EVALUATION OF ANTI-INFLAMMATORY AND ANTI-ASHMATIC ACTIVITY OF *SALVIA HISPANICA* SEED OIL

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Abstract:
Herbal products are often perceived as safe because they are natural. In India, in recent years there is increased research in traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. *Salvia hispanica.,* chia seed oil contains 60% of omega 3 alpha-linolenic acid and 40% of omega 6 linoleni acids. Omega -3 supplementation increases the activity of white cells that gobble up dangerous bacteria. Omega -3 protects the lungs from colds, flus and other respiratory tract infections. Evaluation of anti-inflammatory and anti-asthmatic activity of *Salvia hispanica* seed oil inhibits the percentage contraction at concentration of 100 µg/ml in goat tracheal chain preparation. *Salvia hispanica* seed oil possess significant antihistaminic activity (H1-antagonist) and can be attributed to broncho dilating, anti-inflammatory, activity etc. Hence detailed study needs to be conducted to evaluate the phytoconstituent responsible for the above-mentioned results and their clinical efficacy in the treatment of related diseases.

Keywords: Salvia hispanica, antihistaminic activity, goat tracheal chain

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1. INTRODUCTION:

Asthma is outlined as a chronic disease of the airways. The chronic inflammation is related to airway hyperresponsiveness (an exaggerated airway narrowing response to triggers, like allergens and exercise), that ends up in continual symptoms like wheezing, dyspnea (shortness of breath), chest tightness and coughing.(Fig 1)

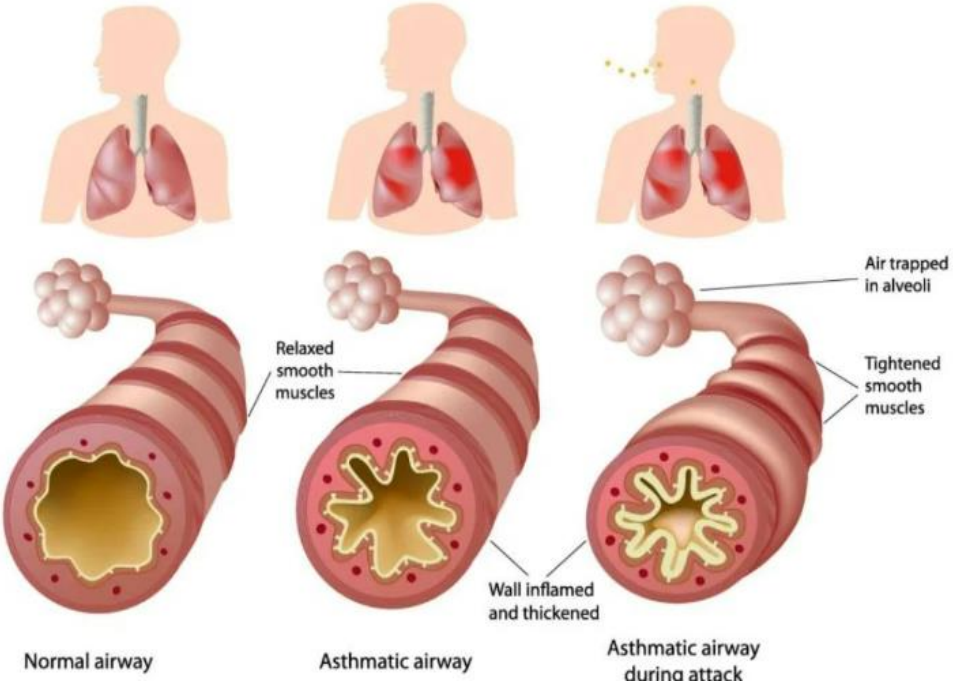

Fig 1:- Bronchospasm constricts the airway

Asthma remains the most common chronic respiratory diseases, affecting approximately 10% of the population (1). According to WHO estimates, there were 417,918 death due to asthma at global level and 24.8 million DALYS attributable to asthma in 2016 (2,3).According to National Family Health Survey-2(NFHS-2) report the estimated prevalence of asthma in India is 2468 per 1, 00,000 (4).Chia seeds are rich in essential fatty acids. Omega -3-fatty acids present in chia seed help to prevent vitamin D deficiency and increases the activity of white cells that gobble up dangerous bacteria .Omega -3-fatty acid also protect the lung from cold, flu and other respiratory tract infection(5).

2. MATERIALS AND METHODS:

2.1 MATERIALS

2.1.1 Chia seed oil:

Extraction Oil from Chia seeds are extracted employing one of the three methods generally used for any oil seeds, (a) compression method involves pressing the seeds at 4 °C or 25 °C in dark. This result in preservation of antioxidant contents, however, oil recovery is limited [6, 7, and 8]. (b) Solvent extraction–involves Soxhlet method using organic solvents like hexane. Though functional characteristics like absorption capacity and emulsifying stability are favoured, this method is least preferred as it poses health issues from the use of hexane [6, 7]. (c) Supercritical fluid extraction–is the most preferable method which uses carbon dioxide at 80ºC resulting in a better purity of ALA. The oil yield is increased with high pressure [9, 10].

2.1.2 Preliminary Phytochemical Screening:

The preliminary phytochemical screening was done for oil extracts of *Salvia hispanica*. The screening tests was performed for various phyto constituents like carbohydrates, proteins, steroids, starch, cellulose, cholesterol, saponins, alkaloids, flavonoids, terpenoids, volatile oils, tannins, glycosides. Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, fatty acids, saponins and glycosides and terpenoids were carried out on the oil extract using standard procedures. (11)

2.1.3 Solubility test:

Oils and fats are soluble in organic solvents like, chloroform, alcohol etc. but are insoluble in water.

2.1.4 Preparation of Kerb's solution

Weigh accurately about NaCl-6.9gm; NaHCO3-2.1gm; MgSO4-1.28gm; KH2PO4-0.16; KCl-0.35gm; CaCl2-0.28gm and dextrose-2gm. Dissolved in distilled water and make upto 1L Then Krebs solution was maintained at 37oC and

gassed with 95% O2 and5% CO2. Tissue was suspended under isotonic solutions

2.1.5 Goat tracheal strip preparation

This method is a modification to the tracheal chain model where the knitting/connecting of the tracheal rings is not performed. In this method, goat tracheal chain was obtained immediately after slaughterhouse of the animals and cut into zigzag fashion thereby exposing large portion of the tissue using the method described by Kulkarni (12). It was suspended in a organ bath of 20 mL containing Krebs-Henseleit solution (Concentration in mM/L as NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 25.0; KH2PO4, 1.2; Glucose, 11.1) maintained at 37 ± 10^{O}C a stream of O2 was bubbled through the organ tube. One end was tied to aerator tube and other attached to isotonic frontal writing lever to kymograph paper on Sherrington rotating drum. Tissue was allowed to equilibrate for 45 min. during which, the bathing solution was changed at every 15 min. under to load of 400 mg (13). The contractile responses of tracheal strip to Histamine were recorded in presence and absence of green tea extracts.

2.2 PHARMACOLOGICAL SCREENING METHODS

2.2.1 ANTI -INFLAMMATORY ACTIVITY

Protein Denaturation Method:

Preparation of control solution: 2ml of egg albumin, 28ml of Phosphate buffer and 20ml of distilled water.

Standard solution: 2ml of egg albumin, 28ml of Phosphate buffer and various concentration of standard drug (Aspirin) (100,200,300,400,500,600 µg/ml).

Test solution: 2ml of egg albumin, 28ml of Phosphate buffer and various concentration of *Salvia hispanica* seed oil (100,200,300,400,500, 600 µg/ml). All the above solutions were adjusted to pH 6.4 using 1N Hcl.

The samples were incubated at 37˚C for 15 mins and heated 70˚C for 5 mins. After cooling the absorbance of the above solution was measured at 560nm using spectrophotometer (14).

2.2.2 ANTIASTHMATIC ACTIVITY

Isolated Goat Trachea Chain Preparation

Isolated adult goat tracheal tissue was obtained immediately after Slaughter house of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37 + 0.5°C. Dose Response Curve (DRC) of histamine (100 µg/ml) in plane Krebs solution and in different doses (10 µg/ml, 50 µg/ml and 100 µg) of *Salvia hispanica* seed oil in ethanol was taken. To record the dose response curve of histamine, in absence and in presence of the Test (Chia seed oil) (15,16).

3. RESULTS AND DISCUSSION:

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

- Solubility test shows presence of oils and fats
- Preliminary Phytochemical screening

Table 1- Preliminary phytochemical screening

SL.NO	EXPERIMENT	INFERENCE
1.	Carbohydrates	+
2	Proteins and Amino acids	+
3	Glycosides	-
4.	Saponins	+
5.	Alkaloids	-
6.	Flavanoids	-
7.	Proteins	-
8	Trepenes	+
9	Phenolic compounds	-
10.	Tannins	-
11.	Sterols and Steroids	+
12.	Fixed oils	+++

3.2 PHARMACOLOGICAL SCREENING METHODS:

In-vitro anti-inflammatory activity:

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied (Table 2). It was effective in inhibiting heat induced albumin denaturation. The study indicated, as the concentration is increased, there is a decrease in absorbance (Fig 2).

Table 2- Protein denaturation (Absorbance Standard VS Test)

Concentration (µg/ml)	Absorbance of standard	Absorbance of test
100	0.542	0.432
200	0.511	0.419
300	0.433	0.363
400	0.379	0.314
500	0.352	0.298
600	0.245	0.211

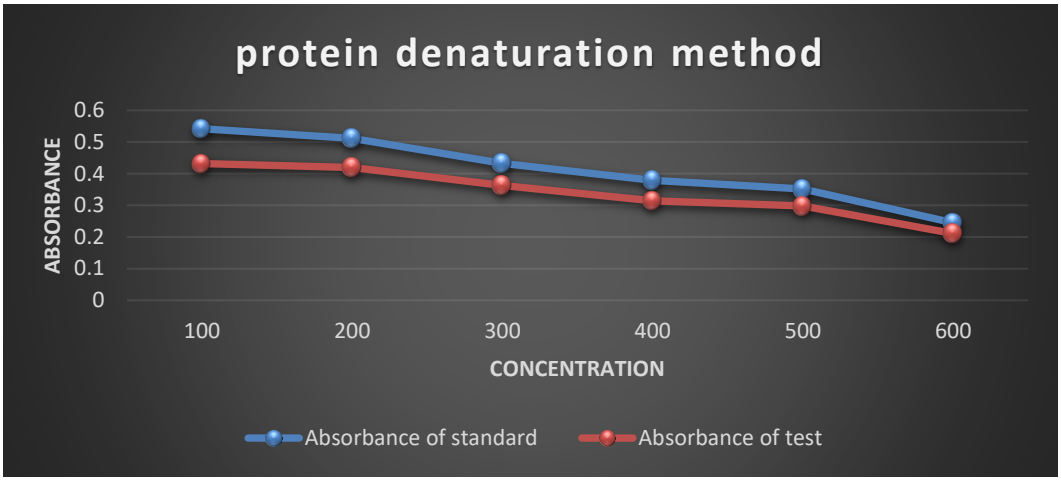

Fig 2 protein denaturation method

3.3 ANTI-ASTHMATIC ACTIVITY

Histamine is autacoids and is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. Besides the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle by acting on H1 – histamine receptor that causes the contraction of intestinal smooth muscle (17).

Histamine is synthesized, store and released by mast cells in the airway wall. In blood, histamine is stored in basophile, the non-mast cell histamine is stored in histaminocytes in the stomach and histaminergic neurons in the brain apart from this number of mediators releases on antigen antibody reaction like kinins and others (18). Although, airway mast cell are likely to be the major cellular source of histamine in asthma there is increasing evidence that basophiles may be recruited to asthmatic airways and may release histamine in response to cytokine histamine releasing factors hence, histamine has multiple effects on airway function that are mediated by specific surface receptors on target cells. H1 – histamine receptor mediate most of the effects of histamine that are relevant to asthma (19).

Certain literatures suggested that H1 – histamine receptor have been demonstrated in animal and human lung, goat tracheal chain and guinea pig ileum are responsible to produced bronchial, smooth muscle contraction.

Table 3- anti asthmatic activity (Dose VS Percentage Response)

S. No	Dose		Percentage of response height				
	In ml	In mg/ml	Histamine	Test1(10µg/ml)	Test 2 (50µg/ml)	Test 3 100(µg/ml)	Std anti histamine
1)	0.1	10	52.1	50.2	52.8	36.8	34.6
2)	0.2	20	55.1	63	65.3	52.6	46.3
3)	0.4	40	68.4	70.1	80.8	68.2	62.8
4)	0.8	80	85.3	83.5	90.2	94.9	97.5
5)	1.6	160	100	100	100	100	100

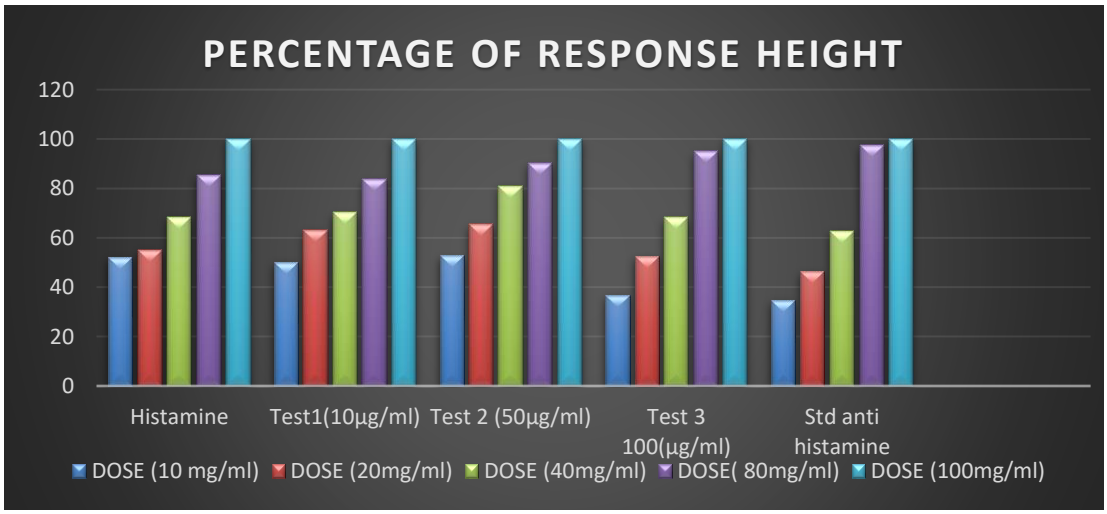

Fig 3 Anti asthma activity dose vs percentage response

Thus in the present study we are using isolated goat tracheal chain preparation. The similar response exhibited by the extract in case of goat trachea chain preparation, which support the above statement that *Salvia hispanica* seed oil are acting on H1 – histamine receptor as antagonists (20).

In the present study, it was observed that three different concentrations (10µg/ml,50 µg /ml,100 µg /ml) of *Salvia hispanica* seed oil inhibits the contractions produced by histamine (100 µg /ml) in the tissue preparations (Table 3). Histamine was taken in different dose levels and DRC was plotted (Fig 3) and histamine was able to produce notable contraction on isolated goat tracheal chain.

4. DISCUSSION:

The study behind this article is anti inflammatory and anti asthmatic activity. While using synthetic drugs like prednisolone, hydrocortisone etc we observed various adverse effects hence we consider natural or herbal remedies to our current study asthma. *Salvia hispanica* seed oil was taken in consideration. *Salvia hispanica* seed oil which consists of omega 3 fatty acid and is useful to control airway inflammatory asthma.

Also we came out with isolated goat tracheal chain preparation to act against histamine which causes bronchoconstriction. the sample extracted from *Salvia hispanica* seed oil was found to reduce the histamic activity by inhibiting H1- receptor which causes inflammation related to asthma. Thus this extract of oil may act as histamine antagonist.

5. CONCLUSION:

Salvia hispanica seed oil is rich in omega 3 fatty acid. Omega-3 protects the lungs from cold, flu and other respiratory tract infections. No doubt you have heard of the benefits of taking cod liver oil during the winter months, chia seed oil also works on the same mechanism (5). The two mechanism such inflammation and immune system are involved in Pathophysiology of asthma.

A study revealed that the presence of omega 3 fatty acid in chia seed oil was already proven for anti-inflammatory and Immunomodulatory activity (21).

Hence on the basis of the previous report we chose *Salvia hispanica* seed oil for current study. The preliminary phytochemical analysis of the *Salvia hispanica* seed oil shows the presence of fatty acids. The dose response curves of *Salvia hispanica* seed oil showed significant H1 receptor antagonistic activity. This oil is well proved for a better anti-asthmatic activity and recommended for future use. It is suggested that the anti-asthmatic effect of the *Salvia hispanica* seed oil could be further evaluated in other experimental animals.

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