In vitro evaluation of Anti asthmatic activity of Pistacia integerrima Gall Extract on Goat Trachea and Chicken ileum

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Abstract- **Bronchial asthma is most common chronic disease in adults and children that is characterized by airway obstruction and bronchial hyper responsiveness. The clinical manifestation of asthma are difficulty in breathing, pain , cough, fast pulse , throat irritation and anxiety. consistent with World Health Organization (WHO) in 2019, Asthma affected people were 262 million and it caused 461,000 deaths. Ayurveda and other Indian literature mention the utilization of plants treatment for various human ailments. Plants and their extracts still provide effective treatment for diseases of all kinds including asthma. Pistacia integerrima belonging to the Anacardiaceae and subfamily Anacardiaceae is one of the widely accepted plants used to cure a vast array of diseases. The normal claims, phytochemical investigations and pharmacological evaluation and a few ayurvedic formulations provide the backbone to make this tree as a plant of Heaven. The different parts of the plant roots, leaves, stems, barks, Galls and fruits contains various bioactive compounds like amino acid, alkaloids, sterols, flavonoids, tannins, saponins,etc. The plant is known to have many biological activities including antibacterial, antifungal, analgesic, antioxidant, phytotoxic, cytotoxicity and antiasthmatic. The in-vitro screening methods are essential to convert the effective drug candidate from preclinical studies to human being. The current research suggests the anti-asthmatic properties of methanolic extract of Pistacia integerrima on isolated goat tracheal preparation and chicken ileum preparation.**

***Keywords*: Asthma, Anacardiaceae, Kakadshingi, Pistacia Integerrima, Maceration, Goat Trachea, Hitamine, Chicken ileum**

INTRODUCTION:

Asthma may be a disease of the lung's airways. It affects 155 million individuals within the world. Its Prevalence and severity among children have increased significantly within the world over the Past 40 year. It varies from 5–30 percent in several population[1]. It affected 14–15 million people in the United States, including estimated 4.8 million children. It is most common chronic disease of childhood. It accounts for about 11 million hospital visits annually and therefore the sixth most frequent reason for visits in ambulatory setting[2]. About 4,70,000 patients are hospitalized and quite 5,000 patients die annually thanks to asthma[1]. Asthma may be a global problem, many man-made drugs are used to treat acute symptoms of asthma, but they're not completely safe for long term use. Hence search has been started once more to look back to traditional medicine which can be used to treat asthma. Ayurveda and other Indian literature mention the utilization of plants treatment for various human ailments. Herbal medicine is currently within the lime light and is given more popularity than ever before as sales figures in some countries, for instance the USA, have risen beyond the expectations of some producers. the explanations for this change are complex but clearly are connected with what could be described as the 'greening of medicine'[7]. Plants and their extracts still provide effective treatment for diseases of all kinds including asthma.

In Ayurveda and Chinese traditional medicine Pistacia integerrima galls and stem bark were used as an honest remedy for the patients having asthma, cough and other allergic conditions. Pistacia integerrima (Family. Anacardiaceae) is named as Kakadshingi in Ayurveda and Indian traditional medicine[7]. It consists of gall-like excrescences formed by insects on the leaves, petioles and branches of the plant. Kakadshingi is employed in indigenous system of medicine (Ayurveda, Unani and Siddha) as a remedy for cough, asthma, fever, respiratory and in liver disorders[7]. Kakadshingi also finds usage within the treatment of children's ear infections, suppress haemorrhage from gums and used to prevent bleeding from nose. Hakims consider galls useful in pulmonary infections, diarrhoea and vomiting[7].

Parts of Pistacia integerrima gives antibacterial, antifungal, anticancer, anti-inflammatory, anti-asthmatic, anti-oxidant properties[5].

Common names of Pistacia integerrima:

Language	Common names[5]
Latin	Pistacia integerrima
English	Crab claw and zebra wood
Urdu	Kakarasingi
Hindi	Kakadasrngi
Bengali	Kankihasringi
Punjabi	Kakar, Kakarsingi
Kashmiri	Kamaladina

Taxonomic positions[5]:

Kingdom	Plantae
Division	Angiospermae
Subdivision	Eudicots
Class	Rosides
Order	Sapindales
Family	Anacardiaceae
Scientific name	Pistacia integerrima

MATERIAL AND METHOD:

Plant Material:

Plant galls extract: A sample of aqueous extract of Pistacia integerrima galls dry powder purchased from Manakarnika Aushdhalay, Chinchwadgaon, Pune, India, on 21/09/2022 under the brand name Kakadshingi. the merchandise FSSAI Lic. No:11517037000444; Batch number is 01/22, it had been approved by QC manager.

Figure 1: Natural Plant of Pistacia integerrima Gall

Figure 2: Galls of the Plant

Preparation of Methanolic extract by Cold maceration (at room temperature)   Method:

Cold Maceration Extraction Method: during this process, the coarsely powdered material of Pistacia integerrima leaf galls is extracted by placing the powder in a stoppered container with the solvent methanol and allowed to stand at room temperature for a different period of time (6h, 12h, 24h, 48h) with frequent agitation until the soluble matter has dissolved[6]. The mixture then is strained, the marc (the damp solid material) is pressed, and therefore the combined liquid is clarified by filtration or decantation after standing[6]. All the extract was evaporated to dryness, weighed and stored for future use.

The Methanolic extract of Pistacia integerrima leaf galls was subjected to the subsequent investigation, *Antiasthmatic activity using Goat Tracheal Chain Preparation and Isolated Chicken Ileum Preparation.*

Figure 3 & 4: Gall Extract Preparation

TESTS FOR DETECTION OF PHYTOCHEMICAL COMPONENTS:

1)Test for Flavonoids

Shinoda Test: (Magnesium hydrochloride reduction test): To the test solution of Pistacia integerrima add few fragments of magnesium ribbon and concentrated hydrochloric acid drop wise, pink scarlet, colour appears after few minutes indicating the presence of flavonoids[3].

Ferric Chloride Test: To the test solution of Pistacia integerrima add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoids[3].

2)Test for Tannins

Ferric Chloride Test: Some amount of extract of Pistacia integerrima was dissolved in distilled water to this solution 2ml of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins[3].

Lead Acetate Test: Some amount of extract of Pistacia integerrima a few drops of lead acetate solution was added. Formation of precipitate indicates presence of tannins[3].

3)Test for Glycoside

Borntrager's Test: To 3 ml of test solution of Pistacia integerrima, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red colour in ammonical layer indicates presence of anthraquinone glycoside.

4)Test for Alkaloids

To the extract of Pistacia integerrima, dilute HCI was added. It was then shaken well and filtered. The filtrate was used to perform following tests:

Mayer's test: To 2-3 ml of filtrate, few drops of Mayer's reagent was added. Formation of white precipitate indicates the presence of alkaloids.

Hager's test: To 2-3 ml of filtrate, few drops of Hager's reagent was added. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test: To 2-3 ml of filtrate, few drops of Wagner's reagent was added. Formation of reddish brown precipitate indicates the presence of alkaloids[3].

Sr No.	Name of phytoconstituent	Presence/ Absence
1.	Flavonoids	+ + -
2.	Alkaloids	+ - +
3.	Glycosides	+ + +
4.	Tannins	- + +

CHROMATOGRAPHIC PURIFICATION:

TLC was performed to isolate the principle components that were presents in most effective extracts of plants the TLC was used by different solvent systems.

Solvent phase

Chloroform:Glacial acetic acid:methanol (4:5:1).

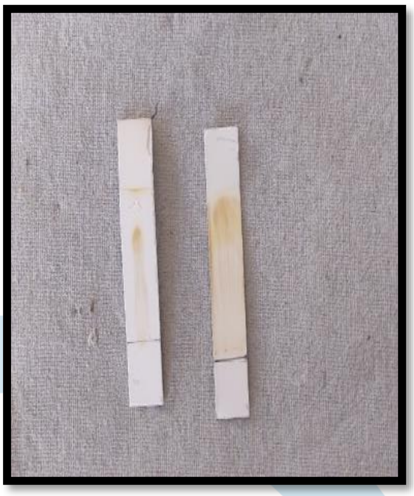
Methods

The purity of every eluted sample was tested by using TLC method. it's a technique used to separate wide range of compounds of biochemical interest. It are often utilized to quantitative as well as qualitative and preparatory work. The petroleum ether extract was subjected to thin layer chromatography about 0.1-0.2 ml of conc. Methanolic extract was loaded on the plate by using capillary. During spotted plates were carefully dried and used for elution purpose. Initially various solvents like benzene, pet ether, chloroform ethanol were tested alone. Later different combinations of solvents chloroform, glacial ethanoic acid, methanol were tested counting on polarity basis. The spotting plate was carefully dried and used for elution purpose.

Development of chromatogram:

The eluted spotted plates were dried at temperature and they were placed in iodine chambers for the development of chromatogram. The Rf values of cleared spot were calculated & proper solvent system was identified and therefore the proper solvent system is Chloroform:Glacial acetic acid:methanol (4:5:1).

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$$



Preparation of Physiological Salt Solution:

Sr. No.	Constituents	Krebs Henseleit salt solution
1.	NaCl	9.6g
2.	KCl	0.35g
3.	MgCl2	0.11g
4.	NaH2PO4.2H2O	0.14g
5.	Glucose	2.0g
6.	NaHCO3	2.10g
7.	CaCl2	0.28g

Isolated Goat Tracheal Chain Preparation:

1) Fresh goat tracheal tissue was collected in oxygenated Krebs-Henseleit solution directly from butcher[4].
2) After separation of individual rings, three rings were tied together in series to form a tracheal chain. In an organ tube, Krebs-Henseleit solution was maintained at 37 ± 1ºC and carbogen was bubbled (1 bubble/sec).
3) Tracheal chain was suspended in the organ tube. Lower end of chain was attached to oxygen delivery tube and the other attached to isotonic frontal writing lever to a rotating drum[4]. The tip of sketch pen and white plain paper were attached to frontal writing lever and rotating drum respectively.
4) The tissue was allowed to relax for 45 min under a load of 400 g during which the bathing solution was changed at every 15 min.
5) The Contractile responses of tracheal strip to histamine (100 µg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml and 1.6ml were recorded in absence and presence of methanolic extract of Pistacia integerrima by using 'Sherrington' Recording Drum with a frontal Writing lever.
6) Similar concentration effect curve was taken in presence of standard Drug Cetrizine (1 µg/ml). The height of response curve was measured to express percentage inhibition.

Figure 5 &6: Preparation of Isolated Goat Tracheal Chain

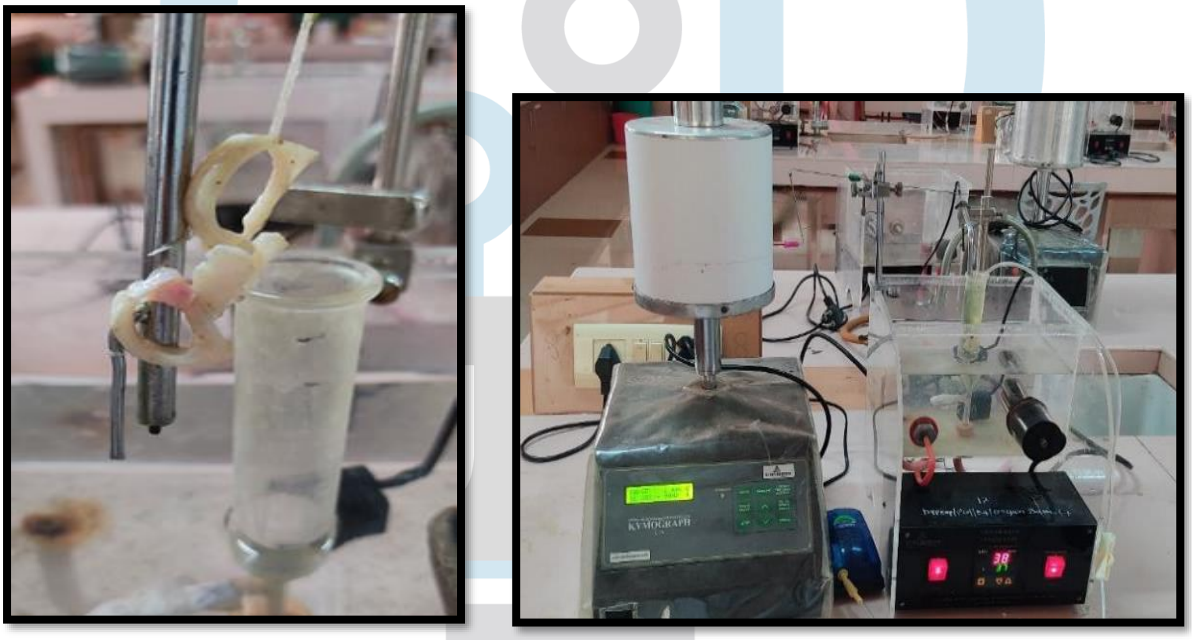


Figure 7: Organ Bath and Kymograph paper on Sherrington Rotating drum

RESULTS AND GRAPHS

Table No.1: Effect of extract of Pistacia integerrima (500 µg/ml) on Histamine induced contraction of Isolated goat tracheal chain preparation.

Sr No	Dose of Histamine	Con. of histamine	Log dose of histamine	Average % response in absence of BP Extract	Average % response in presence of BP Extract

1	0.1	2	0.30	19.04	19.04
2	0.2	4	0.60	42.38	39.047
3	0.4	8	0.90	71.42	71.42
4	0.8	16	1.20	91.42	89.52
5	1.6	32	1.50	100	95

Figure 8: Dose Response Curve of Histamine in the absence and presence of extract of Pistacia integerrima (500 µg/ml) recorded on goat tracheal chain preparation.

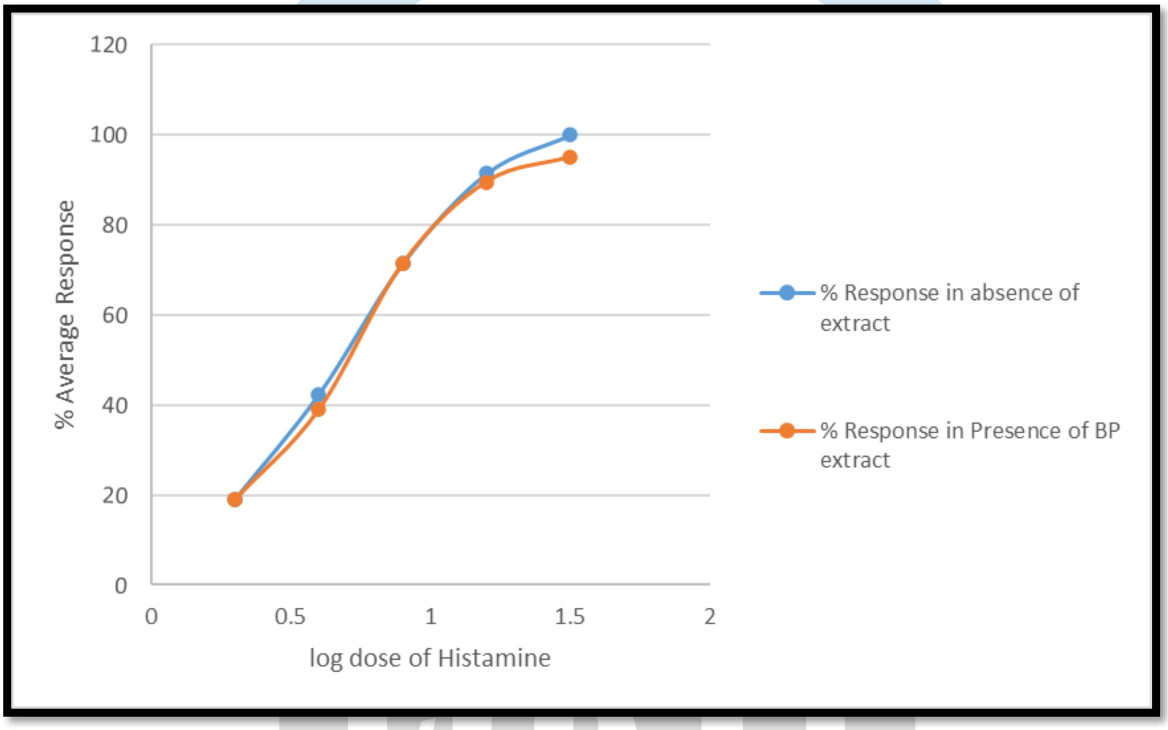


Table No.2: Effect of extract of Pistacia integerrima (1000 µg/ml) on Histamine induced contraction of Isolated goat tracheal chain preparation.

Sr No	Dose of Histamine	Con. of histamine	Log dose of histamine	Average % response in absence of BP Extract	Average % response in presence of BP Extract
1	0.1	2	0.30	19.04	17.6
2	0.2	4	0.60	42.38	36.19
3	0.4	8	0.90	71.42	61.90
4	0.8	16	1.20	91.42	83.33
5	1.6	32	1.50	100	94.28

Figure 9: Dose Response Curve of Histamine in the absence and presence of extract of Pistacia integerrima (1000 µg/ml) recorded on goat tracheal chain preparation.

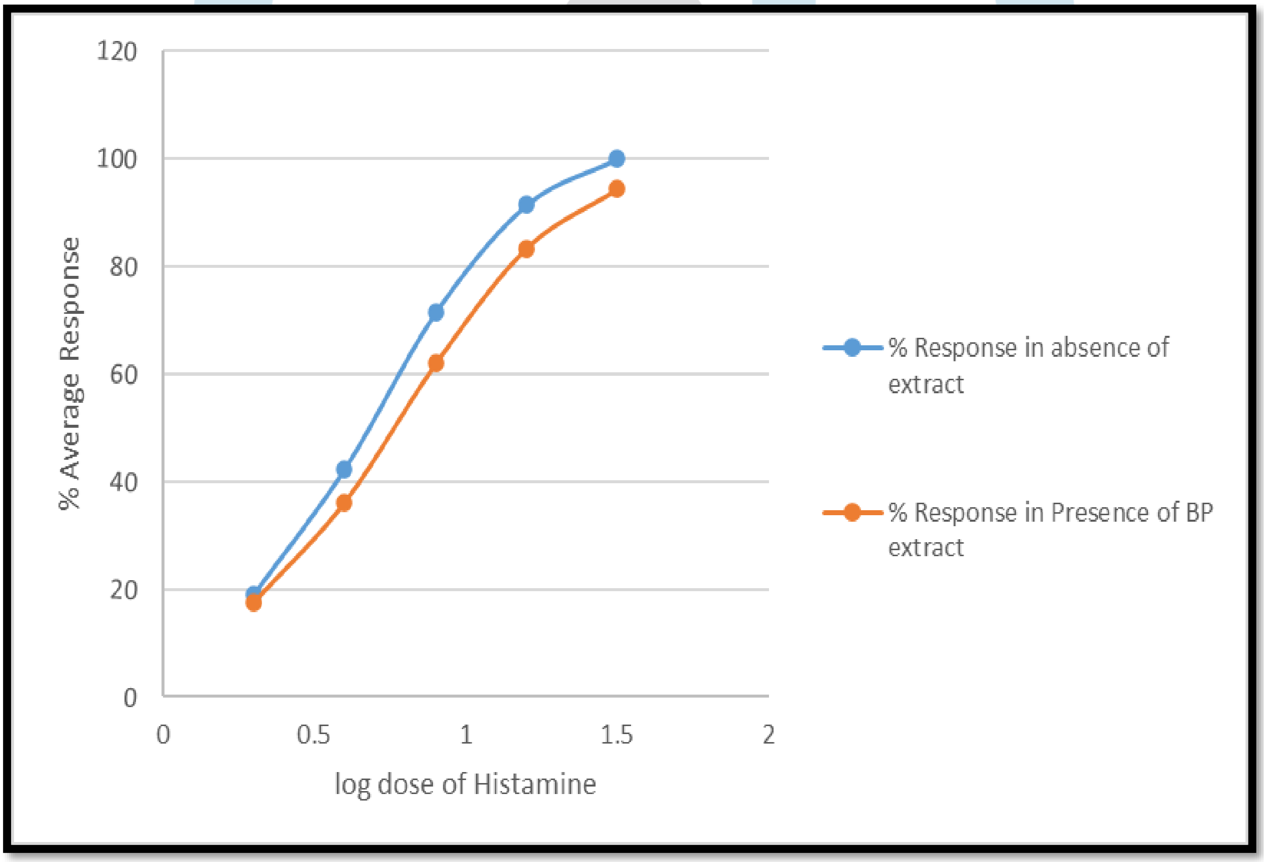
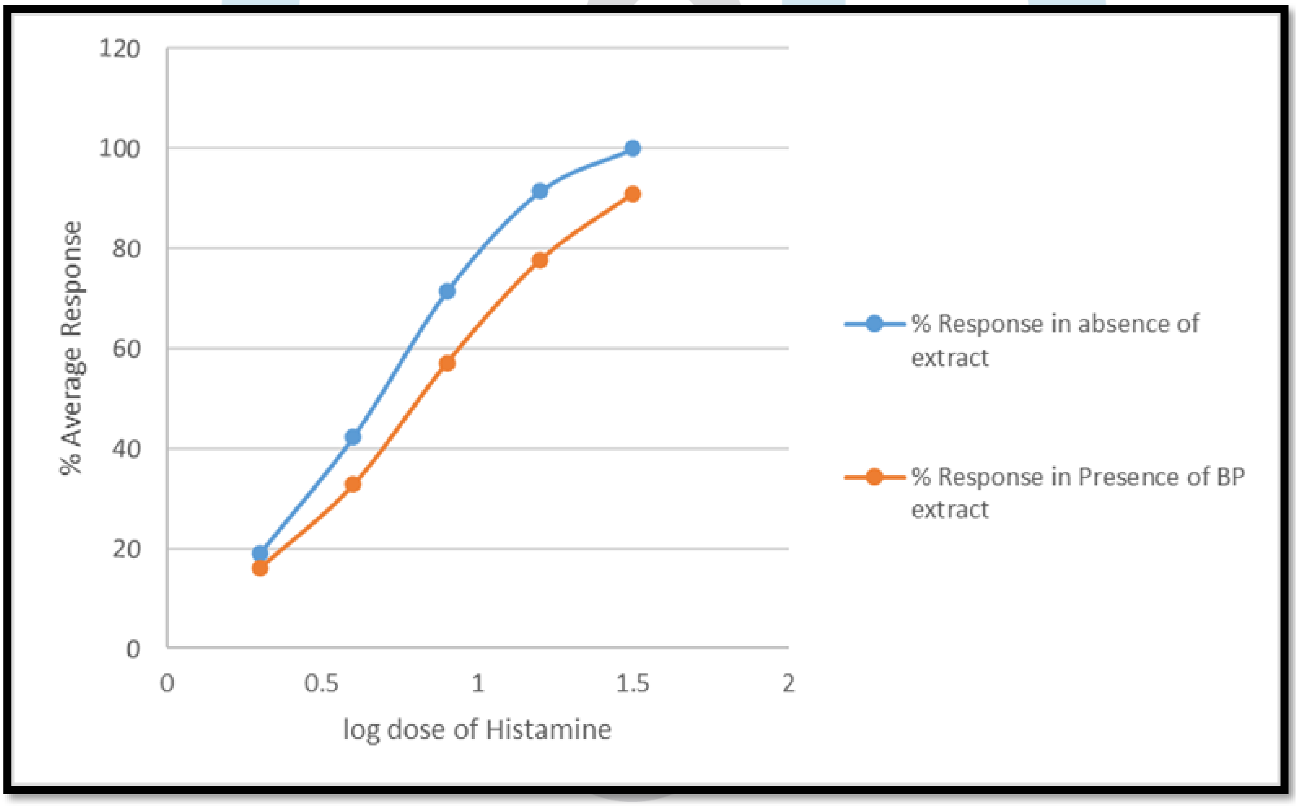


Table No.3: Effect of extract of Pistacia integerrima (1500 µg/ml) on Histamine induced contraction of Isolated goat tracheal chain preparation.

Sr No	Dose of Histamine	Con. of histamine	Log dose of histamine	Average % response in absence of BP Extract	Average % response in presence of BP Extract
1	0.1	2	0.30	19.04	16.19
2	0.2	4	0.60	42.38	32.85
3	0.4	8	0.90	71.42	57.14
4	0.8	16	1.20	91.42	77.61
5	1.6	32	1.50	100	90.95

Figure 9: Dose Response Curve of Histamine in the absence and presence of extract of Pistacia integerrima (1500 µg/ml) recorded on goat tracheal chain preparation.



ISOLATED CHICKEN ILEUM PREPARATION:

1) The fresh chicken ileum was collected from local slaughter house in Tyrodesolution[8] and cleaned off the mesentery. The segment of 2cm long was mounted in a 20ml tissue organ bath and maintained at 37˚C. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min[9].

2) Contact time of 60 sec, and base line of 30sec time cycle were set for proper recording. Dose response curve of acetyl choline was recorded on the kymograph first. Then cumulative concentration-effect curves were recorded on kymograph for Histamine in absence and presence of both aqueous and methanolic extracts of Pistacia integerrima on Kymograph by using Sherrington's Recording Drum[9].

3) The same procedure was carried for concentration-effect curve of histamine in presence of Chlorpheniramine maleate 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[9].

Preparation of Physiological Salt Solution:

Sr. No.	Constituents	Tyrode Solution(g/l)
1.	NaCl	8.0g
2.	KCl	0.2g
3.	MgCl2	0.1g
4.	NaH2PO4.2H2O	0.05g
5.	Glucose	1 or 2g
6.	NaHCO3	1.0g
7.	CaCl2	0.2g

RESULTS AND GRAPHS

Table No.4: Effect of extract of Pistacia integerrima (500 μg/ml) on Histamine induced contraction of Isolated chicken ileum preparation.

Sr No	Dose of Histamine	Con. of histamine	Log dose of histamine	Average % response in absence of BP Extract	Average % response in presence of BP Extract
1	0.1	2	0.30	18.5	18.5
2	0.2	4	0.60	39.5	35
3	0.4	8	0.90	69	69
4	0.8	16	1.20	95	92.5
5	1.6	32	1.50	100	97.5

Figure 10: Dose Response Curve of Histamine in the absence and presence of extract of Pistacia integerrima Gall (500 µg/ml) recorded on chicken ileum preparation.

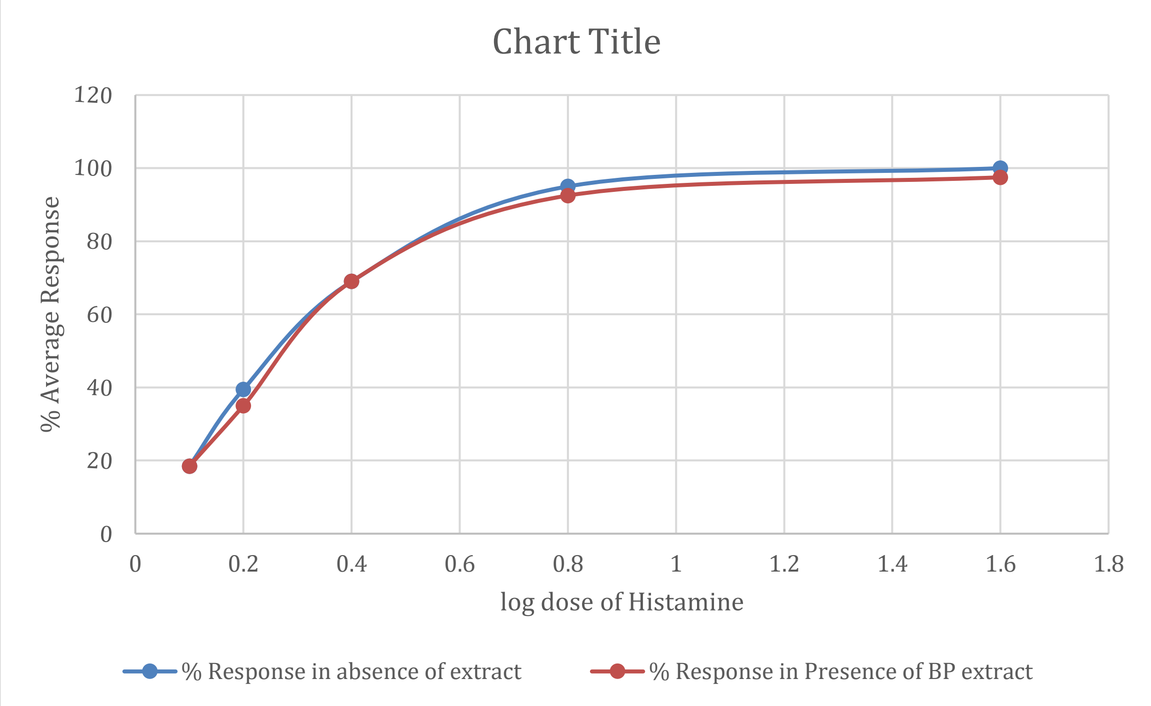
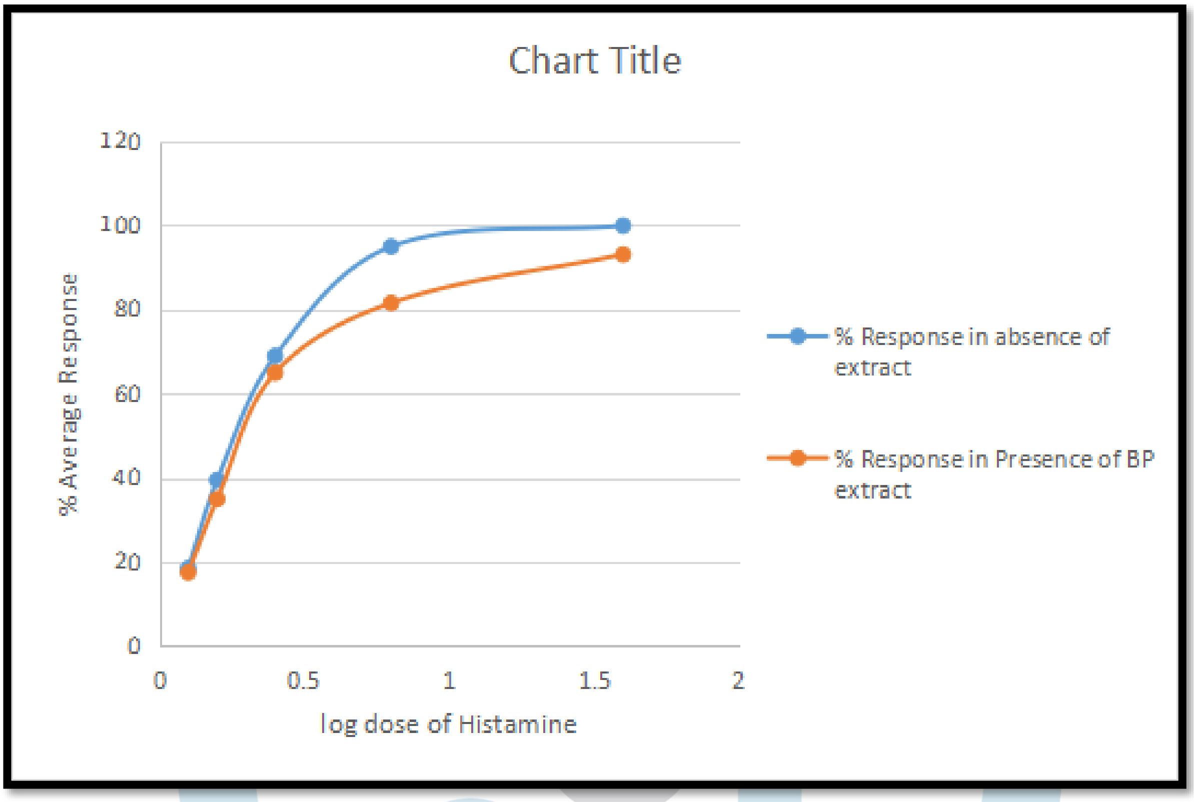


Table No.5: Effect of extract of Pistacia integerrima (1500 µg/ml) on Histamine induced contraction of Isolated chicken ileum preparation.

Sr No	Dose of Histamine	Con. of histamine	Log dose of histamine	Average % response in absence of BP Extract	Average % response in presence of BP Extract
1	0.1	2	0.30	18.5	17.5
2	0.2	4	0.60	39.5	34
3	0.4	8	0.90	69	69
4	0.8	16	1.20	95	81.6
5	1.6	32	1.50	100	93.15

Figure 11: Dose Response Curve of Histamine in the absence and presence of extract of Pistacia integerrima (1500 μg/ml) recorded on chicken ileum preparation.



Asthma is an allergic disease with the utmost clinical and economic effect is an allergic and inflammatory outward sign of respiratory disorders. The illness and mortality of the disease it a worldwide concern. The symptoms of bronchial asthma is characterized by wide blow out narrowing of the bronchial tube due to contraction of the smooth muscle in reply to stimuli subsequently in the release of histamine is used for screening antihistaminic activity. The H1 receptors after stimulation produces well-ordered dose related contraction of isolated Goat trachea. In the current study, Pistacia integerrima significantly inhibited the histamine induced contraction of isolated Goat tracheal chain preparation and chicken ileum indicating its H1 receptor antagonistic activity and supports the antiasthmatic properties of the plant. Bronchoconstriction induced by Histamine is the an immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to spasm in Goat and causes very strong smooth muscle contraction and capillary dilation in cardiovascular system. Bronchodilators can delay the occurrence of these symptoms. The study resulted in deep-rooted the bronchodilator properties of the plant, justifying its claiming in the treatment of asthma. In asthma mostly drugs effective are steroid. Phytochemically the plant discloses the presence of steroidal, flavonoids, nucleus in form of triterpenoids as well various saponin glycosides. The antiasthmatic activity showed by the plant may be because of these chemical moieties. Asthma is essentially characterized by the restriction of obstruction tracheal muscle. The disorder of bronchial asthma is characterized by wide spread narrowing of the bronchial tube due to contraction of the smooth muscle in response to stimuli causing in the release of chemical mediators such as histamine.

CONCLUSION

It can be concluded that the gall extract obtained through solvent extraction by maceration from Pistacia integerrima can be used as raw material for the synthesis of many drugs and thus remain an important source of new therapeutic agent.

Current study resolved that Pistacia integerrima gall extract possess highly substantial anti-asthmatic activity by significantly inhibited the histamine induced broncho constriction of Isolated goat trachea chain preparation and isolated chicken ileum representing its H1 receptor antagonistic activity and support the plants by its anti-asthmatic properties and this proved the traditional claims of anti- asthmatic property of Pistacia intergerrima Gall.

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