EVALUATION OF POLYHERBAL FORMULATION, ROUTACK, IN RODENT MODELS OF ANAPHYLAXIS

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ABSTRACT
Anaphylaxis is a condition presenting with significant morbidity in sensitized individuals. IgE cross linking, degranulation of mast cells and release of preformed mediators are the pathophysiologic sequences responsible behind the anaphylactic turmoil. Adrenaline rescue is the only available therapy which can salvage an individual from an anaphylactic attack. Other concurrent therapies are only supportive. Complementary and alternative medicine (CAM) offers several alternatives that can be explored to tend the development of anaphylaxis. ROUTACK is one such polyherbal formulation containing the whole dried plant or parts of Clerodendron serratum, Saussurea lappa, Solanum xanthocarpum and Tephrosia purpurea. All these ingredients have specific properties that may be useful in ameliorating the pathophysiology of anaphylaxis. We explored the protective effect of this formulation in two different rodent models of anaphylaxis. The models represented the condition developed during an anaphylactic attack. In both the studies, the results suggested that ROUTACK offered significant protective effect against anaphylaxis, albeit at higher doses. The polyherbal formulation exhibited substantial protective effect against triple antigen induced anaphylaxis in rats. ROUTACK was uncovered of its activity against systemic anaphylaxis induced by mast cell secretagogue, compound 48/80. This effect can be translated to the clinical side if the formulation can be incorporated as a dietary supplement in patients who are at risk of developing anaphylaxis.

Key Word: Triple antigen, horse serum, compound 48/80, serum IgE

INTRODUCTION
Specific allergens on first contact with the human mucosal layer lead to sensitization of an individual to that allergen. Next contact with the same allergen may result in a life-threatening response termed as anaphylaxis. It manifests as respiratory distress, intense bronchospasm, vascular collapse, shock and ultimately death. Other mucocutaneous and gastrointestinal effects are also observable and are characteristics of
a systemic anaphylactic reaction. Heterologous proteins are the chief responsible factors as they have the capability of acting as specific allergens. Individuals may differ in their responses towards chronology of the attack but the hallmark symptomatology is the onset of debilitating manifestations within minutes of contact with the antigen[1]. IgE, mast cell histamine, basophils and eosinophilic proteins are the prime pathophysiologic factors eliciting an anaphylactic attack. Binding of IgE on the IgE-specific FcεR1 receptor of mast cells, leading to mast cell degranulation and release of histamine is the main pathway that commands the setting of anaphylaxis. As noted by Bingham et al, 2000[2], the host of pre-formed mediators released on mast cell degranulation are responsible for initiating and sustaining the crisis of an anaphylactic attack. Eosinophilic and basophilic proteins are not innocent bystanders for the event but contribute significantly to the anaphylactic attack by damaging the pulmonary and tracheal tissues as well as by amplifying the inflammatory response[3].

Current therapy of an anaphylactic attack involves parenteral epinephrine immediately on detection of the attack. This may be followed by oral corticosteroids or anti-histaminics as and when required[4]. Prophylactic therapy to prevent the occurrence of an attack is not well established. Though avoidance of the inducing allergen is the best possible measure for prevention, it is not a perpetually exercisable alternative. Complementary and alternative medicine (CAM) offers a broad domain of healing resources. The Ayurvedic Drug Laboratory, Madhya Pradesh, India, honed these resources to develop a polyherbal formulation, ROUTACK. It is a crude powder formulation containing the whole dried plant or parts of Clerodendron serratum (Verbenaceae), Saussurea lappa (Asteraceae), Solanum xanthocarpum (Solanaceae) and Tephrosia purpurea (Fabaceae) in equal proportions. All these plants have reported anti-histaminic, anti-inflammatory, spasmolytic or mast-cell stabilizing properties[5,6,7,8]. These plants have been individually utilized in ayurveda as medications for asthma and bronchitis. Due to these properties we hypothesized that this formulation might be effective in preventing the symptoms of anaphylaxis. Hence this formulation was evaluated in immunologic and non-immunologic models of anaphylaxis.
MATERIALS AND METHODS

Horse serum was purchased from Hi Media Chemicals, Mumbai, India. Triple Antigen (DTP Vaccine) was purchased from local market (Mfg. by Serum Institute of India Pvt. Ltd.). Compound 48/80 was purchased from Sigma Aldrich, St. Louis, MO, USA. The polyherbal formulation ROUTACK was a gift sample from Ayurvedic Drug Laboratory, MP, India. All other reagents and chemicals were of analytical grade. Albino rats of wistar strain (200-230 g) and Balb/c mice (20-22 g) were used for the study. Animals were housed in an air-conditioned room (25±2°C, 30-65% RH) in polypropylene cages having paddy husk as bedding with 12hr light-12 hr dark cycles. They had free access to pelleted diet and tap water. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Pharmacy Department, The M. S. University of Baroda. All experimental procedures were carried out as per CPCSEA guidelines.

Acute toxicity studies of the polyherbal formulation were carried out as per OECD guideline 423 for orally administered chemicals and compounds\cite{9}. Accordingly, 2000 mg/kg was found to be the LD₅₀ for ROUTACK. For further studies, 1/10th and 1/5th dose of LD₅₀ in rats and corresponding doses in mice were selected. The animals were divided into 4 groups. Group I, vehicle control; Group II, standard control; Group III, lower ROUTACK dose group; Group IV, higher ROUTACK dose group.

Triple-Antigen Horse-serum induced anaphylaxis in rats\cite{10}

20 Male wistar rats (200-250 gm) were sensitized by subcutaneous injection of 0.5ml of Horse serum followed by 0.5ml of Triple antigen vaccine containing 2×10^{10} Bordetella pertussis organisms per ml. The sensitized animals were then divided into 4 groups of 5 animals each and dosed for 2 weeks daily between 1600-1800 hrs. Vehicle controls received 1 ml/kg 1% sodium CMC. Standard controls received 10 mg/kg prednisolone sod. phosphate per orally. Test groups received 200 mg/kg and 400 mg/kg of ROUTACK respectively, suspended in 1% sodium CMC per orally. On 14th day, 2 hrs after the last dose, anaphylaxis was induced by i.v. injection of 0.25ml of horse serum and each animal was scored for the characteristic symptoms (Table 1) for 1 hr by a blind observer:
Table 1: Symptom score for Triple-Antigen Horse serum induced anaphylaxis.

Scores for the respective symptoms are indicated on the left hand side. The highest observable score is noted for individual animals.

<table>
<thead>
<tr>
<th>Score</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visual symptoms</td>
</tr>
<tr>
<td>2</td>
<td>Increased rate of respiration</td>
</tr>
<tr>
<td>4</td>
<td>Increased rate of respiration with immobility</td>
</tr>
<tr>
<td>6</td>
<td>Dyspnea for 10 mins</td>
</tr>
<tr>
<td>8</td>
<td>Cyanosis for 10 mins</td>
</tr>
<tr>
<td>10</td>
<td>Dyspnea &amp; Cyanosis for 10 mins</td>
</tr>
<tr>
<td>12</td>
<td>Death</td>
</tr>
</tbody>
</table>

Total score of each group was calculated and thus mean ± SEM was calculated.

Compound 48/80 induced systemic anaphylaxis in Balb/c mice[11]

Balb/c mice (20-22 g) of either sex were divided into 4 groups of 8 animals each. The animals were dosed for 2 weeks daily between 1000-1200 hrs. Dosing pattern was similar to that of shown above, except that the test groups received 400 mg/kg and 800 mg/kg of ROUTACK respectively. 2 hrs after the last dose, anaphylaxis was induced by injection of mast cell degranulator, Compound 48/80 (8 mg/kg in saline, i.p.) & observed for 1 hr. Death was scored as ‘1’ and survival was scored as ‘0’. Sum of deaths were calculated for each group and mean ± SEM was thus found.

Statistical analysis

Mean ± SEM of all the groups were compared using a computer based fitting program (Graphpad Prism ver. 05). Difference between the groups was compared using one-way ANOVA followed by Bon-ferroni’s multiple comparisons test. The results were considered to be statistically significant when p<0.05.
RESULTS

Figure 1 shows the symptomatic score of the animals challenged with 0.5 ml of serum. Prednisolone sod. phosphate was used as the standard drug. As compared to the vehicle control group (CON), the standard control group (PRE) showed significant (***, p<0.001) protective effect. The lower ROUTACK dose group, RO 200, did not show any protective effect upon triple antigen and horse serum induced anaphylaxis in rats (non-significant=ns) but the higher ROUTACK dose group, RO 400, showed quite a significant (*, p<0.05) protective effect. However, the effect of prednisolone was far better. ROUTACK, at higher dose may have a superior protective effect.

![Figure 1](image)

**Figure 1**
Effect of ROUTACK on anaphylaxis induced by horse serum. Results are expressed as mean ± SEM of total scores (n=5). *p<0.05, **p<0.01, ***p<0.001, ns= non significant.

As shown in figure 2, death caused due to anaphylactic shock was scored in compound 48/80 induced systemic anaphylaxis. All the mice administered with compound 48/80 in the vehicle control group (CON) died due to the resultant anaphylactic shock. However, in the PRE group survival score was higher and found to be statistically significant (**, p<0.01). Death score was also lowered in mice administered with 400 mg/kg (RO 400) of ROUTACK but it was non-significant (ns). In
animals treated with 800 mg/kg of ROUTACK (RO 800), a significant (*, p<0.05) decrease in the death score was observed.

![Graph showing effect of ROUTACK on anaphylaxis induced by Compound 48/80.](image)

**Figure 2**

Effect of ROUTACK on anaphylaxis induced by Compound 48/80. Results are expressed as mean ± SEM of total scores (n=8). *p<0.05, **p<0.01, ***p<0.001, ns= non significant.

**DISCUSSION**

Active anaphylaxis induced by Triple antigen & horse serum is a key model to study the symptomatic affects of type I allergy. The globulin fractions of horse serum act as environmental allergens triggering allergic response\(^{[12]}\). High intravenous dose of serum, enhanced with triple antigen results in imperative anaphylactic response. Triple antigen acts as a primer of the immune system so that it acts in a desired fashion and drives a T\(_{H2}\) biased response\(^{[13]}\). All these mechanisms allude to the immunological aspect of anaphylaxis and hence represent an ideal model to understand the pathology of the condition. In our study we found that vehicle control animals exhibited significant morbidity and mortality when the sensitized animals were challenged with the serum. This effect may be attributed to the cascade of reactions occurring during type I allergy. IgE cross linking\(^{[14]}\), degranulation of mast cells\(^{[2]}\), release of mediators and accumulation of inflammatory cells lead to symptoms like respiratory distress, wheezing, cyanosis and ultimately death due to hypoxia. However, the higher doses of the polyherbal
formulation, ROUTACK, significantly prevented the occurrence of the above mentioned symptoms. This effect may be endorsed to the different protective effects found in the ingredients of ROUTACK. C. serratum has anti-histaminic property\[^{15}\], while S. lappa\[^{16}\] and S. xanthocarpum possess spasmylytic constituents\[^{7}\]. T. purpurea has mast cell stabilizing property\[^{8}\]. All these actions in concert may result in the prophylactic effects exhibited by the polyherbal formulation. This shows that ROUTACK possesses a significant immunological property through a myriad range of protective mechanisms.

Compound 48/80 is a non-specific mast-cell degranulator. This effect of compound 48/80 is non-immunologic in character. It acts as a calcium channel ionophore making the membranes of mast cells leaky. For this effect of compound 48/80, prior sensitization is not required\[^{17}\]. This mechanism of compound 48/80 has been explored to study the systemic anaphylactic response. One of the constituents of ROUTACK, T. purpurea, possesses marked mast cell stabilizing activity\[^{18}\]. Further, C. serratum also possesses activity as anti-histaminic and also against other pre-formed mediators\[^{5}\]. All these effects were translated \textit{in vivo} where mice pre-treated with ROUTACK showed a significantly low mortality rate when injected with compound 48/80. However, such an effect was observable only in mice treated with high doses of ROUTACK. This shows that pre-treatment with higher doses of ROUTACK or incorporation of the formulation as a daily dietary supplement may help prevent the development of future attacks.

\textbf{CONCLUSION}

The results obtained with the above studies indicate that ROUTACK possesses significant protective effect against immunological and non-immunological aspects of anaphylaxis. Though the effect was observed only at higher doses, it was evident that the polyherbal formulation significantly prevented morbidity caused due to anaphylaxis. ROUTACK remains a potential candidate for the prophylactic therapy of anaphylaxis. It may serve as a credible combination candidate in terms of prophylaxis. Further studies may lend credence to the molecular mechanisms responsible behind the anti-anaphylactic activity of ROUTACK.

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REFERENCES


