STUDY MECHANISM OF ACTION OF KRISHNA VAJRAHRAKA BHASMA (KVB) IN CHRONIC ASTHMA

Dr. Sarda Kushal D.
Dr. Mrs. Pandit V. A.
Dr. Mrs. Dawane J. S.
Mr. Deshmane G. B.
Dept. of Pharmacology
Medical College,

Bharati Vidyapeeth Deemed University, Pune-411043.

email-kdsarda@gmail.com
Asthma

- Chronic inflammatory disorder
- Characterized by
  - Hyper responsiveness of bronchi
  - Increased secretions
  - Mucosal edema
  - Mucosal plugging
Asthma

Acute

Bronchospasm

Therapy of Acute Attack

Bronchodilators

(β₂ agonists)

Chronic

Bronchospasm

Prophylactic therapy

Chr. Inflammation & hyperactivity of bronchi

Release of mediators

Anti-inflammatory

(Corticosteroids)

(Mast cell stabilizers)
Limitations of asthma prophylaxis

• Corticosteroids:
  (Effective but many adverse effects)

• Mast cell stabilizers:
  (Less adverse effects but less effective)

• So, search continues:
  For a new, effective and safe drug.
Krishna Vajrabhrakra Bhasma (KVB)

- An ayurvedic preparation
- Used very commonly to reduce frequency of acute asthmatic attacks in chronic asthma (> one year duration)
- ‘Mica’ (abhraka) main constituent of KVB
- Efficacy and exact mechanism of action: Not known.
Krishna Vajrabhraka Bhasma

**Source:** College of Ayurveda, BVDU, Pune.

**Studies conducted by us:**

- **Toxicity Testing:**
  - **Acute:** OECD guideline 420
    \[ \text{LD}_{50} > 2000 \text{mg/kg} \]
  - **Subacute:** OECD guideline 423
    No toxicity up to 50mg/100gm
    (Four times human dose)
Krishna Vajrabhraka Bhasma

• Studies conducted by us…

➢ Efficacy testing:

  Bronchial hyper reactivity in Guinea pigs:
  Egg albumin aerosol in Histamine Chamber
  Corticosteroid- positive control
  Parameter used- pre convulsive time
  Result: KVB efficacy comparable to Corticosteroids
AIM

To study the mechanism of action of KVB in Chronic Asthma.

OBJECTIVES

1. To study the effect on Mast cell degranulation.

2. To study Anti – inflammatory activity.
Material and Methods
Exp I - Mast Cell Stabilization in Rats

• Animals:
  – Albino Rats
  – Either sex
  – Weight-150 to 200 gms.

• Sensitization:
  – With egg albumin (20 mg I.P. & 20 mg S.C.)
  – Sensitization period - 21 days

• Treatment: given as per group.
  - Animals divided into 5 groups of 6 animals each.

• KVB dose: extrapolated from human dose
  11mg/ 100gm rat - Low dose
  22mg/100gm rat - High dose
Mast Cell Stabilizing Activity

Studied in 2 parts:

- **Part A:** Single dose treatment
  (3 hrs before challenge)

- **Part B:** Sub-acute treatment
  (daily for 14 days before challenge)
## Mast Cell Stabilizing Activity - Part A (SD)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Route</th>
<th>Interval before challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Vehicle Control (honey)</td>
<td>Oral</td>
<td>3hrs SD</td>
</tr>
<tr>
<td>(II)</td>
<td>Vehicle Control (water)</td>
<td>Oral</td>
<td>3hrs SD</td>
</tr>
<tr>
<td>(III)</td>
<td>KVB low dose (11mg/100gm)</td>
<td>Oral</td>
<td>3hrs SD</td>
</tr>
<tr>
<td>(IV)</td>
<td>KVB high dose (22mg/100gm)</td>
<td>Oral</td>
<td>3hrs SD</td>
</tr>
<tr>
<td>(V)</td>
<td>Sodium cromoglycate (0.25mg/100gm)</td>
<td>Intraperitonial</td>
<td>SD 30 min. before challenge</td>
</tr>
</tbody>
</table>
# Mast Cell Stabilizing Activity - Part B

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment (dose)</th>
<th>Route</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Vehicle Control (honey)</td>
<td>Oral</td>
<td>14 days</td>
</tr>
<tr>
<td>(II)</td>
<td>Vehicle Control (water)</td>
<td>Oral</td>
<td>14 days</td>
</tr>
<tr>
<td>(III)</td>
<td>KVB low dose (11mg/100gm)</td>
<td>Oral</td>
<td>14 days</td>
</tr>
<tr>
<td>(IV)</td>
<td>KVB high dose (22mg/100gm)</td>
<td>Oral</td>
<td>14 days</td>
</tr>
<tr>
<td>(V)</td>
<td>Sodium cromoglycate (0.25mg/100gm)</td>
<td>Intraperitoneal</td>
<td>SD 30 min. before challenge</td>
</tr>
</tbody>
</table>
Mast Cell Stabilization in Rats

- Challenge: Egg albumin 20 mg I.P. to all animals.

- Collection of Mesentery:
  - Animals sacrificed 30 min after challenge
  - Mesentery collected in 10% formalin

- For Observation:
  - Each piece of mesentery stained with toluidiene blue
  - Placed over a neubaur chamber
  - Number of mast cells (Ruptured & Unruptured) counted under high power (40X) microscope.
Mast Cells in mesentery

Parameter: Percentage of unruptured mast cells
Exp II- Anti-inflammatory action in Rats

- **Animals:**
  - Albino Rats
  - Either sex
  - Weight-150-200 gms.

- **Model:** – Granuloma Pouch Technique
  1\(^{st}\) Day – Preparation of Air pouch (20ml of air) on the dorsum between shoulder limbs.
  2\(^{nd}\) Day – Inj. of 0.5ml croton oil in sesame oil.
  3\(^{rd}\) Day – Air withdrawn, adhesions broken.
  3\(^{rd}\) – 9\(^{th}\) Day – Treatment given as per group.
# Anti-inflammatory activity

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment(dose) 3\textsuperscript{rd} to 9\textsuperscript{th} day</th>
<th>Route</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Vehicle Control (Honey)</td>
<td>Oral</td>
<td>7days</td>
</tr>
<tr>
<td>(II)</td>
<td>Vehicle Control (Water)</td>
<td>Oral</td>
<td>7days</td>
</tr>
<tr>
<td>(III)</td>
<td>KVB Low dose (11mg/100gm)</td>
<td>Oral</td>
<td>7days</td>
</tr>
<tr>
<td>(IV)</td>
<td>KVB High dose (22mg/100gm)</td>
<td>Oral</td>
<td>7days</td>
</tr>
<tr>
<td>(V)</td>
<td>Diclofenac sodium (10mg/kg)</td>
<td>Oral</td>
<td>7days</td>
</tr>
</tbody>
</table>

10\textsuperscript{th} Day – Pouch dissected, weighed, exudate measured.
Granuloma Pouch

Parameter: ‘Weight of Pouch’ & ‘Amount of Exudate’
Results

Exp. I- Mast cell stabilization in rats Comparison between KVB single dose & 14 days treatment

**Highly significant p<0.001

Treatment Groups
Results

**Exp. II Anti-inflammatory action**

- **Highly significant** at p<0.001

**Wt of pouch (mg)**

- Treatment Groups
  - Control water
  - Control honey
  - KVB Low dose
  - KVB High dose
  - Diclofenac

**Highly significant** at p<0.001
Results

Exp. II Anti-inflammatory action

Exudate (mL)

- Control water
- Control honey
- KVB Low dose
- KVB High dose
- Diclofenac

** Highly significant at p<0.001
KVB, an ayurvedic preparation, is used to prevent acute attacks in chronic asthma.

Chronic Asthma

Chronic Inflammation

Mast cell stabilizers
(Reduce degranulation
But less anti-inflammatory)

Corticosteroids
(Anti-inflammatory
But lack mast cell destabilizing action)

KVB
Future Studies

Chronic Asthma

Chronic Inflammation

Remodeling

(Hyper-responsiveness of bronchi)

(Irreversible structural changes)

Rasayana Property

KVB

Study the effect of KVB on bronchial remodeling
THANK YOU
Classification of Abhraka

According to colors
- Sweta (White)
- Rakta (Red)
- Peeta (Yellow)
- Krishna (Black)

According to effect of heat:
- Pinak Abhraka
- Nag Abhraka
- Manduka Abhraka
- Vajra Abhraka

Krishna Vajrabhraka
Preparation of
Krisnna vajrabhraka Bhasma

Steps to obtain bhasma

• Shodhan
  Detoxification
• Dhanybhraka Nirman:
  Intermediate step to reduce particle size
• Maran
  Conversion to lusterless light weight smooth fine powder.
Toxicity studies
Degranulation (immediate)
Histamine, Heparin, Proteases, TNFα

Mambrane derived mediators (over min.)
PGs, Leukotrienes, PAF

Vasodilatation

Influx of inflammatory cells

Synthesis & release of mediators

Sm. Muscle hyper reactivity

Cytokine production (over Hrs.)
Interleukins

Bronchoconstriction
Expt. 1 A: % Unruptured cells (S.D. treatment)

<table>
<thead>
<tr>
<th>NO.</th>
<th>CONTROL HONEY</th>
<th>CONTROL WATER</th>
<th>III KVB Low dose</th>
<th>IV KVB high Dose</th>
<th>V Na cromoglycate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.74</td>
<td>13.74</td>
<td>75</td>
<td>87.03</td>
<td>81.45</td>
</tr>
<tr>
<td>2</td>
<td>20.31</td>
<td>18.2</td>
<td>68.42</td>
<td>82.35</td>
<td>87.78</td>
</tr>
<tr>
<td>3</td>
<td>23.89</td>
<td>17.58</td>
<td>75</td>
<td>86.27</td>
<td>85.12</td>
</tr>
<tr>
<td>4</td>
<td>20.31</td>
<td>21.06</td>
<td>69.7</td>
<td>80.77</td>
<td>91.3</td>
</tr>
<tr>
<td>5</td>
<td>20.54</td>
<td>18.5</td>
<td>73.33</td>
<td>76.32</td>
<td>87.78</td>
</tr>
<tr>
<td>6</td>
<td>24.24</td>
<td>20.46</td>
<td>84.44</td>
<td>88.44</td>
<td>84.44</td>
</tr>
<tr>
<td>MEAN ± SD</td>
<td>21.17 ± 2.37</td>
<td>18.27 ± 2.07</td>
<td>74.32 ** ± 2.4</td>
<td>86.31 ** ± 1.42</td>
<td>83.53 ** ± 9.5</td>
</tr>
</tbody>
</table>

** significantly more effective (P<0.05)
Expt. I B: % Unruptured cells (Chronic Pretreatment)

<table>
<thead>
<tr>
<th>NO.</th>
<th>CONTROL HONEY</th>
<th>CONTROL WATER</th>
<th>III KVB Low dose</th>
<th>IV KVB high Dose</th>
<th>V Na cromoglycate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.72</td>
<td>13.42</td>
<td>80.35</td>
<td>85.93</td>
<td>88.33</td>
</tr>
<tr>
<td>2</td>
<td>17.85</td>
<td>16.32</td>
<td>78.57</td>
<td>88.88</td>
<td>87.75</td>
</tr>
<tr>
<td>3</td>
<td>13.55</td>
<td>13.55</td>
<td>79.03</td>
<td>89.65</td>
<td>87.87</td>
</tr>
<tr>
<td>4</td>
<td>12.72</td>
<td>13.72</td>
<td>76.13</td>
<td>88.46</td>
<td>85.24</td>
</tr>
<tr>
<td>5</td>
<td>14.28</td>
<td>13.88</td>
<td>74.62</td>
<td>82.69</td>
<td>87.5</td>
</tr>
<tr>
<td>6</td>
<td>13.24</td>
<td>12.56</td>
<td>71.18</td>
<td>86.66</td>
<td>84.44</td>
</tr>
<tr>
<td>MEAN ± SD</td>
<td>14.22 ± 5.9</td>
<td>13.90 ± 5.9</td>
<td>74.64** ± 4.74</td>
<td>86.55** ± 4.7</td>
<td>87.04** ± 2.37</td>
</tr>
</tbody>
</table>

** significantly more effective (P<0.05)
Results

**Highly significant**

At $p<0.001$
Exp. II Anti-inflammatory action

- Control water
- Control honey
- KVB High dose
- KVB low dose
- Diclofenac

Exudate (mL)

Treatment Groups

*significant at p<0.5
***significant at p<0.001
Results

Comparison of KVB single dose and 14 days Treatment

- Control water
- Control honey
- KVB Low dose
- KVB High Dose
- Na Cromoglycate

% unruptured mast cells

** Highly significant at p<0.001
Results

Comparison between KVB single dose & 14 days treatment

**Highly significant
At p<0.001

- Single dose
- 14 days treatment

Control water 1, Control honey 2, KVB Low dose 3, KVB High Dose 4, Na Cromoglycate 5, Control water 6, Control honey 7, KVB Low dose 8, KVB High Dose 9, Na Cromoglycate 10