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Antiasthmatic Evaluation of Polyherbal Formulations in Laboratory Animals

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ABSTRACT

Asthma is a chronic disease with spastic contraction of smooth muscle in the bronchioles characterized by difficult breathing with wheezing. Asthma, a common, chronic inflammatory disorder of the airways, associated with pronounced health and economic consequences, has been identified as one of the five pressing global lung problems. In the present investigation the three different polyherbal formulations (Formulation I, Formulation 2 and Formulation 3) prepared as per the standard formulas of Ayurvedic proprietary medicines were evaluated for its suitability as an antiasthmatic therapy using passive paw anaphylaxis in rats and histamine induced bronchoconstriction in guinea pigs. Three different doses of each formulation i.e. 1000, 1500 and 2000 mg/kg were used in this regard. Results of the studies revealed that all the formulation I was effective in the significantly reducing duration of catalepsy in clonidine induced catalepsy model at all the time intervals at all doses while formulation II and III showed this activity at higher doses only. Whereas, in milk induced leukocytosis and eosinophilia models, Formulation I, significantly reduced the total leukocyte count at all the doses while Formulation II and III showed this effect at 1500 mg/kg dose only.

Keywords: Asthma, polyherbal formulation, paw anaphylaxis, bronchoconstriction.

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INTRODUCTION

Asthma is chronic inflammatory disorder of the airways is characterized by acute exacerbation of coughing, dyspnea, wheezing and chest tightness particularly early in the morning and at night [1]. Reversible bronchoconstriction, elevated basal airway tone, activation and accumulation lymphocyte (Eosinophils), hypertrophy of smooth muscles and sub mucosal glands, sub mucosal fibrosis, airway wall edema, mucus overproduction and episodes of non-specific airway hyper-responsiveness to specific spasmogens are common manifestations [2,3]. There is a considerable mortality and morbidity due to asthma in the recent years due to various factors [4]. However it is avoidable with appropriate therapy to be taken in time for prescribed time period. The last two decades data have shown dramatic increase in the asthmatic cases [5]. The average cases of asthma ranges from 9.9-33% which is matter of serious concern [6]. Moreover a chief cause includes histamine release which is one of the inflammatory mediators leading to contraction of smooth muscles, vasodilatation, increased vascular permeability, mucus hypersecretion and inflammation [7]. Thus, when allergic disease occurs, affected tissue is infiltrated by cells with a T helper cell 2 (Th2) - type cytokine profile (such as IL-4, IL-5, IL-9 and IL-13 [8], which favors the synthesis of IgE and activation of mast cells, lymphocytes and eosinophils all of which ultimately lead to inflammation and disease [9].

Large number of anti asthmatic drugs belonging to α2 agonists (Ephedrine), corticosteroids (Hydrocortisone, Prednisolone), mast cell stabilizers (Sodium chromoglycate, Kitotifen), methylxanthines (Theophylline, Aminophylline), leucotriene antagonists (Montelukast, Zafirlukast) are widely used in the treatment of asthma but these drugs produce certain serious adverse effects like immune suppression, cardiac abnormalities, central nervous system depression, hyperglycemia etc [1]. None of them seems to be an ideal drug that in turn demands for the search for new drug. On the contrary, world health organization (WHO) has recognized herbal medicine as an essential building block for primary health care and is being increasingly utilised to treat a wide variety of diseases mainly in countries of Indian subcontinent [10].

There is also high prevalence of usage of alternative traditional system of medicines for the treatment of asthma [11]. Ayurveda, an Indian traditional system of medicine offers a unique insight into comprehensive approach to asthma management through proper care of the respiratory tract. More than 400 medicinal plant species have been used ethno pharmacologically and traditionally to treat the symptoms of asthmatic and allergic disorders worldwide. However the scientific documentation of these plants is relatively scanty. Hence there is a growing interest regarding for the preclinical evaluation of various plants used in traditional system of medicine [12,13]. Such preclinical evaluation may direct drug discovery in a systematic way to come out with the ideal theory that may fill the blank spots of the modern medicine and its system [10]. Scientific documentations of various extracts have revealed that, individual extracts are not sufficient to produce the effect comparable to that of the synthetic drugs. Hence combination of various extracts leading to a polyherbal formulation is an ideal

way to enhance the therapeutic effectiveness [14]. However such combination may lead to either synergism or sometimes may show antagonism hence exactly opposite results may be obtained. Based upon the scientific documentations and phytoconstituents reported, appropriate extracts of suitable part in prescribed concentrations can be mixed to get desired polyherbal formulations with certain predictable effects. These predictions later can be confirmed by preclinical evaluation. On the similar ground, we have made three different polyherbal formulations as per the standard formulas of Ayurvedic proprietary medicines [15,16,17] and evaluated for its suitability as an antiasthmatic therapy.

MATERIALS AND METHODS

Plant material:

Leaves of Passiflora incarnata, roots of Picorrhiza kurroa, bulbs of Urgenia martima, rhizomes of Curcuma longa, leaves of Adhatoda vasica, leaves of Ocimum sanctum, roots and rhizomes of Glycyrrhiza glabra and stems of Ephedra sinica were purchased from local vendors. All the plants/parts were identified and authenticated at authorised agency.

Preparation of extract:

All eight medicinal plants were subjected to aqueous extraction through maceration at room temperature. All the extracts were then filtered through filter paper, dried and stored (Rangari, 2009).

Preparation of Polyherbal Formulation:

As per the standard formula of Ayurvedic Proprietary Medicines the following formulations were prepared:

Formulation I	Formulation II	Formulation III
Passiflora incarnata	Passiflora incarnata	Passiflora incarnata
Picrorrhiza kurroa	Picrorrhiza kurroa	Picrorrhiza kurroa
Adhatoda vasica	Urginea maritima	Glycyrrhiza glabra
Ocimum sanctum	Curcuma longa	Ephedra sinica

Chemicals and drugs:

Eosin solution (Qualigens, India); toluidine blue (Research Lab Fine Chem., India), WBC diluting fluid (Qualigens, India) were purchased from respective vendors. The chemicals used for physiological salt solutions and other preparations were of Laboratory grade.

Preparation of drug solution:

Accurately weighed quantities of the powdered extracts were dissolved in distilled water to prepare required formulation. These formulations were stored in the refrigerator.

Animals:

Dunkin-Hatley Guinea pigs weighing 350-400g of either sex, wistar albino rats (120-150 gm) and albino mice (30-50gm) were procured from central animal house Maharashtra College of Pharmacy, Nilanga, District Latur. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). The animals had free access to food (Amrut feed, Chakan oil mills, India) and water ad libitum throughout study. Institutional Animal Ethical Committee approved the protocol. All the experiments were carried out between 9:00- 16:00 hours.

Preliminary acute toxicity test:

Acute toxicity study was performed in healthy albino mice (30-50gm) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development (OECD).

Polyherbal Formulations I, II and II were administered to the mice for oral toxicity study.These mice were then observed for incidence of mortality or any sign of toxicity up to 24 hours after oral administration.

The dosing schedule as per the OECD (guideline 425) was as follows: Only one mouse received a dose at a particular time. First mice received a dose of 175 mg/kg and were observed for 03 hours after dosing for any toxicity signs, survival or death. If the first animal died or appeared moribund, the second animal received a lower dose. The dose progression or reduction factor was 3.2 times of the previous dose. If no mortality was observed in the first animal then the second animal received a higher dose. Dosing of the next animal was continued depending on the outcome of the previously dosed animal for a fixed time interval (03 hours). The test was stopped when one of the stopping criteria was met:

05 reversals occur in any 06 consecutive animals tested.
03 consecutive animals died at one dose level.

Survived animals were observed for outcomes for a period of 24 hours (AOT425 Guidelines) [18].

Phytochemical Analysis of the polyherbal formulations:

Test solutions of the three formulations were prepared in distilled water in order to make the concentration 100 mg/ml. The following procedures were adopted to test for the presence of various chemical constituents in the formulations [19].

Methods for Anti-Asthmatic Activity

Passive Paw Anaphylaxis in rats [20]

Preparation of serum for sensitisation:

Anti serum to egg albumin was raised in rats using aluminum hydroxide gel as an adjuvant. Animals were given three doses of 100 µg (s.c.) of egg albumin adsorbed on 12 mg of aluminum hydroxide gel, prepared in 0.5 ml of saline on 1st, 3rd and 5th day. On 10th day of sensitisation, the blood was collected from the retro orbital plexus, allowed to clot and the serum was separated by centrifugation at 1500 rpm.

Procedure:

66 albino rats of wistar strain were randomly divided into 11 groups each containing 06 rats. Group I Control, (Distilled water) Group II to Group X served as test groups, amongst these Groups II, III and IV received, Formulation I at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Groups V, VI and VII received, Formulation II at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Groups VIII, IX and X received, Formulation III at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Group XI served as standard group and received standard drug Dexamethasone 0.50 mg/kg, i.p. All the animals were passively sensitised with 0.1 ml of the undiluted serum into the left hind paw. The opposite paw received an equal volume of saline. 24 hours after sensitisation these animals were given the drug treatment. 01 hour after treatment of test and reference standard drug, the animals were challenged in the left hind paw with 10 µg of egg albumin in 0.1 ml of saline, and the paw inflammation was measured as volume of displacement in ml at interval of 01, 02, 03 and 04 hours using a digital Plethysmometer.

Histamine induced Bronchoconstriction in Guinea pigs [21]

Overnight fasted guinea pigs were randomly divided into 11 groups each containing 06 animals. Group I Control, (Distilled water) Group II to Group X served as test groups, amongst these Groups II, III and IV received, Formulation I at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Groups V, VI and VII received, Formulation II at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Groups VIII, IX and X received, Formulation III at doses 500 mg/kg, 1000 mg/kg, 1500 mg/kg p.o. respectively. Group XI served as standard group and received standard drug Chlorpheniramine maleate 2 mg/kg, i.p. Prior to drug treatment each animal was

placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The latency of dyspnea (i.e from the time of exposure leading to the appearance of preconvulsion dyspnea) (PCD) was determined. As soon as the PCD was noted, the animal was removed from the chamber and placed in a fresh air. 24 hours later the animals were the drug treatment. These animals were again subjected to histamine aerosol later at interval of 01 hour, 04 hours and 24 hours of drug administration and latency of dyspnea was determined at each interval.

Statistical analysis:

The comparison was made against the vehicle treated control group and the data was expressed as mean ± SEM. The data was analysed by one way analysis of variance (ANOVA) followed by Dunnett's 't' test [22].

RESULTS

Phytochemical Analysis of the polyherbal formulations:

Phytochemical analysis of Formulation I showed presence of alkaloids, glycosides, flavonoids, phenolic compounds, flavonoids and volatile oils whereas glycosides, flavonoids, phenolic compounds, carbohydrates, proteins, volatile oils were present in Formulation II. Formulation III showed presence of saponins, alkaloids, glycosides, flavonoids, tannins and volatile oils.

Acute toxicity assessment:

Oral administration of Formulation I, II and III did not produce any serious toxic effect in mice and no mortality was found up to 2000 mg/kg and thereby reported to be safe. The administration of Formulation I did not show any change in the alertness, touch response and locomotor activity. Formulation II showed reduction in alertness and touch response whereas; formulation III reduced all these three observations. The data was analysed with help of AOT425 Software.

Passive Paw Anaphylaxis in rats

In case of formulation I, the dose of 1500 mg/kg showed significant reduction in inflammation at all intervals but was more significant after 03 and 04 hours ($p<0.01$) as compared after 01 and 02 hour ($p<0.05$) of drug administration. Pretreatment with 1000 mg/kg significantly reduced paw edema at interval of 03 hour ($p<0.01$) as compared to 02 and 04 hour ($p<0.05$) after drug administration. The reference standard i.e Dexamethasone was highly significant at all intervals ($p<0.01$). (Figure 1)

Pretreatment with the dose of 1500 mg/kg of Formulation II showed significant reduction in inflammation at all intervals ($p<0.05$) except at 01 hour interval of drug

administration. The dose of 1000 mg/kg significantly reduced paw edema only at the interval of 03 hour ($p<0.05$) as compared against vehicle treated control group. The reference standard i.e Dexamethasone was highly significant at all intervals ($p<0.01$). (Figure 2)

In case of formulation III, the dose of 1500 mg/kg showed significant reduction in inflammation at all intervals except at 01 hour interval of drug administration. The dose of 1000 mg/kg significantly reduced paw edema only at the interval of 03 hour ($p<0.05$) as compared against vehicle treated control group. The reference standard i.e Dexamethasone was highly significant at all intervals ($p<0.01$). (Figure 3)

Histamine induced bronchoconstriction in guinea pig

The latency of dyspnea of rats pretreated with doses 500, 1000, 1500 mg/kg of Formulation I was found to be 67.96 ± 01.68, 60.73 ± 01.77,53.04 ± 01.77, 01 hour after administration of drug, 69.73 ± 02.16, 87.83 ± 01.04, 53.48 ± 01.44 at interval of 04 hours and 86.99 ± 07.08, 96.60 ± 03.60, 73.15 ± 03.84 at an interval of 24 hours after drug administration respectively.

The dose of 500 mg/kg showed significant ($p< 0.05$) delay in the latency of dyspnea only at 04 hour interval. On the contrary, 1000 mg/kg showed dose dependant effect at the interval of 01 hour ($p<0.05$) and 04 hours ($p<0.01$) after drug administration. The dose 1500 mg/kg was most significant ($p<0.01$) as it showed equipotent effect at all intervals. (Figure 4)

In case of formulation II, only 1500mg/kg was significant at the interval of 04 hours. It delayed the latency of dyspnea to 60.43 ± 01.54 as compared against vehicle treated control group 48.73 ± 02.46. The reference standard was found to be significant at all intervals. However, the significance was more ($p<0.01$) at 01 and 04 hour as compared to 24 hour ($p<0.05$). (Figure 5).

Pretreatment with 1500 mg/kg of Formulation III showed significant increase in the latency of dyspnea at all the three intervals. On the other hand, 1000 mg/kg was effective only at 04 hour interval. The reference standard was significant at all intervals. However, significance was more ($p<0.01$) at 01 and 04 hour interval as compared to interval of 24 hour ($p<0.05$). (Figure 6)

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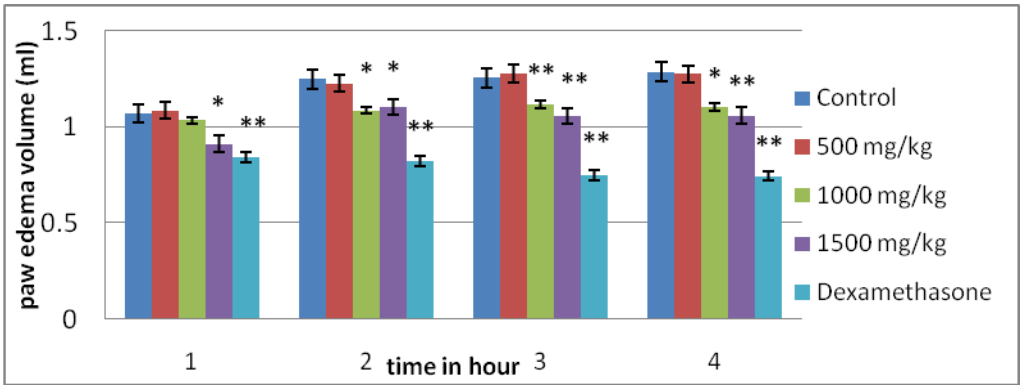

Figure 1: Effect of Formulation I and Dexamethasone on the paw edema volume in rats

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p<0.05, **p < 0.01.

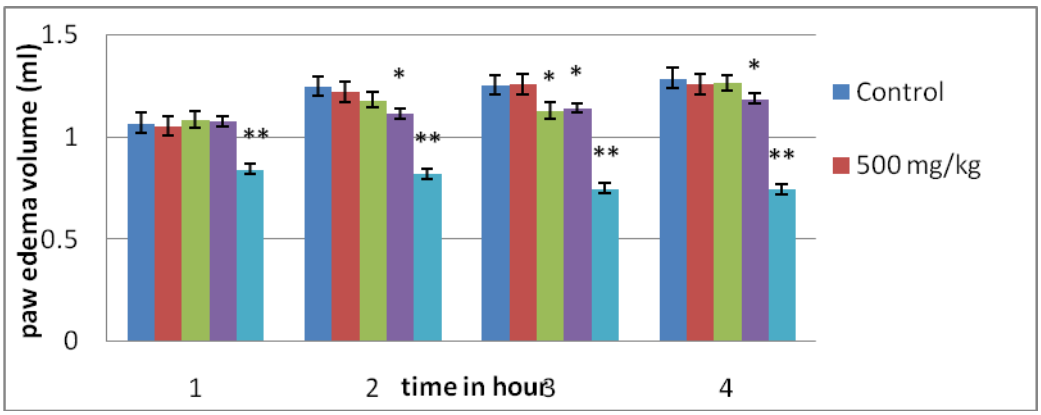

Figure 2: Effect of Formulation II and Dexamethasone on the paw edema volume in rats

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p<0.05, **p < 0.01.

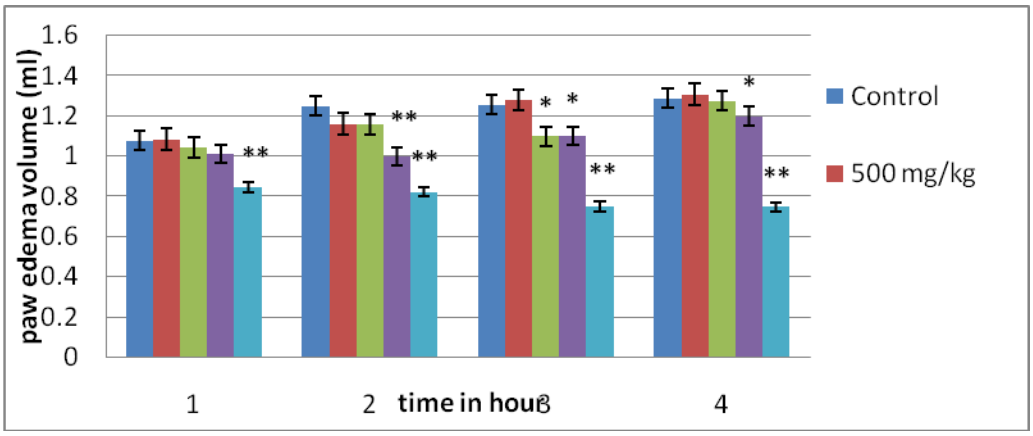

Figure 3: Effect of Formulation III and Dexamethasone on the paw edema volume in rats

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p<0.05, **p < 0.01.

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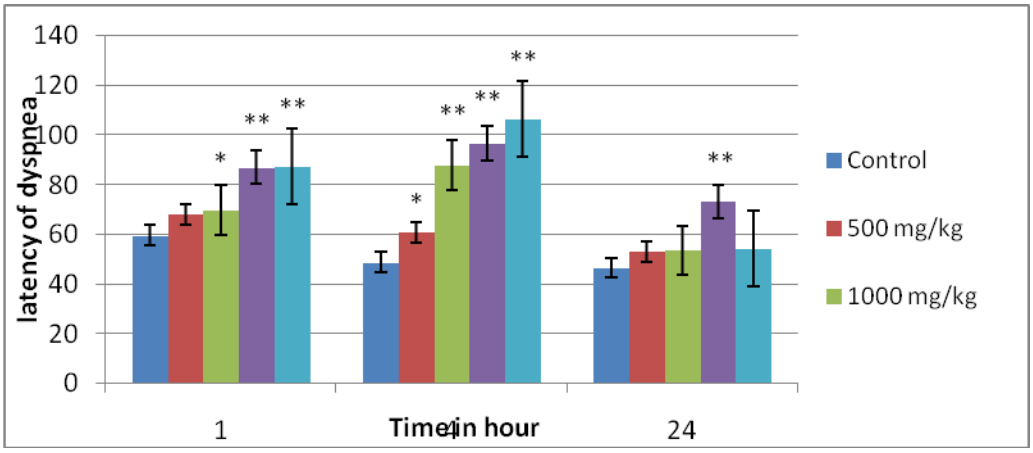

Figure 4: Effect of Formulation I and Chlorpheniramine maleate on latency of dyspnea in Histamine induced bronchoconstriction in guinea pigs.

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p< 0.05, ** p< 0.01, *** p< 0.001

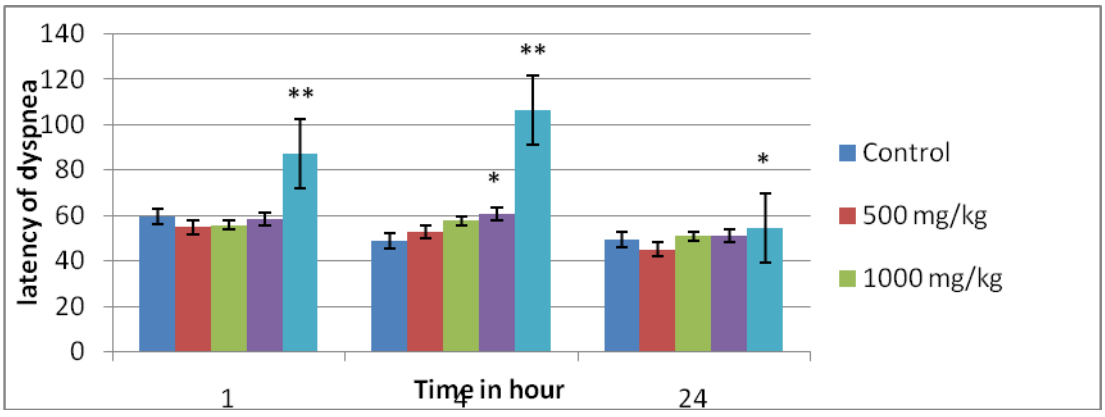

Figure 5: Effect of Formulation II and Chlorpheniramine maleate on latency of dyspnea in Histamine induced bronchoconstriction in guinea pigs.

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p< 0.05, ** p< 0.01, *** p< 0.001

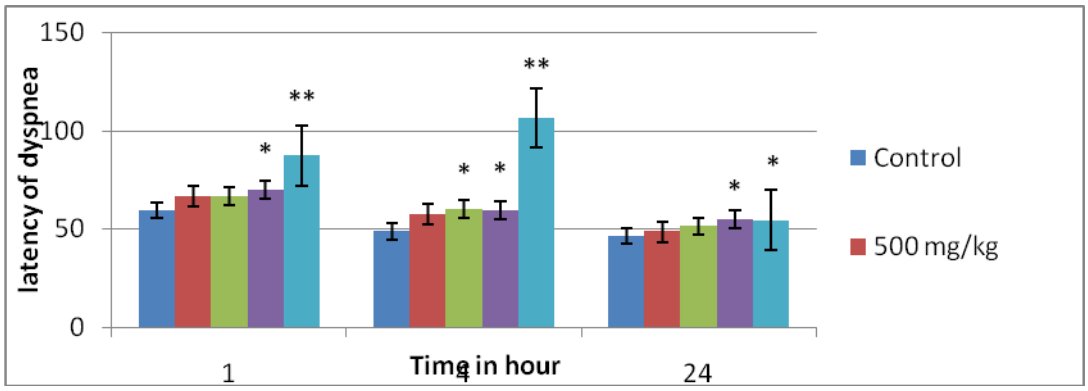

Figure 6: Effect of Formulation III and Chlorpheniramine maleate on latency of dyspnea in Histamine induced bronchoconstriction in guinea pigs.

Results are expressed as mean ± SEM (n = 6). Data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. *p< 0.05, ** p< 0.01, *** p< 0.001.

DISCUSSION

In any polyherbal formulation, pharmacological action exerted by formulation is mainly governed by certain phytochemicals; hence preliminary phytochemical evaluation of all three formulations was carried out. The analysis of Formulation I showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds and volatile oils whereas, glycosides, flavonoids, phenolic compounds, volatile oils were found to be present in Formulation II. Formulation III showed the presence of saponins, alkaloids, glycosides, flavonoids, tannins and volatile oils. Overall alkaloids in formulation I and alkaloid as well as saponins in formulation III was present as different phytoconstituents, rest of all were same. The presence of these different constituents and its interaction with other same phytoconstituents may be responsible to alter the therapeutic profile of individual formulations. The overall presence of phytochemicals is in accordance with the previous published reports wherein antiasthmatic action was established [20,23]. This further supports the selection of suitable plants.

Since pharmacological evaluation showed different levels of significance from formulation to formulation, it may be due to the presence of alkaloids in formulation I and alkaloids as well as saponins in formulation III.

Although the individual constituents of the polyherbal formulations have been documented for its antiasthmatic effect without any untoward effects, however it is essential to test it again for its toxicity profile to establish its safety when used in combination (i.e. polyherbal combination). In light of this, the acute oral toxicity studies of all three formulations were carried out. Our findings indicated that all these formulations were found to be devoid of any serious toxic symptoms and no mortality was found up to the dose of 2000 mg/kg. Also, the administration of Formulation I did not show any change in the alertness, touch response and locomotor activity. Formulation II showed reduction in alertness and touch response whereas; formulation III reduced all these three observations. These results satisfy the first requirement of safety pharmacology. Based on these results and pilot study, three different doses i.e 500 mg/kg, 1000 mg/kg and 1500 mg/kg were selected for the further pharmacological evaluation.

Antigen antibody reaction results in mast cell degranulation which is the first step of asthma, is manifested as bronchoconstriction (role of histamine and leukotriene) and inflammation (role of leucocytes and eosinophils) [24]. The stabilisation of mast cells can be the most important step towards prevention of precipitation of asthma. In case of passive paw anaphylaxis model of asthma, there is immunological stimulation by ova albumin and the antibodies raised against the antigen are injected locally into the paw of rat. Local antigen antibody reaction in the rat paw manifests into the inflammation and paw edema. In the present investigation, the formulation was investigated against passive paw anaphylaxis model so as to

evaluate the immunomodulatory efficacy of the formulations to be useful in allergic asthma [25, 26].

Pretreatment with 1500 mg/kg of formulation I showed significant reduction in inflammation induced by ova albumin at all intervals. Formulation II and III were less significant in reducing paw edema as compared against vehicle treated control group. It is now well known that mast cells are extensively involved in the pathophysiology of bronchial asthma. This suggests the possible use of the dose of 1500 mg/kg of formulation I, towards the prevention of allergic asthma which may be attributed to its mast cell stabilising property. Thus this formulation is suitable to be used as a preventive remedy which is the most important measure in case of possible exposure to allergens [27].

Although mast cell stabilisation is an ideal precautionary measure in asthma management, however, this may not be possible in all clinical cases. Many times degranulation takes place without prior notice and patient exhibits first clinical manifestation i.e. bronchoconstriction. In such case, relief from bronchoconstriction becomes the primary objective of treatment. Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing the precipitation of bronchoconstriction. This further leads to airway hyper responsiveness and bronchial airway inflammation. Also, bronchoconstriction which is mediated by H_1 histaminic action is treated as a major symptom because long term bronchoconstriction leading to hypoxia may result into generalised hypo functioning which if untreated can become a syndrome with serious complications [21]. A study regarding involvement of H_1 and H_2 receptors done in experimental model of asthma using guinea pig documented prominent involvement of H_1 receptors. Since majority types of asthma showed the role of histamine via H_1 receptor, any new formulation to be used in asthma was tested for its H_1 antihistaminic properties [27, 28].

In this study, all three formulations were investigated for their inhibitory effect against histamine induced bronchoconstriction. The study found that formulation I was most effective in this regard as it showed significant delay in the latency of dyspnea. It also showed dose dependent effects suggesting that the preparation is devoid of any interaction or side effects even at large doses. Since severity of asthmatic condition can drastically vary in a single person from season to season and place to place, hence an ideal formulation shall be effective at wider dose range without any dose dependant limitations. This preparation satisfies this particular aspect. Moreover, although it acts through inhibition of H_1 receptor, still it did not show any sedation as recorded during its toxicity test which perhaps could be the best outcome towards patient compliances compared to the present synthetic medication [29]. Present formulation I has not only delayed the latency but this ability was sustained for a longer duration of time hence doses to be administered to provide protection round the clock in a particular season or during specific predictable exposure can be reduced to a greater extent [30,31]. This is an additional benefit of this combination. The increased duration may be attributed to the synergistic combinations of phytoconstituents.

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CONCLUSION

The study validated the composition of formulation I towards management of asthma and probable putative mechanism of action is attributed to antiallergic, immunomodulatory, anti-inflammatory and antistress properties of the combination. The exact role of individual phytoconstituents needs to be illustrated using suitable bio-analytical techniques to extrapolate exact mechanism of this action.

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