ASSESSMENT OF SPASMOLYTIC ACTIVITY OF ALCOHOLIC EXTRACT OF ACICILLEA MILL, RUBIA CORDIFOLIA AND SAUSSUREA LAPPA IN WISTAR ALBINO RATS

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ABSTRACT

The present study was carried out to evaluate the potential of alcoholic extract of anti-inflammatory plants viz. Achillea millefolium, Rubia cordifolia and Saussurea lappa for spasmylocytic activity in rat model using radiotin organ bath. The relaxant effect of all extracts on pre-contraction rat tracheal chain by carbachol (30 µM) had been screened. Isometric contractions of isolated rat tracheas were recorded at 1.4 g resting tension and carbachol dose-response curves performed. EC₅₀ values (27.12, 13.13 and 7.32 µM, respectively) were identified by plotting cumulative concentration response curve and pD₂ values (4.88 ± 0.99, 6.06 ± 1.03 and 7.41 ± 0.97, respectively) were calculated for individual alcoholic extract of plant. All extracts were able to relax carbachol pre-contracted tracheas significantly in a concentration dependent manner. Our results suggested potential role of Achillea millefolium, Rubia cordifolia and Saussurea lappa in asthma for further potential therapeutic and clinical uses.

Keywords: Achillea millefolium, Rubia cordifolia, Saussurea lappa, Spasmylocytic, Tracheal chain.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways but till date the fundamental causes of asthma are not completely known1, numerous antigens or allergens are capable of triggering the acute attacks of asthma. In the recent years, the morbidity and mortality of population due to asthma is increasing, with an estimated 300 million individuals affected worldwide, which will further increased by 100 million by 20252–7. Till date, the cause and permanent cure of asthma are unknown. However, for symptomatic relief a medical practitioner prescribes β-adrenergic receptor agonists, glucocorticoids and anticholinergic agents. Although these medications results in deleterious side effects viz. cardiotoxicity, tachycardia, immunodeficiency and hyperglycaemia, they are still prescribed in the management of asthma8,9. With these perspectives, we approached to traditional system of medicine to find a cure of asthma because these medicines are devoid of such side effects. Present study was carried out to evaluate the potential of three alcoholic extracts on tracheal chain preparations in order to estimate their anti-asthmatic potential10, 27–28. Further the alcoholic extracts of these plants were compared with an atropine derivative, ipratropium bromide.

MATERIALS AND METHODS

Plant Material

Achillea millefolium Linn. (Asteraceae / Comositae) flowers, Rubia cordifolia Linn. (Rubiaceae) roots and Saussurea lappa (Asteraceae / Comositae) roots were collected from local market of Delhi, India and were identified at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. (NISCAIR/RHMD/Consult/-2009-10/1548/121)

Preparation of the Alcoholic Extracts and Qualitative Analysis

The shed dried crude drug was subjected to pulverization and packing into Soxhlet apparatus to get alcohol extract (18 h). The solvent was removed under reduced pressure and crude extract was subjected to qualitative analysis11–12. The extract thus obtained was used for in-vitro organ bath study.

Experimental Animals

Wistar albino rats of either sex weighing 200-230 g were obtained from the animal house of DIPSAR, University of Delhi, India. The animal were housed at an ambient temperature (27 ± 2°C) under a 12 h normal phase light-dark cycle and were fed on standard pellet chow diet (Amrut rat feed, Sangli, India), were accessible ad libitum tap water during the entire study. All the animal experiments were performed according to the guidelines provided by Committee for the Purpose of Controlled and Supervision of Experiment on Animals (CPSCEA), New Delhi, India, approved by Institutional Animal Ethics Committee (IAEC/DIPSAR/2010-II/01) of DIPSAR, New Delhi. Rats were allocated randomly to the groups: Group 1: 30 µMol of carbachol, served as negative control; Group 2: CCRC of ipratropium bromide, served as standard; Group 3-5: CCRC of test extracts. Cumulative relaxation was observed in group 3-5 following a 30 µMol dose of carbachol in 60 mL organ bath.

Spasmylocytic Activity in Isolated Tracheal Strip

Isolation of Trachea and Preparation of Tissue13–15

Euthanized animals were incised on ventral skin region of neck without damaging the tracheal smooth muscles. The exposed portion of the trachea was isolated by giving a transverse cut just below the thyroid cartilage and above the carinal portion of the trachea. The trachea obtained was quickly transferred to a petridish containing freshly prepared modified Krebs Henseleit solution (MKHS) (composition per
Measurement of Tension due to Standard Drugs and Compounds

A pretension of 1.4 g was applied to the tracheal strips in all the experiments conducted followed by a stabilisation period of 30-40 minutes to obtain a constant baseline. Tissues were replenished with the fresh MKHS 2-3 times during stabilisation period. Each dose-response experiment was done on set of 6 strips isolated from 3 different tracheas.

Concentration response curves were obtained for different test and standard compounds in the sequence of concentration 1:3:10:30:100:300:1000:3000 viz. 1 nM: 3 nM: 10 nM: 30 nM: 100 nM: 300 nM: 1000 nM: 3000 nM, allowing sufficient time of 3 minutes to elapse between each concentration. The relaxation due to ipratropium and test drugs was measured on the tracheal muscles, pre-contracted with carbachol (30 µMol). The cumulative increase in muscle relaxation was recorded over a ipratropium bromide concentration ranging from 10\(^{-12}\) to 3 X 10\(^{-7}\)M, whereas for the test compounds CCRCs were obtained in the concentrations ranging from 10\(^{-12}\) to 3 X 10\(^{-4}\) M. % Contraction and % relaxation were obtained from the plots obtained by CCRC of respective drugs. EC\(_{50}\) values were obtained graphically followed by calculation of pD\(_2\) values.

Statistical Analysis

Analysis of each data set was performed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (sigma plot 11, USA). The data was considered to be statistically significant at P < 0.05.

Table 1: Qualitative chemical tests of the extracts of *Achillea millefolium*, *Rubia cordifolia* and *Saussurea lappa*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Tests</th>
<th>Achillea millefolium</th>
<th>Rubia cordifolia</th>
<th>Saussurea lappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hager’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone Glycosides</td>
<td>Bontrager’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Legal’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Froth test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Libermann Burchard’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: pD\(_2\) values of Ipratropium bromide and alcoholic extract of *Achillea millefolium*, *Rubia cordifolia* and *Saussurea lappa*

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Starting concentration in moles</th>
<th>Ending concentration in moles</th>
<th>n</th>
<th>pD(_2)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipratropium bromide</td>
<td>1 X 10(^{-12})</td>
<td>3 X 10(^{-7})</td>
<td>6</td>
<td>8.65 ± 2.82</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>1 X 10(^{-12})</td>
<td>3 X 10(^{-7})</td>
<td>6</td>
<td>4.48 ± 0.99(^{+})</td>
</tr>
<tr>
<td><em>Rubia cordifolia</em></td>
<td>1 X 10(^{-12})</td>
<td>3 X 10(^{-4})</td>
<td>6</td>
<td>6.06 ± 1.03(^{-})</td>
</tr>
<tr>
<td><em>Saussurea lappa</em></td>
<td>1 X 10(^{-12})</td>
<td>3 X 10(^{-4})</td>
<td>6</td>
<td>7.41 ± 0.97(^{-})</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n: number of tissues; pD\(_2\): negative log of EC\(_{50}\) value. Levels of significance: *P < 0.001; **P < 0.01; *P < 0.05 as compared with ipratropium bromide. **P < 0.01; *P < 0.05 as compared with *Achillea millefolium*.
RESULTS
Crude drugs were subjected to qualitative analysis which confirms the presence of essential oil, terpenoids, flavonoids, saponins, proteins and tannins in Achillea millefolium flowers; alkaloids, carbohydrates, anthraquinone glycosides, sterols, flavonoids and protein in roots of Rubia cordifolia; alkaloids, carbohydrates, glycosides, phytosterols, terpenoids, oil and tannin in Saussurea lappa roots (Table 1). The percentage yield for alcoholic extracts of Achillea millefolium, Rubia cordifolia and Saussurea lappa was found to be 10.87 %, 28.92 % and 19.15 %, respectively. CCRC of contraction and relaxation were expressed as a percentage of the maximal response for each substance. Ipratropium bromide and tested compounds produce relaxation in a dose dependent manner in carbachol (30 µM) induced pre-contraction strips. The CCRC of carbachol revealed sealing effect on 100 µM concentration where 100% contraction was achieved therefore, 30 µM carbachol was selected to attain sub-maximal response with the IC50 2.24 µM. The CCRC of ipratropium bromide showed contraction induced by carbachol was completely diminished at 100 nM concentration (Figure 1) with the EC50 2.24 nM and pD2 8.65 ± 2.82 and it was found significant (P < 0.001) when compared to carbachol while the CCRC of alcoholic extract of Achillea millefolium showed contraction induced by carbachol was significantly (P < 0.001) abolished at 100 µM concentration with the EC50 27.12 µM and pD2 4.48 ± 0.99 when compared to ipratropium bromide. The CCRC of alcoholic extract of Rubia cordifolia showed contraction induced by carbachol was completely abolished at 100 µM concentration with the EC50 13.13 µM and pD2 6.06 ± 1.03 which was found to be significant when compared to ipratropium bromide. The CCRC of alcoholic extract of Saussurea lappa showed contraction induced by carbachol was significantly abolished at 100 µM concentration of Saussurea lappa alcoholic extract with the EC50 7.32 µM and pD2 7.41 ± 0.97 (Table 2).

DISCUSSION
Tracheal strips were contracted with carbachol and a dose response curve was obtained to standardise the submaximal concentration to evaluate relaxant effect of standard and test extracts. As it is well established that inflammation and immunomodulation are the key etiological factors for the precipitation of asthma, therefore, the basis for selection of the plant was, whether they possess these activities or not. Achillea millefolium is known to contain bioactive principle triterpenes and sesquiterpenes, which are reported to modulate histamine, bradykinin, MMPs and ILS19. These chemokines play a key role in management of inflammation; therefore, Achillea millefolium was selected for its possible role in treatment in asthma. Rubia cordifolia contains major constituents as anthraquinone glycosides which are known to possess antioxidant, anti-PAF and anti-LTs activities which are involved in asthma20,21. The plant Saussurea lappa is a rich source of sesquiterpene lactones which are reported to modulate inflammatory markers such as iNOS, TNF-α and NF-κB22. Since inflammation is said to have a critical role in pathogenesis of asthma, Saussurea lappa was investigated for its possible cure. Among three plants selected for the study, Saussurea lappa was significantly able to relax the contraction induced by carbachol (30 µM) followed by Rubia cordifolia and Achillea millefolium. pD2 values indicate that all the three alcoholic extracts were not comparable in terms of potency exhibited by ipratropium bromide. However, Saussurea lappa was found to be significantly more potent compared to Rubia cordifolia and Achillea millefolium. However, since all the drugs exhibited the relaxation of 100 % at their higher doses. Therefore, the efficacy for all the three drugs remains the same. Several pathways are known to be involved in the relaxation of the tracheal strips including H2 antagonism23, β2 agonism24, NO induced relaxation25 and prostenoids induced relaxation25. Saussurea lappa has been reported to have anti-peroxidative effects and known to suppress contractions in guinea pig aorta, possibly due to the presence of sesquiterpene lactones26. Sesquiterpenes are known to stimulate the sGC which through activation of cGMP and PKG pathway stimulate extrusion of K+ ions and thereby reduces intrinsic Ca2+ ions, leading to relaxation of smooth muscles26. Further the sequestering of Ca2+ ions is also reported for sesquiterpenes. An elaborated study to delve the mechanism is solicited for the active constituents of these plants for their bronchorelaxant activities.

CONCLUSION
Our results showed that alcoholic extract of Saussurea lappa was more potent and followed by Rubia cordifolia and Achillea millefolium but our studies does not reveal specific mechanism of action for their muscle relaxing activity. Therefore, we suggest further receptor-binding studies to establish the mechanism of action of these compounds. Further investigations are needed to know the active principles from the roots of Rubia cordifolia and Saussurea lappa and flowers of Achillea millefolium that possess anti-inflammatoriy effects.

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REFERENCES


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