INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

ASSESSMENT OF SPASMOLYTIC ACTIVITY OF ALCOHOLIC EXTRACT OF *ACILLEA MILLEFOLIUM*, *RUBIA CORDIFOLIA* AND *SAUSSUREA LAPPA* IN *WISTAR* ALBINO RATS

Nagar Ashish[1]*, Kumar Ashok[1], Gullaiya Sumeet[3], Singh Varun[1], Dubey Vishal[1], Srinivasan B. P.[2]
[1]Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Pushp Vihar, Sector III, M. B. Road, New Delhi, India
[2]Department of Pharmacy, University of Delhi and Director, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi, India
[3]Department of Phamacology, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India
*Corresponding Author Email: ashish0101@gmail.com

Article Received on: 15/05/13 Revised on: 01/06/13 Approved for publication: 21/07/13

DOI: 10.7897/2230-8407.04714
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

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 spasmolytic activity in rat model using radnoti organ bath. The relaxant effect of all extracts on pre-contracted 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 were performed. EC_{50} values (27.12, 13.13 and 7.32 µM, respectively) were identified by plotting cumulative concentration response curve and pD_2 values (4.48 ± 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*, Spasmolytic, Tracheal chain.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways but till date the fundamental causes of asthma are not completely known[1], 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 2025[2-7]. Till date, the cause and permanent cure of asthma are unknown. However, for symptomatic relief a medical practitioner prescribes β_2-adrenergic receptor agonists, glucocorticoids and anticholinergic agents. Although these medications results in deleterious side effects *viz*. cardio-toxicity, tachycardia, immunodeficiency and hyperglycaemia, they are still prescribed in the management of asthma[8-9]. 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 potential[10, 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 *Sassurea 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 analysis[11-12]. 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^{0}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.

Spasmolytic Activity in Isolated Tracheal Strip

Isolation of Trachea and Preparation of Tissue[13-15]

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

litre: NaCl, 6.9 g; KCl, 0.35 g; $CaCl_2$, 0.28 g; $MgSO_4$, 0.28 g; KH_2PO_4, 0.16 g; Na_2CO_3, 0.21 g; glucose, 1.5 g), aerated with carbogen at pH 7.4 $\pm$ 0.05 and temperature of 37°C. The tracheal piece was cleaned of extraneous tissue, cut spirally into 2-3 strips of tissues depending on the length of trachea, one end to other in spiral fashion such that 2 or 3 segments of cartilage separate each turn. The single channel physiography with Poly VIEW software (version 16) (RADNOTI, Monrovia, CA, USA) was calibrated as per the standard operating procedures[16]. The strips were mounted in a tissue bath containing 60 mL of pre-warmed MKHS, connected to force transducer.

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[17]. 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[18], 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[19].

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*

Phytoconstituents	Tests	*Achillea millefolium*	*Rubia cordifolia*	*Saussurea lappa*
Alkaloids	Mayer's test	-	+	+
	Dragendroff's test	-	+	-
	Wagner's test	-	+	+
	Hager's test	-	-	-
Anthraquinone Glycosides	Borntrager's test	-	+	-
Cardiac Glycosides	Legal's test	-	-	+
Saponins	Foam test	+	-	-
	Froth test	+	-	-
Phytosterols	Libermann Burchard's test	-	+	+
Flavonoids	Lead acetate test	+	+	-

Table 2: pD_2 values of Ipratropium bromide and alcoholic extract of *Achillea millefolium*, *Rubia cordifolia* and *Saussurea lappa*

Drugs	Starting concentration in moles	Ending concentration in moles	n	pD_2-value
Ipratropium bromide	1 X 10^{-12}	3 X 10^{-7}	6	8.65 ± 2.82
Achillea millefolium	1 X 10^{-12}	3 X 10^{-4}	6	4.48 ± 0.99***
Rubia cordifolia	1 X 10^{-12}	3 X 10^{-4}	6	6.06 ± 1.03$^{**,\#}$
Saussurea lappa	1 X 10^{-12}	3 X 10^{-4}	6	7.41 ± 0.97$^{*,\#\#}$

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*

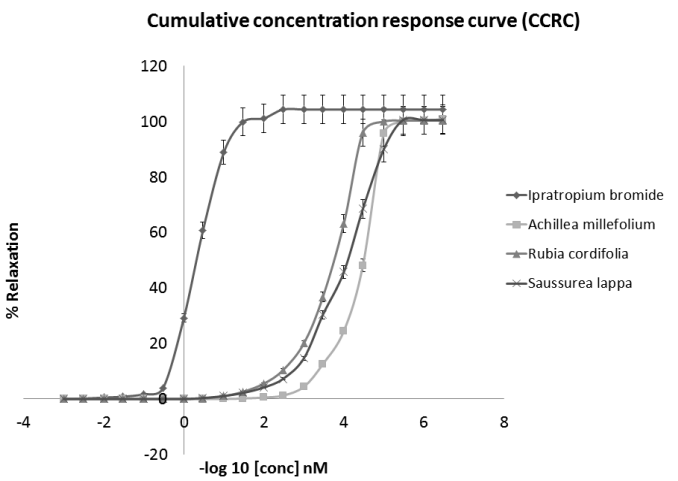

Figure 1: Cumulative concentration-response curves fitted by non-linear iterative regression analysis of Ipratropium bromide, alcoholic extract of flowers of *Achillea mellifolium*, roots of *Rubia cordifolia* and *Saussurea lappa*. The preparations were pre-contracted by carbachol. Responses are expressed as % relaxation of the tension produced by the spasmogen. Vertical bars shows S.E. values, n = 6

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-contracted 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 IC_{50} 2.24 µM. The CCRC of ipratropium bromide showed contraction induced by carbachol was completely diminished at 100 nM concentration (Figure 1) with the EC_{50} 2.24 nM and pD_2 8.65 ± 2.82 and it was found significant ($P < 0.001$) when compared to carbachol while the CCRC of alcoholic extract of *Achillea mellifolium* showed contraction induced by carbachol was significantly ($P < 0.001$) abolished at 100 µM concentration with the EC_{50} 27.12 µM and pD_2 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 EC_{50} 13.13 µM and pD_2 6.06 ± 1.03 which was found to be significant when compare 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 EC_{50} 7.32 µM and pD_2 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 ILs[10]. 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 asthma[20, 27]. 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-ƙB[28]. 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 mellifolium*. pD_2 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 H_2 antagonism[21], β_2 agonism[22], NO induced relaxation[23] and prostonoids induced relaxation[24]. *Saussurea lappa* has been reported to have anti-peroxidative effects and known to supress contractions in guniea pig aorta, possibly due to the presence of sesquiterpene lactones[25]. Sesquiterpenes are known to stimulate the sGC which through activation of cGMP and PKG pathway stimulate extrusion of K^+ ions and thereby reduces intrinsic Ca^{++} ions, leading to relaxation of smooth muscles[26]. Further the sequestering of Ca^{++} 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 mellifolium* 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 mellifolium* that possess anti-inflammatory effects.

ACKNOWLEDGMENT

We are thankful to Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India and Department of Training and Technical Education, Government of NCT Delhi, India for the funding assistance.

REFERENCES

1. WHO (World Health Organisation); c2012. Available from: http://www.who.int/features/qa/46/en/index.html. [cited 2012 Dec 02].
2. Bousquet PJ, Godard P, Daures JP. The public health implications of asthma. Bull World Health Organ 2005; 83: 548-54. PMid:16175830 PMCid:PMC2626301
3. Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D, Jindal SK, *et al.* Prevalence and risk factors for bronchial asthma in Indian adults: A multicentre study. Indian J Chest Dis Allied Sci 2006; 48: 13-22. PMid:16482947
4. Jindal SK. Bronchial Asthma: The Indian Scene. Curr Opin Pulm Med 2007; 13: 8-12. http://dx.doi.org/10.1097/MCP.0b013e32800ffd09 PMid:17133118
5. MHFW (Ministry of Health and Family Welfare); New Delhi; c2009. Available from: http://www.nfhsindia.org/data/india/indch6.pdf. [cited 2010 Jun 29].
6. GIA (Global initiative for asthma/WHO initiative); c2011. Available from: http://www.ginasthma.com/index.asp?l1=1andl2=0. [cited in 2011].
7. World Health Organisation; c2011. Available from: http://www.who.int/mediacentre/factsheets/fs307/en/index.html. [cited 2011 Nov 12].
8. Waldeck B. [beta]-Adrenoceptor agonists and asthma—100 years of development. Eur J Pharmacol 2002; 445(1 and 2): 1–12.
9. Gennari C. Differential effect of glucocorticoids on calcium absorption and bone mass. Br. J. Rheumatol 1993; 32 Suppl 2: 11–4. http://dx.doi.org/10.1093/rheumatology/32.suppl_2.11 PMid:8495275
10. Saeidnia S, Gohari AR, Mokhber Dezfuli N, Kiuchi F. A review on phytochemistry and medicinal properties of the genus *Achillea*. Daru 2011; 19(3): 173-186. PMid:22615655 PMCid:PMC3232110
11. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th ed, Bailliere Tindall, London; 1983. p. 21-22.
12. Lorke D. A New Approach to Practical Acute Toxicity Testing. Arch. Toxicol 1983; 54: 275-287. http://dx.doi.org/10.1007/BF01234480 PMid:6667118

13. Constantine JW. The spirally cut tracheal strip preparation. J. Pharm. Pharmacol 1965; 17: 384–385. http://dx.doi.org/10.1111/j.2042-7158.1965.tb07688.x PMid:14324462
14. Costantin LL *et al*. Localization of Calcium-Accumulating Structures in Striated Muscle Fibers. Sci 1965; 147: 158-160. http://dx.doi.org /10.1126/science.147.3654.158
15. Burka JF, Saad MH. Bronchodilator-mediated relaxation of normal and ovalbumin-sensitized guinea-pig airways: lack of correlation with lung adenylate cyclase activation. Br. J. Pharmacol 1984; 83(3): 645-655. http://dx.doi.org/10.1111/j.1476-5381.1984.tb16218.x PMid:6439271 PMCid:PMC1987096
16. Chang S *et al*. Alteration of the PKC-mediated signaling pathway for smooth muscle contraction in obstruction-induced hypertrophy of the urinary bladder. Lab. Investigation 2009; 89: 823-832. http://dx.doi.org/ 10.1038/labinvest.2009.38 PMid:19381130 PMCid:PMC2702459
17. Morin C *et al*. Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. Am. J. Physiol. Lung Cell Mol. Physiol 2009; 296: L130-L139. http://dx.doi.org/10.1152/ajplung.90436.2008 PMid:18978038
18. Mustafa SJ *et al*. P1 (adenosine) purinoceptor assays. Curr. Protoc. Pharmacol 2009; 45: 4.7.1-4.7.13.
19. Patel KG *et al*. Evaluation of bronchodilator and anti-anaphylactic activity of *Myrica sapida*. Iran Biomed J 2008; 12(3): 191-196. PMid:18762824
20. Kumar S, Agnihotri VK, Thakur S, Verma A, Saxena RC, Soni KK. Some important medicinal plants used in the treatment of asthma - a review. Int. J. Pharma. Sci. Res 2012; 3(10): 500-502.
21. Drazen JM *et al*. Alteration of histamine response by H_2-receptor antagonism in the guinea pig. J Appl Physiol 1980; 48(4): 613-8. PMid:7380687
22. Berber de Vries *et al*. β-Agonist-induced constitutive β_2-adrenergic receptor activity in bovine tracheal smooth muscle. Br J Pharmacol 2000; 131(5): 915–920. http://dx.doi.org/10.1038/sj.bjp.0703664 PMid:11053211 PMCid:PMC1572420
23. Tsong Long Hwang *et al*. YC-1 potentiates nitric oxide-induced relaxation in guinea-pig trachea. Br J Pharmacol 1999; 128(3): 577–584. http://dx.doi.org/10.1038/sj.bjp.0702830 PMid:10516635 PMCid:PMC1571672
24. Sipahi EY *et al*. Nitric oxide and prostanoid-dependent relaxation induced by angiotensin II in the isolated precontracted mouse tracheal muscle and the role of potassium channels. Pharmacol Res 2000; 42(1): 69-74. http://dx.doi.org/10.1006/phrs.2000.0657 PMid:10860637
25. Pandey MM, Rastogi S, Rawat AKS. *Saussurea costus*: Botanical, chemical and pharmacological review of an ayurvedic medicinal plant. J. Ethnopharmacol 2007; 110: 379–390. http://dx.doi.org/10.1016/j.jep. 2006.12.033 PMid:17306480
26. Liu B, Yang J, Wen Q, Li Y. Isoliquiritigenin, a flavonoid from licorice, relaxes guinea-pig tracheal smooth muscle *in vitro* and in vivo: Role of cGMP/PKG pathway. Euro. J. Pharmacol 2008; 587: 257–266. http://dx.doi.org/10.1016/j.ejphar.2008.03.015 PMid:18462716
27. Deshkar N, Tilloo S, Pande V. Phcog Rev.: Review Article: A Comprehensive Review of *Rubia cordifolia* Linn. Pharmacog. Rev 2008; 2(3): 124-134.
28. Madhuri K, Elango K, Ponnusankar S. *Saussurea lappa* (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Orient. Pharm. Exp. Med 2012; 12: 1–9. http://dx.doi.org/10.1007/s13596-011-0043-1

Cite this article as:
Nagar Ashish, Kumar Ashok, Gullaiya Sumeet, Singh Varun, Dubey Vishal, Srinivasan B. P. Assessment of spasmolytic activity of alcoholic extract of Acillea millefolium, Rubia cordifolia and Saussurea lappa in wistar albino rats. Int. Res. J. Pharm. 2013; 4(7):64-67 http://dx.doi.org/10.7897/2230-8407.04714

Source of support: Nil, Conflict of interest: None Declared