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Research Article

DEVELOPMENT AND EVALUATION OF A POLYHERBAL PREPARATION FOR PREVENTING ASTHMA IN RATS

Tulsiani Puja

Department of Pharmacology, B.R. Nahata College of Pharmacy, Mhow-Neemach Road, Mandsaur, Madhya Pradesh, 458001, India

**Corresponding author: pujaonly1@gmail.com*

ABSTRACT

The objective of the research work was to prepare a polyherbal preparation with multi directing mechanisms targeting on Asthma. Most commonly used herbs like *Glycyrrhiza glabra*, *Allium cepa* and *Clerrodendrum serratum* which were collected from Attar bohra shop, Mandsaur with hydroalcoholic (50:50) solvent and further combined in equal proportion so as to prepare a polyherbal preparation (F1). Further F1 at 200 and 400 mg/Kg were evaluated for acute toxicity and Anti asthmatic activity. These herbs have expectorant, anti oxidant and anti inflammatory, anti histaminic effects respectively which are in turn useful parameters against asthma. Guinea pig tracheal chain proved helpful in evaluating the efficacy of the preparation as compared to other marketed ayurvedic and allopathic medications. Also Broncho Alveolar Lavage fluid was observed for eosinophilic and macrophage count estimation. Airway hyper responsiveness in response to Methacholine administration proved beneficial for the study. This was further supported by lung tissue histology. Bronchial muscle relaxation was seen as well as inflammation due to free radicals and cytokines exhibited a marked decline.

Keywords: Anti oxidant, Macrophage, Broncho Alveolar Lavage

1. INTRODUCTION

Asthma leading to a reversible airway constriction thus causing insufficient respiration rate, BHR, decline in Forced Expiratory volume to less than 20%. A disease whose estimated cost expenditure is $12.6 billion. Thus this study was set to prepare a polyherbal preparation (F1) containing the extracts of 3 herbs like *Glycyrrhiza glabra, Clerodendrum serratum and Allium cepa* confined to the temperate regions of Asia. Different parts of the herbs are used in Indian traditional medicine for the treatment of a broad spectrum of ailments including *Glycyrrhiza glabra* widely used in gastritis (inflammation of the stomach) and ailments of the upper respiratory tract; *Clerodendrum serratum* used as an appetiser, digestive, stomachic, blood purifier, alleviates kapha, fever, worms, burning sensation, laxative, cephalalgia and ophthalmia; *Allium cepa* used as an antihelmintic, antiparasitic, diuretic and repellent, in the treatment of asthma, bronchitis, whooping cough, warts, acne, appetite loss and urinary tract disorders. All three are individually proved to be effective in hepatoprotection [1].

In this regard, we are evaluating a significant Asthma protective potential of the polyherbal preparation in the light of inhibition ALT and Eosinophil level with the antioxidant defending system against egg albumin evoked lung damage in rat [2]. In the present research, we have assessed antiasthmatic activity of Polyherbal preparation further by in vivo monitoring the changes in ALT in BALF, weight of the lung, DRC given by tracheal chain through experimental models [3, 4].

2. MATERIAL AND METHODS

2.1. Preparation of polyherbal preparation

Fresh root barks of Glycyrrhiza glabra (300) were dried and crushed in grinder and treated with petroleum ether (5 times the volume of drug) for 24 hours and further treated with 1:5 (drug: solvent) of 50:50 hydroalcoholic solvent for 52 hours [5]. *Clerodendrum serratum* 200 g, while *Allium cepa* 600 g produces a yield of 6.66, 6.5 and 3.33% [6]. The extraction was carried out in a cold room (20+ 1°C). The homogenate was then dried on water bath till semisolid dry extract was obtained which was then shade dried which was afterwards

mixed with carboxy methyl cellulose and water to prepare a polyherbal suspension (20 %).

2.2. Treatment of animals

Adult swiss albino rats, weighing between 100-150 g, were acclimatized to conditions in the laboratory (room temperature, 60-80% relative humidity, day night cycle) for 10 days prior to the commencement of the treatment, during which they received food and tap water ad libitum. Rats were then divided in several groups of 6 rats each. Initially Acute toxicity study and in vitro DPPH (Diphenyl picryl hydrazyl) free radical scavenging activity were performed so as to satisfy with its safety and antioxidant potential and further evaluation of anti asthmatic activity was done through egg albumin as well as somewhere histamine induced asthma models as follows [7]. The normal group was given saline (0.9% NaCl) orally at 10 mg/ Kg body weight dose for once daily and that too for 7 consecutive days.

2.3. Assessment

2.3.1. Acute toxicity test:

The Polyherbal preparation (F1) was found to be safe at 2000mg/kg body weight in rats and guinea pigs. Bronchial hyper reactivity of Guinea pig was checked through histamine chamber; whereas Guinea pig tracheal chains (partially cut trachea in a zig- zag fashion) were used for visualizing the Drug response curve with an antagonizing effect of anti cholinergic and antihistaminic effects of polyherbal preparation over Histamine induced tracheal contraction [8].

After 18 hours of 2 days continuous administration of egg albumin, ALT level was found in blood serum which was inturn obtained from blood withdrawn from retro-orbital route of the rat. Also Broncho Alveolar Lavage Fluid (BALF) was collected by suction through bronchial gavage with 2 ml of Phosphate Buffer Saline (PBS) and around 1 ml of BALF was recovered after 4 lavages.

This BALF was centrifuged (2000 rpm, 6min) and the supernatant was mixed with 1X PBS and then further visualized under the microscope after staining with Eosin dye for eosinophil count [9].

Lung tissue was removed and kept in 10% formalin solution for 48 hours for histopathology and this provided a proof showing the effect of the herbal and standard drug (0.89 mg/Kg) prevention after asthma inducing agent treatment. Finally Statistical analysis of the observations was done.
At the last the preparation was standardized by finding out the sodium content, ash value and florescence in UV that too in different types of solutions (polar, non polar, etc.).

3. RESULTS AND DISCUSSION

Saponins and flavanoids present as a major constituent in the herbal extract was indeed the major reason for antioxidant activity as confirmed by DPPH free radical scavenging activity test thus responsible for treating oxidative stress during asthma, thus depleting the oxides leading to inflammation produced during asthma, an autoimmune disease.

Also the polyherbal preparation decreased the eosinophil count in BALF and a fall in ALT as in comparison with the model control group of experimental animals. Thus it proved to be beneficial in asthma. The probable mechanism of action could be expectorant with anti oxidant, anti inflammatory and anti histaminic effects which were individually present in different herbs targeting for asthma protection [10].

Thus F1 could be administered safely and will prove to be effective in asthma caused by egg albumin or else by histamine. The preparation also proved to be effective in a dose dependent prophylaxis manner for Asthma as in comparison with Salbutamol as a standard drug. Hence it could be concluded that the polyherbal preparation is probably acting through free radical scavenging, expectorant and anti histaminic mechanism and thus could be further studied for its efficacy.

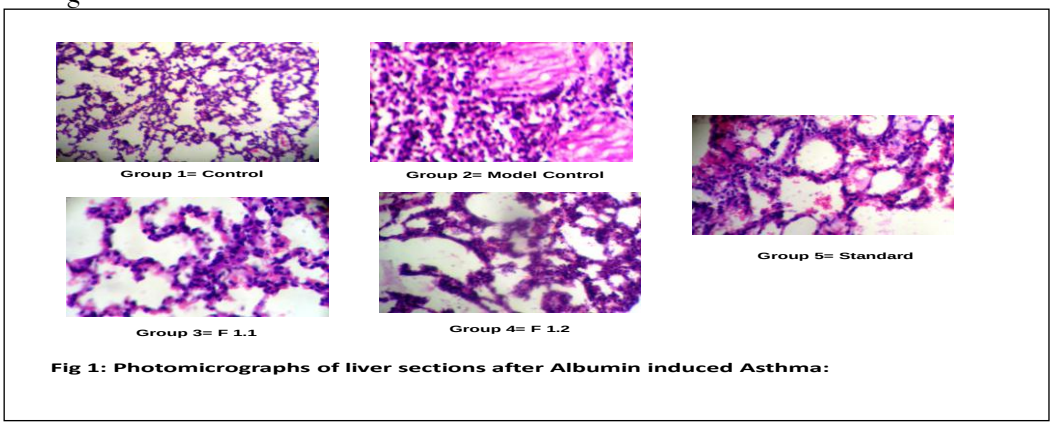

Fig I: Photomicrographs of Lung sections after 1% egg Albumin induced Asthma

Fig: 1= Normal control, 2= Model Control, 3= Polyherbal preparation (200 mg/Kg) {F 1.1}, 4= Polyherbal preparation (400 mg/Kg) {F1.2} and 5= 4 doses of Herb Standard (Salbutamol 0.89 mg/Kg).

Description of the findings for different tissue:

Fig 1= Normal lung tissue architecture, with no inflammation and healthy alveoli tissues.

Fig 2= Moderate congestion with peribronchiolar and interstitial tissue showing heavy acute and chronic inflammation with neutrophil and eosinophil accumulation, presence of hypertrophy and mucous plugs.

Fig 3= Moderate tissue congestion, with acute inflammation in peribronchiolar and interstitial tissues.

Fig 4= Moderate tissue congestion; Fig 5= Mild congession in lung tissue.

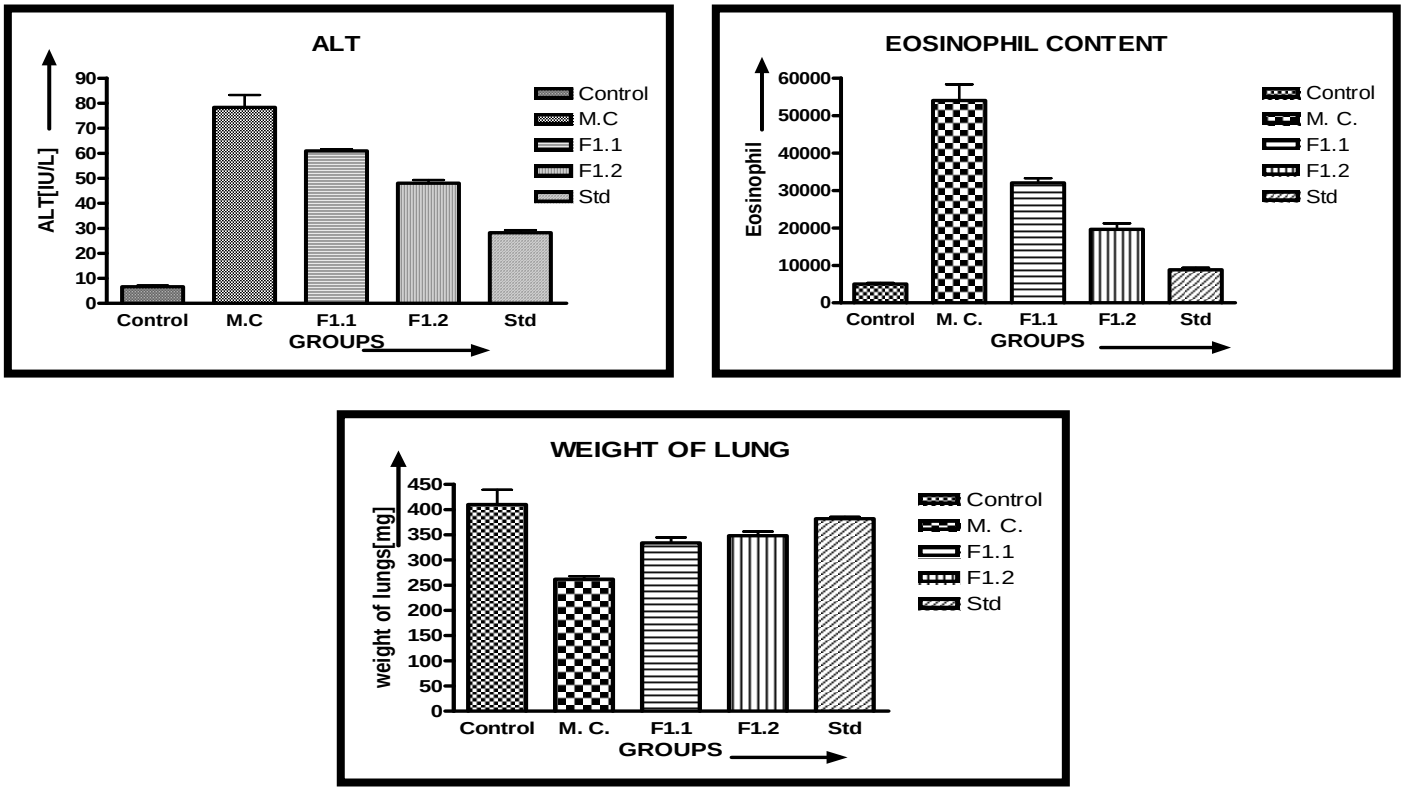

Fig II: Histogram depicting change in ALT, weight of lungs and Eosinophil with pretreatment to egg albumin inhalation

Observation Table 1

S.No.	Groups	ALT (IU/L)	Eosinophil count	Weight of Lung (mg)
1	Control (Saline 10 ml/Kg)	6.64± 0.47	5000.0± 316.0	410.0± 29.15
2	Model Control (Egg albumin=1.5%)	78.32± 4.99***	54000.0± 4301.0*	262.0± 5.83***
3	F1.1 (200 mg/kg of oral polyherbal preparation)	60.94± 0.65***	32000.0± 1265.0*	334.0± 10.77***
4	F1.2 (400 mg/kg of oral polyherbal preparation)	2.48± 1.30***	19600.0± 1631.0*	348.0± 8.60***

** P<0.05; ** P<0.01; *** P<0.001; Dunnett's t test against the respective control; Values in± SEM of 5 animals; Abb. F1.1= Polyherbal preparation Test (200 mg/Kg), F1.2= Polyherbal preparation (400 mg/Kg).*

The above mentioned table depicts clearly a significant fall in ALT , Eosinophil levels while a rise in Lung weight in treated group (400 mg/Kg) as in approximate levels as compared to Standard (Salbutamol), while less in 200 mg/ Kg all in comparison to model control group.

4. CONCLUSION

Finally this polyherbal preparation could be further standardized and marketed out as a new herbal formulation for asthma with minimum side effects and better as compared to individual herbal treatment.

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