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Mast cell stabilizing and bronchodilatory activity of *Nyctanthes arbortristis* bark

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Abstract

Nyctanthes arbortristis Linn (Oleaceae) is one of the well known medicinal plant widely used in folk medicine for the treatment of asthma. Present work was undertaken to check potential of the plant bark in the treatment of asthma. Petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *N. arbortristis* bark (50 and 100 mg/kg, i.p.) were screened for mast cell stabilizing activity in mice and studies on smooth muscle preparation of guinea pig ileum (in vitro) for bronchodilation property. The petroleum ether extract of *N. arbortristis* bark showed maximum protection against mast cell degranulation by clonidine and resisted contraction induced by histamine better than other extracts. All the results are dose dependant. So it can be concluded that petroleum ether extract of *N. arbortristis* bark have bronchodilation and mast cell stabilizing property and can be used in the treatment of asthma..

Keywords: *Nyctanthes arbortristis;* mast cell degranulation; guinea pig ileum; bronchodilation

Introduction

Nyctanthes arbortristis Linn (Oleaceae) is one of the well known medicinal plant. It is commonly called as nyctanthes means night flowering and arbortristis means as it loses its brightness during day time. It is common wild hardy large shrub or small tree, native to India, distributed wild in sub-Himalayan regions and southwards to Godavari. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers (Kiew & Baas, 1984; Nadkarni, 1982; Kirtikar & Basu, 2000). It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs. Leaves are opposite, ovate, acute or acuminate, entire or with few large distant teeth, short bulbous hairs rounded or slig-

ht cuneate. Flowers are small, delightful fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are white. Fruits are a capsules of 1-2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one seeded carpels, reticular veined and glabrous.

Leaves of *N. arbortristis* are responsible for some CNS activities like hypnotic, tranquilizing and local anesthetics (Saxena et al., 2002; Ratnasooriya et al., 2005; Das et al., 2008) and antiast-hmatic activity (Nirmal et al., 2011). Fresh juice of leaves is antimalerial (Badam et al., 1988; Aminuddin et al., 1993). Antifungal activity of the leaves was established against *Alrernaria alternate* (Chauhan, 1978). Aqueous extract of leaves is proved to be hepatoprotective (Chauhan, 1978). Kiew and Baas (1984) isolated an alkaloidal principle named nyctanthin form the leaves. Iridoid glucosides [arbortristosides A (Figure 1), B, C and 6-β-hydroxy-loganin] were isolated from the plant and have antileishmanial activity (Tandon et al., 1991). A new iridoid glycoside along with nyctanthic acid, oleanolic acid, friedelin, 6-β-hydroxy-loganin and arbortristoside A has been isolated from the plant (Rathore et al., 1989). A minor iridoid glucoside, arborside D and its acetyl derivatives were identified from the plant (Singh et al., 1995).

Traditionally the plant is used in the treatment of asthma and cough. Histamine, present in mast cells is responsible for asthma hence present work was undertaken to check mast cell stabilizing property and bronchodilatory property of the extracts of *N. arbortristis* bark and in this way to check its application in the treatment of asthma.

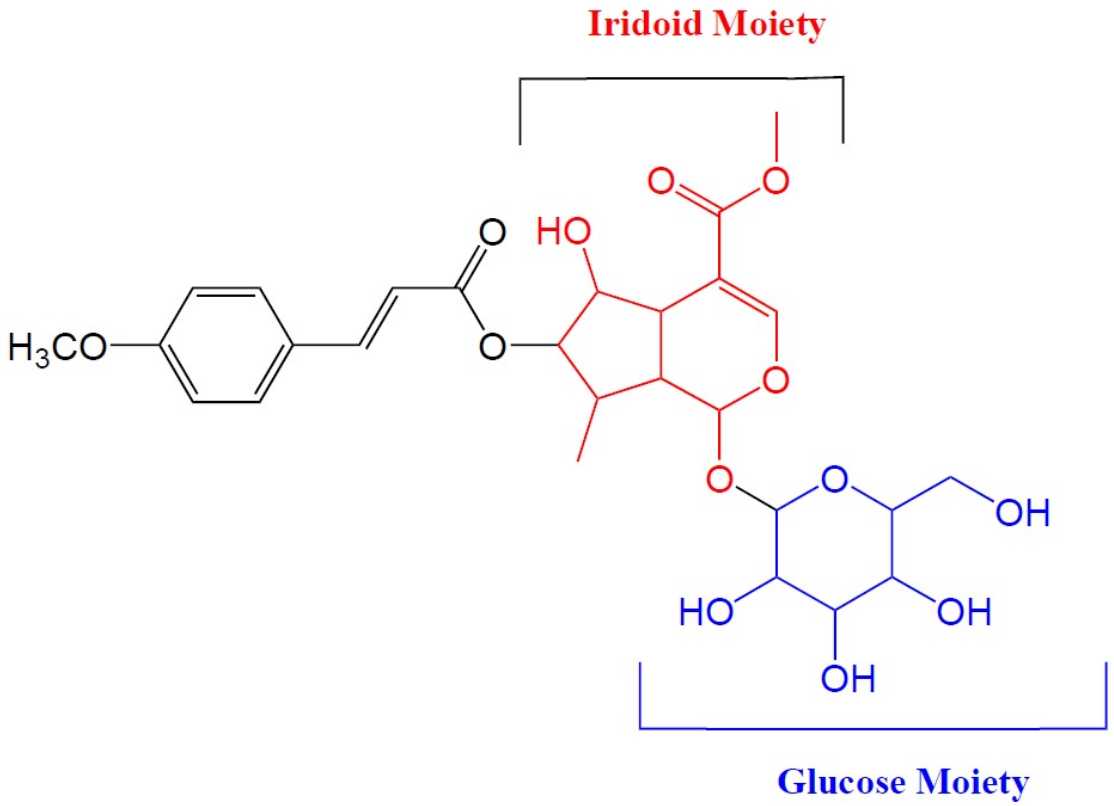

Figure 1. Chemical structure of a typical Iridoid glycoside (Arbortristoside A).

Materials and methods

Plant material

Bark of *N. arbortristis* was collected from Ahmednagar district of Maharashtra in August 2007 and authenticated by Dr. P.S.N. Rao, Botanical Survey of India, Pune, where a sample specimen (voucher number: Nirmal-1) has been deposited.

Extraction

Dried and powdered bark of the plant was extracted successively with various solvents viz. petroleum ether, chloroform, ethyl acetate and ethanol in Soxhlet extractor. The mark left was extracted using water as solvent. Extracts were concentrated by vacuum distillation and then dried in open air to produce respective extracts.

Animals

Male Swiss mice (20-25 g) were used for mast cell stability studies and male guinea pigs (250-350 g) were used for antihistaminic action on isolated ileum. The animals were housed under standard laboratory conditions and fed with standard rodent diet and water *ad libitum*. Rodent diet is composed of crude proteins 16%, crude fats 3.8%, crude fibers 2%, amino acids, vitamins and minerals. The animals were kept in constant temperature (22 ± 2°C), humidity (55%) and light-dark condition (12/12 h light/dark). The experimental protocol was approved by institutional animal ethical committee.

Drugs and chemicals

The following drugs and chemicals were used. Drugs: disodium chromoglycate purchased from commercial source. Chemicals: Petroleum ether (60-80^{o}C) AR, chloroform AR, ethanol AR, ethyl acetate AR, tween 80 AR and RPMI 1640 buffer (pH 7.2-7.4) were purchased from PCL, India.

Mast cell degranulation study

The method was described by Gupta et al (1994) and Lakadawala et al (1980). Mice were divided into twelve groups, six animals in each group. A three day drug treatment schedule was followed as group-I received vehicle (5 ml/kg, i.p.). Group-II was treated with standard drug disodium chromoglycate (200 ug/kg, i.p.). Whereas animals belonging to other groups received petroleum ether extract, chloroform extract, ethyl acetate extract, ethanol extract and aqueous extract of *N. arbortristis* bark (50 and 100 mg/kg, i.p., each). On the fourth day, each animal was injected with 0.9% saline solution (4 ml/kg) in to peritoneal cavity. By gentle massage, peritoneal fluid was collected after 5 min and transferred in to siliconised test tube containing 7-10 ml RPMI 1640 buffer medium (pH 7.2-7.4). This solution was then centrifuged at 400-500 rpm. Pellets of mast cells were washed with same buffer medium twice by centrifugation, discarding supernatant. These cells were challenged with clonidine (50 µg), incubated at 37°C in a water bath for 10 min followed by staining with 1% toludine blue and observed under microscope (45X). A total of 100 cells were

counted from different visual area. Percent protections against degranulations were calculate-ed.

Studies on smooth muscle preparation of guinea pig ileum (in vitro)

Paranjape and Mehta (2008) have described this method. Guinea pigs were sacrificed by a sharp blow over the head, abdomen was opened and ileum was dissected out and a small piece of 2-3 cm was taken from portion situated 15 cm proximal in the ileo-caecal junction and suspended in Tyrode solution (NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, $NaHCO_3$ 1.0, NaH_2PO_4 0.05 and glucose 1 gm/lit) and continuously aerated and maintained at 37 ± 0.5^0C. The tissue was allowed to equilibrate for 30 min under a load of 500 mg. After equilibration period a contact time of 30 sec and 5 min time cycle was followed for recording the response of histamine by using frontal writing liver.

After obtaining a dose response curve of histamine on ileum, petroleum ether extract (100 µg/ml) of *N. arbortristis* bark was added to the reservoir containing Tyrode solution and a response of tissue was recorded for the same dose of histamine (0.1, 0.2, 0.3 and 0.4 µg/ml) to obtain inhibition curve. After several washings, chloroform, ethyl acetate, ethanol and aqueous extracts of *N. arbortristis* bark was added to the reservoir containing Tyrode solution and same procedure was followed to obtain inhibition curve separately. Graph of percentage of maximum relaxation response versus concentration of histamine was plotted to record dose response curve of histamine in presence various extracts of *N. arbortristis* bark.

Results

Mast cell degranulation study

Table 1 show that petroleum ether extract of *N. arbortristis* bark showed maximum protection against mast cell degranulation by clonidine. The results are significant and dose dependant compared with control group. Histopathology of granulated mast cells was also observed (Figure 2).

Table 1. Effect of various extracts of *N. arbortristis* bark on mast cell stabilizing activity.

Treatment	Dose (mg/kg, i.p.)	% protection
Disodium chromoglycate	200 µg/kg	74.00 ± 1.9
Petroleum ether extract	50	47.2±2.7
	100	54.5±1.8
Chloroform extract	50	18.5±1.3
	100	22.4±1.5
Ethyl acetate extract	50	19.5±1.3
	100	25.4±1.5
Ethanol extract	50	27.5±0.9
	100	30.8±0.8
Aqueous extract	50	21.5±1.3
	100	27.4±1.5

All the data is expressed as mean ± SEM, n=6 in each group.

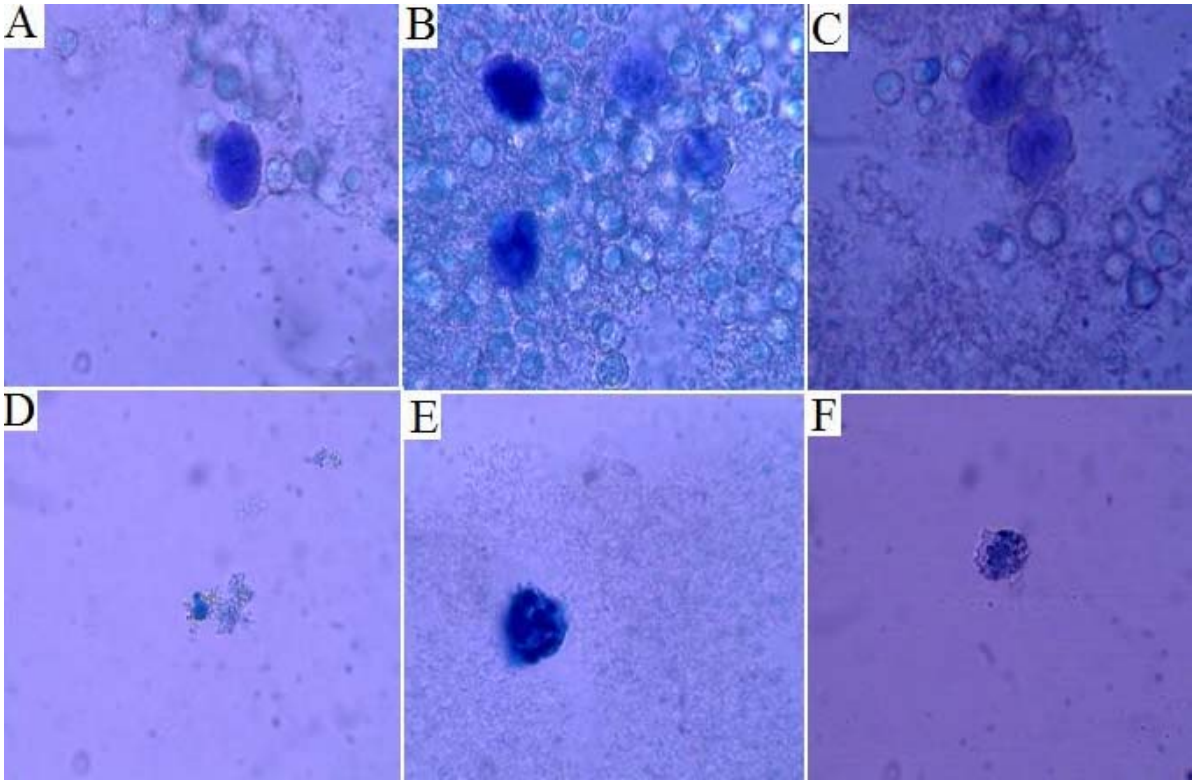

Figure 2. Effect of various extracts of *N. arbortristis* bark on mast cell degranulation (Histopathology). A. Non granulated mast cell, B. Granulated and non granulated mast cells, C. Partially granulated cells, D. Mast cell with degranulation, E. Incomplete granulation, F. Incomplete granulation.

Studies on smooth muscle preparation of guinea pig ileum (in vitro)

Histamine (0.1-0.4 µg/ml) causes contraction of guinea pig ileum. Petroleum ether extract (100 µg/ml) of *N. arbortristis* bark resisted contraction induced by various concentrations of histamine (0.1-0.4 µg/ml) better than other extracts. Petroleum ether extract showed better relaxation of guinea pig ileum in histamine induced contraction than other extracts (Figure 3).

Discussion

Asthma is a chronic inflammatory disease of the airways in which many type of cells play a role, more important being mast cells, eosinophils and T-lymphocytes. Mast cells play the main role in the early stages of type I hypersensitivity reactions. Mast cell releases mediators such as histamine, which plays central role in the pathogenesis of asthma (Geetha et al., 1981). In asthma there is rapid activation of airway mast cells, which in turn rapidly releases pro-inflammatory mediators such as histamine and eicosonoids, which induce contraction of airway smooth muscle and mucus secretion. Mast cells are widely distributed in the connective tissue with a preferential localization adjacent to small blood vessels. The mast cells contain basophile granules literally loaded with active substances which causes vascular and other tissue reactions similar to those characteristic of inflammatory processes (Uvnas, 1969). Lakadawala *et al.* (1980) have shown that clonidine releases histamine from

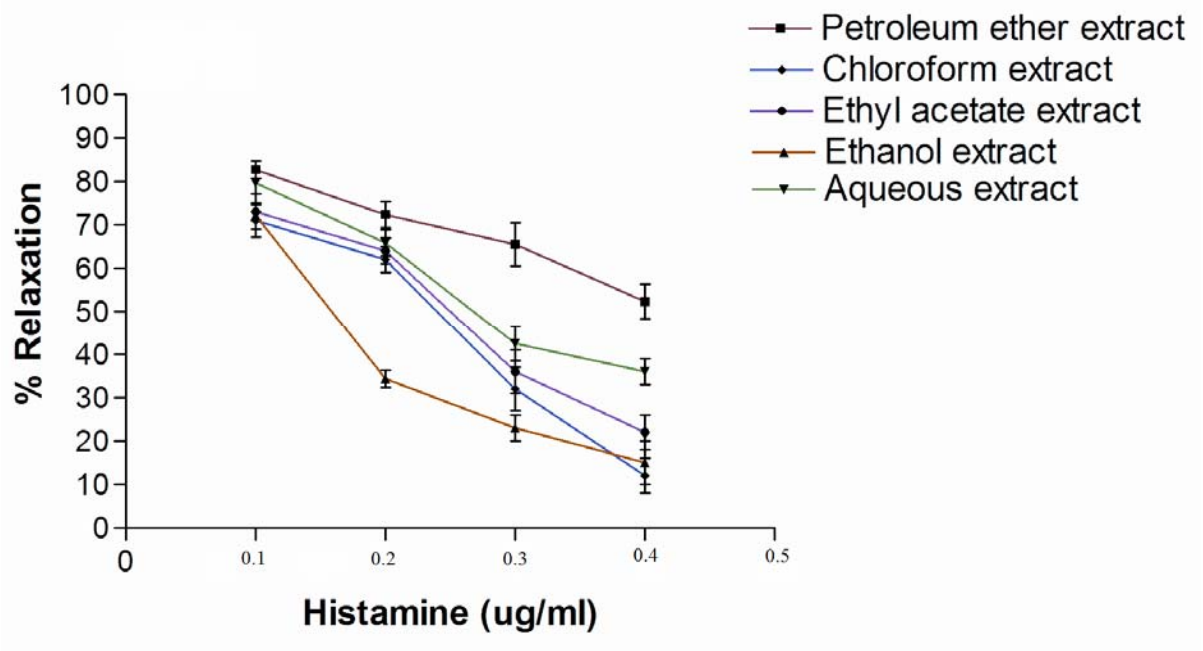

Figure 3. Effect of *N. arbortristis* bark extracts on histamine- induced contraction on guinea pig ileum.

mast cells in a similar manner to a selective liberator like compound 48/80. It is known that disodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate (Bertelli et al., 1973). It has been known that all pharmacological agents that increase intracellular levels of cAMP relax airway smooth muscle and inhibit the release of autocoids from the tissue and basophils (Yuk et al., 2007).

The results of guinea pig ileum indicated that the petroleum ether extract of *N. arbortristis* bark relaxed significantly the ileum muscle strips pre-contracted by histamine which suggests the involvement of β_2-agonists on the relaxation of the tissue. The ability of the petroleum ether extract of *N. arbortristis* bark to inhibit the contraction induced by the bronchoconstrictor histamine suggests a possible role in the treatment of asthma. Furthermore, the relaxation of histamine pre-contracted ileum by the petroleum ether extract of *N. arbortristis* bark indicates their potency in ameliorating established asthma. Airway responsiveness in asthma is attributed in part to changes in autonomic regulation particularly increased parasympathetic activity (Rang and Dale, 2003). Airway obstruction/bronchoconstriction or airway hyper-responsiveness in asthma are believed to be a direct consequence of airway wall inflammation (Holt et al., 1999; Prasad et al., 2000). *N. arbortristis* bark extract exhibited moderate degree of anti-inflammatory activity (Das et al., 2006).

Mechanisms that possibly underlie this anti-inflammatory activity include inhibition of the actions of inflammatory mediators such as histamine, effect on adernocorticoid hormone and immunosuppressi-on. Inhibition of immunosuppression also incorporates inhibition of the activity of proinflammatory mediators. Crude extracts of the plant *N. arbortristis* can show anti-allergic effect because another iridoid glycoside, picroliv is structurally similar to the iridoid glycosides isolated from *N. arbortristis.* Picroliv showed good antiallergic and

immunomodulating effect (Baruah et al., 1998; Sinha et al., 1998; Puri et al., 1992). It is reported that Iridoid glycosides isolated from *N. arbortristis* shows hepatoprotective activity. Again picroliv is exten-sively reported as hepatoprotective compound (Shukla, 1991; Saraswat et al., 1997). This aspect provides a clue to the potential safety of crude extract and its chemical constituents especially the Iridoid glycosides. Picroliv obtained from the roots and rhizomes of *Picrorhiza kurroa*, is reported to increase the nonspecific immune response and to induce a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters (Puri et al., 1992). Picroliv has also demonstrated an ability to prevent the liver damage experienced by laboratory animals as a side effect commonly associated with the use of sodium stiboglucon-ate (Pentostam®) (Mittal et al., 1998).

This proposed mechanism is consistent with previous findings that anti-inflammatory plant principles have shown to act through control of adrenocorticoid hormone and immunosuppression, respectively (Barik et al., 1992; Singh et al., 1997). Inherent anti-inflammatory activity is a desirable property of a putative anti-asthmatic agent, since asthma is a complex chronic inflammatory disease of the airways. The results of this study showed mast cell stabilizing and a relatively potent relaxant (bronchodialatory) effect of petroleum ether extract of *N. arbortristis* bark on the ileum of the guinea pig. These activities justify the traditional use of this plant in the treatment of bronchoconstrictive diseases. Detailed investigation of the efficacy, isolation of the active ingredient and further toxicity study may warrant the development of the plant extract in to proper drug.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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