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Research Article

EVALUATION OF ANTI-CHOLINERGIC AND ANTI-ANAPHYLACTIC ACTIVITY OF SHIRISHADI POLYHERBAL COMPOUND

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ABSTRACT
Asthma is a chronic inflammatory disease of airways with widespread narrowing of air passage which may be relieved spontaneously or as a result of therapy and, clinically it is characterized by paroxysms of dyspnea, cough and wheezing. Inflammation and broncho-constriction are the two major hallmarks in the pathology of Asthma. Shirishadi is a polyherbal drug used in the management of bronchial asthma by Ayurvedic practitioners from decades. Shirisha (*Albezzia lebbeck*), Nagarmotha (*Cyprus rotandus*) and Kantakari (*Solanum xanthocarpum*) are the ingredient herbs of this compound. Hence, the present investigation was undertaken to evaluate the bronchodilator and anti-anaphylactic activity of Shirishadi Polyherbal compound. Experimental models studied were egg albumin induced anaphylaxis in guinea pigs and Anti-Cholinergic activity was studied on Isolated Frog Heart and Frog Rectus Muscle. The extract produced 30± 0.23% inhibition in maximum contraction produced by Acetylcholine which is much less than that produced by standard drug (99.9%), moreover the dose of extract that produced the visible effect is much higher than that used for therapeutic purpose suggesting that antiasthmatic effect of drug is not due to Acetylcholine antagonism activity. Neither Acetylcholine efficacy nor its potency decreases significantly with increasing dose of drug. Drug increased the cardiac tone and stimulate the cardiac contractility but unable to prevail over complete inhibition of heart rate produced by Acetylcholine. The drug produced significant protection against egg albumin induced anaphylactic shock characterized by decrease in intensity and delay in the development of symptoms of dyspnoea, asphyxia and collapse. All these findings reveal the bronchodilator and anti-anaphylactic activity of Shirishadi compound indicating its beneficial use in asthma.

Key words: Shirishadi Polyherbal compound, Anti-cholinergic activity, Anti-anaphylactic activity.

INTRODUCTION

Asthma is defined as a disorder characterized by chronic airway inflammation and increased airway responsiveness to a variety of stimuli. It involves in complex interactions between many cells and inflammatory mediators that results in inflammation, obstruction (partially or completely reversible after treatment or resolves spontaneously), increased airway responsiveness (i.e. hyper-responsiveness) and episodic asthma symptoms[1]. Research indicates that airway hyper-responsiveness is important in the pathogenesis of asthma and that the level of airway hyper-responsiveness usually correlates with the clinical severity of asthma[2]. The available treatment options have major limitations owing to low efficacy, associated adverse events and compliance issues[3]. As a result, there is high prevalence of usage of complementary and alternative medicines for treatment of this disease.

Ayurveda, an ancient system of Indian medicine, has recommended several drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders [4]. *Albizia lebbeck* also known as tree of happiness is extensively used herb in various traditional medicines[5]. In Chinese system of medicine it is used for relieving stress, anxiety & depression. Whereas in Ayurveda (Indian system of medicine) it is told to be Vishaghana i.e. destroying toxins present in body. It is mainly indicated in allergic conditions such allergic rhinitis, allergic asthma, urticaria etc[6]. Research studies had shown that it possess anti-histaminic & mast cell stabilizing property by virtue of which it is supposed to work as anti-asthmatic[7,8]. It also has anti-inflammatory and antioxidant properties. *Solanum xanthocarpum* known as kantakari in Ayurveda is very effective in respiratory tract disorders. It is found to have strong bronchodilator effect along with anti-inflammatory property. *Cyperus rotandus* or Mustaka is thought to have originated in India and then spread from there during the past 2,000 years. Its uses in modern Ayurvedic medicine are primarily for treating fevers and digestive system disorders (diarrhoea, vomiting, indigestion, etc.). Shirishadi is a polyherbal compound successfully use for the management of Asthma from decades. The present study aims to search the probable mode of action of Shirishadi drug in Asthma by using various pharmacodynamic parameters.

Plant collection

The plants *Albizzia lebbeck, Cyprus rotandus* and *Solanum xanthocarpum* were collected from local market of Varanasi. The identification of the drugs was done by Prof.A.K. Singh, Department of Dravyaguna, S.S.U., Varanasi.

Extraction of the plant material and sample preparation

Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug was carried out by Hot percolation method through soxhlet appratus. Thereafter extract was dried using rotatory evaporator and dried extract was put to the process of standradization.The extract was dissolved in distilled water and different concentration of drug was prepared.

Animals

All animals were housed at ambient temperature (22 ± 1°C), relative humidity (55 ± 5%) and 12/12 hlight/dark cycle. Animals had access to standard pallet diet and water given ad libitum. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India

Egg albumin induced anaphylaxis in guinea pigs

Guinea pigs were sensitized by two intra-peritoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48-h interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and

served as control group. Animals of Group II received ethanolic extract of Shirishadi compound (500 mg/kg, p.o., once daily) dissolved in distilled water for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for the onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms (min.) and mortality. The severity score with respect to symptoms was recorded using the method of Gupta *et al*. as: increased respiratory rate, dyspnoea for 10 min., dyspnoea and cyanosis for 10 min and collapse. Guinea pigs remaining alive after the antigen challenge were counted to record the percentage of mortality due to anaphylactic shock by using the following equation :

$$\text{Mortality rate (\%)} = \frac{\text{Number of guinea pigs collapsed}}{\text{Total number of experimental animals}} \times 100$$

Steroidal Effect

25 albino rats of either sex (100 to 150 g) were taken and divided into five groups (n=5 for each group). Twenty animals were treated with escalating doses of Shirishadi compound as 200mg/kg, 500mg/kg, 1g/ kg & 2gm/kg b.wt. respectively for one week. Five control animals were treated with equivalent volume of distilled water. On the seventh day the animals of both the groups were sacrificed and effect of drug on adrenal gland, and spleen was estimating by weighing the organ.

Studies on Isolated Frog Heart and Frog Rectus Muscle for Anti-Cholinergic activity

The isolated frog rectus abdominis

A frog is pitched and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally onto the anterior aspects of the limbs. This exposes the flat whitish muscles of the anterior abdominal wall from their pubic origin to their sternal insertion. The two recti are removed and placed in frog Ringer solution in a shallow dish. They are carefully cleaned and one of them is trimmed to the desired size and mounted in an organ bath of 5ml capacity, at room temperature, aerated with oxygen. For recording purposes, an isotonic lever with a sideways writing point is used tangential to the smoked drum, balanced for a tension of 2.5gm with an extra load of 1gm on the long arm. The latter serves to bring back the lever to baseline, since when the rectus has contracted, it does not relax rapidly even on washing out of the drug. (Table 2 & 3, Fig 1& 2)

Isolated Perfused Frog's Heart

A frog is pithed and pinned it on frog broad. The skin is slit open in the midline throughout the length. The abdominal musculature was slit open in the midline upto the sides of Xiphoid cartilage, pectoral girdle was removed and heart was exposed. Th epericardium was open and few drops of frog's Ringer was dropped over the heart. A thread was passed under the vena cava to tie the cannula and make a small nick in it. Then a small nick is made in one of the aortae. The frog board was fixing on the plane stand and venous cannula is tie to the inferior venacava and perfusion was started. The venous pressure was maintained at 2-4cm of water by altering the height of perfusion bulb and then opening completely the screw clamp. A universal lever was then fixed on the plain stand. A small thin hook was pass through the tip of the ventricle and tie with the free limb of lever. A tension of 4g and magnification of 10 X was maintained. (Table 3)

RESULT & DISCUSSION

Asthma, a chronic relapsing inflammatory disease characterized by hyper-reactive airways, leading to reversible bronchoconstriction. The inflammation causes an associated increase in airway responsiveness to various stimuli[12]. Bronchoconstriction, cough, mucus production and airway hyper-sensitivity to bronchoconstriction mediators are the main clinical manifestations of asthma and these features correlate well with the severity of the disease. Single mediator approach to asthma therapy is difficult as the disease process involved in asthma is complex [13].

Parasympathetic nerves are characterized by their release of neurotransmitter acetylcholine. The vagus nerve sends motor fibres from the brain to smooth muscle cells in the bronchial walls and the stimulation of the vagus nerve releases acetylcholine, which binds to specific "cholinergic" receptors on smooth muscle cells within the bronchial walls and thus constricts the airways.

Thus, it can be concluded that cholinergic stimulation causes broncho-constriction through airway smooth muscle contraction [14].

Evaluation of anti-cholinergic effect of drug both on nicotinic & muscarinic receptors shown that drug does not have significant anti-cholinergic property. To evaluate the efficacy of drug on nicotinic receptor the extract of polyherbal drug was added to perfused isolated frog rectus muscle preparation at different concentration in the presence of graded dose of Acetylcholine. The extract produce 30$\pm$ 0.23% inhibition in maximum contraction produce by Acetylcholine which is much less than that produce by standard drug (99.9%), moreover the dose of extract that produce the visible effect is much higher than that use for therapeutic purpose suggesting that antiasthmatic effect of drug is not due to Acetylcholine antagonism activity. Neither Acetylcholine efficacy nor its potency decreases significantly with increasing dose of drug as shown by left shifting of graph (Fig. 1).

Anaphylaxis is defined as "a serious allergic reaction that is rapid in onset and may cause death". On a pathophysiologic level it is an acute multi-system **type I hypersensitivity reaction**. "True" anaphylaxis is caused by degranulation of mast cells or basophils mediated by immunoglobulin E (IgE), and pseudo-anaphylaxis occurs without IgE mediation [15,16]. Anaphylaxis caused by egg albumin induced a rapid and huge increase of plasma catecholamines especially adrenaline. Anaphylaxis induced by egg albumin is true type with type-1 hypersensitivity reaction induced by IgE mediated immune response mainly against ovaalbumin and ovamucoid. As shown in Table 1, an intravenous challenge of egg albumin resulted in a fatal anaphylactic shock in 70% of the animals in control group characterized by symptoms of dyspnoea, asphyxia and collapse. Pre-treatment with ethanolic extract of Shirishadi compound (100 mg/kg, p.o.) significantly protected the sensitized guinea pigs against anaphylactic shock as the onset of symptoms were delayed, less severe and none of the animals collapsed (Table 1).

Statistical analysis for symptoms by paired *t*-test; values are mean ± SEM; n = 5 in each group; significantly different from control group **P*<0.001.

Anti-anaphylactic activity of drug suggest that it might prohibit the antigen antibody (IgE bound to the surface of mast cell) interaction or stabilize the mast cell and prevent the massive liberation of various inflammatory mediators such as histamine etc. Thus probably it act at three stages in disruption of pathogenesis as 1. Desensitization by preventing antigen –antibody interaction 2. Stabilizing mast

cell membrane 3. Mediator antagonism. This result is accordance with earlier studies on *A. lebbeck* (Tripathi. et al. BHU- 1979) that indicate that the anti-anaphylactic activity of *A. lebbeck* (one of the constituent of Shirishadi compound), besides being due to cromoglycate-like action on the mast cells is also due to inhibition of the synthesis of antibodies and suppression of T-lymphocyte activity [17,18, 19,20].

Seven days treatment produces no significant change in weight of adrenal gland and spleen, proving that there are no evidences that the drug in any way stimulates the adrenal gland. Thus it can be said that anti-anaphylactic activity is not attributed due to stimulation of adrenal gland.

The present findings reveal protection against egg albumin induced anaphylactic shock characterized by decrease in intensity and delay in the development of symptoms of dyspnoea, asphyxia and collapse. In line with this notion, anti-anaphylactic effect of Shirishadi may be due to inhibition of phenomenon of sensitization or non-availability of antibodies on the mast cell surface. All these findings reveal the bronchodilator and anti-anaphylactic activity of Shirishadi indicating its beneficial use in asthma.

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Table 1: Effect of Shirishadi Compound on egg albumin induced anaphylaxis in rats

Group	Pre convulsion time (min)		Percent protection	
	Onset (min)	Duration (min)	Severity (score)	Mortality (%)
Group I (Control)	1.246 ± 0.056	23.66 ± 0.345	9.4 ± 0.306	70%
Group II (Shirishadi compound 100mg/ Kg, p.o.)	2.914 ± 0.088*	7.054 ± 0.131*	3.0 ± 0.615*	0%

Table 2: **Effect of Hydroethanolic extract of Shirishadi Extract on various organ weights after 7days**

Dose/ 100gm of animals	Lung/ 100g bwt N= 3	Liver/ 100g bwt N= 3	Stomach/ 100g bwt N=3	Kidney /100 g bwt. N= 3	Heart/ 100g bwt N=3	Adrenal gland/ 100g bwt N= 3	Testis/ 100g bwt. N=3	Spleen/ 100g bwt N=3
Control	948± 1.56	3200± 0.08	1078±1.78	350±2.05	330± 1.98	7.1±3.67	884±0.56	281± 1.05
200mg	875± 2.34	3600 ± 1.23	1136±2.3	340± 1.56	320± 2.08	7.8±0.68	865± 1.78	286± 0.56
500mg	890± 0.54	3450± 1.54	1010±0.01	300± 0.04	305± 3.56	6.9± 2.36	840±0.47	260± 1.23
1gm	930.5 ± 0.90	3545 ± 3.21	1080 ± 2.78	365±0.64	315± 0.01	7.5 ±1.67	884 ± 0.02	290± 0.56
2gm	950± 1.04	3711± 0.21	1178± 0.08	370± 1.02	327± 1.36	8± 2.98	870±0.05	280± 0.01

Table -3; Competitive Drug Antagonism in Frog Rectus Muscle Preparation

S/No.	Dose of Acetylcholine (10 µg/ml)	Log molar concentration of Acetylcholine	Control % maximum response	Dose of S.E. (mg/ml)	% Inhibition of maximum Ach contraction by Shirishadi compound	% Inhibition of maximum Ach contraction by Shirishadi Compound
1.	0.1ml	7.05	30 ± 2.03	1	2.06 ± 1.76	1.3± 0.05
2.	0.2ml	6.66	56± 1.45	5	4.36± 0.56	5.6± 0.45
3.	0.4ml	6.35	75± 3.05	10	10± 3.12	20.45± 2.13
4.	0.8ml	6.20	89± 1.25	20	24.98± 2.34	32± 4.56
5.	1.6ml	5.75	99.9± 0.98	50	30.2± 0.23	35± 3.12

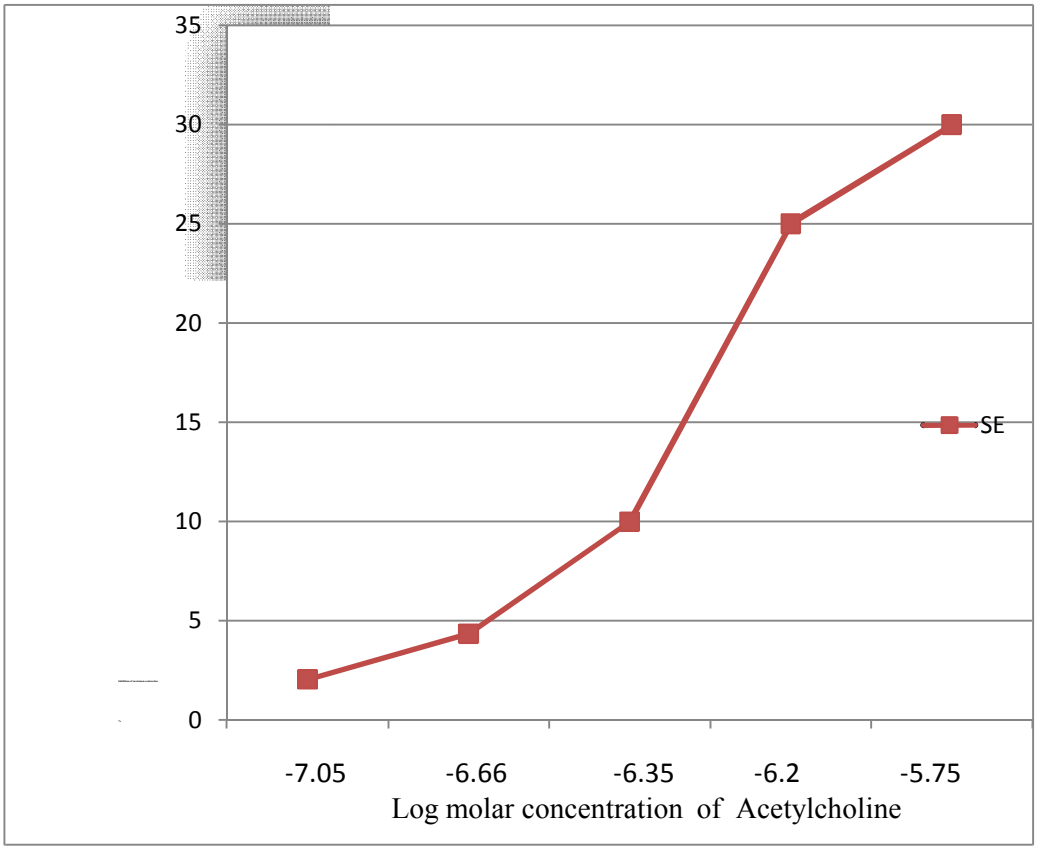


Fig 1: Effect of increasing concentrations of hydroalcholic extract Shirishadi on the cumulative dose responses of Acetylcholine on Frog Rectus muscle preparation

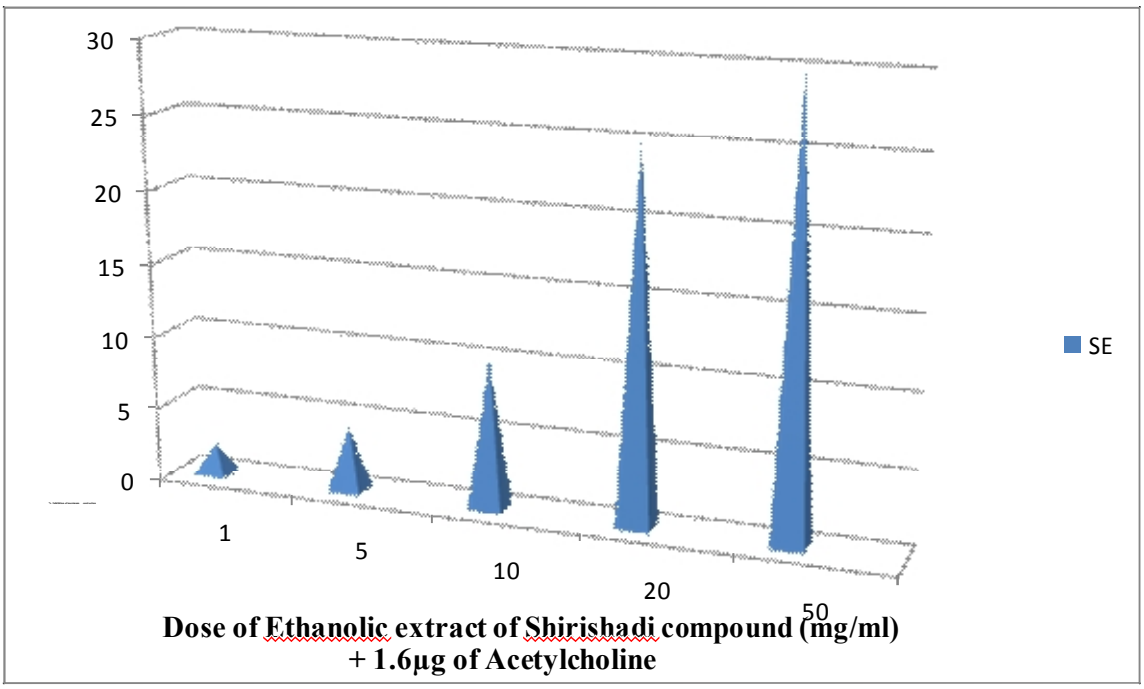


Fig. 2; Competitive Antagonism of Acetylcholine by Shirishadi extract on isolated perused frog rectus muscle

Table- 3; Activity of drug on isolated perfused frog heart:

S.No	Drug	Dose (mg/ml)	Cardiac Rate	Cardiac Rhythm	Cardiac Tone	Cardiac Contractility
1.	Shirishadi Extract	5	42	Regular	Increase	Stimulant
2.		10	46			
3.		20	50			

Normal Heart Rate before administration of Drug= 54/min, Heart rate after Acetylcholine =22/min

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