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RESEARCH ARTICLE

Evaluation of *In Vivo* Anti-histaminic and Anti-anaphylactic Activity of *Siddha* Mono-herbal Formulation *Kanduparangi Chooranam* (*Pygmaeopremna herbacea* - Root Powder)

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Abstract

Background: Current treatment options for allergic diseases often have limitations, including adverse effects and low efficacy, while traditional Indian medicine, specifically *Siddha* formulations, offers potential alternatives. **Aim:** This study evaluates the *in vivo* anti-histaminic and anti-anaphylactic activities of *Kanduparangi Chooranam* (KC), a *Siddha* monoherbal formulation prepared from the root powder of *Pygmaeopremna herbacea*. **Methods:** The study used Wistar rats for mast cell stabilisation and guinea pigs for histamine-induced bronchospasm. KC was given at 100 mg/kg and 200 mg/kg doses. Mast cell stability was measured by intact and disrupted cells, while bronchospasm was assessed by Pre-Convulsive Dyspnea (PCD) time. Data were analysed using one-way ANOVA and Newman-Keuls tests. **Results:** Mast cell stabilisation: KC significantly increased the percentage of intact mast cells compared to the sensitised control group ($p<0.01$). The protective effect was observed at both 100 mg/kg and 200 mg/kg doses, with no significant difference between the two. Histamine-induced bronchospasm: KC significantly prolonged the PCD time in guinea pigs compared to the control group ($p<0.001$), demonstrating its potential bronchodilatory effect. Similar to mast cell stabilisation, both KC doses showed comparable efficacy. **Discussion:** The study suggests that KC stabilises mast cells by inhibiting degranulation and reducing histamine release. Its ability to prolong PCD time indicates anti-histaminic properties. Similar efficacy at both doses suggests 100 mg/kg may be sufficient for therapeutic use. **Conclusion:** *Kanduparangi chooranam* shows strong anti-histaminic and anti-anaphylactic properties, making it a potential treatment for allergies and asthma. Further studies and clinical trials are needed to confirm its efficacy and explore its role in immune modulation.

Major Findings: In this research, *kanduparangi chooranam* exhibited significant anti-histaminic and anti-anaphylactic properties by stabilising mast cells and prolonging PCD time in guinea pigs. Both 100 mg/kg and 200 mg/kg doses showed comparable efficacy, suggesting its potential as a natural treatment for allergic conditions.

Keywords: Anti-histaminic, Anti-anaphylactic, Bronchospasm, *Kanduparangi Chooranam*, *Pygmaeopremna herbacea*, *Siddha* Medicine

Abbreviations: PCD: Pre-Convulsive Dyspnea; LD: Lethal Dose; SEM: Standard Error of Mean; ANOVA: Analysis of Variance; NaCl: Sodium Chloride; KCl: Potassium Chloride; $CaCl_2$: Calcium Chloride; $NaHCO_3$: Sodium Bicarbonate

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1. Introduction

Even though the population's overall health has improved recently, the prevalence of allergies and asthma has increased[1]. Significant morbidity and a negative financial impact are caused by allergic disorders. The causes of the rise in upper and lower respiratory tract allergy illness prevalence have been determined by a number of epidemiological studies[2]. Some of the hypothesized causes include rising environmental contamination, a significant shift in the distribution of genes that makes people more likely to produce too much IgE, changing lifestyles, and an increasing understanding of the illnesses[3].

The function of mast cells, immunoglobulins, lymphocytes, and different autacoids in the etiopathogenesis of allergic disorders has been extensively studied during the past several decades. The management of allergic illnesses remains far from acceptable despite the extensive research on the topic[4].

Due to their limited effectiveness, related side effects, and difficulties with compliance, the present treatment options for upper and lower respiratory tract allergic disorders have significant limitations[5]. The Indian medical system, AYUSH, has detailed several medications made from natural sources that are capable of helping treat allergic diseases and bronchial asthma[6].

Kanduparangi chooranam, derived from the root of *Pygmaeopremna herbacea* (Roxb) Moldenke, is a traditional *Siddha* formulation known for its wide range of therapeutic applications. This herbal remedy possesses a bitter and astringent taste, with hot potency and post-absorptive pungent effects. Rich in medicinal compounds such as acid resin, icetexane, sirutekkone, and scutellarein, it exhibits multiple pharmacological actions. The presence of bioactive phytochemicals, including alkaloids, saponins, quinones, lignans, terpenoids, and glycosides, enhances its medicinal potential. These attributes make it valuable in managing inflammatory disorders, allergic reactions, and liver-related ailments, while also promoting overall health and immunity[7,8]. The effects of the *Siddha* formulation of *kanduparangi chooranam* on histamine-induced bronchospasm in guinea pigs, active anaphylaxis, and mast cell stabilisation in rats have been studied in this study.

2. Materials and Methods

2.1 Preparation of the Trial Drug

Pygmaeopremna herbacea root was purchased, cleaned, and crushed into a fine powder in accordance with the directions provided in the *Siddha* classical textbook.

2.2 Animals for Mast Cell Stabilising Activity[9]

Male and female Wistar rats, weighing between 175 and 200 grams, were maintained under typical laboratory conditions. The animals were maintained under controlled conditions of 22 ± 2°C temperature, 60 ± 5% relative humidity, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and had free access to drinking water.

2.3 Animals for Histamine-Induced Bronchospasm[10]

Guinea pigs of either sex, weighing 400–600 grams, were kept under standardised environmental conditions with a maintained temperature of 22 ± 2°C, relative humidity of 60 ± 5%, and a 12-hour light/dark cycle. They were given a regular pellet diet and had unrestricted access to water.

2.4 Chemicals Used in Mast Cell Stabilising Activity and Histamine-induced Bronchospasm

Histamine and horse serum were procured from Sigma Chemicals; toluidine blue was obtained from Loba-Chemie, Mumbai; and the IgE ELISA kit was sourced from Orion Diagnostics, Espoo, Finland. A triple antigen preparation containing 20,000 million *Bordetella pertussis* organisms was sourced from the Serum Institute of India Ltd., Pune. All other required chemicals and reagents were procured from Hi-Media Laboratories Ltd., Mumbai.

2.5 Ethical Clearance of this Research Study

The study protocol received approval from the Institutional Animal Ethics Committee and was conducted in accordance with the guidelines established by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India. [Approval No.: IAEC/C.B.S.BHARATH CHRISTIAN/M. D(S)/TNMGRMU/84/2020].

2.6 Statistical Analysis

Data from various experiments were presented as mean ± standard error of the mean (SEM) and statistically evaluated using one-way ANOVA, followed by the Newman-Keuls post hoc multiple comparison test. A p-value less than 0.05 was considered indicative of statistical significance. Data analysis was performed using GraphPad Prism software, version 4.0.

2.7 Mast Cell Stabilising Activity

2.7.1 Treatment protocol

A total of 24 rats were randomly divided into four groups, each comprising six animals. Group I served as the normal control and received only the vehicle (water). Group II was designated as the sensitized control. Group III received *kanduparangi chooranam* at a dose of 100 mg/kg body weight orally, serving as a treatment group. Group IV, also a treatment group, was administered *kanduparangi chooranam* orally at a dose of 200 mg/kg body weight.

Table 1, presents the grouping of experimental animals used for evaluating the Mast Cell Stabilising Activity. In Groups II to IV, sensitisation was achieved through the subcutaneous injection of 0.5 ml horse serum along with 0.5 ml of a triple antigen preparation containing 20 billion *Bordetella pertussis* organisms, administered once daily over a period of 14 days. On the 14th day, two hours after the final dose, the animals were humanely sacrificed, and the intestinal mesentery was carefully excised for mast cell analysis.

Mesenteric tissues along with portions of the intestine were carefully excised and immersed in Ringer-Locke solution (containing $CaCl_2$ – 2.2 mM, KCl – 5.6 mM, NaCl – 154 mM, $NaHCO_3$ – 6.0 mM, and glucose – 5.55 mM per litre of distilled water) maintained at 37°C. To induce mast cell activation, the samples were incubated with 5% horse serum for 10 minutes. Subsequently, the mast cells were stained with 1.0% toluidine blue and examined under a microscope to assess and count both intact and degranulated mast cells.

Table 2, illustrates the effect of *kanduparangi chooranam* on mast cell stabilization in histamine-sensitized rats.

2.8 Histamine-induced Bronchospasm in Guinea Pigs

2.8.1 Treatment Protocol

Bronchospasm was experimentally induced in guinea pigs by exposing them to a 1% histamine aerosol delivered at a constant pressure of 1 kg/cm² inside a Plexiglas aerosol chamber measuring 24 × 14 × 24 cm. The study comprised three groups, with six animals in each group. Group I served as the control. Group II received the *Siddha* formulation *kanduparangi chooranam* orally at a dose of 100 mg/kg body weight, while Group III was administered the same formulation at a dose of 200 mg/kg body weight, also by oral route. Both Groups II and III acted as treatment groups.

Table 3 presents the grouping of animals used in the mast cell stabilising activity study in Histamine-induced Bronchospasm in Guinea Pigs models. On Day 0, before initiating any treatment, the animals were subjected to a 1% histamine aerosol within an exposure chamber maintained at a constant pressure of 1 kg/cm². The key parameter evaluated was Pre-Convulsive Dyspnea (PCD), determined by measuring the time elapsed from the start of histamine exposure to the onset of respiratory difficulty leading to convulsions. Once signs of PCD appeared, the animals were immediately taken out of the chamber and allowed to recover in normal atmospheric conditions. This initial measurement was recorded as the baseline

Table 1. Group of animals in the mast cell stabilising activity

Groups	Control	Sensitization status	Administration
I	Negative control	No sensitization	Normal saline 0.5 ml/kg
II	Sensitized control	Sensitization was carried out by administering a subcutaneous injection of 0.5 ml horse serum along with 0.5 ml of a triple antigen preparation containing 20 billion *Bordetella pertussis* organisms, once daily for a duration of 14 days.	-
III	Treatment control 1		*Kanduparangi Chooranam* at the dose of 100 mg/kg body weight.
IV	Treatment control 2		*Kanduparangi Chooranam* at the dose of 200 mg/kg body weight

(Day 0). Subsequent PCD readings were taken on Days 1 and 5, two hours after oral administration of the test formulation, following the same experimental protocol.

Table 4 illustrates the effect of *kanduparangi chooranam* on histamine-induced bronchospasm in guinea pig models.

3. Results and Discussion

3.1 Mast Cell Stabilising Activity

The mast cell stabilising potential of *kanduparangi chooranam* (KC) was evaluated in histamine-sensitised rats by analysing the percentage of intact and degranulated mast cells. The findings are summarised in Table 2. In the normal control group (Group I), a significantly higher proportion of intact mast cells (82.68 ± 2.55) and a lower percentage of degranulated mast cells (15.20 ± 0.88) were observed, indicating normal physiological conditions. Conversely, the sensitised control group (Group II) exhibited a notable decrease in intact mast cells (13.25 ± 0.94) and a substantial rise in degranulated mast cells (84.60 ± 2.55), confirming the successful induction of mast cell degranulation.

Table 2. Effect of *kanduparangi chooranam* on mast cell stabilisation in histamine-sensitised rats

Groups	Mast Cells	
	Intact	Disrupted
Normal Control	82.68±2.55	15.20±0.88
Sensitized Rats	13.25±0.94	84.60±2.55
KC 100mg/kg	65.20±2.30*a	34.30±1.40*a
KC 200mg/kg	61.45±2.15*a	32.10±1.24*a

Values are expressed as Mean±S.E.M. *a significantly different from sensitized control at p<0.01

3.2 Effect on Histamine-induced Bronchospasm

The impact of *kanduparangi chooranam* on histamine-induced bronchospasm in guinea pigs was assessed by recording the *Pre-Convulsive Dyspnea (PCD)* time at different time points (Day 0, Day 1, and Day 5). The findings are summarised in Table 4. On Day 0, there was no significant variation in PCD time among the groups, indicating that all animals exhibited a comparable baseline response to histamine aerosol exposure. The control group demonstrated a PCD time of 174.50 ± 2.20 seconds, whereas the treatment groups receiving 100 mg/kg and 200 mg/kg recorded PCD times of 180.25 ± 2.38 and 185.18 ± 2.48 seconds, respectively.

3.3 Effect of *Kanduparangi Chooranam* on Mast Cell Stabilising Activity

Administration of *kanduparangi chooranam* at a dose of 100 mg/kg (Group III) led to a significant increase in the number of intact mast cells (65.20 ± 2.30) and a reduction in degranulated mast cells (34.30 ± 1.40) compared to the sensitised control group (p<0.01). Similarly, the 200 mg/kg dose (Group IV) produced a comparable effect, with 61.45 ± 2.15 intact mast

Table 3. A group of animals in the mast cell stabilising activity

Groups	Control	Bronchospasm-inducing agent	Administration
I	Control	Animals were exposed to a 1% histamine aerosol delivered at a constant pressure of 1 kg/cm^2 within a Plexiglas aerosol chamber measuring 24 × 14 × 24 cm	Normal saline 0.5 ml/kg
II	Treatment control 1		*Kanduparangi chooranam* at the dose of 100 mg/kg body weight
III	Treatment control 2		*Kanduparangi chooranam* at the dose of 200 mg/kg body weight

Table 4. Effect of *kanduparangi chooranam* on histamine-induced bronchospasm in guinea pigs

Groups	Pre-Convulsive Dyspnoea (PCD) time in Seconds		
	Day 0	Day 1	Day 5
GP 1	174.50±2.20	265.25±4.30	224.45±4.20
Kanduparangi Chooranam 100mg/kg	180.25±2.38	224.40±3.70	416.30±7.10*a
Kanduparangi Chooranam 200mg/kg	185.18±2.48	220.32±3.40	407.15±6.70*a

Values are expressed as Mean ±S.E.M. *a significantly different from control on day 5 at p<0.001.

cells and 32.10 ± 1.24 degranulated mast cells, both of which were significantly different from the sensitised control ($p<0.01$). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences. The results demonstrated that both treatment groups (KC 100 mg/kg and KC 200 mg/kg) exhibited statistically significant improvements in mast cell stabilisation compared to the sensitised control ($p<0.01$). However, no significant difference was observed between the two treatment doses ($p>0.05$), suggesting a dose-independent effect.

3.4 Effect of *Kanduparangi Chooranam* in Histamine-induced Bronchospasm

On Day 1, the control group exhibited a slight increase in Pre-Convulsive Dyspnea (PCD) time (265.25 ± 4.30 seconds), whereas the treatment groups showed a moderate rise (224.40 ± 3.70 seconds and 220.32 ± 3.40 seconds for the 100 mg/kg and 200 mg/kg doses, respectively), indicating an initial effect of the treatment. By Day 5, a significant increase in PCD time was observed in the treatment groups compared to the control. The control group showed a slight decline in PCD time (224.45 ± 4.20 seconds), while the treatment groups demonstrated a substantial prolongation: 416.30 ± 7.10 seconds for the 100 mg/kg dose and 407.15 ± 6.70 seconds for the 200 mg/kg dose. Both treatment groups exhibited statistically significant differences compared to the control on Day 5 ($p<0.001$).

One-way ANOVA followed by Tukey's post hoc test was used to compare PCD times across the groups. On Day 5, both treatment groups receiving *kanduparangi chooranam* demonstrated a highly significant increase in PCD time compared to the control group ($p < 0.001$), indicating improved tolerance to bronchospasm. However, the difference between the 100 mg/kg and 200 mg/kg dosage groups was not statistically significant ($p > 0.05$), indicating that the therapeutic response may have reached a plateau at the lower dose.

The significant prolongation of PCD time in the treatment groups suggests that *kanduparangi chooranam* effectively alleviates histamine-induced bronchospasm in guinea pigs. The observed bronchodilatory effect may be attributed to its potential antihistaminic and mast cell-stabilising properties. The absence of a dose-dependent effect beyond 100 mg/kg indicates that this dosage is sufficient to achieve maximum therapeutic benefits. Further investigations are needed to clarify its precise mechanism of action and to assess its potential clinical applications in respiratory disorders.

In sensitised animals, antigenic stimulation triggers mast cell degranulation, a key feature of anaphylactic reactions. The findings of this study demonstrate that *kanduparangi chooranam*, a traditional *Siddha* formulation, provided substantial protection against mast cell degranulation following antigenic challenge. This stabilising effect may be attributed to its bioactive compounds, which either inhibit antigen–antibody interactions responsible for mast cell disruption or suppress IgE antibody production, thereby preventing degranulation.

The results further indicate that *kanduparangi chooranam* exhibits potent mast cell-stabilising activity, as evidenced by the increased proportion of intact mast cells and decreased degranulation in treated rats. This protective effect is likely mediated by its ability to regulate histamine release. Further research is warranted to elucidate its precise mechanism of action and explore its potential applications in the management of allergic disorders.

4. Conclusion

The findings of this study underscore the significant mast cell-stabilising and anti-histaminic properties of *kanduparangi chooranam*, highlighting its potential as a natural therapeutic agent for allergic and inflammatory respiratory conditions. Its ability to inhibit mast cell degranulation and improve bronchospasm tolerance suggests a promising role in managing asthma and related disorders. The observed effects indicate that lower doses may be sufficient for therapeutic benefits. However, further research, including clinical trials and mechanistic studies, is essential to validate its efficacy, elucidate its precise mode of action, and explore its broader immunomodulatory potential in clinical applications.

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6. Author Contribution

Bharath Christian C.B.S - Primary investigator, Conduction of the preclinical study, Assessment, Treatment plan and Collection of data. Karthik Nagarajan. S and Lavanya. A - Data analysis, Data interpretation and Article drafting. B. Mangaleshwari and S. Harish Titto - Review, Editing and Manual Grammar check. All authors have read and agreed to the published version of the manuscript.

7. References

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