ANTI-ASTHMATIC AND ANTI-TUSSIVE ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF NYCTANTHES ARBORTRISTIS LINN.

Kumkum Mathur*1, A K Mathur2, R Issrani1, S R Ambani3, M Goyal1

1Lachoo Memorial College of Science and Technology, Jodhpur, India
2Dr S N Medical College, Jodhpur, India
3All India Institute of Medical Sciences, Jodhpur, India

ABSTRACT

Ethnopharmacological relevance: Nyctanthes arbor-tristis L. of family Oleaceae (NATL) is has been reported for use in respiratory disorders such as asthma and bronchitis. It has been scientifically evaluated for anti-allergic and antihistaminic properties; however, potential of NATL as anti-asthmatic and anti-tussive has not been evaluated till date.

Material and method: The pharmacological studies were performed using guinea pigs. Anti-asthmatic activity was evaluated using 0.1% histamine and 2% acetylcholine induced asthma. Anti-tussive activity was evaluated using citric acid induced cough model.

Result: The NATL has depicted anti-asthmatic effects against histamine and acetylcholine cocktail induced asthma. When compared with control group all treatment groups viz. atropine, mepamine, NATL 100, 200 and 300 mg/kg were found offering statistically significant (P<0.05) increases in preconvulsive time and produced 210.4, 229.9, 170.1, 215.0 and 238.8% increase in latency in convulsion respectively. The NATL have depicted anti-tussive effects against citric acid induce coughing, it increases the latency of onset of cough and frequency of coughing.

Conclusion: Based on the results of present study it can be concluded that NATL possesses anti-asthmatic potential.

Keywords: Nyctanthes arbor-tristis; anti-asthmatic; anti-tussive; histamine; acetylcholine; guinea pig.

INTRODUCTION

Bronchial asthma is one of the major respiratory diseases affected around 235 million people of the world. It is a chronic disease of the air passages of the lungs, which inflames and narrows them. The strongest risk factors for developing asthma are inhaled substances and particles that may provoke allergic reactions or irritate the airway. Lack of treatment of asthma causes deaths in low and lower middle income countries. However, appropriate management of asthma can enable people to enjoy a good quality of life (WHO, 2013).

The asthma is managed by using inhaled corticosteroids and bronchodilators. This therapy controls symptoms in most of the cases. However, their prolonged use has been accompanied by side-effects such as osteoporosis, esophageal candidiasis, upper respiratory tract infections and pharyngitis (Colleen et al., 1999). Therefore, development of either new alternative or complementary therapies for this disease is becoming important.
In India, several ethnomedicinal plants are used for the treatment of Bronchial asthma (Kasirajan et al., 2007). *Nyctanthes arbor-tristis* L. of family Oleaceae (NATL), has been reported for uses in respiratory disorders such as asthma and bronchitis (Gupta et al., 1993; Anonymous, 1997). It has also been reported to be having antiallergic and antihistaminic properties (Saxena et al., 1984; Saxena et al., 1987; Gupta et al., 1993; Gupta et al., 1995; Anonymous, 1997; Nair et al., 2005; Chatterjee et al., 2007; Nirmal et al., 2012a, and 2012b). The present study was initiated to scientifically evaluate anti–tussive and anti-asthmatic effects of ethanolic extract of leaves of *Nyctanthes arbor-tristis*.

**MATERIALS AND METHODS**

2.1 Collection and preparation of plant material
The fresh leaves of *Nyctanthes arbor-tristis* L. were collected in October 2006 from Jodhpur, India. Herbarium of plant was identified by Taxonomist of Botanical Survey of India (BSI), Jodhpur, Rajasthan. A voucher specimen was submitted in the BSI.

2.2 Extract preparation
The shade dried leaves of *Nyctanthes arbor-tristis* L were crushed and kept in a closed vessel with ethanol (absolute alcohol) so as to avoid evaporation of ethanol and allowed to stand for seven days with occasional shaking. Seven days later the liquid was filtered and marc (the solid residue) was pressed to recover as much solution as possible. The liquid so obtained was mixed well and then ethanol was allowed to evaporate. The dried extract obtained after complete evaporation of ethanol was collected. It was then kept in desiccators because of its hygroscopic nature.

2.3 Animals
Guinea pigs of either sex (150–200 g) were used for anti-asthmatic and antitussive experiments. Animals were housed at room temperature (22–24 °C) and constant humidity (50–60%) under a 12 h light-dark cycle. The experiments were performed during the light portion (0800-1600 h). The experimental protocol and procedures used in this study were approved by IAEC of Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan.

2.4 Anti-asthmatic activity against 0.1% histamine and 2% acetylcholine induce asthma
For performing anti-asthmatic test, guinea pigs were subjected to the sensitivity test. Guinea pigs were placed in a histamine chamber and sprayed with the mixture of 0.1% histamine and 2% acetylcholine chloride (1:1, w/v) under the average pressure of 450 ± 50 mm Hg for 15 sec. The time to onset of respiratory distress and tumble (preconvulsive time) was recorded and guinea pigs with preconvulsive time of less than 120 s were selected.

The eligible guinea pigs were randomly divided into six groups (n = 10), 1.5 h before the histamine and acetylcholine challenge different groups were treated orally with saline (control group), atropine 2 mg/kg, mepyramine 8.0 mg/kg and NATL 100, 200 and 300 mg/kg. Each guinea pig was observed for maximum of 6 min for the occurrence of convulsion and tumble (Xu et al., 1991).
2.5 Anti-tussive activity of ethanolic extract of leaves of Nyctanthes arbor-tristis against citric acid induced cough.
To screen the sensitivity, guinea pigs were placed in a histamine chamber and sprayed with 17.5% citric acid (w/v) under the average pressure of 450 ± 50 mmHg for 1 min (Chen, 1994). The period from the start to the onset of cough (latent period of cough) and frequency of cough was recorded. The frequency of cough between 10 and 30 were considered to be eligible. 1.5 h before the citric acid exposure, different groups were orally administered with saline (control group), codeine phosphate 20 mg/kg and NATL 100, 200 and 300 mg /kg. Each guinea pig was observed for 5 min for latent period of cough and frequency of cough.

2.6 Statistical analysis
Results were statistically analyzed by ANOVA, followed by the multiple comparisons by Turkey test. The results were expressed as mean ± standard error mean (SEM). P < 0.05 was considered significant.

RESULTS

3.1 Anti-asthmatic effect
The NATL have depicted anti-asthmatic effects against histamine and acetylcholine cocktail induced asthma (Table 1). When compared with control group all treatment groups viz. atropine, mepyamine, NATL 100, 200 and 300 mg/kg were found offering statistically significant (P<0.05) increases in preconvulsive time (latency in convulsion). Mepyamine, NATL 200 and 300 mg/kg were found to have statistically significant (P<0.05) increases in preconvulsive time (latency in convulsion) as compared to atropine treated group.

Table 1: Anti-asthmatic activity of Ethanol extract of leaves of Nyctanthes arbor-tristis against the mixture of 0.1% histamine and 2% acetylcholine induce asthma.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Preconvulsion Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>2 ml</td>
<td>106.23 ± 3.52</td>
</tr>
<tr>
<td>Atropine</td>
<td>2</td>
<td>95.80 ± 3.79</td>
</tr>
<tr>
<td>Mepyamine</td>
<td>8</td>
<td>87.25 ± 4.71</td>
</tr>
<tr>
<td>NATL I</td>
<td>100</td>
<td>88.35 ± 5.86</td>
</tr>
<tr>
<td>NATL II</td>
<td>200</td>
<td>86.35 ± 3.75</td>
</tr>
<tr>
<td>NATL III</td>
<td>300</td>
<td>89.42 ± 4.92</td>
</tr>
</tbody>
</table>
Guinea pigs were placed in a histamine chamber and sprayed with the mixture of 0.1% histamine and 2% acetylcholine chloride (1:1, w/v) under the average pressure of 450 ± 50 mm Hg for 15 sec. The time to onset of respiratory distress and tumble (preconvulsive time) was recorded. Values are expressed as the Mean ± SEM of ten observations.

“*” p<0.05 Statistical significance in comparison to control, comparisons are made between: control versus atropine, mepyamine, NATL 100, 200 and 300 mg/kg.

“#” p<0.05 Statistical significance in comparison to atropine, comparisons are made between: atropine versus mepyamine, NATL 100, 200 and 300 mg/kg.

NATL: Ethanolic extract of Leaves of *Nyctanthes arbortristis* Linn.

### 3.2 Anti-tussive effect

The NATL have depicted anti-tussive effects against citric acid induced cough (Table 2). As compared to control group all treatments viz. codeine phosphate, NATL 100, 200 and 300 mg/kg offered statistical significant (P<0.05) increases in latency in onset of cough. However, decrease in frequency of coughing was found statistically significant (P<0.05) only in NATL 200 and 300 mg/kg group when compared to control group (Figure 1).

*Figure 1: Antitussive activity of Ethanolic extract of leaves of *Nyctanthes arbortristis* Linn.*

against citric acid induce cough.
Guinea pigs were placed in a histamine chamber and sprayed with 17.5% citric acid (w/v) under the average pressure of 450 ± 50 mmHg. Each guinea pig was observed for 5 min for frequency of cough. Values are expressed as the Mean ± SEM of ten observations.

“*” p<0.05 Statistical significance in comparison to control, comparisons are made between: Control versus codeine phosphate, NATL 100, 200 and 300 mg/kg.

**DISCUSSION**

In the present study the ethanolic extract of leaves of *Nyctanthes arbor-tristis* L. (NATL) has been found to possess anti-asthmatic and anti-tussive activity. NATL has shown protection against histamine and acetylcholine aerosols induced asthma. The effect was comparable with conventional antihistaminic mepyramine and was more than that of atropine. The protection offered by the extract might be due to antagonism of H1 receptors, which produces prophylactic antiallergic effect (Gailen and Marshall, 2000). There is also a possibility of direct bronchodilation through atropine like activity (Thomas, 1977).
Similar results were also observed by Saxena et al (2002) where the extract protected the animal from bronchospasm and convulsions caused by histamine aerosol. NATL showed anti-tussive effect against the citric acid induced cough. Coughing is associated with asthma (Silbert, 1961). Anti-tussive effect of NATL possibly helps in the effective management of bronchial asthma and respiratory disorders in the ethnic community. The pharmacological action of NATL could be due to presence of phytoconstituents such as arbortristoside A and C. These constituents have been demonstrated the mast cell stabilizing activity in rats, which is an important target of newer anti asthmatic drugs (Gupta et al, 1995). Based on the results of present study it can be concluded that NATL possesses the anti-asthmatic potential. Although, the physiology of rodents may be different from that of humans but further studies and clinical trials may prove potential clinical implication of the present study and may provide alternative safe and economic way out to relieve the patients from asthma.

REFERENCES


