In–Vitro Antihistaminic Efficacy of Ethanolic Extract of Phyllanthus Amarus Schumach. and Thonn. - A Research Article

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***Abstract*—Current treatments for allergic-mediated conditions are often linked to a range of side effects. The traditional use of plant extracts in healthcare offers a promising potential for discovering new pharmaceutical compounds. The aim of the present study was to assess the anti-histaminic efficacy of Phyllanthus amarus Schumach. and Thonn. using the ethanolic extract of its fresh leaves and stem. The experiment was conducted in smooth muscles of isolated chicken ileum as it is rich in histamine receptors. The pharmacological effects observed in the experimental in-vitro model are believed to be attributed to the alkaloids, flavonoids, tannins, lignans and other chemical components present in the plant extract. These results indicate that the ethanolic extract of the fresh leaves and stems of P. Amarus demonstrates notable anti-histaminic effects.**

***Index Terms*—Phyllanthus amarus Schumach. And Thonn., Antihistaminic efficacy, Chlorpheniramine maleate, Histamine, DRC**

I. INTRODUCTION

Allergy is an exaggerated immune response to harmless substances, ranging from mild symptoms to severe reactions like asthma and anaphylaxis [1]. The World Health Organization reports that about 20% of the global population suffers from IgE-mediated allergies [2]. Mast cells play a key role in allergic reactions, triggering microvascular leakage and tissue swelling upon activation. These cells release mediators like histamine, prostaglandin D2, and leukotriene C4, with histamine being a primary factor in the development of allergic diseases [3].

The primary treatments currently available for IgE-mediated allergies include anti-IgE serum, antihistamines, and mast cell stabilizers, though antihistamines and stabilizers are linked to side effects like dry mouth, sedation, dizziness, and tinnitus. As a result, ongoing research is focused on developing new antiallergic therapies with fewer adverse effects [2]. Studies shows that many traditionally used plants possess pharmacological properties that may provide alternative treatments for allergic conditions with fewer side effects than conventional therapies [4].

Phyllanthus amarus Schumach & Thonn, a member of the “Phyllanthaceae” family, is native to tropical and subtropical regions and is known by names such as black catnip, carry me seed, Gale-of-wind and child pick-a-back [5][6]. This species belongs to the Phyllanthus genus which is an annual, glabrous herbaceous plant with slender, leaf-bearing branches reaching 30 to 60 cm in height, distichously arranged subsessile leaves that are elliptic-oblong and obtuse, and yellowish to whitish flowers. Phyllanthus amarus contains a variety of phytochemical constituents with significant medicinal potential, including alkaloids (such as epibubbialine, isobubbialine, and securinine), flavonoids (including quercetin, rutin, kaempferol),lignans (such as phyllanthin, hypophyllanthin, niranthin,), tannins (including ellagitannins like corilagin and tannin precursors such as ellagic acid, gallic acid, and gallocatechin), triterpenoids (like like lupeol, phyllanthenol, phenazine and its derivatives), steroids (such as amarosterol A, amarosterol B, Estradiol), and volatile

oils (including linalool
and phytol) [5] [7]. Phyllanthus amarus is used in traditional medicine for its diverse therapeutic properties, including treatment for jaundice, asthma, infections, and its antiviral effects against hepatitis B [2] [9].

A review of the literature reveals that no scientific studies have been conducted to determine antihistaminic efficacy of ethanolic extract of Phyllanthus amarus Schumach, and Thonn.using in-vitro experimental models.

Hence, this present study aims to investigate the antihistaminic efficacy of the ethanolic extract of Phyllanthus amarus Schumach. and Thonn. on isolated chicken ileum.

II. MATERIALS AND METHODS

1. Plant Material Collection and Authentication

Fresh leaves and stem of Phyllanthus Amarus Schumach. and Thonn. were gathered from Ernakulam district, Kerala state during the month of November and authenticated by Dr. Justin R Nayagam, Assistant Professor, Department of Botany, Union Christian College, Ernakulam, Kerala.

2. Processing of sample

The fresh leaves and stem of the plant were harvested, thoroughly washed with distilled water, and then placed in the shade to dry for 30 days. Then the shade-dried aerial parts were ground using a mechanical grinder to produce a coarse powder.

3. Preparation of Plant Extract

35 grams of this coarse powder was packed in to Soxhlet apparatus and then subjected to extraction successively with 400 ml of ethanol. The extraction was carried out for 48 hours; by keeping the extraction temperature at 78°C. The extraction was continued until the colour of the solvent in the siphon tube become colourless. An electric oven was used to evaporate the ethanol at a low temperature at the conclusion of the experiment to produce a crude extract that weighted 11.47g and had a yield of 32%. The extract was kept in a refrigerator at 4°C until it was needed for further use.

III. PHYTOCHEMICAL ANALYSIS [10]

3.1 Tests for Alkaloids

Mayer’s test

A 3 ml sample of ethanolic extract was mixed with 3 ml of 1% hydrochloric acid and heated on a steam bath. Subsequently, Mayer's reagent was added to the solution, resulted in the formation of turbidity, which indicates the presence of alkaloids.

Dragendorff test

To 2 ml of the extract solution, Dragendorff's reagent (a potassium bismuth iodide solution) was added. An orange-brown precipitate which is formed indicating the presence of alkaloids.

3.2 Tests for Flavonoids

Shinoda Test

To 2 ml of the extract solution, a few magnesium turnings, 5 ml of 95% ethanol, and a few drops of concentrated hydrochloric acid were added. The development of a red colour indicated the presence of flavonoids.

Sodium hydroxide test

Aqueous sodium hydroxide was added to the plant extract solution, resulting in the development of a yellow colour. This colour gradually fades upon the addition of dilute acetic acid, indicating the presence of flavonoids.

3.3 Tests for Tannins

Ferric chloride test

To 2 ml of the ethanolic extract, 2 ml of distilled water was added, followed by a few drops of $FeCl_3$ solution. The formation of a green precipitate indicates the presence of tannins.

Lead acetate test

When a few drops of lead acetate ($Pb(C_2H_3O_2)_2$) solution are added to the extract, the formation of a white precipitate of lead tannate formed which confirmed the presence of tannins.

3.4 Tests for Lignans [11]

Fehling’s Test

To 2 ml of the extract solution few drops of Fehling’s solution A and Fehling’s solution B was added and boiled for 5 min on boiling water bath. The formation of red precipitate indicates the presence of lignans.

Benedict’s test:

To 2 ml of the extract drops of Benedict’s reagent added, boiled for 5 minutes. The formation of orange coloured precipitate indicates the presence of lignans.

3.5 Tests for Steroids and Triterpenoids [12]

1.Liebermann - Burchard test:

To 2 ml of extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was

then added carefully. Formation of green colour on upper layer and deep red colour on lower layer indicates the presence of steroids and triterpenoids respectively.

IV. PROCUREMENT OF CHICKEN ILEUM [13]

For conducting the experiment, fresh chicken ileum was collected from slaughter house. It was kept at room temperature with sufficient aeration in freshly prepared Tyrode solution. The research was done at the Chemists College of Pharmaceutical Sciences and Research in Ernakulam Kerala, India, in the Pharmacology laboratory of the Department of Pharmacology.

V. CHEMICAL REAGENTS AND DRUGS

Histamine (Biovision), Chlorpheniramine maleate (Joncpm), Ethanol (Spectrum Chemicals)

VI. EXPERIMENTAL DESIGN

6.1 Drug Preparation and Serial Dilutions [13]

A solution of $1x10^{-1}$ g/ml was prepared by dissolving 100 mg of the extract in 100 ml of distilled water. From this, 20 ml was transferred and diluted to 100 ml with distilled water. Another 20 ml of this solution was then taken and diluted to 100 ml with distilled water. For the conventional medications, histamine (100 mg/ml) and

chlorpheniramine maleate were prepared using the same dilution procedure. Tyrode solution was made by dissolving the following components in one liter of water: 8 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 1 g $NaHCO_3$, 1 g NaH2PO4, 0.1 g MgCl2, and 2 g glucose.

6.2 Evaluation of antihistaminic activity on isolated chicken ileum [13]

The experiment was conducted using Sherrington Rotating Drum. Fresh chicken ileum was collected, placed in a beaker containing freshly prepared tyrode solution at 37°C, and then aerated. A portion of the ileum measuring 2-4 cm was excised, mounted, and kept at 37°C in a 35-cc organ bath containing tyrode solution with oxygen supply. The kymograph and its attachments were set up so that the tissue would be properly tensioned. Before starting the medication infusions, the tissue was given 15 minutes to acclimatise.

Histamine dose response curve (DRC) were established in the following order:

1. Histamine alone
2. Histamine in presence of ethanolic extract of Phyllanthus amarus Schumach. and Thonn. Leaves and stem.
3. Histamine in presence of Chlorpheniramine maleate.

VII. STATISTICAL ANALYSIS

All data obtained were represented as mean ± standard error of mean values.

VIII. RESULTS

Phytochemical analysis

The chemical screening tests indicated the presence of, alkaloids, flavonoids, and tannins, lignans, steroids and triterpenoids in the ethanolic extract of P.amarus Schumach and Thonn.(Table 1)

Assessment of Anti-histaminic activity

DRC of Histamine

Dose of 0.4 ml,0.8 ml ,1.6 ml, 3.2ml was administered to the isolated chicken ileum and the DRC was recorded. The contraction produced in the smooth muscles of chicken ileum cause an increase in height of the response. (Fig 1)

DRC of histamine in presence of P.amarus

Dose of 0.4ml, 0.8ml,1.6ml,3.2ml was administered to the isolated chicken ileum by keeping the dose of P. amarus (0.4ml) as constant, P. amarus inhibits the activity of the histamine causes decrease in height of the response. (Fig 2)

DRC of histamine in presence of Chlorpheniramine maleate

Dose of 0.4 ml, 0.8 ml, 1.6ml , 3.2ml was administered to the isolated chicken ileum by keeping the dose of chlorpheniramine maleate (0.4ml) as constant , Chlorpheniramine maleate inhibits the activity of the histamine which cause a decrease in height of the response .(Fig 3)

Table 1: Phytochemical analysis of leaves and stems of P. amarus .[14][15]

Phytochemicals	Ethanolic extract
Alkaloids	+
Flavonoids	+
Tannins	+
Lignans	+
Triterpenoids & Steroids	+

"+" Indicates presence of the phytochemical constituents of P. amarus.

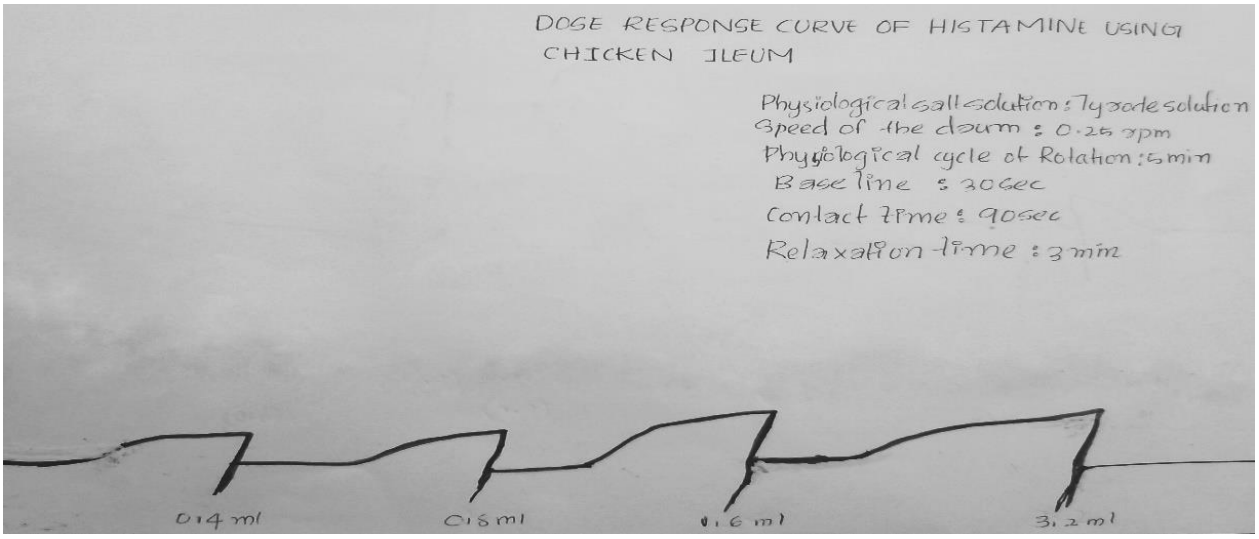


Fig 1: DRC of Histamine

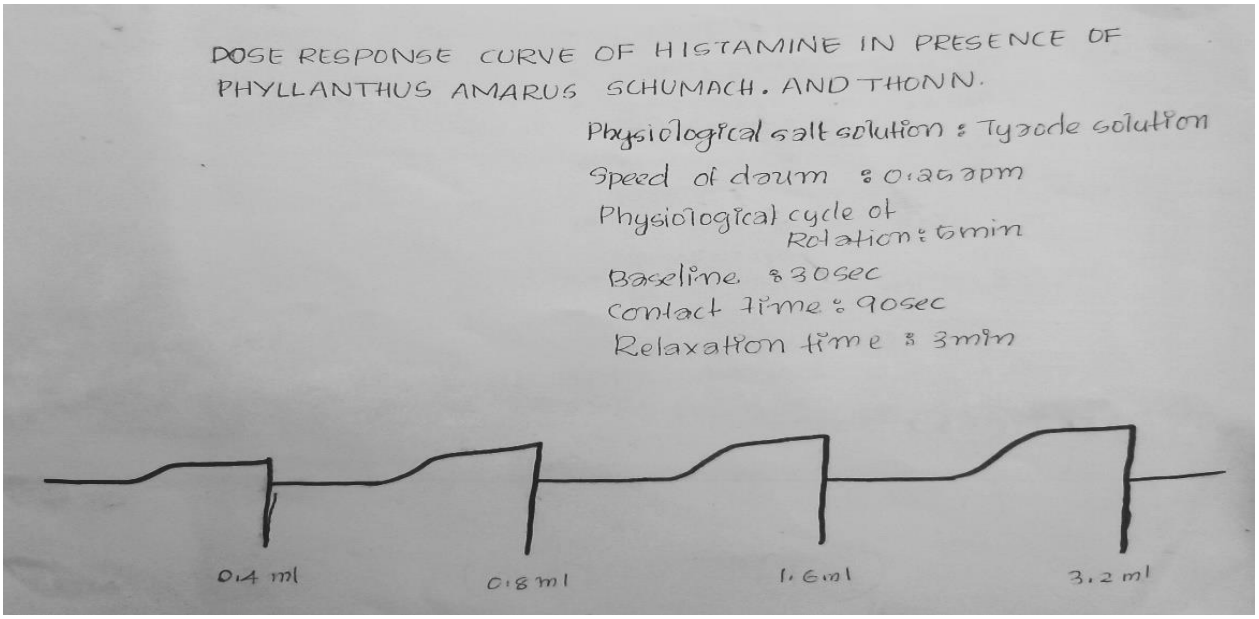


Fig 2: DRC of Histamine in presence of P. amarus

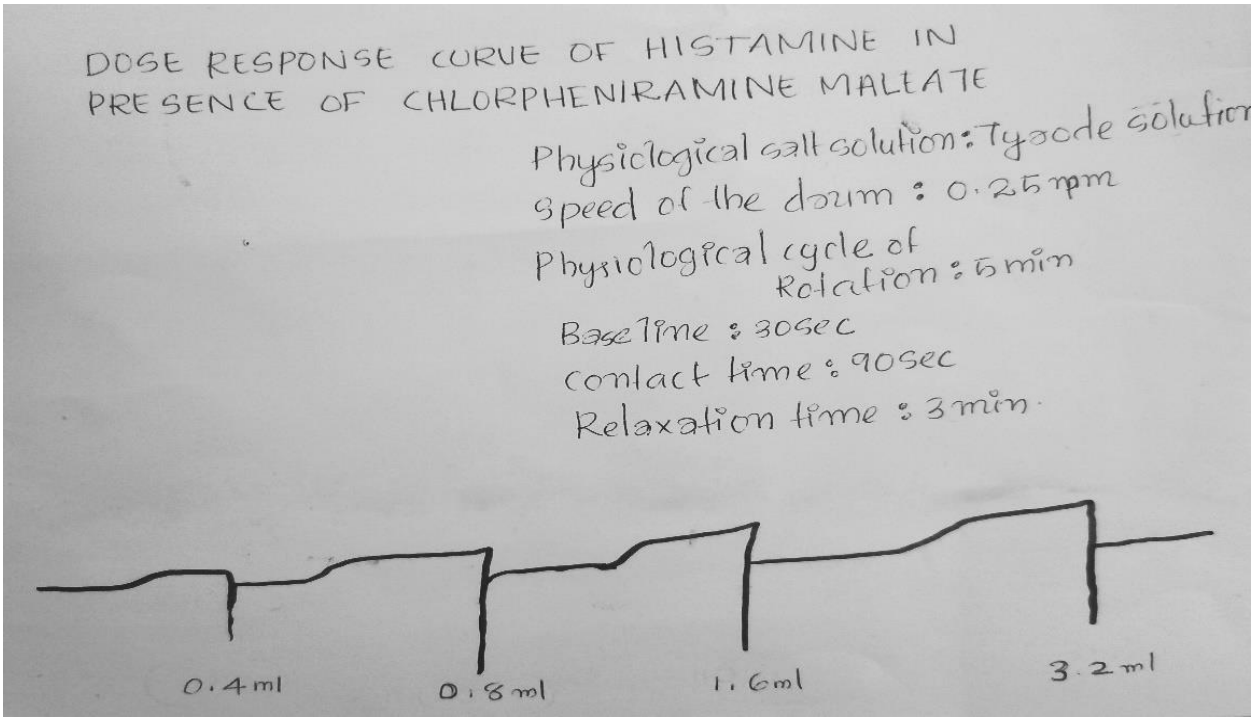


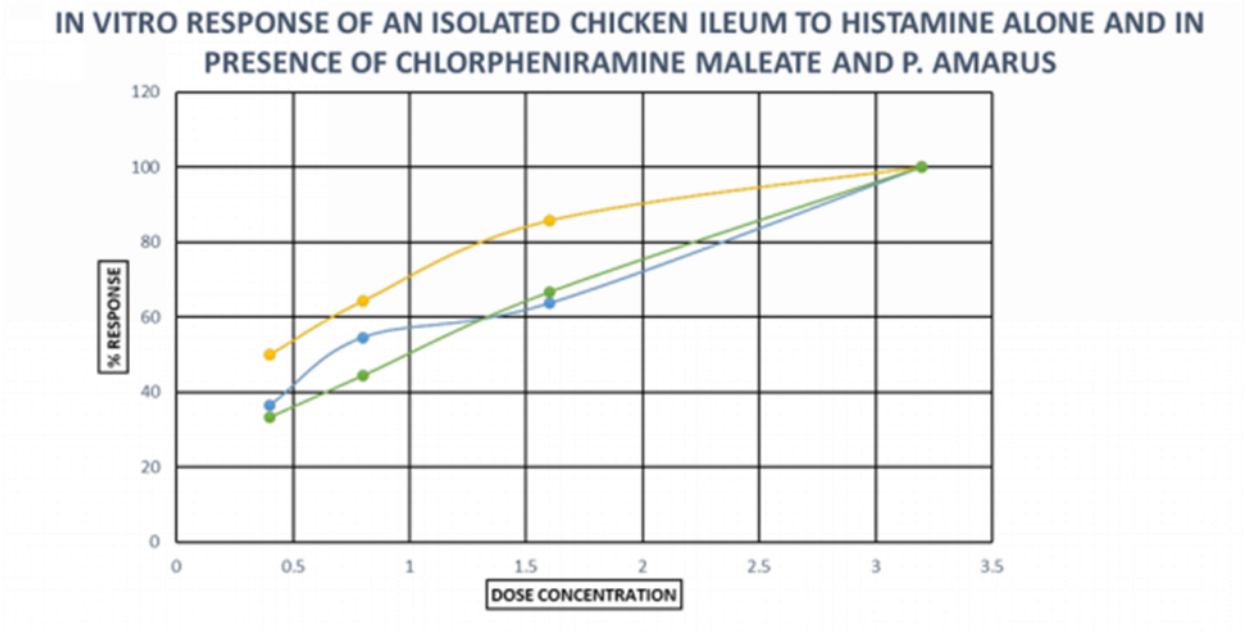
Fig 3: DRC of Histamine in presence of Chlorpheniramine maleate

Sl. No:	Dose of Histamine (ml)	Response of Histamine alone		Response of Histamine in presence of P. amarus		Response of Histamine in presence of Chlorpheniramine maleate	
		Height in mm ±SEM	% Response	Height in mm ±SEM	% Response	Height in mm ±SEM	% Response
1	0.4	7 ±0.881	50.00	4± 0.333	36.36	3 ±1.452	33.33
2	0.8	9± 1.154	64.28	6± 0.577	54.54	4 ±1.547	44.44
3	1.6	12 ±1.201	85.71	7± 0.666	63.63	6± 0.881	66.66
4	3.2	14± 0.236	100.00	11±0.881	100.00	9± 1.201	100.00
Mean			74.99		63.63		61.10

Table: 2 Invitro responses of an isolated chicken ileum to Histamine alone and in the presence of P. amarus and Chlorpheniramine maleate

IX. DISCUSSION

Dose response curve of histamine alone, Dose response curve of Histamine in presence of P. amarus and Dose response curve of Chlorpheniramine maleate was compared. The height of dose response curve of P. amarus and Chlorpheniramine maleate was showed as decreased compared to that of the height of Histamine. It indicates that P. amarus is having antihistaminic efficacy .



Response of Histamine alone percent response
Response of Histamine in presence of P. Amarus percent response
Response of Histamine in presence of Chlorpheniramine Maleate percent response

The Anti-histaminic activity was evaluated by plotting dose response curve. Using the mean height obtained from the DRC, corresponding percentage response was calculated. The mean percentage response of Histamine, Phyllanthus amarus Schumach. and Thonn., Chlorpheniramine maleate was found to be 74.99%,63.63%, 61.10% respectively.

X. CONCLUSION

Antihistaminic properties of ethanolic extract of Phyllanthus amarus Schumach. and Thonn. leaves and stem were studied in comparison with Chlorpheniramine maleate. Histamine is an allergic mediator which produces contraction in smooth muscles. Antihistaminics inhibits histamine and relaxes all smooth muscles. Standard methodology was used to conduct the preliminary phytochemical analysis, which identified the presence of alkaloids, flavonoids, and tannins, lignans, steroids and triterpenoids. Antihistaminic activity was studied invitro using isolated chicken ileum. Isolated chicken ileum is an intestinal smooth muscle and it contains number of receptors such as, histaminic, muscarinic, adrenergic, serotonergic, and GABAergic receptors. Contraction induced by histamine were recorded as dose response curve (DRC), which suggests that Histamine increases contraction in a dose dependent manner. Antihistaminics relaxes all visceral smooth muscles. Antihistaminic activity of ethanolic extract of Phyllanthus amarus Schumach. and Thonn. was compared with Standard Chlorpheniramine maleate. Comparison of mean percentage response of Phyllanthus amarus Schumach. and Thonn. with that of Chlorpheniramine maleate proved significant antihistaminic activity of the extract.

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