Research Article

PHARMACOLOGICAL INVESTIGATION OF ANTIASTHMATIC ACTIVITY OF FRUIT EXTRACT OF *PHYLLANTHUS EMBLICA*

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Accepted on: 10-10-2012; **Finalized on:** 30-11-2012.

ABSTRACT

The present investigation was carried out to evaluate antiasthmatic activity of fruit extract of *Phyllanthus emblica*. Antiasthmatic activity of methanolic extract of fruits of *P. emblica* was evaluated in guinea pigs and rats. Bronchodilating activity of fruit extract was evaluated against 0.1% histamine aerosol induced bronchospasm in guinea pigs. Methanolic extract of *P. emblica* in dose of 200, 400 and 600 mg/kg were administered orally. The mast cell stabilizing activity of methanolic extract of *P. emblica* fruits (2, 4 and 6mg/ml) and anti-anaphylactic activity of methanolic extract of *P. emblica* fruits (200, 400 and 600mg/kg) were investigated against compound 48/80-induced mast cell degranulation and egg albumin induced anaphylaxis in rats, respectively. Anti-inflammatory activity of methanolic extract of *P. emblica* fruits (200, 400 and 600mg/kg) was investigated against carageenan induced paw edema in rats. Treatment with methanolic extract of *P. emblica* showed significant (**P<0.01) protection against histamine aerosol induced bronchospasm in guinea pigs. Methanolic extract of *P. emblica* fruits dose dependently protected the mast cell degranulation induced by compound 48/80. Significant inhibition (*P<0.05, **P<0.01) of egg albumin sensitized paw edema was observed and significant inhibition (*P<0.05, **P<0.01) carageenan induced paw edema was observed by administration of methanolic extract of *P. emblica* as compared to control. All these results suggest that methanolic extract of *P. emblica* fruits has not only bronchodilating activity but also mast cell stabilizing activity, antianaphylatic activity & anti-inflammatory activity, which could be helpful in preventing asthmatic attacks.

Keywords: Antiasthmatic activity, *Phyllanthus emblica,* Bronchodilating activity, Anti-inflammatory activity.

INTRODUCTION

Bronchial Asthma according to the GINA guidelines final update November 2006 is clearly defined as: "A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning." These episodes are associated with airflow obstruction within the lung that is often reversible either spontaneously or with treatment.[1] Asthma is reported in 1.2% to 6.3% adults in most of countries. The overall burden of asthma in India is estimated at more than 15 million patients. Asthma in adults is generally reported as 2.7 to 4.0% in most European countries, 12% in England, 7.1% in United States and higher 9.5 to 17.9% in Australia.[2] Many synthetic medicines are used to treat acute symptoms of asthma, but they are not completely safe for long term use.[3] Herbal drug, as per World Health Organization, today about 80 % of people in developing countries still relays on traditional drug based largely on species of plants and animals for their primary health care.[4]

Medicinal plant selected for the present investigation is *Phyllanthus emblica (P. emblica)/ Emblica officinalis (E. officinalis)* of family Euphorbiaceae. It is easily available & well known plant in India. All parts of plant are useful because they have great medicinal value: Fruits are reported to be appetizer, diuretic, antibacterial, antifungal, antioxidant, laxative and antidiabetic; Leaves have antipyretic, anti-inflammatory and antiulcer activity; Seeds have antipruritic action; Roots are used as analgesic for toothache, Bark has astringent and antigonorrhoeal action. Chemical constituents of *P. emblica* fruits are flavonoids, saponins, tannins, glycosides & phenolics which have antiasthmatic activity. Traditionally fruit juice is used with other ingredients in asthma.[5] However, so far no studies have been reported, and evaluating antiasthmatic activity of fruits of *P. emblica* so, present investigation was carried out to investigate antiasthmatic activity of fruits extract of *P. emblica*.

MATERIALS AND METHODS

Collection and identification of plant

Fruits of *P. emblica* were collected from forest of Wan Vikas Nigam, Baroda, Gujarat (India). Fruits were identified and authenticated by Dr. P. S. Nagar, Botany Department of M.S. University, Baroda, Gujarat, India. A voucher specimen (Number: BCP/2012-01/09) was deposited in 'BARO' the herbarium of department of Botany, M.S. University, Baroda. Certificate of authentication was submitted in Baroda College of Pharmacy. Shade dried fruits' extract was used for the study.

Preparation of Extract

Fruits were cut and shade dried and powdered to 40 mesh and stored in air-tight container till further use. Methanolic extract was prepared with the help of Soxhlet apparatus and solvent was evaporated at 40°C in rotary evaporator.[6]

Identification Tests for Major Constituents

Test for Tannins: Extract was treated with few drops of 1% ferric chloride solution. Colour was observed.[7]

Test for Flavonoids: Extract was added to 5-6 drops of conc. HCl and 1.5 ml of methanol solution. Colour was observed.[7]

Test for Phenolics: Two ml of ethanol was added to the extract and few drops of ferric chloride solution. Colour was observed.[7]

Test for Saponins: Two ml of distilled water was added to extract and shaken well and observed for frothing.[7]

Test for Glycosides: Extract was mixed with 2 ml chloroform. Then 2ml acetic anhydride and 2 drops of conc. H_2SO_4 were added from the side of test tube. Colour was observed.[7]

TLC identity test: Mobile phase was prepared by a mixture of 20 volumes of toluene, 45 volumes of ethyl acetate, 20 volumes of glacial acetic acid and 5 volumes of formic acid. Test solution was prepared by 2 g of the coarsely powdered substance under examination refluxed with 50-75 ml of methanol for 15 minutes, cooled and filtered. The residue was refluxed further for two times with 75 ml of methanol, cooled and filtered. Combined all the filtrates and concentrated under vacuum to 50 ml. Reference solution was prepared by 0.4 g of the coarsely powdered fruit refluxed with 50-75 ml of methanol for 15 minutes, cool and filter. The residue was refluxed further for two times with 75 ml of methanol, cooled and filtered. Combined all the filtrates and concentrated under vacuum to 10 ml. 10 µl of each solution as bands 10 mm by 2 mm applied to the silica gel GF254 plate. The mobile phase allowed to rise 8 cm. The plate was dried in air and examined in ultraviolet light at 254 nm and 365 nm, sprayed with anisaldehyde sulphuric acid reagent. R_f value calculated and compared of both standard and test solution.[8]

Animals

Guinea pigs of either sex were acquired from Anand Agricultural University, Anand and Wistar albino rats of either sex were acquired from Flair Labs, Surat. Animals were then acclimatized to the experimental room having ambient temperature (23±2°C), controlled humidity (55±5%) conditions, and 12:12 hour light and dark cycle. Animals were caged in polypropylene cages with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. Study was conducted after obtaining approval (Protocol no. 984/11/15) by Institutional Animal Ethics committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Acute toxicity study (OECD 423)

Six female wistar rats weighing 150-175 gm were maintained under standard husbandry conditions, were used for two sets of experiments in groups of three animals. Both of the groups were fasted overnight before the test. The first group was administered 2000 mg/kg body weight of freshly prepared methanol extract of *Phyllanthus emblica* suspended in 1% carboxy methyl cellulose (CMC), while the other group was given an equivolume of 1% carboxy methyl cellulose solution. The animals were observed individually immediately after dosing and then at least once during first 30 min, periodically during the first 24 hours and daily thereafter, for total of 14 days for behavioural, neurological and autonomical changes. Animals observed for mortality.[9]

Bronchodilating activity on guinea pigs

The guinea pigs were kept in a closed chamber and exposed to an aerosol of 0.1% histamine hydrochloride and time for preconvulsion dyspnoea was noted. As soon as preconvulsion dyspnoea (PCD) commenced, animals were removed from the chamber and placed in fresh air to recover. This time for PCD was taken as basal value. After 15 days of wash out period the same animals were randomly divided into four groups each containing six animals. Group-I received 1% sodium CMC, Group-II received Ketotifen 1mg/kg P.O. (Standard), Group-III, IV and V received methanolic extract of *P. emblica* in divided doses of 200mg/kg, 400mg/kg and 600mg/kg respectively. The suspension of drugs was prepared in 1% CMC administered orally. Two hours after the drug treatment, animals were exposed to histamine aerosol and times for PCD noted. The effect of drug was calculated by following formula:

$$\% \text{ increase in PCD time} = [1-T1/T2] \times 100$$

T1 = time for PCD onset on day 0 and T2 = time for PCD onset after drug treatment.[10-15]

Mast cell stabilization activity

The animals were sacrificed and the pieces of mesentry were collected in petri dish containing Ringer Locke solution and then subjected to treatment schedule: Petri dish no. 1 - Ringer Locke solution (Positive control); Petri dish no. 2 - 0.1ml of Ketotifen fumarate (10 µg/ml); Petri dish no. 3 - 0.1ml of test agent in 1% sodium CMC (*P. emblica*, 2mg/ml); Petri dish no. 4 - 0.1ml of test agent in 1% sodium CMC (*P. emblica*, 4 mg/ml); Petri dish no. 5 - 0.1ml of test agent in 1% sodium CMC (*P. emblica*, 6 mg/ml). Each petri dish was incubated for 15 min at 37°C. Later, compound 48/80 (0.1ml, 10 µg/ml) was added to each petri dish and again incubated for 10 min. at 37°C. After that, all pieces were transferred to 4% formaldehyde containing 0.1% toluidine blue and kept a side for 15-20 min. After staining and fixation of mast

cells, mesentry pieces were transferred through acetone and xylene two times and mounted on slides. All the pieces were examined under the high power of light microscope. Percentage protection of the mast cells in the control group and the treated groups was calculated by counting number of degranulated mast cells from total of at least 100 mast cells counted. Percentage inhibition of mast cell degranulation for each treatment was calculated by following formula:[16-20]

$$\%inhibition\ of\ MCD = \frac{1 - Number\ of\ degranulated\ mast\ cells}{Total\ no.\ of\ mast\ cells} * 100$$

Passive paw anaphylaxis

Albino Wistar rats were given subcutaneously three doses of 100 mg of egg albumin on day 1, 3 and 5. On day 10 of sensitization, blood was collected and centrifuged to separate serum. Animals were divided in to five groups (n=6). Group-I received 1% sodium CMC, Group-II received Dexamethazone 0.27mg/kg, p.o. (Standard), Group-III, IV and V received methanolic extract of *P.emblica* in divided doses of 200mg/kg, 400mg/kg and 600mg/kg respectively. Prior to drug treatment animals were sensitized with serum. 24 hrs, after the drug treatment animals were again challenged with 10 mg of egg albumin and inhibition of edema was noted.[21-24]

Anti-inflammatory activity

Albino wistar rats of either sex weighing 200-250 g were divided into five groups of six animals each. Group-I received 1% sodium CMC, Group-II received Diclofenac sodium 20mg/kg P.O. (Standard), Group-III, IV and V received methanolic extract of *P.emblica* in divided doses of 200mg/kg, 400mg/kg and 600mg/kg respectively. Animals of different groups were treated with respective drugs and subsequently 1 hr after treatment, 0.1 ml of 1% carageenan was injected subcutaneously into the plantar region of right hind paw to induce edema. The paw volume was measured initially and at 1, 2, 3 and 4 hrs after carageenan injection using plethysmometer. Increase in paw volume was noted.[25-28]

Statistical analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by Dunnet's t-test through GraphPad prism computer software version 4.03 to find out the level of significance. Data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The present study was carried out on methanolic extract of dried fruits of *P. emblica,* collected from local market of vadodara. The extractive value of *P. emblica* in methanol was found to be 29% w/w.

Identification Tests for Major Constituents

Methanolic fruit extract of *P. emblica* was found to have tannins, phenolics, saponins, flavonoids and glycosides. (Table 1).

Table 1: Identification Tests for Major Constituents

Tests	Observation	Result
Test for Tannins	Blue-Black colour	+
Test for Phenolics	Blue colour	+
Test for Flavanoids	Pink-tomato red colour	+
Test for Saponins	Frothing	+
Test for Glycosides	First red, blue and finally green colour	+

+ Presence

Preliminary phytochemical screening of *P. emblica* revealed the presence of saponin, flavonoids, and glycosides. Saponins are reported to possess mast cell stabilizing, antiallergic and antihistaminic activities. Glycosides isolated from various plants are reported to have antiasthmatic activity through several mechanisms i.e spasmolytic activity by relaxation of ileum, and antiallergic activity. Several flavonoids have been shown to possess smooth muscle relaxant and bronchodilator activity. The flavonoids are also reported to inhibit mast cell degranulation in several studies.[29]

TLC Identity Test

R_f value of test solution was found to be 0.40 that was compared with R_f value of reference solution i.e. 0.41. Green colour was observed that showed the presence of gallic acid.

Acute Toxicity Study

Acute toxicity was performed at dose 2000mg/kg.

There were no changes observed in body weight. There were no behavioural, neuronal and autonomical changes. Mortality was not observed.

Bronchodilating activity on guinea pigs

Pretreatment with methanolic extract of *P. emblica* (200, 400 and 600mg/kg, p.o.) to guinea pigs caused significant increase in Pre-Convulsive Dyspnoea (PCD) time as compared to control group of animals when exposed to histamine (0.1%) aerosol (Table 2, Fig. 1). In the histamine aerosol study, prior treatment of *P. emblica* (200, 400 and 600 mg/kg, p.o.) protected the animals to a significant extent (**$P < 0.01$) from the development of asphyxia produced by histamine aerosol. This confirms that it has antihistaminic activity.

Table 2: Effect of methanolic extract of *P. emblica* on histamine induced bronchospasm in guinea pigs

Treatment Group (n=6)	% Increase in PCD time (Mean ± S.E.M.)
Control	1.32 ± 0.03
Ketotifen (1mg/kg, p.o.)	88.18 ± 2.08**
Methanolic extract of *P. emblica* (200mg/kg, p.o.)	28.09 ± 1.96**
Methanolic extract of *P. emblica* (400mg/kg, p.o.)	32.98 ± 1.20**
Methanolic extract of *P. emblica* (600mg/kg, p.o.)	44.12 ± 2.93**

All values are expressed as mean ± S.E.M., n=6 in each group. ** P<0.01 significantly different when compared to control (i.e. group-I); Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t- test.

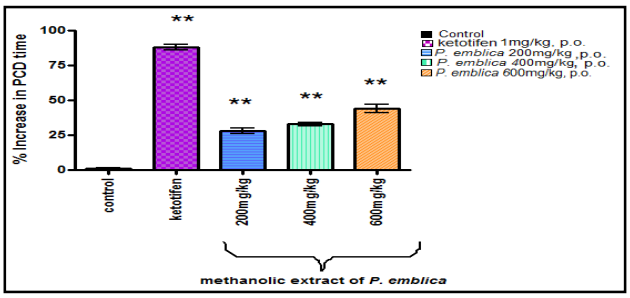

Figure 1: Bronchodilating activity of *P. emblica* on histamine induced bronchospasm in guinea pigs

Mast cell stabilization activity

Ketotifen (10µg/ml) as a reference standard produced an inhibition of 75.16% as compared to control group. Methanolic extract of *P. emblica* (2, 4 and 6mg/ml) produced dose dependent inhibition of mast cell degranulation (Table 3, Fig. 2). The % inhibition of mast cell degranulation was significantly (**P<0.01) found to be 46.16 ± 2.02, 51.66 ± 2.07 and 62.16 ± 2.60 % with 2mg/ml, 4mg/ml and 6 mg/ml of methanolic extract of *P. emblica* respectively. Ketotifen fumarate, a known mast cell stabilizing agent, also brought significant 75.16 ± 2.05 % (**P<0.01) inhibition in degranulating mast cells.

Table 3: Effect of methanolic extract of *P. emblica* Compound 48/80 induced rat mesentric mast cell degranulation

Treatment groups	% Inhibition of mast cell degranulation (Mean ± S.E.M.)
Control	16.16 ± 1.138
Ketotifen (10µg/ml)	75.16 ± 2.05**
Methanolic extract of *P. emblica* (2mg/ml)	46.16 ± 2.02**
Methanolic extract of *P. emblica* (4mg/ml)	51.66 ± 2.07**
Methanolic extract of *P. emblica* (6mg/ml)	62.16 ± 2.60**

All values are expressed as mean ± S.E.M., n=6 in each group. ** P<0.01 significantly different when compared to standard Control group (i.e. group-I); Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t- test.

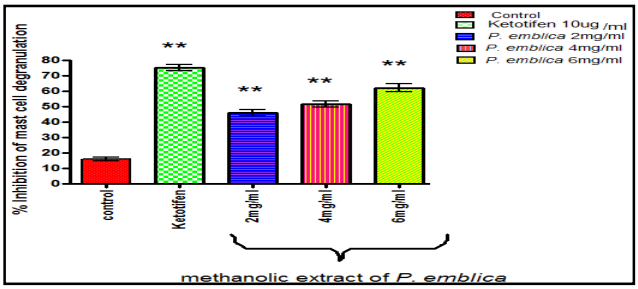

Figure 2: Rat mesentric Mast cell stabilizing activity of *P.emblica*

Passive Paw anaphylaxis

Egg albumin (100mg/ml) was found to induce anaphylactic reaction by increasing paw edema volume. Treatment with test drug (*P.emblica* 200mg, 400mg and 600mg/kg) caused dose dependent decrease in paw edema volume as compared to control (Table 4, Fig. 3). Pre-treatment with methanolic extract of fruits of *P. emblica* (200, 400 and 600mg/kg, p.o.) significantly inhibited (*P<0.05, **P< 0.01) the increase in paw volume when comparable to egg albumin sensitized and dexamethasone administered groups.

Table 4: Effect of methanolic extract of *P. emblica* on egg albumin induced passive paw anaphylaxis

Treatment Groups	Paw edema volume (ml) (Mean ± S.E.M.)			
	1hr	2hr	3hr	4hr
Control	0.93 ± 0.11	0.76 ± 0.09	0.62 ± 0.08	0.52 ± 0.10
Dexamethasone (0.27mg/kg, p.o.)	0.33 ± 0.07**	0.27 ± 0.02**	0.23 ± 0.03**	0.20 ± 0.02**
Methanolic extract of *P. emblica* (200mg/kg, p.o.)	0.62 ± 0.04*	0.52 ± 0.04*	0.43 ± 0.05	0.34 ± 0.04
Methanolic extract of *P. emblica* (400mg/kg, p.o.)	0.54 ± 0.09**	0.45 ± 0.05**	0.37 ± 0.04*	0.29 ± 0.02*
Methanolic extract of *P. emblica* (600mg/kg, p.o.)	0.45 ± 0.06**	0.38 ± 0.05**	0.27 ± 0.03**	0.26 ± 0.04*

All values are expressed as mean ± S.E.M., n=6 in each group. *P<0.05, ** P<0.01 significantly different when compared to control (i.e. group-I); Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t- test.

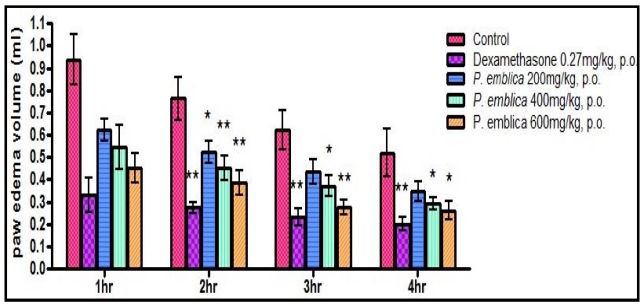

Figure 3: Effect of *P. emblica on* egg albumin induced passive paw anaphylaxis.

Anti-inflammatory Activity

Methanolic extract of *P. emblica* at the dose of 200 mg/kg, 400 mg/kg and 600mg/kg significantly decreased rat paw edema volume), which was comparable to that of control group (Table 5, Fig. 4). Methanolic extract of fruits of *P. emblica* showed significant decrease (*P<0.05, **P<0.01) in paw volume which was increased by carageenan.

Table 5: Effect of methanolic extract of *P. emblica* on carageenan induced inflammation in rats

Treatment Groups	Paw edema volume (ml) (Mean ± S.E.M.)			
	1hr	2hr	3hr	4hr
Control	0.22 ± 0.06	0.87 ± 0.17	1.13 ± 0.17	1.42 ± 0.17
Diclofenac Sodium (20mg/kg, p.o.)	0.10 ± 0.05	0.23 ± 0.08**	0.26 ± 0.10**	0.30 ± 0.11**
Methanolic extract of *P. emblica* (200mg/kg, p.o.)	0.20 ± 0.04	0.53 ± 0.14	0.59 ± 0.13*	0.82 ± 0.10*
Methanolic extract of *P. emblica* (400mg/kg, p.o.)	0.18 ± 0.02	0.39 ± 0.11*	0.51 ± 0.12*	0.66 ± 0.14**
Methanolic extract of *P. emblica* (600mg/kg, p.o.)	0.14 ± 0.032	0.33 ± 0.09*	0.41 ± 0.15**	0.43 ± 0.18**

All values are expressed as mean ± S.E.M., n=6 in each group. *P<0.05, ** P<0.01 significantly different when compared to control (i.e. group-I); Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t- test.

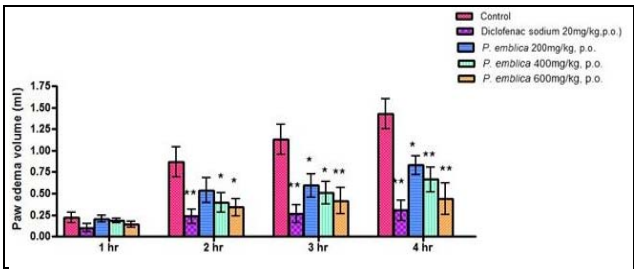

Figure 4: Effect of *P. emblica* on carageenan induced paw edema

Acknowledgement: The authors sincerely thank Baroda College of Pharmacy (Vadodara, India) for providing the necessary facilities to carry out this research work.

CONCLUSION

Methanolic extract of *P. emblica* fruits showed bronchodilating activity, mast cell stabilization activity, antianaphylatic activity and anti-inflammatory activity in the present study suggestive of its potential in prophylaxis and management in asthma. Based on these observations, *P. emblica* can be said to have potential in treatment of asthma and other allergic conditions. However, further studies with other experimental models, especially the role of cytokines are warranted to substantiate the anti-asthmatic and anti-allergic activity of *P. emblica.*

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