contains alkaloids, flavonoids, and triterpenes as major phytochemicals. *Pranacare* has been traditionally used to treat various medical conditions including asthma. Our study sheds light on the mechanisms of action by which *Pranacare* manifests its anti-asthmatic effects. Indeed, *in vivo* and *ex vivo* analyses reveal that *Pranacare* can potently inhibit histamine- and acetylcholine-induced bronchospasm, mast cell degranulation, as well as histamine-, acetylcholine-, and 5HT-induced smooth muscle contraction. Yet, our study does not rule out the possibility that *Pranacare* may manifest its anti-asthmatic activity through other molecular and cellular mechanisms.

We anticipate that *Pranacare* may be more employed in the future for the management of asthmatic attacks and other allergic conditions. Future studies should also focus on identifying and isolating that the main active principles and other phytochemical constituents within *Pranacare* that mediate its anti-asthmatic activity.

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