EXPERIMENTAL EVALUATION OF ANTI DIABETIC ACTIVITY OF SWARNAMAKSHIKA BHASMA

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ABSTRACT:
Swarnamakshika identified under Maharasa Varga is considered as Chalcopyrite Mineral which contains iron (Fe), Copper (Cu) and Sulphur(S) as major components along with other trace elements of therapeutic importance. Swarnamakshika possess Rasayanagrya, Dusadhya Rogahara, Sakalamayaghna and Mehahara properties. Present research work has been taken to evaluate anti-diabetic activity of Swarnamakshika Bhasma (SMB) on Albino rats. Five groups viz Normal Control, Diabetic Control, Glibenclamide (GLB, 10mg/kg, p.o.) SMB (0.45mg/0.2kg ,p.o) and SMB+Honey (0.9mg/0.2kg ,p.o) containing 6 rats in each group are subjected for Acute (i.e. single day) and chronic (15days) anti-diabetic study on Alloxan (ALX) induced diabetic rats and compared statically by Anova Test. Blood Glucose level of the drug was evaluated by Oral Glucose tolerance test (1st Day), and Fasting Blood Glucose Estimation (1,3,5,7,9,11,13,15-Days ) for evaluation of Anti-Diabetic activity. Results showed that SMB and SMB+HONEY possess significant anti-hyperglycemic activity. Histo-pathological Studies showed that SMB has regenerated islets of langerhans, and relatively increased granulated and normal β-cells of pancreas.

Key words: Anti-Diabetic Activity, Histo-pathological Study, Swarnamakshika Bhasma, Serum Glucose Level.

INTRODUCTION: Rasashastra deals with variety of Metals and Minerals which play an important role in Ayurveda Therapeutics. Swarnamakshika is one such mineral of Maharasa Varga used by Vaidyas since Samhita Period as it has wide range of therapeutic activity. For scientific re-validation of data and to generate facts and figures of effect of SMB on Diabetes present research work was planned. Diabetes Mellitus is a metabolic disorder caused by complex interactions of genetics, environmental factors and life style choices characterized by Polydipsia, Polyuria, polyphagia, Hyperglycemia, Glycosurea, and Hyperlipidemia. Several new molecules are being developed with new progressive researches in contemporary medical System. But none of these are free from untoward effects in the body, therefore it was felt that Swarnamakshika Bhasma can be proved as an effective Anti-Diabetic drug as per the literatures of Rasashastra.

Preparation Of Swarnamakshika Bhasma:
SMB was prepared as per the reference of Rasa Ratna Samuchaya at PG DEPT of Rasashasra&B.K. Ayurveda
Mahavidyalaya, Hubballi. Bhasma siddhi lakshanas obtained after ten Gaja Putas. Then SMB was subjected for different analytical procedures to ensure the quality of the Bhasma.

After assessed by all these analytical parameters, genuine sample of SMB was taken for experimental study for evaluation of Anti-Diabetic activity on Albino rats.

**ANTI-DIABETIC STUDY:**

Study was performed under two phases viz Single-dose one-day study and Multiple-dose fifteen-day study to evaluate short term and long term Anti-Diabetic effect of test drug on Alloxan induced Albino rats in two different doses.

Male Wistar rats weighing 150–200 gm were used for the present study. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET’s College of Pharmacy, Dharwad, India EG.No.112/1999/CPCSEA).

**MATERIALS:**

**Drugs:** Test drug- *Swarnamakshika Bhasma*, Standard drug- Glibenclamide


**Animals Used:** Wistar strain Albino Rats.

**Equipments:** Animal cage, Day night cycle chamber, weighing balance.

**Glass wares:** Glass Beakers, Test tubes, Stirrer, Measuring jar, 18” needle & Disposable Syringe.

**Chemicals and Reagents:** Normal Saline, 10% Formalin, Bouin Holland solution, Chloroform, Paraffin wax.

**METHOD:** The suspensions of SMB (TEST-1) and *SMB with Honey* (TEST-2) were prepared by using distilled water and administered orally to experimental animals. Fasting Blood glucose level was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was introduced by a single i.p. injection of 120mg/kg Alloxan monohydrate. After 48 hours, the hyperglycemic rats (Glucose level >200 mg/dl) were separated and divided into 5 different groups comprising of 6 rats each for anti-diabetic study. The treatment was started from the same day except normal control and diabetic control groups for a period of 15 days.

Blood glucose levels were estimated in both groups by using a glucose oxidase-peroxidase reactive strips and a Glucometer (S D Check Gold Blood Glucose Meter, Standard Diagnostics, and Korea).

**Experimental design for Single-dose one-day study**

The experimental rats were divided into five groups of six each and treated as follows:

**Group-1**- Normal control – Normal food and Distilled water (10ml/kg)

**Group-2**- Diabetic control - Alloxan Monohydrate (120mg/kg)

**Group-3**- Alloxan Monohydrate+GLB (10mg/kg, p.o.)

**Group-4**- Alloxan Monohydrate+SMB (0.45mg/ 0.2kg, p.o.)

**Group-5**- Alloxan Monohydrate+SMB+Honey (0.9 mg/0.2 kg, p.o.)

Blood samples were collected at 0, 1, 2 and 4 h after drugs administration [single-dose one-day study].

**Experimental design for Multiple-dose fifteen-day study:**

...
The experimental rats were divided into five groups of six each and treated as follows

**Group-1**- Normal control – Normal food and Distilled water (10ml/kg)

**Group-2**- Diabetic control - Alloxan monohydrate (120mg/kg)

**Group-3**- Alloxan monohydrate+GLB (10mg/kg, p.o.)

**Group-4**- AlloxanMonohydrate+SMB (0.45mg/ 0.2kg, p.o.)

**Group-5**- AlloxanMonohydrate+SMB+Honey (0.9 mg/0.2 kg, p.o.)

Fasting Blood samples were collected at 1, 3,5,7,9,11,13 and 15 days after drug administration (Multiple-dose fifteen-day study).

**RESULTS:**

**Organoleptic characters:** *SMB* was smooth, tasteless odourless bright red in colour.

**Physical test:** PH-7.32 ± 0.10, Total Ash-3.52%, Acid insoluble ash: 27.36%, water soluble ash: 2.97%, loss on drying at 110°C: 0.21%, Loss on ignition at 1000°C: 1.7%.

**Chemical tests:** Estimation of sulphur: 12.16 %, Estimation of copper: 19.5%, Estimation of iron: 31.08%.

**Solubility:** Water-3.5 %w/w, Nitric acid-80 % w/w, Hydrochloric acid-92 % w/w.

**Particle size analysis by lazer diffraction method:** 4.46 μm (Mean particle size).

**X-ray diffraction study:** Compound identified as, raw *Swarnamakshika* cufes2. Compounds identified as, *Shodhita Swarnamakshika* cufes2 Compounds identified as *SMB-cufes*2 & fe2o3

Stastical evaluation of the data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way Anova followed by Turkey’s post-test using Graph Pad Prism version 5.0, USA.

**TABLE NO. 1 EFFECT of Five Groups ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (SINGLE-DOSE ONE-DAY STUDY)**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 Minutes</th>
<th>30 Minutes</th>
<th>60 Minutes</th>
<th>120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>117±7.37</td>
<td>134.7±5.5</td>
<td>126±6.5</td>
<td>113.3±2.8</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>550.3±4.61</td>
<td>600±0</td>
<td>593.3±5.7</td>
<td>590±2.08</td>
</tr>
<tr>
<td>GLB</td>
<td>570.7±9.09</td>
<td>591±8.54</td>
<td>600±0</td>
<td>531.3±8.08</td>
</tr>
<tr>
<td><em>SMB</em></td>
<td>575.5±5</td>
<td>595±4.35</td>
<td>480±3.4</td>
<td>381.7±4.3</td>
</tr>
<tr>
<td><em>SMB + HONEY</em></td>
<td>591.3±10.2</td>
<td>600±0</td>
<td>567.3±2.3</td>
<td>502±6.42</td>
</tr>
</tbody>
</table>

1. Diabetic control compared to GLB (P > 0.05)  
2. Diabetic control compared to SMB (P > 0.05)  
3. Diabetic control compared to SMB+HONEY (P > 0.05)

**TABLE NO.2 EFFECT OF Five Groups ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (MULTIPLE-DOSE FIFTEEN-DAY STUDY)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st Day</th>
<th>3rd Day</th>
<th>5th Day</th>
<th>7th Day</th>
<th>9th Day</th>
<th>11th Day</th>
<th>13th Day</th>
<th>15th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>122.5+1 .72</td>
<td>129+7.7</td>
<td>114.9+3 .7</td>
<td>124+5. 2</td>
<td>119.3+ 10</td>
<td>118.8+1 7</td>
<td>105+2. 6</td>
<td>123+1  2</td>
</tr>
</tbody>
</table>
Diabetic Control   | 585+10 | 596+2.0 | 597.3+3 .4 | 564.3+ 1.7 | 589.8+ 0.5 | 597+4.7 | 559.5+ 3.2 | 531+7.6
Glibenclamide | 418+8.1 | 581+14.4 | 434.3+1 3.9 | 468.3+13 | 507+5.7 | 425.5+1 0 | 458.8+ 6.7 | 375.5+ 7.1
SMB | 596+4.1 | 449.3+1 3.2 | 146+7.1 | 113.5+10 | 136.3+9.9 | 111.3+1 .1 | 115+17 | 123+5.2
SMB + Honey | 597+3.4 6 | 592+3.4 | 107+10.9 | 598.5+3 | 201.5+3.8 | 173.8+1 .0 | 138.3+12 | 135+10

1. Diabetic control compared to GLB (P > 0.05)
2. Diabetic control compared to SMB (P < 0.001)
3. Diabetic control compared to SMB+HONEY (P < 0.01)

GRAPH NO.1 SHOWING THE EFFECT OF SMB AND SMB+HONEY ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (SINGLE-DOSE ONE-DAY STUDY)

GRAPH NO.2 SHOWING THE EFFECT OF SMB AND SMB+HONEY ON SG LEVELS IN ALX-INDUCED DIABETIC RATS (MULTIPLE-DOSE FIFTEEN-DAY STUDY)

Histo-pathological Examination: The whole pancreas, kidney and liver from each animal were removed after sacrificing the animals, collected and preserved in 10% formalin solution. The samples were submitted to Hubli Diagnostic Lab Pvt Ltd. (Hubli, Karnataka) India for Histo-pathological examination.
### TABLE NO.3 SHOWING HISTO-PATHOLOGIC CHANGES IN PANCREAS

<table>
<thead>
<tr>
<th></th>
<th>NORMAL CONTROL</th>
<th>DIBETIC CONTROL</th>
<th>GLB</th>
<th>SMB</th>
<th>SMB+HONEY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lining epithelium</strong></td>
<td>Normal</td>
<td>Hypertrophic</td>
<td>Hypertrophic</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td>Normal</td>
<td>Abundant amount of pink granular material(++)</td>
<td>Moderate amount of pink granular material(++)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Nuclei</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>ISLETS OF LANGERHANS</strong></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>cells</strong></td>
<td>Normal</td>
<td>Absent</td>
<td>Decreased</td>
<td>Normal appearing cells with regenerative activity</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>ducts</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Intestinal connective tissue</strong></td>
<td>Normal</td>
<td>Congestion</td>
<td>Congestion</td>
<td>Congestion</td>
<td>Congestion</td>
</tr>
</tbody>
</table>

### TABLE NO.4 SHOWING HISTO-PATHOLOGIC CHANGES IN LIVER

<table>
<thead>
<tr>
<th></th>
<th>NORMAL CONTROL</th>
<th>DIBETIC CONTROL</th>
<th>GLB</th>
<th>SMB</th>
<th>SMB+HONEY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Architecture</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Hepatocytes</strong></td>
<td>Normal</td>
<td>Ballooning degeneration (++)</td>
<td>Ballooning degeneration (++)</td>
<td>Ballooning degeneration (++)</td>
<td>Ballooning degeneration (++)</td>
</tr>
<tr>
<td><strong>Portal triads</strong></td>
<td>Normal</td>
<td>Portal vein dilated</td>
<td>Portal vein dilated</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Central view</strong></td>
<td>Normal</td>
<td>dilation</td>
<td>Central vein dilation</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Kupffer’s cells</strong></td>
<td>Normal</td>
<td>Hyperplasia prominent</td>
<td>Hyperplasia</td>
<td>Hyperplasia</td>
<td>Hyperplasia</td>
</tr>
<tr>
<td><strong>Sinusoidal spaces</strong></td>
<td>Normal</td>
<td>Congestion</td>
<td>Congestion</td>
<td>Congestion</td>
<td>Congestion</td>
</tr>
<tr>
<td><strong>others</strong></td>
<td>Normal</td>
<td>Fatty Change (++)</td>
<td>Fatty Change (+)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
TABLE NO.5 SHOWING HISTO-PATHOLOGIC CHANGES IN KIDNEY

<table>
<thead>
<tr>
<th></th>
<th>NORMAL CONTROL</th>
<th>DIBETIC CONTROL</th>
<th>GLB</th>
<th>SMB</th>
<th>SMB+HONEY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glomerulus-Capillary tuft</strong></td>
<td>Normal</td>
<td>Severe congestion</td>
<td>Mild congestion</td>
<td>Moderate congestion</td>
<td>Mild congestion</td>
</tr>
<tr>
<td><strong>Bowman’s capsule</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Bowman’s space</strong></td>
<td>Normal</td>
<td>Reduced</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Proximal convoluted tubes</strong></td>
<td>Normal</td>
<td>Hypertrophic abundant pink granular material(++)</td>
<td>Hypertrophic pink granular material(++)</td>
<td>Mild(+)</td>
<td>Mild(+)</td>
</tr>
<tr>
<td><strong>Distal convoluted tubes</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Henle’s loop</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Collecting tubes</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Interstitial connective tissue</strong></td>
<td>Normal</td>
<td>Congestion (++) Round cell infiltration(+)</td>
<td>Congestion (+)</td>
<td>Congestion (+)</td>
<td>Congestion (+)</td>
</tr>
</tbody>
</table>

**DISCUSSION:** SMB was prepared by giving 10 Gaja Putas. 45% yield of SMB was obtained. Then Amrutikarana was also carried out with Panchamrita to enhance the therapeutic merits and to eradicate shishta doshas. Panchamrita is having Snigdha, Mridu, Shlakshna guna and Sheeta veerya (except madhu), helps to remove the Rukshata and teekshnata of Bhasma. The total ash 1.6% in SMB indicative of the presence of organic matter in the final product which probably imported during Shodhana or Bhavana procedure. Acid insoluble ash is 1.6%, which indicates the easy absorption of drug. The low acid insoluble acid ash values facilitate absorption in gut. Loss on ignition at 1000°C: 1.6 % shows organic material in the bhasma. Loss on drying shows the end product contain 0.49% of moisture in SMB which is within normal limits. The mean value of total percentage of particles is 4.46 μm, 100% of the particle size is within the range of 0.0-8.83μm. The falling of total range under 0.0 to 8.83 μm indicating fineness of Bhasma. In XRD analysis, only component identified as CuFeS₂ peaks.

Administration of single dose of GLB, SMB and SMB+Honey in diabetic rats showed reduction in SG levels at different time intervals compared to base values i.e. at 0 Minutes of the same group. The SMB (0.45mg/0.2kg) has shown a significant Anti-Diabetic effect in rats at 120 minutes after oral administration compared to Glibenclamide and SMB+Honey (0.9mg/kg) in single dose one day Anti-Diabetic module. But oral administration of SMB, SMB+Honey and GLB not caused a statistically significant reduction.
Long term administration of SMB and SMB+Honey to diabetic rats for 15 days showed marked fall in SG levels compared to base values i.e. at 1st day. SMB (0.45 mg/0.2kg) and SMB+HONEY (0.45 mg+0.9mg/0.2kg) showed significant reduction i.e (P<0.001), (P<0.01) respectively. These data suggest that efficacy of both is higher when compared to standard GLB (10mg/kg) (P>0.05). Multiple-dose fifteen-day study revealed that the SMB and SMB+Honey showed potent anti-diabetic activity compared to GLB

Histo-pathological examination showed that SMB has the potency to regenerate islets of langerhans, and relatively increased granulated and normal β-cells. However, the expansion was better with SMB+Honey than SMB which possibly regenerated β-cells.

Anti-Diabetic activity of The SMB & SMB+Honey might be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells along with regeneration of pancreatic β-cell.

CONCLUSION: Swarnamakshika is a Chalco-pyrite mineral included under Maharasa Varga has got equal importance in both Dehavada and Dhatuvada. SMB was prepared by giving ten Gaja Putas and the nearly 45% of yield obtained.X-RD report of ashdhitha Swarnamakshika showed compound copper pyrite (CuFeS2) with cubic crystal system, Shodhita Swarnamakshika showed compound copper pyrite (CuFeS2) with cubic crystal system, Swarnamakshika Bhasma showed compound ferric oxide (Fe2O3) with Rhombohedral crystal system along with compound copper pyrite (CuFeS2) with cubic crystal system The mean value of total percentage of Swarnamakshika bhasma particles is 4.46 μm, 100% of the particle size is within the range of 0.0-8.83μm. This shows the superiority of Pharmaceutical Procedure. Swarnamakshika Bhasma has got considerable Anti-Diabetic activity in single dose one-day study. Whereas in Multiple dose 15 days study SMB and SMB+ Honey both showed highly significant Anti-Diabetic activity. The P-value of both was P<0.001, P<0.01 respectively. Histo-pathological studies showed that Swarnamakshika Bhasma has the potential to regenerate islets of langerhans and relatively increases granulated and normal β-cells which proves Anti-Diabetic activity of SMB.

REFERENCES:

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B.K., AMV, Hubballi. R.G.U H.S University, Bengaluru., India.

**Source of support**: Nil

**Conflict of interest**: None

Declared

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**Figure 1**: (A) Photomicrographs of normal healthy control group’s rat showing normal globules of acini with normal islet cells (NIC), (H&E, x400) (B) : Photomicrographs of diabetic control group’s rat showing damaged islet cells (DIC), (H&E, x400) (C) : Photomicrographs of standard (Metformin 0.5 g/mg) treated group’s rat showing moderate expansion of islet cells (MEIC), (H&E, x400) (D) : Photomicrographs of ethanolic extract (100 mg/kg) treated group’s rat showing partial restoration of islet cells (PRIC), (H&E, x400) (E) : Photomicrographs of ethanolic extract (200 mg/kg) treated group’s rat showing partial restoration of islet cells (PRIC), (H&E, x400)