Evaluation of Antiasthamic Activity of Electrohomeopathy Formulation Pettorale on Experimental Animals

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INTRODUCTION

Although modern medicine has revolutionized health care, the healing process has always been too complex to be explained by the use of medicines and technological advances alone. There has always been the gestalt of therapy that involves medicines and other components. Those components have reflected in recent years a trend of increasing use of alternative medicines. Primary health care for 80% of the world population is dependent on alternative medicine. ¹

Electrohomeopathy could be a alternative branch of the medical system, projected by Dr. Count Ceasare Mattie of Italy in 1865. Since then it is comprehensively practiced in many countries by the electrohomeopathic practitioners. ², ³

C.C. Mattie allocated 114 medicinal plants in the individual league based on their curative properties and entitled as Scrophulo, Canceroso, Angiotico, Fabrifugo, Vermifugo, Venereo, Limphatico, Pettorale and a series of Electricities. ⁴

The Pettorale group is considered to be specific for respiratory system disorders and further subdivided into four sub groups based on their specific affinity and named as Pettorale – 1, Pettorale – 2, Pettorale – 3 and Pettorale – 4. The Pettorale group which was used as antiasthmatic comprises spagyric essence of different plant combination like Adiantum Capillus Veneris, Allium Cepa, Drosera Rotundifolia, Eucalyptus Globulus, Phellandrium Aquaticum, Uruguaga Ipecacuanha, Hyoscyamus Niger. Polygala Amara and Hydrastis Canadensis. It is widely used by local practitioners for bronchial asthma. Pettorale was used for the symptoms include breathlessness / breathing difficulty accompanied by cough or troublesome flatulence which worsens in the evening and dyspnoea. There is no proper scientific evidence for the efficacy of the drug for the antiasthmatic activity. Hence, the present study is undertaken for the pharmacodynamic evaluation of anti-asthmatic effect of Pettorale in different animal models.

MATERIALS AND METHODS

Drugs and Chemicals

Pettorale of NEHM company is purchased from a local Electrohomeopathy store and used as the test drug. Histamine (SIGMA ALDRICH, USA), Haloperidol (CRO Pharma, India), Egg albumin (SIGMA ALDRICH, USA), and WBC diluting fluid (International Biological Laboratories, India) Chlorophenaramine melate (Menarini India Pvt Ltd) were used in the current study.

Animals

Swiss mice (18-25g), wistar rats (150-200g) and Guinea pigs (400-500g) were used in this experimental study. All the animals were issued from the departmental animal house. The animals were kept under proper atmospheric conditions for at least 1 week before the experiment started. The study was conducted as per the guidelines set by the Institutional Animal Ethics Committee.
conditions at temp. 25±3°C, 45-55% relative humidity and light and dark cycle of 12 hr. They were given free access to food and water and kept under strict hygienic conditions. Approval from the animal ethical committee was taken before the experimental work. (Notification no: 1171/Pol/Re/5/08/CPCSEA).

**Phytochemical investigations**

The Pettorale group was tested for various chemical constituents with the help of qualitative chemical tests. [5]

**Acute Toxicity Study**

An acute toxicity study was carried out using albino rats (150-200g) as per OECD toxicity guideline 420. Pettorale was administered as a starting dose level of 5mg/kg to one rat and the dose was increased up to 2000mg/kg and no evidence of toxicity was found. It was concluded that the drug can be unclassified as per GHS toxicity ranking. Special attention was given during the first 4 hours to all the animals for toxicity signs and behavioural changes, then up to 24 hours and finally kept aside and observed for following 14 days and the LD50 was calculated. [6]

**Histamine induced bronchospasm in guinea pigs**

The method was followed to Taur et al. suggested method for experimentally induced bronchospasm by histamine aerosol exposing to guinea pig but with partial modification. [7]

Total 15 guinea pigs were randomly divided into five groups, three in each group (n = 3). Group I was served as control and administered with vehicle (Normal saline) 10ml/kg orally. While Group II, standard group receives Chlorpheniramine maleate 2 mg/kg and remaining Group II, III and IV animals received Pettorale (100,200 and 400 mg/kg) respectively. After one hour of drug treatment each animal was placed in a histamine chamber and exposed to 0.5% histamine aerosol to induce experimental bronchial asthma. The onset of anaphylaxis was observed. This PCT was noted. These animals were subjected to histamine challenge one hr. after receiving the drug and again the PCT was noted. Animals which resist exposure to histamine aerosol for 15 min were considered to be perfectly protected. Then the guinea pigs were removed immediately from the histamine chamber. [8] The onset of anaphylaxis, duration of recovery and percentage of protection were calculated statistically. [9]

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<th>Table 1: Experimental design for histamine induced bronchoconstriction.</th>
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**Haloperidol induced catalepsy in rats**

Total 30 wistar rats were randomly divided into five groups containing six in each group (n=6). The control group received normal saline (5ml/kg), standard group received Chlorpheniramine maleate 2 mg/kg and test groups received Pettorale at a dose of 100,200 and 400 mg/kg respectively. All the animal groups received haloperidol 1mg/kg intraperitonealy one hour after the drug administration and duration of catalepsy was measured at 30 minutes after haloperidol administration. [10]

**Egg albumin induced paw anaphylaxis in rats**

The wistar rats of either sex were given three doses of 100µg of egg albumin on 1st, 3rd and 5th day. On 10th day of sensitization blood was collected and centrifuged to separate serum, and was stored at 20°C until it was used for experiment. Then another group of 30 fresh rats were randomly divided into five groups containing six in each group (n = 6). Control group received saline (2ml/kg), standard group received Chlorpheniramine maleate 2 mg/kg and test groups received Pettorale at a dose of 100, 200 and 400 mg/kg. Prior to drug treatment (half an hour before) animals were treated with above mentioned serum subcutaneously. 24hr after drug treatment animals were again administered with 10µg egg albumin and paw volume was calculated at 1, 2, 3 and 4hr intervals. [11]

**Milk-induced leucocytosis and eosinophilia in mice**

Total 36 mice were divided into six groups containing six each (n = 6). Blood samples were withdrawn through retro-orbital plexus. Total leucocyte and eosinophil count was determined for each group before treatment of drug and again after 24hrs of milk injection. Group I received normal saline (5ml/kg). Group II received vehicle and milk (4 ml/kg). Group III received Chlorpheniramine maleate (2 mg/kg) and followed by milk 4ml/kg. Group IV, V and VI received single doses of Pettorale (100,200 and 400mg/kg) respectively followed by milk 4ml/kg subcutaneously one hr after administration of Pettorale. Total leucocyte count (TLC) and eosinophil count was done 24hr after milk administration and compared with control, standard and milk induced group. [12]

**Statistical analysis**

The results are expressed as mean ± SEM. One-way ANOVA followed by Tukey’s t-test was applied for the statistical analysis. p<0.05 value was considered to be significant.
RESULT AND DISCUSSION

Results

Phytochemical Screening:
The Electrohomeopathic formulation Pettorale showed the presence of alkaloids, glycosides, carbohydrates, amino acids, proteins, steroids and terpenoids.

Acute oral toxicity study
The Electrohomeopathic formulation Pettorale did not show any mortality, morbidity and any significant changes in the general behavior of rats up to a dose of 2500 - 5000 mg/kg. Therefore, the dose of medicine could be select up to one tenth of the highest tolerable dose for the present study. 13

Effect of Pettorale on histamine induced bronchospasm

Electrohomeopathic medicine Pettorale significantly delayed the onset of histamine induced bronchospasm and exhibited quick recovery which was compared with control and standard. Therefore, the anti-asthmatic activity of catalepsy may be allotted to its anti-histaminic, anti-allergic and anti-inflammatory properties. Pettorale initiated a significant (p<0.05) increase in onset of anaphylaxis and decrease in extent of recovery in dose-dependent manner. (Fig. 1)

Effect of Pettorale on haloperidol induced catalepsy

Electrohomeopathic medicine Pettorale manifested a dose dependent significant (p<0.05) decrease in duration of catalepsy which was well compared with control and standard. Pettorale exhibited a dose dependent significant reduction in cataleptic score 90 minutes after haloperidol administration. (Fig. 2)

Effect of Pettorale on passive paw anaphylaxis

There is significant (p<0.05) reduction in paw oedema volume caused by egg albumin after administration of Pettorale which was compared with control and standard. But it was not related to dose dependent. (Fig. 3)
Effect of Pettorale on passive paw anaphylaxis

Values are expressed in mean±SEM (n=6), one-way ANOVA followed by Dunnett’s t-test. P<0.05 is considered to be significant.

**Effect of Pettorale in milk induced leucocytosis and Eosinophil count in mice**

Negative control group (milk induced) significantly increased the total leucocyte count as well as eosinophil count when compared to standard and control. Pettorale showed significant (p<0.05) dose dependent reduction in TLC and eosinophil count (Fig. 4 and Fig.5)

**Figure 3**: Effect of Pettorale on passive paw anaphylaxis Values are expressed in mean±SEM (n=6), one-way ANOVA followed by Dunnett’s t-test. P<0.05 is considered to be significant.

**Figure 4**: Effect of Pettorale on milk induced leucocytosis Values are expressed in mean±SEM (n=6), one-way ANOVA followed by Dunnett’s t-test. P<0.05 is considered to be significant.

**Figure 5**: Effect of Pettorale on milk induced Eosinophil Values are expressed in mean±SEM (n=6), one-way ANOVA followed by Dunnett’s t-test. P<0.05 is considered to be significant.
DISCUSSION

Asthma is a chronic inflammatory disease of the respiratory tract in which the numerous immune cells, including eosinophils, neutrophils, macrophages, T-lymphocytes, mast cells and epithelial lining play key roles. Investigations of herbal-based interventions have shown that “herbs contain a large number of naturally occurring chemicals that have biological activity”. Herbalists claim that the action of herbs is described according to the way it might affect human physiology. They also suggest that the constituents of herbs, “bioflavonoids,” alkaloids, and essential oils, have “members that are anti-allergic, anti-histaminic, anti-asthmatic, anti-inflammatory, etc.” Electrohomeopathic drug petrollea contains a blend of different plant extracts which is a rich source of different phytoconstituents with a variety of potential biological activities and for bronchodilatation activity. Pettorarella possesses steroidal Saponoins which inhibits the release of several mediators of the phlogistic agents such as serotonin, histamine, prostaglandins, and bradykinin by inhibiting the biosynthetic pathways of inflammatory mediators. In the reference standard group, we used the standard anti-histaminic drug Chlorpheniramine maleate against histamine-induced bronchospasm.

The histamine-induced bronchospasm is a conventional model of an antigen-induced airway blockade. Inhalation of histamine causes hypoxia which leads to smooth muscle contraction. The prominent effect of histamine showed a severe bronchoconspasm in animals. Histamine is one of the chief inflammatory mediators in the immediate phase of asthma. This causes airway hyper responsiveness and bronchial airway inflammation. As Guinea pig contains histaminergic receptors in ileum and tracheal smooth muscle, it is highly sensitive to histamine. Further, histamine is synthesized and released by mast cells in the airway wall and by circulating and infiltrating basophiles. In this study, histamine is produced bronchoconstriction in guinea pigs, but Pettorale induced animals produces a significant (p<0.05) reduction in onset of anaphylaxis in test groups when compared to control and standard and duration of recovery was also decreased significantly (p<0.05) but in dose-dependent manner. So, we can conclude that anti-histaminic activity of Pettorale may be responsible for its anti-asthmatic activity (Fig – 1).

Haloperidol induced catalepsy by inhibiting dopamine (D2) receptors and inhibits dopamine secretion. Dopamine is an agonist of adrenalin and adrenalin is a physiological antagonist of histamine. So, decrease in dopamine level by haloperidol increase the level of histamine. Pettorale administrated animals showed significant (p<0.05) protection against haloperidol induced catalepsy which was clearly reflected by the comparison between control and standard. This further justifies the antiasthmatic activity of Pettorale (Fig.-2).

Active paw anaphylaxis is another in-vivo model to evaluate the modulatory effect on IgE antibody mediated immune hyperactivity using egg albumin as antigen. There is a significant increase in paw oedema of egg albumin control group. Both standard and Pettorale exhibited significant inhibition of egg albumin induced paw edema but not related to dose dependent. The oedema inhibitory effect of the Pettorale may be due to the egulation of IgE mediated Type 1 hypersensitivity.

Bronchial asthma is a chronic inflammatory disease in which leukocytes play a vital role. More important being mast cells, eosinophils and T-lymphocytes. In the present study, the TLC and eosinophil count were observed. After parental administration of milk there is increase in TLC and eosinophil and this stressful condition can be normalized by administration of antistress or adaptogenic drugs. Pettorale administrated animals showed a significant protection against milk induced leucocytosis and eosinophilia which was clearly monitored by the comparison between control and standard. (Fig - 4 and Fig - 5). Thus it can be credited that Pettorale also possesses antiallergic and anti-asthmatic properties.

CONCLUSION

The anti-asthmatic activity of Pettorale may be attributed to its anti-histaminic, anti-allergic and anti-asthmatic properties. Further studies are in progress in our laboratory to explore its active compounds and detailed mechanism of action.

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CONFLICT OF INTEREST

Nil

REFERENCES


