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In-Vitro and In-Vivo Assessment of Anti-Asthmatic Activity of Polyherbal Ayurvedic Drug

[1]Kajaria Divya*, Kajaria Ankit[2], Tripathi. J.S[1], [1]tiwari. S.K[1]
[1]*Dept. of Kayachikitsa,Faculty of Ayurveda,Institute of Medical Sciences, B.H.U.- 221005, Varanasi, India.*
[2]*Dept. of Biomedical Sciences,Indian Institute of Technology, B.H.U.-22005,Varanasi, India.*

Abstract: *About 80% of asthmatic turn to alternative or complementary therapies typically in conjunction with their regular allopathic medication. The role of complementary and alternative medicine in adult asthma treatment is limited because these approaches have been insufficiently researched and their effectiveness is largely unproven. In the present study in –vivo and in-vitro effectiveness of a polyherbal Ayurvedic drug is evaluated for its anti-asthmatic activity. For in –vitro assessment of anti-asthmatic property of drug antiinflammatory, analgesic, immunomodulator effect, and antihistaminic, anti-cholinergic, mast cell stabilizing activity, anti-anaphylactic activity and bronchodilator effect were screen on animal models. Evaluation of Effect of Drug Distribution on Lung Mechanics is also evaluated using MATLAB. In a randomised,open, placebo controlled trial the effects of drug was compared with placebo medication (normal saline) in 60 adults with mild to moderate asthma as an adjunct to conventional treatment. Animal studies showed that drug possess significant mast cell stabilizing activity, immunomodulator activity, bronchodilator activity and anti-anaphylactic activity. Insignificant anti-cholinergic activity was found in the drug. There was significant improvement found in pulmonary function test (including FEV1, FVC and PEFR)in the group treated with polyherbal drug .Improvement remain constant in consecutive follow-ups signifies that there is no reverse bronchoconstriction after discontinuation of drug. This study signifies that polyherbal drug (Shirishadi) may prove beneficial future alternative remedy for asthma and its effect is similar to that of modern contemporary drug when given through nasal route.*

Key Words*: Shirishadi polyherbal compound, immunomodulation, MATLAB, PFT.*

I. Introduction

Now progress in western medical research has reached a bottleneck, as single compound drugs are costly to create, synthesis and manufacture. To see real and sustained progress in research it is necessary to utilize traditional remedies to develop advanced medicines. During the past decade there has been a paradigm shift from utilizing single target drugs to multi-target drugs. The concept of multi-targeted therapy represents the conventional herbal medicine treatments that often employ multicomponent plant tissues extract as natural product mixtures. However very few phytomedicinal products have clear or systematic documentation comparable to that of chemically synthesized drugs as single chemical compound. In present study we aim to justify the use of self experienced polyherbal drug in the management of asthma and explore its pharmacodynamic properties. *Shirishadi* is a polyherbal drug having equal quantity of three herbs namely *Shirisha (Albizia lebbeck), Nagarmotha (Cyprus rotandus)* and *Kantakari (Solanum xanthocarpum).* All the three herbs were collected from the local market of Varanasi (U.P, India) and then identified by Prof.A.K.Singh (Dravyaguna deptt of Ayurveda, IMS,BHU). Hydroethanolic extract of the three herbs were extracted out by hot percolation using soxhlet apparatus. *Shirishadi* polyherbal compound is a self experienced Ayurvedic drug use for the management of allergic respiratory diseases. In Ayurveda *Shirisha* (*Albizia lebbeck*) is told to be *Vishaghana* i.e. destroying the toxins present in the body. It is mainly indicated in allergic conditions such allergic rhinitis, allergic asthma, urticaria etc[1]. *Solanum xanthocarpum* known as *Kantakari* in Ayurveda and is very effective in respiratory tract disorders[2]. It is found to have strong bronchodilator effect along with anti-inflammatory property[3]. *Cyprus rotundus* 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 (diarrhea, vomiting, indigestion, etc.).

II. Material And Methods

Aminopyrine – 50mg/Kg, oral administration, Carrageenin, Pentazocin- 10mg/Kg bwt, I.P. administration, 1hour before aceti acid injection, Endomethacin- 25mg/Kg bwt, oral administration, 1hour before carrageenin injection, Acetic acid- 0.7% acetic acid at a dose of 0.1ml/10 g given Intraperitonealy, Cyclophosphamide (Sigma, life science)- The Cyclophosphamide was suspended in 0.5% Carboxy methyl cellulose solution in distilled water. The solution was administered intraperitoneally at a dose of 30 mg/kg b/w, Ova albumin – Intraperitoneal injection of 0.5ml and 10% w/v solution, Atropin sulphate (E.Merck,Germany),

Anesthetic ether (BDH, India), Dexchlorpheniramine maleate (Schering) dissolved in distilled water to make desired concentration, Histamine dihydrochloride (Sigma Chemical Co. U.S.A.) dissolved in distilled water to make desired concentration, Mepyramine melate- (10 mg/kg= 0.1% Soln.), Acetylcholine chloride (Sigma Chemical Co. U.S.A.), Ketotifen -1 mg/kg, oral administration.

Analgesic and Anti-inflammatory Activity (Winter et al. 1962)

For the present experiment, carrageenin suspension was prepared as a homogenous suspension of powder in 0.9% sodium chloride solution (sterile normal saline) with the help of mortar & pestel. A volume of 0.1ml of suspension was injected through a 26 gauge needle into the plantar surface of the right hind paw below the plantar aponeurosis 1h after the oral administration of test materials. The volume of hind paw of the rats upto the ankle joint was measured plethysmographically, by the mercury displacement method. The volume was measured 1h, 2h ,3h, 4h & 24 after the administration of drug. The extract was administered at 50, 200 and 500 mg/kg body weight. Endomethacin 25 mg/kg body weight was used as standard antiinflammatory agent.

Table No. 1 : Anti-inflammatory activity of crude extract of *Shirishadi* compound by carrageenin induced rat paw edema

	% Increase in Paw Volumes (ml × 1000) ± SEM (percent inhibition)				
Groups	1h	2h	3h	4h	24h
Control	1.78 ± 0.77	3.0 ±0.15	3.61 ± 0.20	4.0± 1.2	1.93 ± 0.11
Standard (Indomethacin 25mg/kg)	0.72 ± 3.7 59%	0.69 ± 0.60 77%	0.67 ± 0.66 81%	0.65 ± 0.37 92%	0.66 ± 0.52 66%
Shirishadi 50mg/ 100gm bwt	1.06 ± 0.68 40%	0.99 ± 0.66 67%	0.93 ± 0.33 74%	0.89 ± 0.33 77%	0.78 ± 0.41 59%
Shirishadi 200mg/100gm bwt	1.0 ± 1.0 43%	0.97 ± 0.14 67.66%	0.92 ± 0.24 74.28%	0.88 ± 0.57 79%	0.77 ± 0.24 60%
Shirishadi 500mg/100gm bwt	0.95 ± 0.33 46.6%	0.95± 0.37 68%	0.83 ± 0.66 77%	0.76 ± 0.63 81%	0.66 ± 0.33 65%

Values are mean±SEM (N=3).

Acetic acid induced writhing test

Pentazocin and aspirin were used as standard analgesic agents. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min,10 min after the application of acetic acid.

Table No 2: Effect of *Shirishadi* Extract on acetic acid induced writhing response in rodents: (Saha *et al*., 2007)

Groups	**Dose of Drug**	**Writhing**[b]	**% Inhibition**
Control	--	15.6 ± 0.50	--
Standard	Pentazocin 10mg/Kg, i.p.	4.3 ± 0.66 **	71.2%
	Aspirin 25 mg/Kg, i.p.	8.0 ± 1.15 **	63.5%
Shirishadi	200mg/100gm bwt, p.o.	7.66 ± 0.88 **	65.6%
	500 mg/ 100gm bwt, p.o.	6.33 ± 0.88 **	70.9%
One way ANOVA	F	46.94	--
	df	4,12	--
	P	<0.001	--

[a] 1hr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 minutes after the injection, the number writhing was counted for 10 min.

[b] Values are mean ± SEM (n = 5, for control & 3in drug treated groups); One-way ANOVA; ***P*<0.001, compared to control.

Radiant heat tail-flick method

The central analgesic activity of the plant material was studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails by using a Medicraft Analgesiometer Mask-N (D'Amour and Smith,1941) and described previously (Saha *et al*., 2007).Briefly, the current intensity passing through the naked nicrome wire was maintained at 5 ampere. The distance between the heat source and the tail skin was 1.5 cm and cut-off reaction time was fixed at 10 second to avoid any tissue damage. Pentazocin was used to compare the analgesic effect of the plant extract.

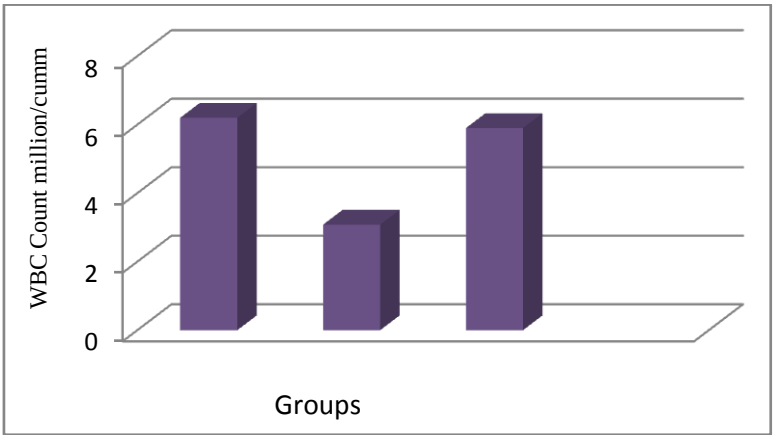
Table No 3: Effects of crude extract[a] on radiant heat tail-flick response in rodents: (D'Amour and Smith, 1941)

Groups	Dose of Drug	Reaction Time (sec)		
		30 (mins) (% elongation)	**60 (mins) (% elongation)**	**120 (mins) (% elongation)**
Control	--	4.65 ± 0.15	4.82 ± 0.16	4.98± 0.20
Standard	Pentazocin 10mg/ kg bwt, i.p.	8.65 ± 0.71**	6.45 ± 0.45**	5.89 ± 0.37**
Shirishadi Compound	200 mg/100gm bwt, p.o.	7.41 ± 1.17** (58.1%)	7.10 ± 0.30** (56.3%)	6.08 ± 0.21** (49%)
	500 mg/100gm bwt, p.o.	7.98 ± 0.12** (61.1%)	7. 57 ± 0.45** (59%)	6.75 ± 0.15** (54%)
One way ANOVA	F	68.5	27	5.34
	P	< 0.001	< 0.001	< 0.001
	df	7,40	7, 40	7,40

a .per oral administration of vehicle and crude extract, radiant heat intensity was 5 amp.
b morphine was administered sub-cutaneously.
c Values are mean ± SEM (n =5); One-way ANOVA; df = 7, 40; ***P*<0.01, **P*<0.05 compared to control.

Immunomodulator Activity (S.Govindan ,2004 & R.E.Fleming,1997)

The animals were divided into the 3 groups containing 6 animals (mice) in each group. Group1 (Control group) received Carboxy Methyl Cellulose (CMC) for 14 days and group 2 (Challenge group) received CMC for 10days, on 11th, 12th and 13th day Cyclophosphamide intraperitonially at a dose of 30mg/kg b/w. Groups 3 (Test group) received ethanolic extract of the drug at a dose of 500mg/kg body weight orally for 14 days. On days 11,12 and 13th day Cyclophosphamide solution was given intraperitonially at a dose of 30mg/kg b/w one hr after the administration of the extract.



All values are mean±SEM, n=6.
***P<0.001 when compared with control group and, ###P<0.001, when compared with Cyclophosphamide treated group (Students t test).
b. P<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

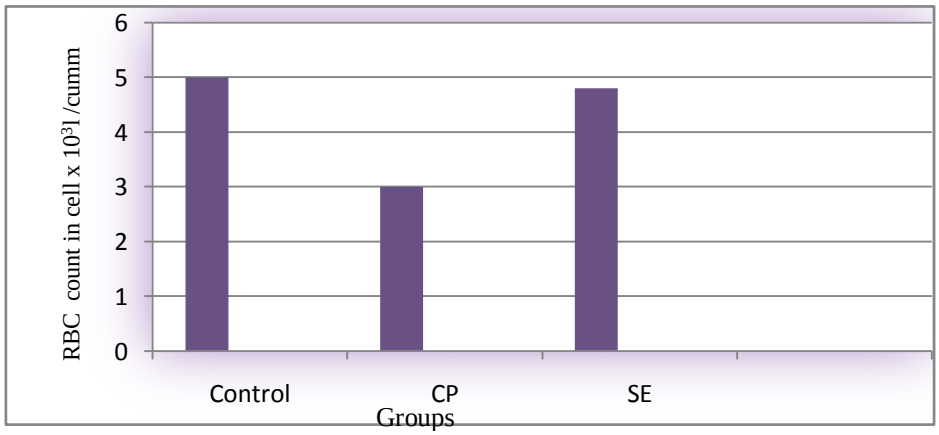


Figure 2. Effect of ethanolic extract of *Shirishadi* Compound on RBC count.
All values are mean±SEM, n=6.

***P<0.001 when compared with control group and , ###P<0.001, when compared with Cyclophosphamide treated group (Students t test).
b. P<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

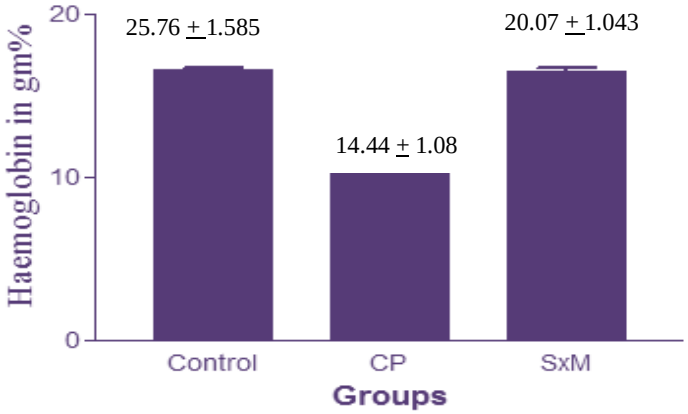


Figure 3. Effect of ethanolic extract of *Shirishadi* Compound on Haemoglobin estimation.
All values are mean±SEM, n=6.
***P<0.001 when compared with control group and , P<0.001, when compared with Cyclophosphamide treated group (Students t test).
b. P<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

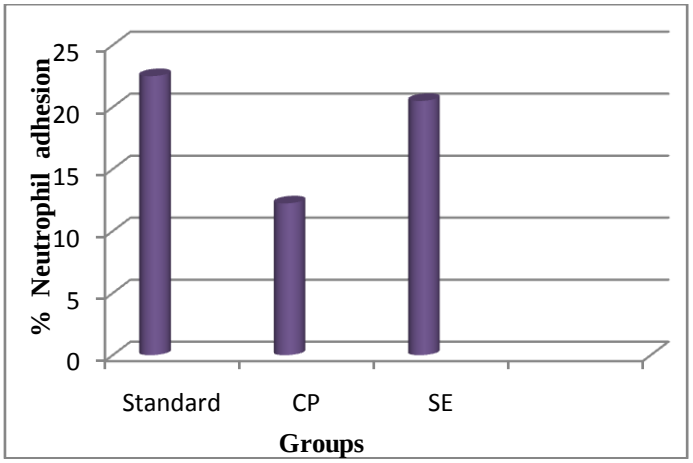


Figure 4. Effect of ethanol extract of *Shirishadi* Compound on neutrophil adhesion test on Cyclophosphamide treated rats.
All values are mean±SEM, n=6.
**P<0.01 when compared with control group,P<0.001 when compared with Cyclophosphamide treated group.(Students t' test).
b. P<0.01 when compared with Cyclophosphamide treated group (one way Anova).

Egg albumin induced anaphylaxis in guinea pigs

Guinea pigs were sensitized by two intraperitoneal 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.

Table No 4 : Effect of *Shirishadi* Compound on egg albumin induced anaphylaxis in rats.(Gupta *et al*)

Group	Pre convulsion time (min)			Percent protection Mortality (%)
	Onset (min)	Duration (min)	Severity (score)	
Group I (Control)	1.246 ± 0.056	23.66 ± 0.345	9.4 ± 0.306	70%
Group II *Shirishadi* compound 500mg/ Kg, p.o.	2.914±0.088*	7.054±0.131*	3.0 ± 0.615*	0%

Study on Isolated Frog Rectus Abdominis Muscle preparation

A frog is pithed and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally upto 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. A standard solution of Ach is added to the bath and a slow contraction is recorded on the slow moving drum for exactly 90sec. The drum is stopped and the bath fluid is replaced by fresh Frog- Ringer. An extra 1g load is used to extend the muscle to its original length.

Table No 5 : 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. & B.E.(mg/ml)	% Inhibition of maximum Ach contraction by *Shirishadi* compound
1.	0.1ml	7.05	30 + 2.03	1	2.06 + 1.76
2.	0.2ml	6.66	56+ 1.45	5	4.36+ 0.56
3.	0.4ml	6.35	75+ 3.05	10	10+ 3.12
4.	0.8ml	6.20	89+ 1.25	20	24.98+ 2.34
5.	1.6ml	5.75	99.9+ 0.98	50	30.2+ 0.23

All the values are mean + SDE ,where n=3.

Study on Isolated Perfused Frog's Heart

A frog was pithed and pinned on frog broad to expose 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 aorta. The frog board was fixed on the plane stand and venous cannula was tied 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 passed through the tip of the ventricle and tied with the free limb of lever. A tension of 4g and magnification of 10 X was maintained.

Table No 6 : Activity of Polyherbal drugs 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			
		10	52			
		20	54			

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

Bronchodilator effect (Sheth et al., 1972)

Guinea pigs of either sex weighing 350 - 500 g were selected and randomly divided into four groups each containing four animals. The drugs were dissolved in distilled water and administered orally through intubation canula. The single dose treatments were given one and half an hour before the study. Group I was administered 0.5% CMC (control), Group II: Mepyramine melate (10 mg/kg= 0.1% Soln.) (standard),Group III: Alcoholic extract of *Shirishadi* compound(100 mg/kg), Group IV: Alcoholic extract of *Shirishadi* compound (200 mg/kg).

One and half hour later the animals were exposed to 0.2% histamine aerosol and time for pre convulsion state (PCD) was noted for each animal. The end point for PCD was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air to recover. This time for PCD was taken as day 0 value. After 15 days of wash out period, the animal of group III, IV,V & VI were again given same schedule of drug and exposed to histamine aerosol and the time for PCD was noted. The % increase in time of PCD was calculated using the following formula.

Percentage increase in time of PCD = $1 - T_1/T_2$ X 100

Where: T1= time for PCD onset on day 0, T2 = time for PCD onset on day 15.

Table No 7 : Effect of polyherbal formulations on histamine-aerosol in guinea pigs

Treatment	Dose (mg/kg)	Onset of convulsion in sec.	Protection (%)	% Increase in preconvulsion time
Control	Saline, 1.0 ml/kg	91.45± 0.093	0	0
Mepyramine	10mg/Kg	1028.0 ± 4.553	90***	33.93± 3.12
Shirishadi Compound	100	800 ± 0.396	80**	27.85± 3.96***
Shirishadi Compound	200	860 ± 0.674	86***	36.13± 3.68***

n=6 in each group; ***P* < 0.01, ****P* < 0.001 vs. control; (χ2 test with Yate's correction).

Assessment of Anti-histaminic activity of Polyherbal compounds on Isolated Guinea Pig ileum

Overnight fasted guinea pigs of either sex weighing 400 - 600 g were sacrificed using cervical dislocation method. The lower most 10cm of ileum was removed from the abdomen and placed in a shallow dish containing warm Tyrode solution. Ileum lumen was cleaned by passing through warm 0.9% saline and then segments about one inch in length, were made. The mesentric attachment and blood etc. were carefully cleaned and the tissues was mounted in a thermostatically controlled Dale's organ bath (temp. 37± 0.5^0 C) containing 20 ml Tyrode's solution under basal tension of 500mg. The composition of solution in mM was NaCl, 137; CaCl2, 1.8; KCl, 2.7; glucose, 5.55; NaHCO3, 11.9; MgCl2, 1; NaH2PO4, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on a Student physiograph (Bio Devices) using isotonic transducer, which exerted a basal tension equivalent to 500 mg load on tissue. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min. Increasing concentration of histamine were added to the bath and the control cumulative concentration- response curve was constructed.

Table No 8 : Results obtained from guinea pig ileum preparation after treatment with *Shirishadi* Extract.

S.No.	Dose of Histamine (10 µg/ml)	Log molar concentration of Histamine	Control % maximum response	Standard % maximum response	Conc. of *Shirishadi* Extract (mg/ml) + 1.6ml of Histamine (10µg/ml)	% Inhibition of maximum Histamine contraction
1.	0.1ml	7.08	32.56 ± 1.023	12.55 ± 1.560	0.5mg	20.98± 2.28
2.	0.2ml	6.79	51.91± 1.450	23.10 ± 2.065	1.0mg	31.56 ± 2.01
3.	0.4ml	6.48	78.26 ± 2.030	35.23 ± 1.020	10mg	43.05± 1.31
4.	0.8ml	6.18	92.90± 2.560	42.65 ± 1.67	20mg	65.78 ± 1.20
5.	1.6ml	5.88	99.58 ± 1.051	51.32 ± 1.25	50mg	83.78± 1.43
					80mg	65.78 ± 1.20

Values are expressed as mean±SEM (n=6). *p<0.05 when compared to control group, **p<0.05 when compared to standard group.

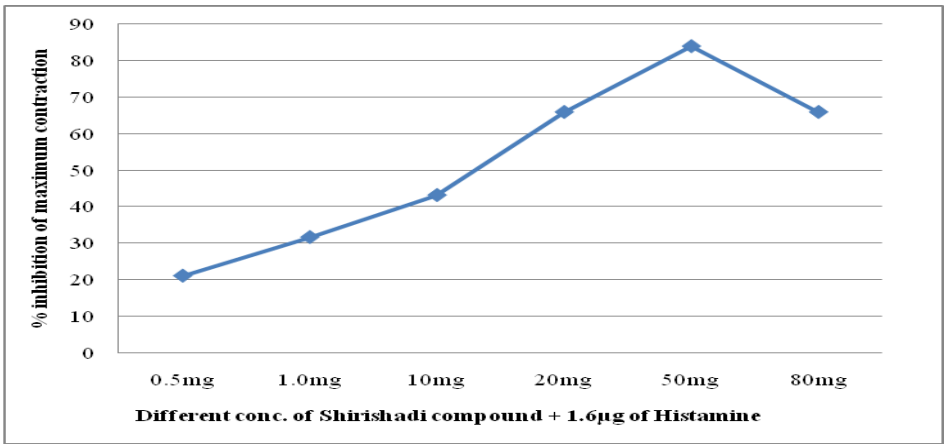


Fig. 19 : % inhibition of contraction produced by maximum dose of histamine in the presence of different concentration of *Shirishadi* Compound.

Studies on Compound 48/80 Induced Rat Mesentric Mast Cell Degranulation

Rats were sacrificed by cervical dislocation and their abdomen was opened wide. Small intestine from jejunum to proximal 2/3 of ileum along with the entire mesenteric attachment was removed into a beaker containing Ringer Lockes solution at 37^0 C, with continuous oxygenation through a capillary tube. Piece of suitable size to include 2-3 arcades of mesenteric vessels were made and the mesentery was then cut out in one piece by gentle dissection. Five to eight good mesenteric pieces can be obtained per animal. The mesentery were collected in petri dish containing Ringer Locke solution and then subjected to the following treatment schedules. Petri dish no. 1 - Ringer Locke solution (Positive control),Petri dish no. 2 - 0.1ml of Ketotifen fumarate (10 µg/ml),Petri dish no. 3 - 0.1ml of test agent in Tween-80 (*Shirishadi* compound, 500 µg/ml),Petri dish no. 4 - 0.1ml of test agent in Tween-80 (*Shirishadi* compound, 1000 µg/ml)

Each petridish was incubated for 15 min at 37°C. Later Compound 48/80 (0.1 ml, 10 µg/ml) was added to each petri dish and again incubated for 10 min. at 37°C. After that, all pieces were transferred to 4% formaldehyde solution containing 0.1% toluidine blue and kept a side for 20 to 25 min. After staining and fixation of mast cells, mesentery pieces were transferred through acetone and xylene two times and mounted on slides. All the pieces were examined under the high power of light microscope.

Table No 9 : Effect of Polyherbal compounds on Compound 48/80 induced rat mesentric mast cell degranulation

Treatment groups	Concentration (µg/ml)	% inhibition of degranulation
Positive control	-	9.83±1.58
Kitotifen	10	80.67±3.30*
Shirishadi compound	500	60.87±1.19*
Shirishadi compound	1000	66.83±2.19*

Results are expressed in mean ± SEM (n=6).

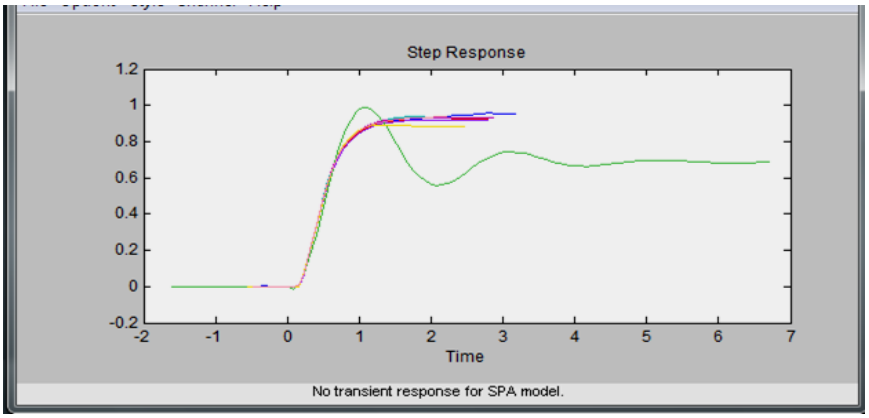
*p<0.001 as compared to Positive control group (One way ANOVA followed by Tukey's multiple range test).

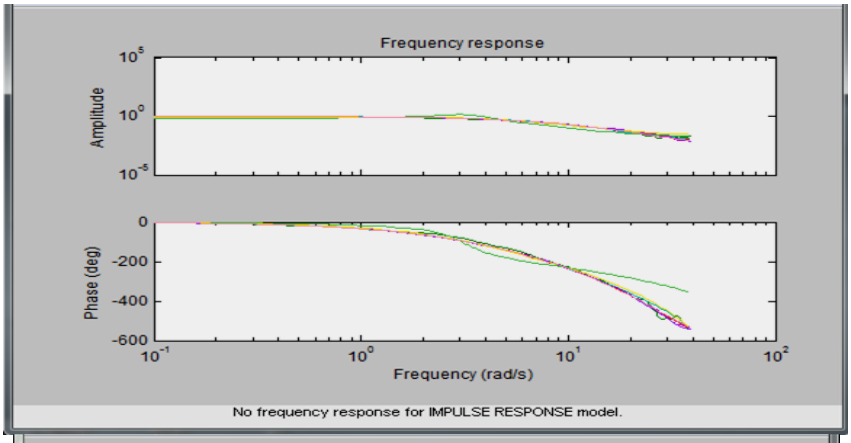
LUNG REMODELLING

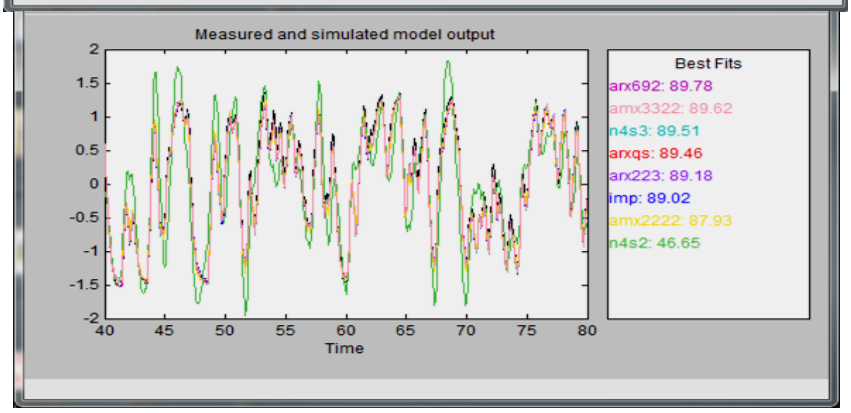
After giving medicine (*Shirishadi*) in the dose of 2.5ml-B.D. for 15 days in patients of moderate asthma with FVC< 1.50 ± 0.45, PEFR< 120± 20 and FEV1< 1.20±0.20, we had taken Volume versus Time variation through graphical method.

The parameters which are used to evaluate the effect of medicine in term of mechanics are volume, pressure, airways resistance and compliance. To evaluate the effect of drug we had fitted a suitable MATLAB model using following models and assumptions:

- We estimates the following types of models and adds the following to the System Identification Tool GUI (Graphical User Interphase) in MATLAB with default names:
- IMP — Step response over a period of time .
- SPAD — Frequency response over a range of frequencies .
- ARXQS — Fourth-order autoregressive (ARX) model .







Clinical Study

An open, randomized and control clinical study on 60 (out of which patients drop-out due to various reasons) known patients of Bronchial Asthma showed the following improvement in pulmonary function test:

Table No. 10: Clinical Study of *Shirishadi* compound on PEFR (Peak Expiratory Flow Rate) in Bronchial Asthma

Groups	**PEFR Mean ± S.D.**				**Within the group comparison paired t test**				
	BT	**AT**	**F1**	**F2**	**BT-AT**	**BT-F1**	**AT-F1**	**BT-F2**	**AT-F2**
Group I	120± 38.0	210± 69.6	207± 65.6	197± 61.8	90± 58.4 t=6.71 p<0.001	87.3± 54.9 t= 6.92 p<0.001	77.6± 49.7 t=6.80 p<0.001	2.63± 9.3 t=1.22 p>0.05	12.3± 13.3 t=4.03 p<0.001
Group II	138± 72.2	194± 80.0	141± 60.7	138± 58.1	55.6± 29.6 t=7.50 p<0.001	3.12± 31.9 t=0.39 p>0.05	52.51±32. 76 t=6.41 p<0.001	0.62± 24.3 t=0.10 p>0.05	56.2± 32.0 t=7.02 p<0.001
Group III	141.0± 44.3	134± 48.9	134± 48.8	131± 43.8	6.5±10.5 t=1.94 p>0.05	7.0± 9.48 t=2.33	0.50± 6.85 t=0.23	10.0± 11.54 t=2.73	3.50± 14.5 t=0.761

						p<0.05	p>0.05	p<0.05	p>0.05
Between the Group comparison One Way ANOVA	F=0.44 P>0.05	F=2.12 P=>0.05	F=3.50 P=<0.05	F=3.15 P<0.05					
POST HOC TEST SIGNIFICANT PAIRS (P<.05)	None	None	(1,3) (2,3)	(1,3) (2,3)					

Table No. 11: Clinical Study of *Shirishadi* compound on FVC (Forced Vital Capacity) in Bronchial Asthma

Groups	**FVC Mean + S.D.**				**Within the group comparison paired t test**				
	BT	**AT**	**F1**	**F2**	**BT-AT**	**BT-F1**	**AT-F1**	**BT-F2**	**AT-F2**
Group I	1.55±0.53	2.16±0.55	2.17± 0.49	2.18± 0.50	0.60± 0.44 t=5.90 p<0.001	10.3± 42.1 t=1.06 p>0.05	9.70± 42.2 t=1.00 p>0.05	0.62± 0.55 t=4.90 p<0.001	.0016± 0.27 t=0.25 p>0.05
Group II	1.18±0.75	2.10±0.80	1.49± 0.71	1.54± 0.73	0.92± 0.32 t=11.4 p<0.001	0.31± 0.47 t=2.50 p<0.05	0.61± 0.43 t=5.71 p<0.001	0.36± 0.42 t= 3.4 p<0.001	0.56± 0.35 t=6.26 p<0.001
Group III	1.07±0.53	1.03±0.48	1.04± 0.43	1.02± 0.45	0.003± 0.13 t=0.914 p>0.05	2.70± 0.16 t=0.50 p>0.05	0.001± 7.47 t=-0.46 p>0.05	0.004± 0.17 t=0.79 p>0.05	0.0006 ±9.7 t=0.194 p>0.05
Between the Group comparison One Way ANOVA	F=1.00 P>0.05	F=6.63 P<0.001	F=0.88 P=<0.001	F=8.01 P<0.001					
POST HOC TEST SIGNIFICANT PAIRS (P<.05)	None	(1,3) (2,3)	(1,3) (2,3)	(1,3) (2,3)					

Table No. 12 : Clinical Study of *Shirishadi* compound on FEV1 (Forced Expiratory Volume)in Bronchial Asthma

Groups	**FEV1 Mean + S.D.**				**Within the group comparison paired t test**				
	BT	**AT**	**F1**	**F2**	**BT-AT**	**BT-F1**	**AT-F1**	**BT-F2**	**AT-F2**
Group I	1.45± 0.60	2.09± 0.61	2.06± 0.63	1.9± 0.60	0.61± 0.48 t=5.50 p<0.001	0.58± 0.50 t=5.00 p<0.001	0.003± 0.12 t=1.17 p>0.05	0.50± 0.50 t=4.38 p<0.001	.10± 0.12 t=3.79 p<0.001
Group II	1.13± 0.42	1.60± 0.57	1.36± 0.49	1.40± 0.47	0.46± 0.31 t=5.9 p<0.001	0.22± 0.31 t=2.8 p<0.01	0.24± 0.30 t= 3.13 p<0.001	0.26± 0.21 t=3.5 p<0.001	0.20± 0.26 t=3.07 p<0.001
Group III	1.14± 0.51	1.10± 0.49	1.09± 0.54	1.16± 0.42	0.003± 0.009 t=1.2 p>0.05	0.004± 0.22 t=0.62 p>0.05	0.002± 0.14 t=-0.62 p>0.05	0.0009± 0.21 t=0.13 p>0.05	0.006± 0.14 t=1.36 p>0.05
Between the Group comparison One Way ANOVA	F= 2.39 P>0.05	F=5.31 P<0.001	F=6.50 P=<0.001	F=5.49 P<0.001					
POST HOC TEST SIGNIFICANT PAIRS (P<.05)	None	(1,3) (2,3)	(1,3) (2,3)	(1,3) (2,3)					

III. Discussion

Pharmacodynamic Screening of Drug Screening the activity of drug on antiasthmatic parameters such as anti-histaminic, anti-cholinergic, bronchodilator,mast cell stabilizing activity, anti PAF activity,anti-inflammatory and analgesic activity and immunomodulator effect to assess probable mode of action of drug shows that the drug possess anti-histaminic property. The non-competitive and preventive type of antagonism with dose dependent response was obtained. There was right shift of the dose response curve in the presence of drug showing the efficacy of drug. Though the anti-histaminic activity is present in the drugs but the dose at which the response obtained is much more than those use for the therapeutic purpose. Brochodilation produced against histamine induced bronchoconstriction confirm the anti-histaminic activity of drugs. No anticholinergic activity was encountered in polyherbal drug. Evaluating anti-cholinergic activity on isolated perfused heart showed that drug was unable to prevent the acetylcholine induced cardiac arrest though the time-span of cardiac arrest was significantly reduced and the heart rate regains its rythmicity and contractility in presence of polyherbal drug but the cardiac arrest was not totally prevented.

The *Shirishadi* extract produces 30±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.

Egg induced anaphylactic response was significantly prohibited by the polyherbal drug and there is 0.00% mortality in group treated with polyherbal drug against 70% mortality in control group (untreated group). The 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. The % inhibition of MCD (mast cell degranulation) was found to be 60.87% and 66.83% in 500, and 1000 µg/ml of *Shirishadi* compound. Prior exposure to Polyherbal compounds produced significant ($p<0.001$) reduction in the Compound 48/80-induced mast cell degranulation. 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. Evaluation of effect of hydroethanolic extract of *Shirishadi* on Cyclophosphamide induced immunosuppression indicated good protection by increasing all the hematological parameters. WBC count, RBC count, and % hemoglobin values observed were better than untreated control groups. Administration of Cyclophosphamide produced a significant decrease in the Total Leukocyte Count from 6.2±0.081 to 2.98±0.214, RBC count from 5.02±0.116 to 3.02±0.152, and % hemoglobin from 15.49±0.081 to 9.32±0.153 ($P<0.01$). *Shirishadi* treated group showed WBC count =5.92± 0.52, RBC count =5.64± 1.53, Hbgm%= 14.48± 0.93 and Neutrophil adhesion 26.07± 1.043($p<0.001$).

In the carrageenan-induced rat paw edema test for acute inflammation, the extract of *Shirishadi* compound in doses of 50mg, 200 mg and 500 mg/kg body weight showed 77% and 79% and 81% inhibition of edema, at the end of 4h ,which is comparable to that of standard (endomethacin) i.e. 92%. In the acetic acid induced writhing test the extract of *Shirishadi* compound (200 and 500 mg/kg body weight) showed a significant ($p<0.001$) reduction in the number of writhes with 65.6% and 70.9% of inhibition respectively . In radiant heat tail-flick test *Shirishadi* crude extract produced 58.1% ($p<0.001$) and 61.10% ($p<0.001$) elongation of tail flicking time 30 minutes after oral doses of 200 and 500 mg/kg body weight respectively . After 60 minutes the extract showed 56.3% ($p<0.001$) and 59.00% ($p<0.001$) elongation of tail flicking time.

Study of change in Lung Mechanics through Parametric model: Graphical variation obtained after administration of drug *Shirishadi* was changed using MATLEB model to evaluate the action of drug on various parameters. The graphical variations strongly confirm that drug decrease the airway resistance, increase the lung volume, increases the lung compliance.

***Clinical study*:** Evaluation of Pulmonary function test showed significant improvement in FVC, PEFR and FEV1 in group treated with polyherbal compound. The most striking fact observed is that the changes were remained constant throughout follow-up, showing no reverse bronchoconstriction. *Shirishadi* compound was found to be more effective in allergic asthma. Treatment with Polyherbal compound decreases the frequency of attack as well as severity of attack on exposure to allergens.

IV. Conclusion

It can be concluded that Polyherbal compounds *Shirishadi* has potent antiasthmatic activity. It can be further concluded that these Polyherbal compound can be used as 'Therapeutic Agent' in the management of acute attack of Asthma as well as chronic persistent Asthma. The trial gives a direction for searching new route of herbal drug administration.

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