COMPARATIVE STUDY OF ANTI-HISTAMINIC & ANTI-INFLAMMATORY ACTIVITY OF TALISADI CHOORNA & GUTI CONTAINING SYNTHETIC VANSHALOCHAN

Dr. Atul Gaikwad¹* and Dr. Nandini More²

¹Assistant Professor, Department of Rasashastra & Bhaishajyakalpana Vigyan, College of Ayurved, Bharati Vidyapeeth Deemed University, Dhankawadi, Pune-411043.
²Associate Professor, Department of Rasashastra & Bhaishajyakalpana Vigyan, College of Ayurved, Bharati Vidyapeeth Deemed University, Dhankawadi, Pune-411043.

ABSTRACT
Shwas is a disease of pranvaha srotas, which can be co-related with Asthma. Histamine and submucosal inflammation are counted among the causative factors of Asthma. Thus, for a formulation to be effective in Asthma, has to possess both anti-histaminic and anti-inflammatory activity. In the present study Anti-Histaminic activity was evaluated using‘Protection against histamine induced bronchospasm’ model and Anti-Inflammatory activity was evaluated using‘Cotton wool Granuloma’ model. From this study it is evident that Talisadi Choorna and Guti both have significant Anti-Histaminic and Anti-Inflammatory activity. When both the activities of Talisadi Choorna and Guti were compared, no significant difference in efficacy was observed.

KEYWORDS: Talisadi Choorna, Talisadi Guti, Synthetic Vanshalochan, Anti-histaminic, Anti-inflammatory.

INTRODUCTION
Asthma occurs mainly due to exposure to allergic particles and infective sources.¹ It is estimated that around 300 million people in the world currently suffer from asthma and India has about 15 to 20 million asthmatics², and 15 million suffer from tuberculosis per year.³ Asthma and Tuberculosis can be broadly correlated with shwaas⁴ and rajyakshma⁵ vyadhi, which are mentioned in ayurvedic samhitas. Talisadi Choorna and Guti though mentioned in
the treatment of rajyakshma vyadhi is also routinely used in shwas vyadhi. Vanshlochan is one of the ingredients of Talisadi but it is not available abundantly, so its synthetic substitute is used routinely. Work on standardization of Talisadi Choorna and Guti containing synthetic vanshlochan is available. But no evidence of efficacy of synthetic substitute of vanshlochan is available. Thus it was worth to work on Talisadi Choorna and Guti containing synthetic vanshlochan to evaluate its efficacy in allergic as well as inflammatory pathology with comparative efficacy study of choorna and guti. As preparation of guti makes choorna laghu is mentioned.

**Aim**

To Study antihistaminic and anti-inflammatory action of standardized Talisadi Choorna and Guti containing Synthetic Vanshlochan.

**Objectives**

- To study the Anti-Histaminic activity of Talisadi choorna and Guti.
- To study the Anti-Inflammatory activity of Talisadi Choorna and Guti.
- To compare efficacy of Choorna and Guti.

**ANTI-HISTAMINIC STUDY**

**Materials**

*Animal model used for the study:* Activity against histamine induced bronchospasm.

**Drugs**

Talisadi Choorna and Guti containing Synthetic Vanshlochan.

**Animals**

For study guinea pigs were used because their biological systems function are similar to humans and they are very sensitive to histamine.

**Details of groups, dose and route of administration for Guinea pigs**

<table>
<thead>
<tr>
<th>Species of animals</th>
<th>Guinea Pig (Cavia porcellus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Source of animals</td>
<td>Government of Maharashtra Institute of veterinary and biological products</td>
</tr>
<tr>
<td>3. Number of animals</td>
<td>18</td>
</tr>
<tr>
<td>4. Number of groups</td>
<td>Total number of groups 3. Group 1- Talisadi Choorna Group 2- Talisadi Guti</td>
</tr>
</tbody>
</table>
Group 3- Control

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Age of animals</td>
</tr>
<tr>
<td>6.</td>
<td>Sex of animals</td>
</tr>
<tr>
<td>7.</td>
<td>Weight of animals</td>
</tr>
<tr>
<td>8.</td>
<td>Dose administration</td>
</tr>
<tr>
<td>9.</td>
<td>Route of administration</td>
</tr>
</tbody>
</table>

Animal Identification

The animals were marked on head, back and tail using picric acid. Appropriate labels were attached to the cages indicating the study number, test substance code, group number and sex, dose, and cage number.

Environmental Conditions

Temperature: Maximum: 27 ºC, Minimum: 25ºC
Mean Relative Humidity: 70 %
The photoperiod was 12 hours artificial light and 12 hours darkness, Light hours being 06:00 to 18:00

Housing

i. Caging: Stainless steel guinea pig cages covered with stainless steel grid top were used.
ii. Water Bottle: Each cage was supplied with stainless steel water bowl (Capacity – 300ml)
iii. Housing: 3 animals of 400-700 grams of same sex per cage.
iv. Room Sanitation: Each day, floor of the experimental room was swept and all work tops and floor were mopped with disinfectant solution.

Diet and Water

i. Diet: Rodent pellet diet (manufactured by 'Pranav Agro Industries Ltd...’) was provided ad libitum.
ii. Water: Drinking water filtered through ‘Aqua guard’ water filter system was provided ad libitum.

Histamine: Histamine in the form of 2/4 imidazyl ethylamine diphosphate salt was used for preparation of 2% histamine solution.

Honey: Honey was used as ‘anupan’ in study groups and to reduce bias a control group is maintained on only honey.
**Spatula:** For oral administration of drug steel spatula was used instead of canula. Administration of drug through canula sometimes leads to entry of drug into lungs which may increase the mortality rate. According to Ayurvedic administration technique the medicine is licked with honey. To maintain uniformity in administration process the animals were made to lick the drug. It was observed that they did like the taste and consumed all doses cheerfully. Precaution was taken to see that each one finishes complete dose without wasting any amount.

**Nebulizer:**

Pulmo-mist Nebulizer Compressor MODEL MDI  
Size: 20cm x 11.5cm x 19cm  
Weight: 1.8kg  
Sound level < 54Db  
Electrical requirement: 0.7AMPS, 230VAC, 50Hz  
Compressor pressure: 25PSI  
Maximum flow: 0.5 ml/min

**Methodology:** 7,8,9

Permission of Institutional Animal Ethics Committee (IACE) was taken before experimentation.

The experiment was conducted in following manner.

1) Dose calculation  
2) Preparation of 2% histamine solution  
3) Administration of dose  
4) Histamine challenge

**1. Dose calculation**

In routine practice majority of vaidyas prescribe Talisadi choorna 2 grams a day. For present study minimum dose of 2 grams for 24 hours was selected to assess the efficacy of Talisadi Choorna. The dose for guinea pigs was extrapolated from 155 mg/kg body weight as this was administered only once in a day.
Human dose, dose calculating factor and mg/kg dose of Talisadi Choorna and Guti for Guinea pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>For 70kg man in 24 hours</th>
<th>Dose calculating factor</th>
<th>For 400gm Guinea pig in 24 hours</th>
<th>For 1 kg Guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.C.</td>
<td>2 gram</td>
<td>0.031</td>
<td>62 mg</td>
<td>155 mg</td>
</tr>
<tr>
<td>T.G.</td>
<td>2 gram</td>
<td>0.031</td>
<td>62 mg</td>
<td>155 mg</td>
</tr>
</tbody>
</table>

Groups, animals per group and dose of each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>No. of animals</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control(Honey)</td>
<td>6</td>
<td>One drop</td>
</tr>
<tr>
<td>II</td>
<td>Talisadi Churna</td>
<td>6</td>
<td>Wt. of Animal X 0.155</td>
</tr>
<tr>
<td>III</td>
<td>Talisadi Guti</td>
<td>6</td>
<td>Wt. of Animal X 0.155</td>
</tr>
</tbody>
</table>

Here 0.155 was dose calculating Factor

Animals were grouped and coded: In each group 3 males and 3 females were taken. In each cage 3 animals of the same sex either male or female were kept. Cages were labeled and the animals were marked for identification.

2. Preparation of Histamine solution

2% histamine solution was prepared. 2gm weighed crystalline powder of Histamine (2/4 imidazolyl ethylamine Diphosphate salt) was added to 100ml distilled water in a beaker and allowed to stand still, till it dissolves.

3. Administration of dose

a. Guinea pigs were weighed daily prior to dosing.
b. Depending on weight dose of each guinea pig was calculated and measured.
c. Measured drug dose of each guinea pig was put on spatula and two drops of honey were mixed with it properly.
d. Oral administration of drug suspension was carried out using steel spatula.
e. For control group only two drops of honey were given.
f. Same procedure was carried out for 21 days for each guinea pig.

4. Histamine Challenge

a. On 22nd day 2% Histamine solution was prepared.
b. 10ml of 2% Histamine solution was taken in Nebulizer and its tip was attached to histamine chamber.
c. Each guinea pig was kept in histamine chamber.
d. Nebulizer was started and challenge was given to each guinea pig.
e. Time required to produce pre convulsive dysnoea (PCD) for each guinea pig was noted.
f. Unused Solution in Nebulizer was also measured.

**Food Consumption:** For recording the food consumption per cage 90 g of standard Guinea pig food pellets (purchased from Amrut laboratory manufactured by Pranav Agro Ind. Ltd., Sangli) were kept in feed tray of the animals. The unconsumed food was weighed and replaced with fresh 90g of food in each tray every day. The time of filling the food tray was noted down and kept constant throughout the study. The animals’ had access to Aqua guard filtered water and food ad *libitum*.

**Observations:** General health of the animals in terms of body weight, behaviour, skin, hair, and food consumption was carefully noted. Cages were cleaned every day.

**ANTI-INFLAMMATORY STUDY**

**Materials**

A. **Drugs**

Standardized Talisadi Choorna and Guti containing synthetic Vanshlochan.

B. **Animals**

**Details of groups, dose and route of administration for Rats**

<table>
<thead>
<tr>
<th>Species of animals</th>
<th>Wistar Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Source of animals</td>
<td>Animal House, Dept. of Pharmacology, BVU Medical College, Pune</td>
</tr>
<tr>
<td>3. Number of animals</td>
<td>18</td>
</tr>
<tr>
<td>4. Number of groups</td>
<td>Total number of groups were 3. Group 1- Talisadi Choorna Group 2- Talisadi Guti Group 3- Control</td>
</tr>
<tr>
<td>5. Age of animals</td>
<td>Adults</td>
</tr>
<tr>
<td>6. Sex of animals</td>
<td>Either</td>
</tr>
<tr>
<td>7. Weight of animals</td>
<td>150-300 gms</td>
</tr>
<tr>
<td>8. Dose administration</td>
<td>Single therapeutical dose 7 days</td>
</tr>
<tr>
<td>9. Route of administration</td>
<td>Oral</td>
</tr>
</tbody>
</table>

C. **Instruments**

Cotton pellets, Scalpel, Desiccator, Scissors, Surgical Needle, Thread, Forceps, Needle Holder, Syringe, Petri dish, Digital balance, Oven.
D. Chemicals
Picric acid, Ethanol, anesthetic ether, Honey.

Methodology: 7,8.9
1. Preparation of cotton pellets
Raw cotton was used for preparation of pellets. Pellets were rolled from cotton with the help of forceps. 18 pellets were formed having uniform weight of 10 mg.

2. Anesthesia procedure
Anesthetic ether was used to induce anesthesia to rats. Cotton swab drenched in ether was kept in desiccator, and then rat was introduced in the desiccator. After five minutes the animal got anaesthetized.

3. Insertion of cotton pellet
Back hairs of animal were trimmed and then back skin was shaved with the help of blade. Skin was disinfected using 70% ethanol, vertical incision of 2cm was taken with surgical blade in the lumbar region. By a blunted forceps subcutaneous tunnel was formed and cotton pellet was inserted.

4. Closure of incision
Simple thread and cutting needle were used for suturing incision.

5. Dose calculation
In routine practice majority of vaidyas prescribe this medicine 2 grams a day. For present study minimum dose of 2 gram in 24 hours is selected to assess the efficacy of Talisadi Choorna and Guti. The dose for Rats was extrapolated from 0.180 mg/kg body weight as this was administered only once in a day.

Human dose, dose calculating factor and mg/kg dose of Talisadi Choorna and Guti for Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>For 70kg man in 24 hours</th>
<th>Dose calculating factor</th>
<th>For 1gm Rat in 24 hours</th>
<th>For 1kg Rat in 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.C.</td>
<td>2 gram</td>
<td>0.181</td>
<td>0.180 mg</td>
<td>180 mg</td>
</tr>
<tr>
<td>T.G.</td>
<td>2 gram</td>
<td>0.181</td>
<td>0.180 mg</td>
<td>180 mg</td>
</tr>
</tbody>
</table>
Groups, animals per group and dose of each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>No. of animals</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control(Honey)</td>
<td>6</td>
<td>One drop</td>
</tr>
<tr>
<td>II</td>
<td>Talisadi Choorna</td>
<td>6</td>
<td>Wt. of Animal X 0.180</td>
</tr>
<tr>
<td>III</td>
<td>Talisadi Guti</td>
<td>6</td>
<td>Wt. of Animal X 0.180</td>
</tr>
</tbody>
</table>

1. Treatment
Animals were treated with Talisadi choorna and Guti for seven days. Two drops of honey was used as a vehicle for administration of drug. Hence, control group was fed with two drops of honey.

2. Removal of cotton pellet
Incision was taken in the lumbar region. Granulomatous capsule formed around the cotton pellet was removed intact.

3. Drying of granuloma capsule
Granuloma capsule was collected in sterilized Petri dish and weighed. Then it was kept in the oven at 40 degree temperature, for 24 hrs. Capsule was again weighed after 24 hrs and kept back in the oven, this was done till a constant weight was obtained. This procedure was done to dry up the fluid present in the capsule.

4. Calculation of granulation tissue formed
Weight of cotton pellet was subtracted from the final weight of capsule. Thus weight of granulation tissue formed was determined.

This procedure was done for all the animals including control group.

Observations
General health of the animals in terms of body weight, behaviour, skin, hair, and food consumption was carefully noted. Cages were cleaned and Cotton Bed was prepared every day.

RESULTS
Mean time required for PCD in each group for Anti-Histaminic activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean time required for PCD(sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51</td>
</tr>
<tr>
<td>Talisadi Choorna</td>
<td>182</td>
</tr>
<tr>
<td>Talisadi Guti</td>
<td>159</td>
</tr>
</tbody>
</table>
Mean weight of dried Granuloma pouch in each group for Anti-inflammatory activity

<table>
<thead>
<tr>
<th>Group</th>
<th>weight of dried Granuloma pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>519</td>
</tr>
<tr>
<td>Talisadi Choorna</td>
<td>173</td>
</tr>
<tr>
<td>Talisadi Guti</td>
<td>177</td>
</tr>
</tbody>
</table>

Statistical Analysis

Unpaired 't'- test was applied for analysis as sample was less than thirty and comparison of two groups was to be done.

Values obtained after applying unpaired ‘t’- test for Anti-Histaminic activity.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>t&lt;sub&gt;table&lt;/sub&gt;</th>
<th>t&lt;sub&gt;cal&lt;/sub&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T.C. - Control</td>
<td>182</td>
<td>32.36</td>
<td>18.76</td>
<td>2.57</td>
<td>6.95</td>
<td>1.97x10^-5</td>
</tr>
<tr>
<td>2</td>
<td>T.G. - Control</td>
<td>159</td>
<td>9.64</td>
<td>5.59</td>
<td>2.57</td>
<td>19.20</td>
<td>1.59x10^-9</td>
</tr>
<tr>
<td>3</td>
<td>T.C. - T.G.</td>
<td>182</td>
<td>33.58</td>
<td>19.48</td>
<td>2.57</td>
<td>1.178</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Interpretation

1. When mean values of time required for pre convulsive dyspnoea of guinea pigs treated with Talisadi choorna and control were compared,

\[ t_{table} < t_{cal} \]

\[ 2.57 < 6.95 \]

Hence, Talisadi choorna was effective against histamine induced bronchospasm.

2. When mean values of time required for pre convulsive dyspnoea of guinea pigs treated with Talisadi guti and control were compared,

\[ t_{table} < t_{cal} \]

\[ 2.57 < 19.20 \]

Hence, Talisadi Guti was effective against histamine induced bronchospasm.

3. When mean values of time required for pre convulsive dyspnoea of guinea pigs treated with Talisadi choorna and guti were compared,

\[ t_{cal} < t_{table} \]

\[ 1.178 < 2.57 \]

Hence, Talisadi Choorna and Guti were equally effective against histamine induced bronchospasm.
Values obtained after applying unpaired ‘t’- test for Anti- inflammatory activity.

<table>
<thead>
<tr>
<th>SN</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>$t_{table}$</th>
<th>$t_{cal}$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T.C. - Control</td>
<td>173</td>
<td>45.41</td>
<td>26.33</td>
<td>2.57</td>
<td>13.14</td>
<td>6.19x10^{-8}</td>
</tr>
<tr>
<td></td>
<td>T.G. - Control</td>
<td>177</td>
<td>67.82</td>
<td>39.33</td>
<td>2.57</td>
<td>8.69</td>
<td>2.83x10^{-6}</td>
</tr>
<tr>
<td>2</td>
<td>T.C. - T.G.</td>
<td>173</td>
<td>64.63</td>
<td>37.49</td>
<td>2.57</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation
1. When mean values of Granulation tissue formed in rats treated with Talisadi Choorna and control were compared,

\[ t_{table} < t_{cal} \]
\[ 2.57 < 13.14 \]

Hence, Talisadi Choorna possessed anti-inflammatory activity.

2. When mean values of Granulation tissue formed in rats treated with Talisadi Guti and control were compared,

\[ t_{table} < t_{cal} \]
\[ 2.57 < 8.69 \]

Hence, Talisadi Guti possessed anti-inflammatory activity.

3. When mean values of Granulation tissue formed in rats treated with Talisadi Choorna and Guti were compared,

\[ t_{cal} < t_{table} \]
\[ 0.45 < 2.57 \]

Hence, Talisadi Choorna and Guti possessed equal anti-inflammatory activity.

Images of Anti-Histaminic Study

- Daily weighing of Guinea Pigs
- Administration of Drug
Images of Anti-Inflammatory Study

Guinea pig in Histamine chamber

Histamine Challenge

Insertion of Cotton Pellet

Closure of Incision

Administration of drug

Excision of Granuloma
DISCUSSION

Anti-histaminic study

Histamine plays an important role in pathology of asthma. Trigger factors like exposure to cold air, dust, smoke and allergens leads to mast cell degranulation, which leads to release of various autacoids. Histamine is one of them, it simulates smooth muscle, which leads to broncho-constriction and finally dyspnoea. Hence a drug to be effective in bronchial asthma has to possess anti-histaminic action. In this study 3 groups of guinea pigs were maintained. Two groups were treated with Talisadi choorna, guti and one group was treated with honey. Control group was treated with honey as it was used as vehicle for administration of Talisadi choorna and guti. When animals were subjected to histamine challenge, those treated with T.C and T.G showed greater resistance to histamine as compared to control. The time interval required for pre-convulsive dyspnoea was greater in those treated with choorna and guti than control. This suggests that choorna and guti are effective. But when choorna and guti are compared there was no significant difference.

Anti-inflammatory study

In asthma sub mucosal inflammation is present, repeated attack of allergens leads to persistence of inflammation. This repeated persistence of inflammation leads to reconstruction of the bronchioles and narrowing of bronchus. Exudates formed during inflammatory process further add to blockage of the bronchiolar lumen and leads to asthma. Thus a drug to be effective in asthma has to posses’s anti-inflammatory activity. In this study three groups of rats were maintained. Two groups were treated with Talisadi choorna, guti and one group was treated with honey. Control group was treated with honey as it was used as vehicle for administration of Talisadi choorna and guti. If a drug has anti-inflammatory activity it inhibits formation of granulation tissue formed during process of inflammation. After this study it was found that rats treated with choorna and guti developed less
granulation tissue as compared to those treated with honey. This suggests that Talisadi choorna and guti has anti-inflammatory activity. But when activity of choorna and guti was compared no significant difference was observed.

CONCLUSION

- Mean Time required for pre-convulsive dyspnoea was greater in guinea pigs treated with Talisadi Choorna and Guti as compared to control. Thus, Talisadi Choorna and Guti are effective against histamine induced bronchospasm. Hence, we may say Talisadi Choorna and Guti have significant Anti-Histaminic Activity.
- Comparison of Anti-Histaminic activity of Talisadi Choorna and Guti showed statistically non-significant difference.
- Mean weight of granulation tissue formed in rats treated with Talisadi Choorna and Guti was less than control. Hence, Talisadi Choorna and Guti have significant Anti-Inflammatory Activity.
- Comparison of Anti-Inflammatory activity of Talisadi Choorna and Guti showed statistically non-significant difference.

REFERENCES

2. http:/www.who.int/respiratory