Study of in-vitro antihistaminic efficacy of amaranthus spinosus linn leaves extract

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Abstract:

Antihistamines inhibit histamines from causing allergic reactions... Amaranthus Spinosus Linn is a widely available plant that inhibits mast cell-mediated anaphylactic reactions. It also possesses laxative, diuretic, anti-diabetic, antipyretic, anti-snake venom, antileprotic, anti-gonorrheal, and expectorant effects. It also have anti-inflammatory, immunomodulatory, anti-androgenic, and anthelmintic properties. The soxhlation method was used to prepare the ethanolic extract. In vitro research was conducted using isolated chick ileum. Chick ileum was suspended at 37 °C with a sufficient oxygen supply in an organ bath containing Tyrode solution. The effects of extract from Amaranthus spinosus Linn leaves on histamine-induced ileum contractions were investigated and contrasted with those of chlorphenramine maleate. For both chlorphenramine maleate and the ethanolic extract of Amaranthus spinosus Linn leaves, the mean percentage response was computed. From this study it was concluded that Amaranthus spinosus Linn extract have significant antihistaminic activity compared to Chlorpheniramine.

Keywords:

Antihistaminic,Amaranthus spinosus linn leaves

1. Introduction:

A class of pharmaceuticals known as antihistamines is used to treat disorders mediated by histamines. The H-1 and H-2 classes of histamine receptors are the two primary subclasses. H-1 receptor-binding antihistamine medications are typically used to treat allergic rhinitis and allergies. First- and second-generation agents are used to further categorize H-1 antihistamines. The central nervous system (CNS) is more easily penetrated by first-generation H-1 antihistamines than by second-generation H-1 antihistamines. Histamine (an endogenous chemical messenger) induces an increased level of vascular permeability, which leads to fluid moving from capillaries into the surrounding tissues. The overall outcome of this is increased swelling and dilation of vessels. Antihistamines stop this effect by acting as antagonists at the H-1 receptors. The clinical benefit is a reduction in allergy symptoms and any related symptoms.[1]

Chlorpheniramine Maleate (CPM), also known as chlorphenramine, is a potent alkyl amine first-generation H1 antihistamine that has been used since the 1950s. CPM is a widely popular drug commonly used to treat allergic conditions, given its antihistamine properties. Although mainly used in over-the-counter treatment for cough and colds, various studies discuss a wide range of CPM's clinical uses, such as treating asthma, plasma cell gingivitis, chronic urticaria, and depression, among others. This antihistamine is usually taken orally; however, intravenous, intramuscular, and subcutaneous routes have been documented CPM is absorbed by the small intestine with a bioavailability (F) of 25-50%, displaying a peak serum concentration between 1-6 hours and a half-life ranging between 2–43 hours due to its variable first-pass hepatic metabolism . [2]

Amaranthus spinosus Linn. (Family Amaranthaceae) is a common Indian plant with therapeutic benefits. It is also known as'spiny amaranth', 'pig weed', and "Kate wali Chaulai (Kanatabhajii)" in Hindi.This upright spinous herb, native to tropical America, ranges in color from green to purple and can be annual or perennial. The plant is utilized in Ayurvedic medicine for its antipyretic, laxative, and diuretic properties.In addition to its culinary benefits, it is also used to treat digestive issues, bronchitis, biliousness, galactagogue, haematinic, stomachic, nausea, flatulence, anorexia, blood disorders, burning feeling, leucorrhoea, leprosy, and piles. Phytochemical studies highlight its potential as a valuable therapeutic plant. This plant contains a variety of nutrients, including alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponin, betalain, b-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins, and carotenoids.

Amaranthus spinosus. Linn is an erect, monoecious perennial that can grow up to 1 m. The stem is terete or obtusely angular, glabrous or slightly pubescent, green, reddish-brown, glabrous, and branching. The leaves are simple and alternating, with no stipules. The petiole is about the same length as the leaf blade. The flowers are terminal and axillary, spike-like, erect, thin and elongated. They have remote axillary spikes at the base, lower clusters, pistillate, and above staminate. Bracteoles are scarious, ovate, and faintly spiny-tipped, often longer than or equal to the tepal. The seed is about 1 mm in diameter, shiny, compressed, black or brownish-black in colour.[3]

2. Materials and methods:

2.1. Plant materials:

Fresh *Amaranthus Spinosus Linn* leaves were gathered from Ernakulam district, Kerala state.

2.2. Animals:

For the investigation, fresh chicken ileum was collected from slaughter house. It was kept at room temperature with adequate aeration in freshly prepared Tyrode solution. The research was done at the Chemists College of Pharmaceutical Sciences And Research in Kerala, India, in the pharmacology laboratory of the department of pharmacology.

2.3. Preparation of plant extract:

The Amaranthus Spinosus Linn was shade dried at room temperature and was subjected to size reduction to a coarse powder by using dry grinder. 35 grams of this coarse powder was packed in to soxhlet apparatus and was subjected to extraction sequentially with 400ml of ethanol. The extraction was carried out for 48 hours; 78°C was kept as the extraction temperature. 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%.[30]

3. Identification test:

3.1. Test for flavonoids [26]:

1. Shinoda test: To dried powder or extract, added 5 ml 95% ethanol, few drops of concentrated hydrochloric acid and 0.5 g magnesium turnings.Pink color indicates the presence of flavonoids.
2. To small amount of residue, add lead acetate solution. Yellow colored precipitate is formed which indicates the presence of flavonoids.
3. Alkaline Reagent test:To the test solution add few drops of sodium hydroxide solution. Intense yellow color is formed, which turns to colorless on addition of few drops of dilute acid which indicates the presence of flavonoids

3.2. Test for tannins [27]:

1. Ferric chloride test: To 2-3 ml of aqueous or alcoholic extract, add few drops of 5% ferric chloride solution. Deep blue or black color is formed which indicates the presence of tannins.
2. Gelatin test:To 2-3 ml of aqueous or alcoholic extract, add gelatin solution. White precipitate is formed which indicates the presence of tannins.

3.3. Test for steroids and triterpenoids [27]:

1. Salkowoski test: To 5 ml of extract add 2 ml of chloroform and 3 ml of concentrated sulphuric acid along the sides of the test tube. Formation of reddish brown color is formed which indicates the presence of Steroids and Triterpenoids.

3.4. Test for proteins [27]:

1. Ninhydrin test: Two drops of 0.2% freshly prepared ninhydrin solution added to 1 mL of extract, Formation of purple color. which indicates the presence of proteins.
2. Biuret Test: Take 2 ml of sample in a test tube; add 2 ml of sodium hydroxide solution and 5-6 drops of copper sulfate solution. Appearance of bluish violet color.which indicates the presence of proteins.

3.5. Test for amino acids [27]:

1. Millon's Test: Take 2 ml of sample; add 2-3 drops of Millon's reagent and shake well Formation of white precipitate, on heating turns to brick red color which indicates the presence of amino acids.

3.6. Drug preparations and dilutions:

For the preparation of the stock solution, 100 mg of the plant extract is weighed and diluted with 100ml of distilled water to makeup 1000µg/ml. From the above prepared stock solution, 20 ml was pipetted out and diluted with 100 ml of distilled water to makeup 200µg/ml. Tyrode solution was prepared per liter of water by the dissolution of the following substances: NaCl-8g; KCl-0.2g; CaCl2-0.2g; NaHCO3-1g; NaH2PO4-1g; MgCl2-0.1g and glucose-2g. [30]

3.7. In vitro study of the effect of *Amaranthus spinosus Linn* on isolated chicken ileum:

Chicken ileum was suspended in bath containing Tyrode solution maintained at 37±0.5°C. A stream of air was bubbled through the organ tube (1bubble/sec). An S-shaped aerator was linked to one end of the ileum, and an isotonic frontal writing lever was coupled to the other end to a drum.The tissue was allowed to equilibrate for 45 min. Contact times of 60 sec, and base line of 30sec time cycle were opted for proper recording. Cumulative concentration-effect curves were recorded on kymograph for Chlorpheniramine Maleate (1µg/ml) in absence and presence of ethanolic extract of Amaranthus Spinosus L. (200µg/ml) on Kymograph by using Sherrington's Recording Drum. The same procedure was carried for concentration-effect curve of CPM in presence of Histamine as a standard drug. The percentage inhibition of extract and standard drug was calculated and graph was plotted by taking log dose verses height of response curve. Chlorpheniramine maleate dose responses were established in the following order:

1. Histamine alone
2. Chlorpheniramine maleate in presence of histamine.
3. Chlorpheniramine maleate in the presence of ethanolic extract of Amaranthus Spinosus Linn leaves. [30]

4. Result and discussion:

Table. 1: In vitro response of an isolated chick ileum to histamine alone and in the presence of Chlorpheniramine and Amaranthus extracts

SL No:	**Dose of Histamine (ml)**	**Response of Histamine alone**	**Response of Histamine in presence of Amaranthus**	**Response of Histamine in presence of Chlor-Pheniramine**

		Height in mm ± SEM	% Response	Height in mm ± SEM	% Response	Height in mm ± SEM	% Response
1	0.1	7.6±0.88	43.00	1.43±0.04	47.00	1.48±0.02	37.00
2	0.2	11.00±1.15	62.00	1.92±0.01	64.00	2.22±0.05	55.00
3	0.4	13.3±1.26	75.00	2.20±0.05	73.00	3.37±0.02	84.00
4	0.8	16.00±0.57	90.00	2.53±0.01	84.00	3.43±0.11	85.00
5	1.6	16.33±0.33	92.00	2.56±0.01	85.00	3.64±0.01	91.00
6	3.2	17.66±0.33	100.00	3.00±0.06	100.00	3.99±0.01	100.00
Mean			77.00		75.50		75.33

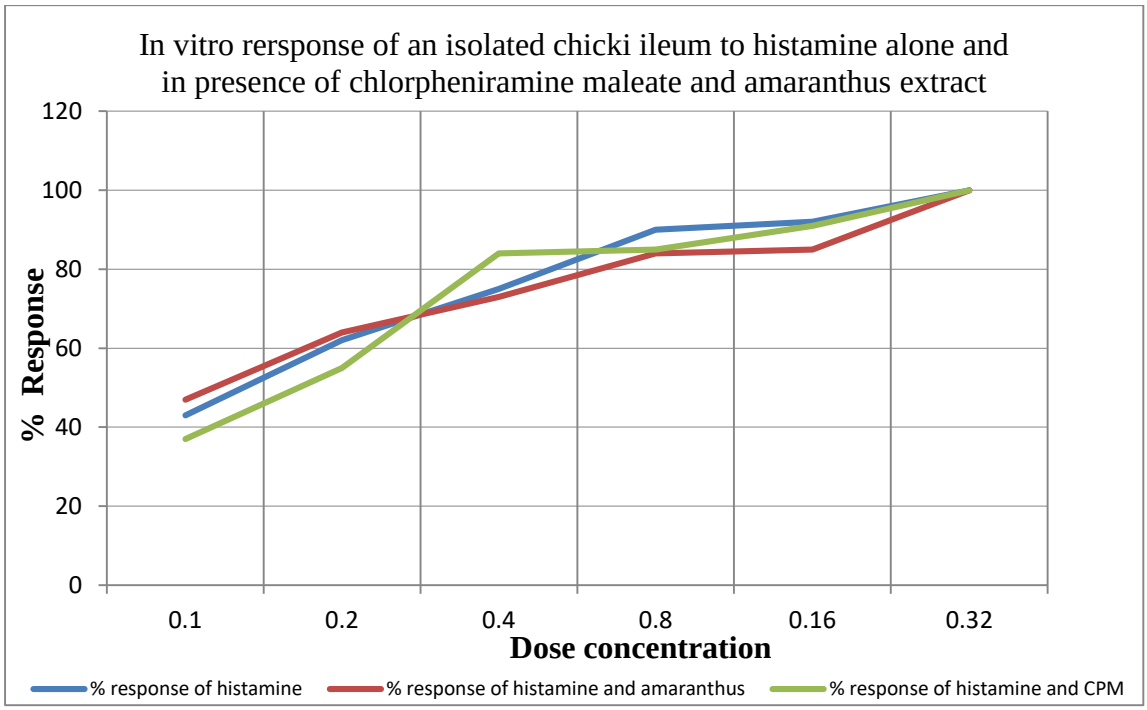

Figure. 1: Plot of in vitro responses of an isolated chick ileum to histamine in presence of Chlorpheniramine maleate and Amaranthus extract

The antihistaminic activity was evaluated by plotting dose response curve. Using the mean height obtained from DRC, corresponding percentage response was calculated. The mean percentage response of histamine, *Amaranthus Spinosus Linn*, Chlorpheniramine Maleate was found to be77.00%, 75.50%, 75.33% respectively. From the observed data, the mean percentage response of Amaranthus *Spinosus Linn* was found to be 1.50% less than that of histamine

5. Conclusion:

Anti-histaminic properties of ethanolic extract of Amaranthus spinosus linn leaves was studied in comparison with Chlorpheniramine maleate. Histamine is an essential molecule that has a role in a variety of body functions. It increases gastric acid secretion, causes inflammation, dilates blood vessels, influences muscle contractions in the intestines and lungs, and regulates your heart rate. It also facilitates the transmission of signals between nerve cells and the movement of fluids through blood vessel walls. When your body detects an allergy as a threat, it releases histamine. Histamine causes capillaries to expand and widen, resulting in allergic symptoms. Anti- histaminic is a class of drugs used to treat disorders mediated by histamines. They act as inverse agonists, binding to the H1 receptor to reduce histamine-induced inflammation. Botanist authenticated the collected plant specimen, and using the soxhlation process, an ethanolic extract was made from the dried, powdered leaves of Amaranthus spinosus linn. Standard methodology was used to conduct the preliminary phytochemical analysis, which identified the presence of flavonoids, tannins, amino acids, steroids and triterpenoids.

Anti-histaminic activity was studied in vitro using isolated chick ileum. Isolated chick ileum isan intestinal smooth muscle and it contains of a number of receptors such as muscarinic, histaminic, adrenergic, and serotonergic and GABAergic receptors. Contractions induced by Histamine were recorded as dose response curve (DRC) which suggests that histamine increase contraction in a dose dependent manner. Antihistaminic medications inhibit the effects of histamine, which tends to relax visceral smooth muscle rather than contract it. Anti-histaminic activity of the ethanolic extract of Amaranthus spinosus linn was compared with standard chlorpheniramine maleate. Comparison of mean percentage response of Amaranthus spinosus linn with that of chlorpheniramine maleate proved significant Anti-histaminic activity of the extract.

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