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Antiallergic and Anti-asthmatic Activities of the Alcoholic Extract of *Terminalia arjuna* and Arjunolic Acid

M.V.V. Prasad[1], N. Anbalagan[2], A. Patra[3], G. Veluchamy[4], and K. Balakrishna[4]*

[1]*Department of Pharmaceutical Chemistry, Dr. HCT College of Pharmacy, Bangalore-571 502, Karnataka, India*
[2]*Department of Pharmaceutical Chemistry, C. L. Baid Metha College of Pharmacy, Thorapakkam, Chennai-600 096, Tamil Nadu, India*
[3]*Department of Chemistry, University College of Science, Kolcutta-700 009, West Bengal, India*
[4]*Central Research Institute for Siddha, Arumbakkam, Chennai-600 106, India*

Abstract – In the present study, the alcoholic extract of Terminalia arjuna (TA) and Arjunolic acid (AA) were studied for its anti-asthmatic and anaphylactic activity. Treatment with TA (250 & 500 mg/kg) and AA (50 &100 mg/kg) has shown significant protection against mast cell disruption in rats induced by compound 48/80. TA and AA also protected the guinea pig against histamine as well as acetylcholine induced bronchospasm. Both TA & AA exhibited better protection against histamine release than against acetylcholine release. Anti-asthmatic and anaphylactic activity may be possibly due to membrane stabilizing potential and inhibition of antigen induced histamine and acetylcholine release.
Keywords – Anti-asthmatic, anti-histaminic, terminalia arjuna, and arjunolic acid

Introduction

Asthma is a chronic disease, involving hypersensitivity reaction of the lower respiratory system producing obstruction of the airways (Barnes *et al.*, 1996). It is a disease that does not discriminate race, age and gender and about 155 million were affected world wide (Cokson *et al.*, 1999). Despite the advancements in asthma therapy, the number of deaths from asthma is rising (Keating *et al.*, 1984) and this necessitates use of newer drugs to treat asthma.

Terminalia arjuna (Roxb. ex DC) (Family-Combretaceae) is known as Arjuna in Indian Medicine. In the traditional systems of medicine, it is used in the treatment of diseases such as cardiac failure, cirrhosis of liver, asthma, bronchitis, cough, tumors, inflammations, diabetes, piles, leucorrhoea (Nadkarni and Nadajarni, 1976; Tripathy *et al.*, 1996; Sharma *et al.*, 2001).

The plant has been shown to contain tannins, triter-penoids, flavonoids, gallic acid, ellagic acid, mannitol and phytosterols (Tripathy *et al.*, 1996; Sharma *et al.*, 2001). The arjunolic acid is the major triterpenoid constituent. Pharmacological studies showed cardiac, antifertility, antioxidant, antibacterial, antifungal, anti HIV and anti neoplastic activities (Tripathy *et al.*, 1996; Sharma *et al.*, 2001).

As the plant has not been screened for anti-asthmatic property, we have made a preliminary study on this activity for both alcoholic extract of the stem bark of the plant as well as on arjunolic acid.

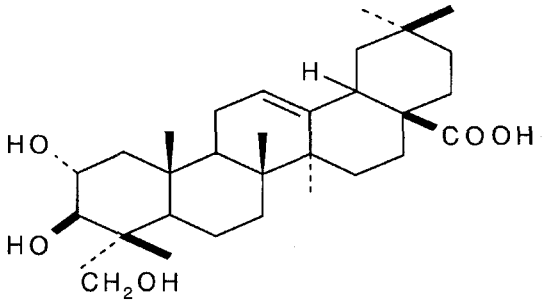
Materials and Methods

Plant material – The stem bark of the plant was collected from Palayamkottai, Tamil Nadu, India, during October 2003 and was authenticated by Dr S. Usman Ali, Department of Botany, Central Research Institute for Siddha, Arumbakkam, Chennai-106. (Voucher specimen no. 128/03, deposited at the herbarium of the institute.)

Extraction – Dried and powdered stem bark of the plant (2 Kg) was extracted with alcohol in the cold (48 hr). The extract was filtered and distilled to a syrupy mass and dried in vacuum (Yield 150 gm).

Isolation of arjunolic acid (1) – The alcoholic extract of the plant material (150 gm.) was chromatographed over silica gel (100-200 mesh) and eluted with solvents of increasing polarity. Elution of the column with chloroform - methanol (9:1) gave crude arjunolic acid which was recromatographed over silica gel. The compound (1), obtained was crystallized from aqueous methanol. (m.pt.

*Author for correspondence
Fax: +914424960425; E-mail: anbupharm@yahoo.com



Arjunolic acid [1]

Fig. 1. Arjunolic acid[1].

338°C, (decomposing), yield 2.5 gm). It gave a single spot on TLC over silica gel G with chloroform - methanol (9:1) as the developing solvent ($R_f - 0.5$) The structure was further confirmed by comparison of spectroscopic data with the reported values (Lalith Jayasinghe *et al.*, 1993).

Animals – Male albino rats (120-150 gm) and guinea pigs (400-600 gm) of either sex were obtained from animal house, C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained at room temperature of 25 ± 2°C with relative humidity of 75 ± 5%, under 12 hours dark and light cycle. The animals were given standard lab diet and water ad *libitum*.

Mast cell stabilizing activity - Compound 48/80 (Sigma Chemicals Co., USA), a condensation product of p-methoxy-N-methyl phenyl amine was used as potent histamine releaser in the non-immunological type of mast cell degranulation.

Thirty-six albino rats were taken and grouped in to six containing six rats in each group. Group I received only 1% Sodium Carboxy Methyl Cellulose [SCMC] suspension and served as solvent control. Group II and III animals received the alcoholic extract of *Terminalia arjuna* [TA] 500 mg and 250 mg/kg/bw/po in 1% SCMC suspension respectively. Group IV and V animals received arjunolic acid [AA] 50 mg and 100 mg/kg/bw/po in 1% SCMC suspension respectively. Group VI animals received Disodium cromoglycate [DCG] 10 mg/kg/bw/po in 1% SCMC for six days. On the seventh day, all the animals were sacrificed; intestines were removed and kept in Ringer-Locke Solution. Mast cells from normal and treated groups were incubated with compound 48/80 (1 μ gm/ml) at 37°C for 10 minutes. After incubation, mast cells were stained with toluidine blue (0.1%) and percent degranulation was counted under a high power microscope (Gupta *et al.*, 1995).

Allergic asthma and histamine, acetylcholine aerosol test – Experimental bronchospasm was induced by exposing the guinea pigs to histamine acid phosphate 0.25% or acetylcholine chloride 10% under a constant pressure 40 mm/Hg from the inbuilt nebulizer of the histamine chamber (Jena *et al.*, 1994; Mitra *et al.*, 1999).

Prior to drug treatment, the animals were placed in the histamine chamber and exposed to aerosol of histamine acid phosphate (0.25%) or acetylcholine chloride (10%) under a constant pressure of 40 mm Hg from the inbuilt nebuliser. The preconvulsive time (PCT) was determined from the time of exposure to the onset of dyspnoea leading to the appearance of convulsion whish is known as preconvulsive dyspnoea (PCD). As soon as the PCD were noted, the animals were removed from the chamber and placed in fresh air. Two and half hours later, the animals of group I (n=10) received 1% SCMC suspension, out which five animals exposed to histamine acid phosphate and five exposed to acetylcholine chloride. Group II (n=10) received alcoholic extract of *Terminalia arjuna* (250 mg/kg/bw/po), out which five animals ex-posed to histamine acid phosphate and five exposed to acetylcholine chloride. Group III (n=10) received alcoholic extract of *Terminalia arjuna* (500 mg/kg/bw/po), out which five animals exposed to histamine acid phosphate and five exposed to acetylcholine chloride. Group IV (n=10) received arjunolic acid (50 mg/kg/bw/po), out which five animals exposed to histamine acid phosphate and five exposed to acetylcholine chloride. Group V (n=10)) received arjunolic acid (100 mg/kg/bw/po), out which five animals exposed to histamine acid phosphate and five exposed to acetylcholine chloride. Group VI (n=5) received chlorpheniramine maleate (2 mg/kg/bw/po) and Group VII (n=5) received atropine sulphate (2 mg/kg/bw/po). The animals were subjected to histamine or acetylcholine challenge. One and a half hours after receiving the drug and the PCT was noted. Protection afforded by the drug is calculated by $(1\text{-}T_1/T_2\times100)$ where T_1 is a mean of control preconvulsion time

Table 1. Effect of alcoholic extract of Terminalia arjuna and Arjunolic acid on compound 48/80 induced degranulation of mast cells

Groups (mg/kg/bw)	Treatment	Dose	%degranulation
I	Vehicle	-	62.32 ± 13.81
II	TA	250	51.12* ± 3.01
III	TA	500	39.09* ± 2.41
IV	AA	50	42.18* ± 2.86
V	AA	100	33.62* ± 2.08
VI	DCG	50	22.62* ± 2.38

Significant level: *p<0.01 when compared to control.

Table 2. Effect of the alcoholic extract of Termininalia arjuna and Arjunolic acid on histamine and acetycholine induced bronchospasm

Group	Treatmet [n]	Dos [mg/kg]	Increase in mean time of exposure to histamine [in seconds]	Increase in mean time of exposure to histamine [in seconds]	%Protection against histamine challenge	%Protection against acetycholine challenge
I	1%SCMC	0.1 ml	8.2 ± 2.4	3.4 ± 6.2	---	---
II	TA[10]	250	265*.4 ± 6.2	72*.6 ± 4.8	28	25
III	TA[10]	500	3.78*.5 ± 5.5	96*.8 ± 3.9	40	32
IV	AA[10]	50	4.63*.5 ± 6.1	120*.1 ± 8.5	49	40
V	AA[10]	100	5.93*.2 ± 1.8	148*.4 ± 6.6	64	51
VI	CM[5]	2	751*.4 ± 2.5	NT	81	---
I	AS[5]	2	NT	210*.0 ± 5.4	---	72

N.T = Not Treated. All Values are mean ± SEM. *p <0.01 when compared to control (Dunnett's multiple comparisions).

and T_2 is post-treatment convulsion time.

Statistical analysis – The results are expressed in mean SEM. Student 't' test used in case of mast cell stabilization activity and one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was used in bronchospasm test.

Results and Discussion – Mast cells present in lungs release mediators such as histamine, acetylcholine, Slow Reacting Substances [SRS] etc., and are responsible for the constriction of bronchial smooth muscles, which in turn causes mucosal edema and viscid secretion and all of them collectively responsible for bronco constriction (Nagarajan *et al.*, 1996).

Under experimental condition, control group that received only compound 48/80 (p-methoxy-N-methyl ph enethyl amine) per se produced about 62% of degranulation (Table 1). But the animals treated with 250 & 500 mg/kg/bw of alcoholic extract of Ter-minalia arjuna exhibited 51% & 39% degranulation respectively. Whereas the triterpenoid arjunolic acid 50 & 100 mg/kg/bw shown 42% and 33% of degranulation respectively. The standard drug Disodium chromoglycate 50 mg/kg/bw produced on ly 22% of degranulation. The above findings reveals that the alcoholic extract of Terminalia arjuna and arjunolic acid has significant mast cell stabilization activity especially arjunolic acid exhibited comparatively better stabilization activity than the alcoholic extract of Terminalia arjuna.

When an antigen reacts with Ig E present on the surface of the mast cells, promotes antigen-antibody reaction, th ereby releasing substances like histamine, acetyl choline, SRS, serotonine etc., (Person *et al.*, 1982). Histamine is the most implicated mediator in broncho constriction, that accompanies asthma (Summers *et al.*, 1981). Acetylcholine on its own can cause bronco constriction by activating efferent cholinergic fibers, secondary to the stimulation of the sub-epithelical mediators such as histamine (Akah *et al.*, 2003; Flenley *et al.*, 1990). The alcoholic extract of *Terminalia arjuna* at 250 & 500 mg/kg/bw showed 28% & 40% pro-tection respectively, against histamine challenge and 25% & 32% protection respectively against acetylcholine challenge (Table 2). But arjunolic acid 50 & 100 mg/kg/bw showed 49% & 64% protection re spectively, against histamine challenge and 40% & 51% protection res-pectively against acetylcholine challenge. The sta-ndard drugs chlorpheniramine maleate [CM] (2 mg/kg/bw) produced 81% protection against hi stamine challenge and Atropine sulphate[AS] (2 mg/kg/bw) produ ced 72% protection against acetylcholine challenge.

The above study suggests that both alcoholic extract of *Terminalia arjuna* and arjunolic acid showed greater % of protection against histamine challenge than against acetylcholine challenge.

All this findings reveal that the antiasthmatic and an tianaphylactic activity of *Terminalia arjuna* and arjunolic acid may be due to the mast cell stabilizing potential and inhibition of antigen induced histamine and acetylcholine release.

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