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EVALUATION OF ANTI-HISTAMINIC ACTIVITY OF MALLA SINDOORA IN GUINEA PIGS

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ABSTRACT

Allergic disorders especially allergic bronchial asthma is of global healthcare concern. *Malla sindoora* is well-known arsenic containing *Kupipaka* preparation ,which is indicated in text in conditions of *shwasa.* Hence an effort was made to evaluate the effect *of Malla sindoora* in allergic disorders like Bronchial Asthma. Study was conducted in 2 phases. **1st phase –** histamine induced bronchospasm in Guinea pigs. **2nd phase-** histamine induced contraction of smooth muscle by using isolated guinea pig tracheal chain preparation. In histamine induced bronchospasm, *Malla sindoora* (MS) at both doses (lower limit & upper limit) showed significant response i.e. delayed onset of proconvulsive time (PCT) at all interval of time with maximum action at 30 min, sustained protective action till 180min, reduction after 240 min and less at 300min yet significant, compared to control. *Malla sindoora* with *Anupana* (*Shunti swarasa +Madhu*) showed better response than *Malla sindoora* plain at all intervals. *Malla sindoora* significantly inhibited the histamine-induced contraction of isolated Guinea pig tracheal chain preparation. *Malla sindoora* with *Anupana* showed more relaxation than *Malla sindoora* without *Anupana*. Hence it can be concluded that *Malla sindoora* is very effective antihistaminic drug and it will impart therapeutic benefit in allergic conditions like Bronchial Asthma. *Anupana* augments the action of *Malla sindoora*.

Keywords: Antihistaminic, *Malla sindoora,* PCT, *Anupana,* Bronchial asthama, allergic disorders

INTRODUCTION

Globally, the incidence of allergic disorders is increasing alarmingly.[1] The allergic disorders especially Asthma, Allergic Rhinitis etc are threatening the quality of human life to a great extent. Unfortunately, in spite of spending millions of rupees on research for effective antihistaminic drugs, biomedicine suffers a major drawback in comprehensive allergy control.

Rasashastra, the mysterious yet scientifically validated offshoot of Ayurveda is having the credential of revolutionizing the Indian pharmaceuticals and therapeutics, because of the synthesis of unique Herbo mineral preparations, which are having long shelf life, high therapeutic value in low dose, quicker action, highly potent, safe etc. Certain herbo mineral formulations might have the potential of providing comprehensive and safe Allergy management. *Malla*

sindoora[2] is one such *Kupipakwa* preparation which is classically indicated in *shwasa, kasa*, etc. *The* wide spectrum of indications of the drug also includes the symptoms of that of allergic diseases of respiratory system. Hence *Malla sindoora* as per the reference of *Rasatantra Sara va Siddaprayoga Sangraha,* which is composed of *Parada* (Mercury*), Gandhaka* (Sulphur*)*and *Malla* (Arsenic trioxide) is chosen for the present study. It is prepared and experimentally evaluated for its anti-histaminic property. Thus, an attempt was made to validate the classical indications of the drug to the current biomedicine scenario.

METHODOLOGY

Preparation of test Drug: *Malla sindoora* was prepared in Dept of Rasashastra and Bhaishajya Kalpana; GAMC, Bengaluru as per the reference of *Rasatantra sara* Va *Siddha prayoga Sangraha*. *Malla sindoora Kajjali prepared from Hingulottha Shuddha parada, Shuddha Gandhaka and Shuddha Malla* at (1:1:1 ratio) is filled in *Kachakupi* and given *Kramagni* in Vertical Muffle Furnace to obtain *Malla sindoora*[1]

Experimental animals: Adult healthy Guinea pigs of either sex weighing about 250-400gms were procured from Sree Siddhaganga College of Pharmacy, Tumakuru. They were fed and housed as per OECD guidelines. The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Animal protocol was obtained from Institutional Animal Ethics Committee (IAEC) with reference no: SSCPT/IAEC.Clear/168/2016. The experimental study was carried out in Sree Siddhaganga College of Pharmacy Tumakuru, Karnataka-572102

Dose of the Standard and Trial drugs

- Based on various research publications available the dose of standard drug Chlorpheniramine maleate was fixed as 2mg/ kg body weight of Guinea pigs.
- Human Dose of the trial drugs was converted to animal dose based on standard dose converting formula[3]

$$\text{Animal dose (mg/kg)} = \frac{\textbf{Human Km}}{\textbf{Animal Km}} * \textbf{HED } (\textbf{mg/kg})$$

Where, Human Km = 37; Animal Km (for Guinea Pigs) = 8

- Dose of *Malla sindoora* as mentioned in classics is ½ to ¼ *ratti* (62.5 mg to31.25)[4].2 62.5 mg was taken as maximum dose for an average human body weight of 70 kgs and 31.25 mg was taken as minimum dose. Therefore, the maximum animal dose by applying dose conversion factor was fixed as 4.81mg /kg and minimum dose as 2.40 mg /kg.

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Preparation of Stock solution

- Plain drug: 20 mg of drug (MS) was made into suspension in 10 ml of distilled water. Each ml will contain 2 mg of drug.
- Drug with vehicle: 20 mg of drug + 10 ml of vehicle (7.5 ml of *Ardraka swarasa*+ 2.5 ml of *Madhu*).

Each ml will contain 2mg of *malla sindoora*. Vehicle dose was approximated considering the approximate human dosage of vehicle (*Ardraka Swarasa* dose: ½ tsp to 2 tsp [5] (about 10ml) and honey approximately 2-5 ml) so that vehicle dosage should not exceed or inadequate of human equivalent dose

Route of administration

- The drugs were administered through rabbit oral gavaging needle.

Vehicle: Group 3, 4 was administered with MS with Distilled water

Group 5, 6 was administered with MS with *Ardraka swarasa* and honey as *anupana*

EXPERIMENTAL TRIAL PROPER:

After determining the effective dose and dosage form of the trial drugs actual experimental trial was carried out.

Study design

Study was conducted in 2 phases.

- 1st phase – histamine induced bronchospasm in Guinea pigs

- 2nd phase- histamine induced contraction of smooth muscle by using isolated guinea pig tracheal Chain preparation.

Phase 1

Table 1: Showing grouping for Histamine induced bronchospasm in Guinea pigs

Group No.	Grouping	Dosing
1.	Control	Q. S
2.	Standard (Chlorpheniramine maleate)	2mg/kg body weight
3.	Low dose of *Malla Sindoora*	2.40mg/kg body weight
4.	High dose of *Malla Sindoora*	4.81mg/kg body weight
5.	Low dose of *Malla sindoora* with *Ardraka swarasa*+ honey *anupana*	2.40 mg/kg body weight
6.	High dose of *Malla sindoora* with *Adraka swarasa*+ honey *anupana*	4.81mg /kg body weight

Procedure:

Overnight fasted guinea pigs were divided into 6 groups, each containing 5 animals. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2% Histamine aerosol. The pre-convulsive time (PCT) was determined from the time of exposure to the onset of convulsions. As soon as the PCT were noted, the animals were removed from the chamber and placed in fresh air.24 hours later the animals of Groups 3 and 4 received *Malla sindoora* stock solution in distilled water and Groups 5 and 6 received *Malla sindoora* with *anupana* in minimum and maximum doses as mentioned in table no. 1.Group 2 received Chlorpheniramine maleate. These animals were then subjected to histamine aerosol after 30min, 60min, 120min, 180min, 240min and 300min of drug administration and PCT was determined. The protection offered by treatment was calculated by using the following formula.

Percentage protection = $(1 - T1/T2) \times 100$

Where; T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.

- Then the results were subjected to statistical analysis.

Phase 2

Effect of *Malla sindoora* on histamine induced contraction of smooth muscle by using isolated guinea pig tracheal chain preparation.

Procedure:

Guinea pigs of either sex weighing 250-400 g were sacrificed using cervical dislocation method. The trachea was rapidly dissected free from surrounding tissues and placed in a petridish containing oxygenated Kreb's solution. Trachea was cut into individual rings and tied together in series to form a chain and suspended in organ tubes filled with 20 ml kerb's solution of the composition: NaCl 5.9, KCl 0.35, and CaCl2 0.28, MgSO4 0.11, NaHCO3 2.1, KH2PO4 0.16 and glucose 2.0 g/L, in plexi glass which was continuously aerated with 95% O2 and 5% CO2 at 37°C±2°C.One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to a force transducer. The tissue was allowed to equilibrate for 45 mins under uniform tension of 1.5 g. The response of trachea was recorded by using student's biopac and force transducer. A dose response curve for histamine was taken in variant molar concentration. After obtaining a maximal dose response curve of histamine, the tissue is washed and the trial drug *Malla sindoora* stock solution .5ml was added and allowed it to remain for 10 minutes. Later the histamine was added (dose being fixed by

the maximal response curve) and the change in curve was noted. Again the tissue is washed, the organ bath is filled with Kreb's solution and the procedure is repeated for next dose of test drug. Procedure was repeated for all test drug stock solutions i.e. MS 0.5ml, MS 1ml, MS+V 0.5ml, MS+V 1ml.The height of contraction due to histamine after each addition of test drugs were measured and tabulated. The percentage reduction in the height was calculated.

Observation and results

Phase 1: Table 2: Average values of Pre-convulsive dyspnoea time (PCT) in seconds

GROUP	G-1 (control)	G-2 (Standard)	G-3 - MS1	G-4: MS2	G-5 MS1+V	G-6 MS2+V
30 min	16.80± 0.7348	99.80± 3.184	125.2±2.147	135.0±2.588	143.2±2.800	150.2±1.068
60 min	18.40 ± 0.6782	130.0 ±1.612	110.6±1.030	118.4±2.064	119.0±2.366	128.6±1.990
120 min	18.00 ±0.5477	148.6 ±2.379	108±1.222	116.2±1.319	119.8±3.o23	127.0±2.214
180 min	17.40 ±0.7483	148.6 ±2.379	103.8±2.010	112.6±7.021	120.4±2.619	125.0±2.168
240 min	17.00 ±0.8944	61.60 ±0.9274	39.80±0.5831	45.20±0.8602	51.20±1.744	53.20±0.3742
300 min	17.00 ±0.8944	53.40 ±0.9274	36.40±1.661	39.80±0.8602	44.0±1.00	45±1.414

Values are expressed as mean±SEM; MS1-*Malla sindoora* minimum dose, MS2-*Malla sindoora* maximum dose, V- Vehicle (*Madhu+Ardraka Swarasa*)

Table 3: % protection in different groups at different interval of time

	G-1 Control	G-2 Standard	G-3 MS1	G-4 MS2	G-5 MS1+V	G-6 MS2+V
30 min	0	83.1%	86.5%	87.5%	88.2%	88.8%
60 min	0	85.8%	83.3%	84.4%	84.5%	85.6%
120 min	0	87.8%	83.3%	84.5%	84.9%	85.8%
180 min	0	88.29%	83.88%	84.54%	85.5%	86.8%
240 min	0	72.4%	57.2%	62.3%	66.7%	68.04%
300 min	0	68.1%	53.2%	57.2%	61.3%	62.2%

Table 4: Summary of comparison between the groups at different intervals of time

	30 min	60 min	120min	180 min	240 min	300 min
Standard Vs Control	*** ↑	*** ↑	*** ↑	*** ↑	*** ↑	*** ↑
MS low dose Vs Standard	*** ↑	*** ↓	*** ↓	*** ↓	*** ↓	*** ↓
MS high dose Vs Standard	*** ↑	** ↓	*** ↓	*** ↓	*** ↓	*** ↓
MS low dose+Vehicle Vs Standard	*** ↑	** ↓	***↓	*** ↓	*** ↓	*** ↓
MS high dose+Vehicle Vs Standard	*** ↑	NS ↓	*** ↓	*** ↓	*** ↓	*** ↓
MS low dose Vs Control	*** ↑	***↑	*** ↑	*** ↑	***↑	*** ↑
MS high dose Vs Control	*** ↑	*** ↑	*** ↑	***↑	***↑	***↑
MS low dose+Vehicle Vs control	*** ↑	*** ↑	*** ↑	***↑	***↑	***↑
MS high dose+Vehicle Vs control	*** ↑	*** ↑	*** ↑	***↑	***↑	***↑
MS high dose Vs MS low dose	NS↑	* ↑	NS↑	NS↑	**↑	NS↑
Ms high dose+Vehicle Vs MS low dose +Vehicle	NS ↑	** ↑	NS↑	NS ↑	NS ↑	NS ↑
MS low dose+Vehicle Vs MS low dose	***↑	* ↑	** ↑	*** ↑	*** ↑	** ↑
MS high dose+Vehicle Vs MS high dose	*** ↑	** ↑	** ↑	** ↑	*** ↑	* ↑

Interpretation

↑: increased PCT (increased protection) of the formal group in comparison with the latter

↓: decreased PCT (decreased protection) of the formal group in comparison with the latter group

* Significant ($p< 0.05$)

** Very significant ($p< 0.001$)

*** Highly significant ($p<0.0001$)

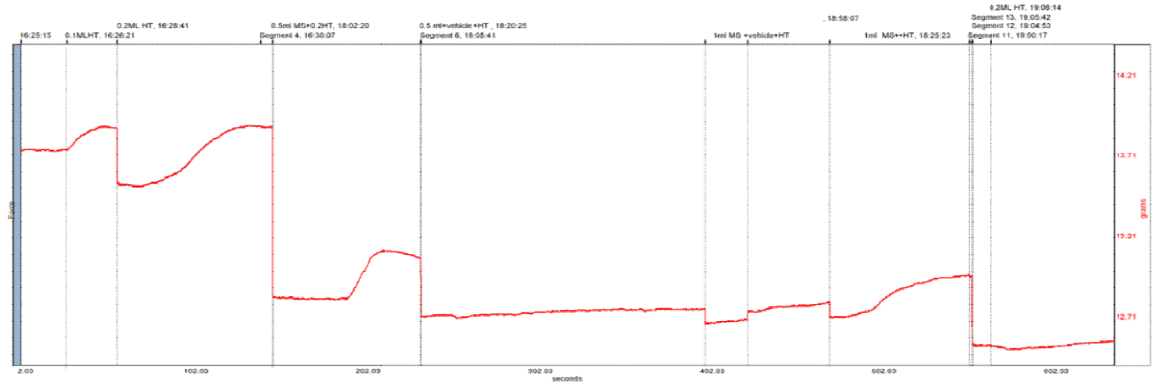
Phase 2: Effect of *Malla sindoora* on histamine induced bronchospasm in isolated guinea pig tracheal chain

Trial -1 Table No 5: Details of Contractile response of trachea in Trail 1

Drug	Base	Height	difference	contraction	Inhibition
histamine 0.1 ml	13.7451	13.8977	0.1526		
histamine 0.2 ml	13.5253	13.9038	0.3785	100	-100
0.5ml MS +HT	12.4267	12.5701	0.1434	37.88639	62.11361
1 ml MS +HT	12.3779	12.4784	0.1005	26.55218	73.44782
0.5ml MSV+ HT	12.7136	12.7499	0.0363	9.590489	90.40951
1ml MSV +HT	12.7175	12.736	0.0185	4.887715	95.11229

MS- *Malla sindoora*, MSV-*Malla sindoora* with vehicle. HT-Histamine

Graph 1: Showing contractile response of Trachea for the Test drugs in Students biopac Trial 1



Trial 2, Table 6: Details of Contractile response of trachea in Trial 2

Drug	Base	Height	Difference	Contraction	Inhibition
Histamine 0.2	12.7685	12.9404	0.1719		
Histaminen0.2 ml	12.1215	13.8122	1.6907	100	
0.5ml MS+HT	12.2887	12.9817	0.693	40.98894	59.02
1ml MS+HT	12.0666	12.5495	0.4829	28.56213	71.44
0.5ml+HT MSV	12.0483	12.386	0.3377	19.97398	80.027
1ml MSV +HT	12.4267	12.6901	0.2634	15.57935	84.42

Graph 2: Showing contractile response of Trachea for the Test drugs in Students biopac in Trial 2

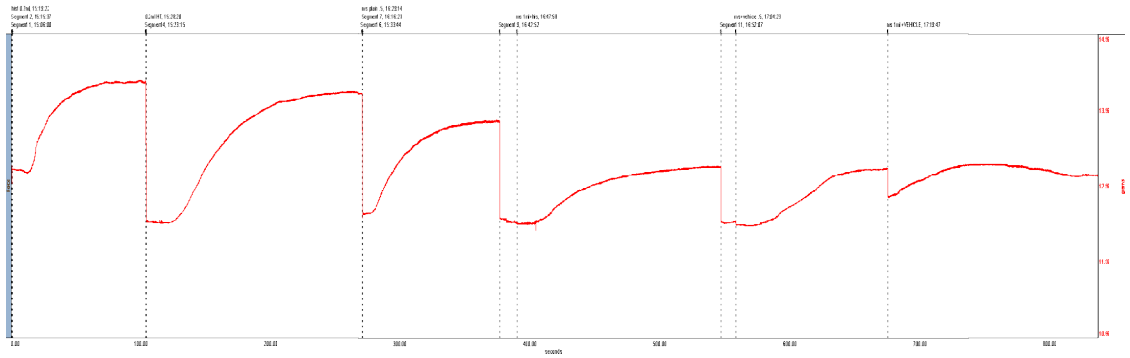


Table 7: Details of Contarctile response of trachea in Trial 3

Drug	Base	Height	Difference	Contraction	Inhibition
Histamine 0.2	13.8676	14.0415	0.1739		
Histaminen0.2 ml	13.2305	14.913	1.6825	100	
0.5ml MS+HT	13.1483	13.586	0.4377	26.01486	73.986
1mlMS +HT	13.5267	13.7901	0.2634	15.65527	84.345
0.5ml MSV +HT	13.3802	13.5755	0.1953	11.60773	88.39
1ml MSV +HT	13.4779	13.5084	0.0305	1.812779	98.188

Graph 3: Showing contractile response of Trachea for the Test drugs in Students in Trial 3

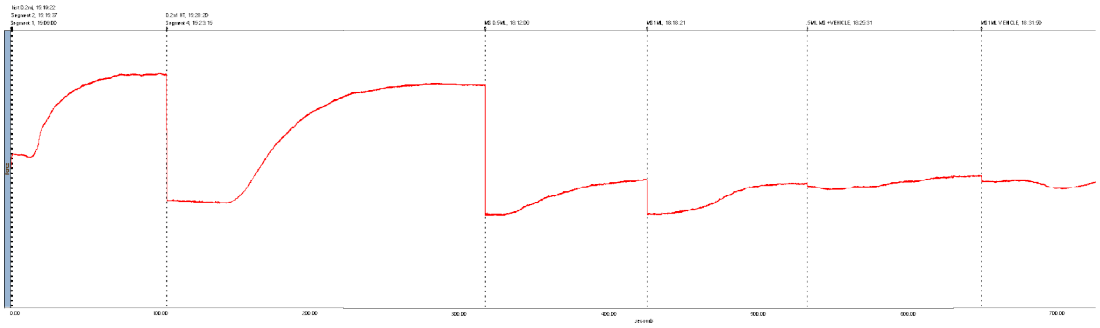


Table 69: showing results of Average % relaxation of Trachea

Sl.No.	**Test Drug**	**Average % of inhibition of contraction**
1.	Histamine	-
2.	*Malla Sindoora* stock solution 0.5ml	65.038 %
3.	*Malla Sindoora* stock solution1ml	76.40 %
4.	*Malla Sindoora* + Vehicle 0.5ml	86.27 %
5.	*Malla Sindoora*+ Vehicle 1ml	92.57 %

DISCUSSION

Experimental Model

- Here in the present study in vivo method of histamine induced bronchospasm in guinea pig and histamine induced contraction of smooth muscle by using isolated guinea pig trachea were followed to evaluate the efficacy *Malla Sindoora* and *Malla sindoora*+ Vehicle for its anti-histaminic activity because of sensitivity and close anatomical and physiological association, which exists between tracheal and bronchial musculature. Further, the great strength of this model is the direct anaphylactic bronchoconstriction upon antigen challenge.

- Receptor pharmacology in guinea pig more closely matches that of human receptor pharmacology than other commonly used species.

Efficacy of Test Drugs on histamine induced bronchospasm in guinea pig

- There was significant response in all trial groups i.e. delayed onset of pre-convulsive dyspnoea/ pre-convulsive dyspnoea time (PCT)
- *Malla sindoora* showed maximum protection at 30 minutes (both plain & vehicle groups) This might be due to the high solubility of arsenic accounting to its quick action, and as *rasaoushadhis* are said to be *aashukari* accounting to the quicker action of drug
- At all intervals *malla sindoora* higher dose showed better protection than low dose. But the difference is statistically insignificant except at 60 and 120 secs. Possible implication is that range of dose given for *Malla sindoora* is not drug dependent (Drug is equally effective in both doses) but host factor need to be considered to fix the dose (*prakriti, bala, vaya* etc)
- MSV (vehicle groups) showed better protection than MS plain in all intervals and difference between them is statistically highly significant, substantiating the claim of *acharyas* that *anupana* / *sahapana* potentiates the action of main drug and counteracts possible ill effects
- All treatment groups exhibited maximum protection at 30 min; protection was sustained till 180 mins. Reduced after 240 mins, but still persistent even after 300 min. in comparison with control. Accordingly, fixation of time of administration can be done. TID dosage or QID administration of drug can be effective during acute phase of asthmatic attack.

Effect of *Malla sindoora* on histamine induced contraction of smooth muscle by using isolated guinea pig tracheal chain preparation.

In the present study, both MS and MSV significantly inhibited the histamine-induced contraction of isolated Guinea pig tracheal chain preparation, indicating antihistaminic activity.MS with vehicle at high dose showed more % of inhibition of contraction than at low dose and also when compared to MS plain

The contraction of tracheal or bronchial smooth muscle *in vitro* has often been utilized for the study of contractile/dilator responses of agonists as well as antagonist. Both tracheal chain and strips preparations are suitable for screening the activity of a drug on respiratory smooth muscles.[6] Spasmogens such as histamine, acetylcholine and barium chloride produce dose dependent contraction of tracheal chain preparation. The guinea pig tracheal muscle has H1, M3 and β2 receptors. The stimulation of H1/ M3 receptors causes contraction of bronchial smooth muscle.

Histamine is released from mast cells and basophils by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation. Histamine can provoke bronchoconstriction; it may also be responsible for bronchial hypersensitivity which is a common feature of asthma. Mast cells with their mediator can be regarded as centre for initiation and mediation of early phase of allergic reaction and may be responsible for initiation of chronic allergic reaction.

Probable mode of action of *Malla Sindoora*

Hypersensitivity in *Ayurveda*: Concept of *Ojovyapat* and *dushi visha*. No straight forward reference of hypersensitivity is found in *Ayurveda.* However can be understood under the concept of *ojovyapat* and *dushivish*[7] Allergy can be understood as state of altered or confused state of Immune system. Altered state of Immunity can be related to *Ojovyapat.* There are 3 types of *Ojus*. *Para ojus, Apara ojus and third one Upadhatu ojus, upadhatu of shukra. Para and apara ojus* does function of *Vyadhi utpadaka pratibandhakatwa* (Innate and acquired Resistance of the body), *Upadhatu Ojus* does the function of *Vyadhi bala virodhitwa* i.e. Humoral and cell mediated immunity; Functions including the protective response involving immune cells, (wbc, eosinophils, mast cells, T lymphocytes Etc) blood vessels and molecular mediator (BCL-2, 8-Isoprostane etc). Their altered function can be understood as *ojovyapat*.

Continuous practice of *Garavisha, viruddha* or erroneous medication can lead to *ojovyapat. Dooshi visha* is less potent *visha* of any sort having its primary affliction with the *raktadhatu* and depending on the site of *Khavaigunya* produces different diseases. *Dooshi visha* in *rakta* when lodges in *amashaya* can produces secondary *doshaa dusti* and produce *amashayottha kapha vata vyadhi* like *Shwasa*.

Ojovyapat is potent cause of Immune modulated hypersensitive disorders. Long standing less potent

visha(dooshi visha) or *gara visha* predisposes to this condition. *Visha* because of its antagonistic properties of *ojus* can afflict *ojus* leading to *ojovyapat*. So along with breaking the pathogenesis of *Shwasa*, *Vishahara* and *ojo vardhaka* properties help to counteract primary cause of Hypersensitivity

Malla is having *vishahara* property (*vruschikadi visha pranut* as per *Rasa Tarangini*). *Gandhaka* is also having property of *vishahara*. *Parada* is best *rasayana, ojovardhaka, balya*. Hence these properties help to counteract the *dooshivisha* and *ojovyapat.*

Probable mode of action of *Malla Sindoora* in *Shwasa*

Overall pharmacodynamics of *Malla sindoora* is *Vata kaphagna*, *Deepana, pachana, Katu rasa, Ushna veerya.*[8]

Shwasa is because of Morbid *Kapha* obstructing *Vata*. *Shwasa* can be *niddnarthakara roga* of many other diseases and can also because of *visha*. *Amashayastha dushivisha* can cause secondary *doshadusti* and can lead to *amashayastha kapha vata vikaras* like *Shwasa*. (*Visha hara* property and *ojo vardhaka* property help to counteract the primary pathogenesis of *Visha* and *Ojovyapat*)[9]

Origin of *Shwasa* is from *aamashaya* which is *pitta sthana* (Correction of *Pitta* or *Agni* by *deeepana guna*). *Samana vayu (Udaka and annavaha srotovichari*) has role in the pathogenesis of *Shwasa*. *Aagantu dosha* first causes vitiation of *Samana Vayu*, then causes vitiation of *Malarupi Kapha (Rasamala*) in respiratory passage and obstruction to *prana* in *Pranavaha srotas*. (*pachana* property of drugs corrects the *Samana dusthti*). *Viguna prana, samana vayus* cause *poorvarupa* of *Shwasa*. *Prana, udaka* and *annavaha sroto dushti* causes signs and symptoms of *Shwasa*. *Saamana dusti* in respiratory passage cause *Kapha vitiation* and obstruction to *Prana*. Hence treatment principle is removing *malaroopi kapha* from *pranavahasrotus*, clearing the passage of *prana* and normalizing *Samana* in *pranavaha srotus*. *Kapha vatagna* property helps in removal of *Kapha* and normalizing *Vata*[10]. Drug has direct action on *aagantu* and *sthanika dosha (vata* and *Kapha*). *Ushna veerya*: Removes the *Sroto Sankocha* leading to dilatation and thus helps in normalizing *Vata*, Liquify *Kapha* & remove *Sroto-Avarodha*. Once *sroto avarodha* is removed, it makes *vatanulomata*, hence, decreases the *Ati-Pravriti* of *Shwasavega* and normalizes the function of *Pranavaha Srotas*.

Deepana: correct the *Samana dusti* and corrects *Agni. Udakavaha and annavaha sroto dusti* is corrected by *Deepana* and *pachana* property of drug.

***Ardraka swarasa* (*Anupana*)** is having *Katu rasa, ushna veerya, Shothahara and Vatakaphahara Deepana.* Hence *counteract the pathogenesis of Shwasa.*[11]

Madhu is having properties of *chedana (*liquefy *kapha). Vishahara (*counteract *dooshivisha), Sukshamarganusari, Yogavahi* helps in quicker action and targeted drug delivery [12]

Probable mode of action of *Malla sindoora*: Modern science view (on the ground of modern researches on arsenicals) *Malla sindoora* is *Sindoora* Kalpana containing AS_2O_3. Researches done on Arsenic trioxide provide valuable inputs to analyse the probable drug action.

1) Apoptosis of pulmonary eosinophils and reduction of eosinophil recruitment: Studies have found that As_2O_3 promotes apoptosis of pulmonary eosinophils in a guinea pig model of asthma.[13] As_2O_3 also reduces eosinophil recruitment. Low dose of As2O3 is proved to be effective with relative safety; it also has potential value in treating asthma

2) Downregulating eotaxin expression:

Eotaxin is an eosinophil-selective chemoattractant that has been identified as a potent activator of eosinophils, inducing eosinophils to generate superoxide and release granule proteins. Early studies suggested that eosinophil recruitment in allergic reactions was regulated by Th2 lymphocytes and that eotaxin production was T cell-dependent. Research found that administration of As_2O_3 in OVA-immunized mice abrogated airway eosinophil recruitment by downregulating eotaxin expression but did not alter serum IgE or IL-5 levels in bronchoalveolar lavage fluid (BALF). Furthermore, the development of AHR (airway hyperresponsiveness) and cellular infiltration into the airway were reduced by treating mice with As_2O_3. [14]

3) Induction of T cell apoptosis and decrease of interleukin-4 release in T cells may be by the mechanism of down-regulation of Bcl-2 expression

The prolongation of eosinophil survival is important in the pathogenesis of asthmatic airway inflammation. Apoptosis of eosinophils may be clinically relevant in asthma, promoting the removal of airway

eosinophils and contributing to clinical improvement. Among apoptosis suppressing genes, bcl-2 prevents apoptosis either through altering cell cycle rates or by activating antioxidant associated mechanisms.[15,16] Interleukin (IL)-5, a cytokine that attracts, activates, and prolongs the survival of eosinophils, is important in causing eosinophilic inflammation in the asthmatic airway and contributing to eosinophil viability in the sputum of asthmatic patients during attacks.[17]It inhibits eosinophil apoptosis by upregulation of bcl-2.[18]

Studies showed that Arsenic trioxide treatment significantly decreased interleukin-4 release and down-regulated Bcl-2 expression in asthmatic patients, while it only slightly affected healthy controls. These results suggest that arsenic trioxide induces T cell apoptosis and decreases interleukin-4 release in T cells of asthmatic patients in vitro and that down-regulation of Bcl-2 expression may be an important mechanism.[19]

5) Reduction in oxidative stress evidenced by reduction of 8-Isprostone

Oxidative stress has an important role in the pathogenesis of asthma. Study shows that oxidative stress is increased in asthmatic subjects as reflected by 8-isoprostane concentrations [20]

In a study conducted on the Role of arsenolite on 8-isoprostane of asthmatic mice plasma, Results showed that Lung function improved after treating with dexamethasone or arsenolite. The WBC of asthmatic mice were significantly higher than those in control group, and decreased after treating with dexamethasone or arsenolite; 8-Isoprostane of plasm in asthmatic mice was higher than that of control group, and decreased after treating with dexamethasone or arsenolite. Study concluded that there is oxidant stress status in asthmatic mice. Arsenolite could lighten airway obstruction, reduce airway high response and redress oxidant stress status in asthmatic mice.[21]

6) Inhibition of NF-KB and abrogation of allergen-induced airway hyperresponsiveness and inflammation [22]

Overactivation of nuclear factor κB (NF-κB) orchestrates airway eosinophilia but does not dampen airway hyperresponsiveness in asthma. NF-κB repression by arsenic trioxide (As_2O_3) contributes to apoptosis of eosinophils (EOS) in airways. A study conducted, provides evidence that As_2O_3 abrogates allergen (OVA)-induced airway eosinophilia by modulating the expression of IκBα, an NF-κB inhibitory protein, and decreases the airway hyperresponsiveness

CONCLUSION

In phase 1 of experiment: significant response was observed in all trial groups i.e. delayed onset of pre-convulsive dyspnea/ pre- convulsive dyspnea time (PCT). Maximum protection was offered at 30-minute interval (better than standard) in all trail groups. And protection was significantly high, up to 180 min in all groups. Protection Reduces after 240 mins and minimal at 300 minutes yet exhibited significant protection in comparison with control even after 300 minutes. *MS* with vehicle group showed better response than *MS* without vehicle group at both doses, which is statistically significant at all intervals (p=0.001). MS high dose offered better protection than low dose, but difference between them is statistically insignificant.

In phase 2: All test drugs both showed significant relaxation in histamine induced tracheal chain contraction

Thus, it can be concluded that Test drug *Malla sindoora* is very effective Antihistaminic drug at both doses and *Anupana* augments drug action

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