



EVALUATION OF ACUTE TOXICITY STUDY AND ANTI-ASTHMATIC ACTIVITY OF ZEAL HERBAL GRANULES

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ABSTRACT

Objective: To evaluate the Acute toxicity study and Anti-asthmatic activity of Zeal Herbal Granules. **Materials & Methods:** In the present study, acute toxicity study was carried out as per OECD guideline 423. Anti-asthmatic activity of Zeal Herbal Granules was investigated against compound 48/80-induced mast cell degranulation. Percentage mast cell degranulation was calculated at different concentration level i.e. 1, 10, 100 and 1000µg/mL. **Results:** Zeal Herbal Granules showed significant protection of rat mesenteric mast cells from disruption caused by compound 48/80. Significant dose dependent effect was observed in percentage mast cell degranulation at different dose level of Zeal Herbal Granules in comparison to negative control. 26.83% mast cell degranulation was observed at 100µg/mL dose level of Zeal Herbal Granules. **Conclusion:** The present study revealed that Zeal Herbal Granules has significant anti-asthmatic activity against compound 48/80-induced mast cell degranulation comparable to that produced by Ketotifen fumarate. There was no lethal and toxic reactions found among the tested animals. Zeal Herbal Granules can be a safe and effective drug for patient with asthmatic complaints.

Keywords: acute oral toxicity study, Anti asthmatic, Compound 48/80, Mast cell degranulation, Ketotifen fumarate, Zeal Herbal Granules.

INTRODUCTION

Asthma is the common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm¹. It may be caused by various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine, etc.² It is thought to be caused by a combination of Genetic factor, Environmental factors (like tobacco, hygiene hypothesis, volatile organic compounds, phthalates), Gene-environment interactions, Exacerbation, Socioeconomic factors³. The prevalence of asthma has increased significantly since the 1970s. As of 2010, 300 million people were affected worldwide. In 2009 asthma caused 250,000 deaths globally.⁴ As per a report, there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to herbal medicine. Selection of scientific and systematic approach for the biological evaluation of herbal formulations based on their use is very important for herbal medicine.⁵ Zeal Herbal Granules is one such Polyherbal formulation containing mainly the ingredients of Yashtimadhu (*Glycyrrhiza glabra*)⁶, Tamala (*Cinnamomum tamala*)⁷, Vasa (*Adhatoda vasica*)⁸, Tulsi (*Ocimum sanctum*)⁹, Shunthi (*Zingiber officinale*)^{10,11}, Bibhitaki (*Terminalia bellerica*)^{12,13}, Pippali (*Piper longum*)^{14,15}, Marich (*Piper nigrum*)^{16,17}, Amalaki (*Emblica officinalis*)^{18,19}, Kasamard (*Cassia occidentalis*)^{20,21}, Kantakari (*Solanum xanthocarpum*)²²⁻²⁵, Twak (*Cinnamomum zeylanicum*)²⁶, Haridra (*Curcuma longa*)²⁷, Soma (*Sarcostemma acidum*)²⁸, and Antmool (*Tylophora asthmatica*)²⁹⁻³¹. Few of the individual ingredients of Zeal Herbal Granules are well reported in Ayurvedic text and modern scientific literature having anti-asthmatic activity but no scientific evidence was available for combination of them. In the light of above background, the present study aimed at evaluation of a polyherbal formulation Zeal Herbal Granules for possible anti-asthmatic action using experimental animal model.

MATERIALS AND METHODS

Drugs and chemicals

Compound 48/80 was purchased from Sigma aldrich (Cat no. 159026, Lot no. 7930), ketotifen fumarate and toluidine blue were procured from local market. Histamine solution was freshly prepared in normal saline (NaCl, 8.5 g/l). All the other chemicals were of analytical grade. The Product sample of Zeal Herbal Granules was procured from Vasu Healthcare Pvt Ltd, Vadodara.

Experimental Animals

Healthy Swiss albino mice (25-30g) of either sex were selected for acute oral toxicity study and Wistar albino rats (180-210g) were taken to evaluate anti-asthmatic activity. All the experimental animals were fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed under standard conditions of temperature (22±2°C), relative humidity (60±5%) and 12:12 light/dark cycle. They were divided in groups of six animals each. The saline fed group served as control and one group was treated with a standard drug. Before experimentation, the animals were kept on fast for 24 h but water was given ad libitum. During experiments, animals were also observed for any alteration in their general behavior. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Sigma Institute of Pharmacy (SIP/IAEC/03/2012-13). All the experiments and the care of the laboratory animals were conducted according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Acute Oral Toxicity Study

The acute toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines no. 423, received from Committee for the purpose of Control and

Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India.³²

Anti-Asthmatic Activity Of Zeal Herbal Granules On Compound 48/80 Induced Mast Cell Degranulation

Six rats were taken for the study. Normal saline containing 5units/ml of heparin was injected in the peritoneal cavity of rat lightly anaesthetized with ether. After a gentle abdominal massage, the peritoneal fluid containing mast cell was collected in centrifuge tubes placed over ice. Peritoneal fluid of 6 rats were collected and centrifuged at 2000rpm for 5 min. Supernatant solution was discarded and the cells were washed twice with saline and re-suspended in 1 ml of saline.³³ 7 samples were prepared in different test tubes for each rat's peritoneal fluid. In the below manner 6 sets of such 7 samples were prepared.

Test tube no. 1: 0.1ml peritoneal fluid

Test tube no. 2: 0.1ml peritoneal fluid + 0.1ml compound 48/80

Test tube no. 3: 0.1ml peritoneal fluid + 0.1ml compound 48/80 + 0.1ml of 10µl/ml of ketotifen fumarate

Test tube no. 4: 0.1ml peritoneal fluid + 0.1ml compound 48/80 + 0.1ml of test agent in saline (1µl/ml of polyherbal granules)

Test tube no. 5: 0.1ml peritoneal fluid + 0.1ml compound 48/80 + 0.1ml of test agent in saline (10µl/ml of polyherbal granules)

Test tube no. 6: 0.1ml peritoneal fluid + 0.1ml compound 48/80 + 0.1ml of test agent in saline (100µl/ml of polyherbal granules)

Test tube no. 7: 0.1ml peritoneal fluid + 0.1ml compound 48/80 + 0.1ml of test agent in saline (1000µl/ml of polyherbal granules)

Each test tube was incubated for 15 minutes at 37° C with peritoneal fluid and respective drug treatment. The aliquots were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue. Then slides were dried in air and mast cells were counted using randomly selected high power objective fields (X450). The percent degranulations of the mast cells were calculated by following formula.

$$\% \text{ inhibition of Mast Cell Degranulation} = [1 - \text{Number of degranulated mast cells} / \text{Total number of mast cells}] \times 100$$

Statistical analysis

The results were expressed as mean values \pm S.E.M. (standard error of mean). The significance was evaluated by one way ANOVA, followed by Tukey's multiple comparison test. The results were considered statistically significant when $P < 0.05$.

Table 1: Effect of ZHG on body weight of mice at 2,000 mg/kg body weight

Product	Dose	Mean body weight (g)		
		0 day	7 th day	14 th day
ZHG solution	2000 mg/kg	27.47 \pm 0.98	27.73 \pm 0.97	28.44 \pm 0.74

Table 2: Effect of ZHG on % Mast cell degranulation

Treatment	Concentration	% Mast cell degranulation
Normal control	-	01.08 \pm 0.31
Negative control	-	83.16 \pm 0.47
Positive (Standard) control (Ketotifen fumarate)	10µg/ml	24.83 \pm 0.47**
Test-1(Polyherbal formulation)	1µg/ml	55.00 \pm 0.73**
Test-2(Polyherbal formulation)	10µg/ml	44.50 \pm 1.06**
Test-3(Polyherbal formulation)	100µg/ml	35.50 \pm 0.43**
Test-4(Polyherbal formulation)	1000µg/ml	26.83 \pm 0.60**

All value represented as Mean \pm S.E.M, where n=6

Significance at the level of ** $p < 0.001$ as compared to Negative Control

RESULTS AND DISCUSSION

During the acute oral toxicity study, the animals did not show any signs of toxicity and mortality at 2000mg/kg single dose administration of Zeal Herbal Granules (ZHG). The body weight was not significantly increased or decreased and is tabulated in Table 1.

Anti-asthmatic activity of zeal herbal granules by studies on compound 48/80 induced mast cell degranulation

In normal control group 01.08 \pm 0.31% mast cell degranulation was observed. Negative control treated with compound 48/80 showed 83.16 \pm 0.47% mast cell degranulation. Pre incubation of rat peritoneal mast cells with test drug (Polyherbal Granules) at 1µg/ml, 10µg/ml, 100µg/ml, 1000µg/ml for 15min resulted in significant reduction of % mast cell degranulation in dose dependent manner 55.00 \pm 0.73%, 44.50 \pm 1.06%, 35.50 \pm 0.43% and 26.83 \pm 0.60% respectively (Table 2). Pre incubation of rat peritoneal mast cells with Ketotifen fumarate at 10µg/ml for 15min resulted in significant reduction of % mast cell degranulation was 24.83 \pm 0.47%. Zeal Herbal Granules at concentration 1000µg/ml showed significant anti-asthmatic effect

comparative to reference standard drug. The results are as tabulated in Table 2.

CONCLUSION

Based on available results, it can be concluded that a Zeal Herbal Granule is a safe and effective polyherbal formulation that possesses mast cell stabilizing properties. It can be helpful in the treatment of patient with asthma.

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