Dose finding study of *Sahaj Vati* (comprised of *Shilajeet, Haridra, Guggul* and *Chitrak*) in validated animal model of obesity

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Received 22 February 2017, revised 23 October 2017

Efficacy of *Sahaj Vati* containing *Shilajeet*, *Guggul*, *Haridra* and *Chitrak* has been evaluated at dose of 100, 200 and 400 mg/kg body weight after screening the appropriate diet induced obesity model (monosodium glutamate and cafeteria diet induced). It has been observed that both monosodium glutamate and cafeteria diet increases body weight but cafeteria diet also causes dyslipidemia. At the dose of 400 mg/kg, body weight of animal was significantly decreased with increased total cholesterol, low-density lipoprotein and triglycerides and insignificant decrease of body weight occurs at dose of 100 mg/kg along with increased triglycerides. At the dose of 200 mg/kg body weight was significantly decreased along with the significant increase of high-density lipoprotein and insignificant decrease in very low-density lipoprotein, low-density lipoprotein, and triglycerides. On the basis of present finding we conclude that monosodium glutamate is more suitable for induction of obesity whereas cafeteria diet for obesity associated with dyslipidemia and *Sahaj Vati* at the dose of 200 mg/kg body weight is more appropriate for reduction of body weight.

Keywords: Monosodium glutamate, Cafeteria diet, *Sahaj vati*, IPC Int. Cl. ²: A61K 36/00, A01D 20/25, A61K 9/00, A61K 38/00, A61K 39/00, A61K 48/00

Obesity has risen tenfold in the past four decades in world¹ and United State Food and Drug Administration (USFDA) recommended developing plant derived drugs as an excellent alternative to synthetic drugs as they can be develop at much faster rate and cheaper prices². The animal studies have leading role in drug development during 20th century and gained increasing attention of researchers in the 21st century³. The success rate in clinical evolution remains low because of lack of suitable animal models used for the assessment of target validity⁶,⁷. This indicates that only adequately designed animal models can contribute to our knowledge of biology and medicine, including the discovery and development of new drugs, so it is of utmost importance to ensure that the chosen model is fit-for-purpose. We prepared *Sahaj Vati* comprised of *Shilajeet, Guggul, Haridra & Chitrak* and supposed to be work in obesity, for moving to forward it is basic question that what will be animal model and at which dose it may be effective. To address this question we have searched for an available animal model for obesity.

Obese animal models can be sub-divided into two types: dietary-induced and genetic. Dietary induced animal model will ideally mimic as possible to the human condition due to similarities between the characteristics exhibited and the specific to human condition (face validity). DIO animals are remarkably consistent with those seen in obese patients in the clinic⁸ thus this model appears to have excellent predictive validity for drug development. Obesity can be induced by monosodium glutamate, cafeteria and high fat diet in dietary induced model, so which animal model will be appropriate for induction of obesity and to find the answer of this question we have plan to select animal model at top priority.

There is big question after model validation that what will be appropriate dose, i.e., dose finding study. Dose-finding (DF) trials are Phase I studies with the objective to determining the optimal biological dose

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(OBD) of a drug. These studies are typically conducted by administering sequentially rising doses to successive groups of individuals and different doses of a drug are tested against each other to establish which dose works best and/or is least harmful. The typical dose-finding study may include four groups: a placebo, low-dose, medium-dose and a high-dose group. Thus after finding a suitable model, we have gone for dose finding study to find the dose of Sahaj Vati.

Materials and methods

Procurement and authentication
All the ingredients of Sahaj Vati, i.e., Shilajeet, Haridra, Chitrak and Agnimantha were procured from local Ayurvedic market (Goladinanath) of Varanasi, Uttar Pradesh except Guggul which was procured from Jaipur, Rajasthan. Guggul, Haridra, Chitrak and Agnimantha were authenticated by Prof NK Dubey, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India vide voucher specimens No. Zinziber. 2014/2, Plumbazina. 2015/1 and Bursera. 2015/1, respectively and kept in the herbarium of Laboratory of Herbal Pesticide, Department of Botany, Banaras Hindu University, Varanasi, India and Shilajeet was authenticated by Prof AK Chaoudhary, Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, vide voucher specimen No. Shila.var.2013/3, kept in the museum of Department of Rasa Shastra, Faculty of Ayurveda, I.M.S., B.H.U., Varanasi, India.

Preparation of Sahaj Vati
Before preparation of Sahaj Vati, Haridra and Chitrak were dried in an oven at a temperature of 40°C and powdered by the milling process and purification and potentiation (Shodhan) of Shilajeet and Guggul was carried out. In this way, Suddha Shilajeet and Suddha Guggul were obtained. Sahaj Vati was prepared in two steps, in first step Suddha Shilajeet; Suddha Guggul was dissolved in decoction of Haridra and Chitrak and in second step levigation (bhavana) of Agnimantha Kwatha was provided. In this way after seven times of levigation with decoction of Agnimantha Sahaj Vati was prepared.

Cafeteria diet
The cafeteria diet was prepared with milk chocolate (10 g), peanuts (10 g), corn starch crackers (5 g), sugar (5 g) and conventional diet (15 g). The ingredients were mixed and divided into pellets, weighing approximately 10 g each. It has been reported that its contents carbohydrate, protein, fat and dietary fiber by 56 %, 14.8 %, 18.7 % and 3.2 % respectively, in this way the nutritional value of cafeteria diet is to 4.23 K cal/g.

Animals
Albino rats of female sex weighing between 140-180 g were used for model validation as well as a dose finding study. The animals were housed in polypropylene cages at an ambient temperature of 25 °C ± 1°C and 45-55 % relative humidity; with a 12:12 h light/dark cycle and commercial food pellets and water ad libitum were provided. Before starting the experiment animals were kept for acclimatization to laboratory conditions for at least one week before using them for the experiments. Principles of laboratory animal care (NIH publication number # 85-23, revised in 1985) guidelines were always followed and prior approval of Institutional Animal Ethical Committee (Dean/2015/CAEC/1269 dated 23.06.2015) of Banaras Hindu University was obtained before commencing experiments.

Model validation
Eighteen albino rats of female sex weighing between 140-180 g were divided into three groups named as A, B, and C for control, cafeteria and monosodium glutamate group, respectively. Only conventional animal diet and tap water was provided to group A and group B received cafeteria diet and tap water whereas monosodium Glutamate (Batch No. CIBB2E001 manufactured by Titan Biotech Ltd. Bhiwadi, Rajasthan) was given orally at dose of 10 mg/kg body weight for 10 days to group C with conventional diet and tap water. After 14 days, body weight, food and water intake and blood sugar and lipid profile were estimated.

Protocol for dose finding study
Thirty albino rats of female sex weighing between 140-180 g were divided into five groups named as A, B, C, D and E for control, obesity control and three dose level of Sahaj Vati, respectively. Only conventional animal diet and tap water was provided to group A, whereas Monosodium Glutamate (Batch No. CIBB2E001 manufactured by Titan Biotech Ltd. Bhiwadi, Rajasthan) were given orally at a dose of 10 mg/kg body weight for 10 days to group B, C, D & E with conventional diet and tap water. In group C, D & E, Sahaj Vati was given at dose 100, 200 &
400 mg/kg body weight. The experiment was carried out for 14 days after that food and water intake and blood sugar and lipid profile were estimated.

**Blood collection methodology**

At the end of study, i.e., on 15th day, blood was collected through retro-orbital technique for biochemical estimations and all animals were fasted overnight prior to blood collection.

**Statistical analysis**

Results were expressed as mean ± SD and significance level were analyzed by analysis of variance (ANOVA) using SPSS 16.0; IBM Corporation.

**Results**

**Model validation**

Body weight and food intake significantly increased in group C whereas water intake was more in group B as compared to group A (Table 1). Serum cholesterol was significantly increased in B & C group, whereas triglyceride was significantly increased in group B as compared to A (Table 2).

**Dose finding study**

Significant decrease in body weight was observed in group E as compared to group A (control) and B (positive control) (Table 3). Serum cholesterol and high-density lipoprotein was significantly increased in D & E groups as compared to group A & B. Serum triglyceride, Low-density lipoprotein, and very low density lipoprotein were increased in group B & C whereas insignificantly decreased in D & E group as compared to group A.

HDL was significantly increased in C, D and E as compared to group A (Table 4).

**Discussion**

Homology exists between the genomes of rodents and humans thus indicating that rodent animal model will be major tool for designing of new formulation. So selection of suitable model will be necessary before conducting experiment. Therefore, before conducting dose finding of Sahaj Vati study we assessed the suitability of animal model for obesity in laboratory condition. In survey it was observed that diet induced obesity (DIO) animal model have excellent predictive validity and also exhibit dietary behavior in the development of obesity regulates through inter-relationship among hormonal and the autonomic nervous system. Further, it closely resembles the reality of obesity in humans and feasible as well as reproducible in nature. Thus induction of obesity was performed by changing diet (cafeteria diet & monosodium glutamate induce diet), to find appropriate model for induction of obesity.

Monosodium glutamate significantly increased body weight whereas food intake and blood sugar was significantly increased by cafeteria diet with significant increase in triglyceride, this is also supported by previous researches. Monosodium glutamate (C5H8NO4Na), food additive improves the taste and mask the bad taste of stale products and increases the risk of being overweight irrespective of the total calorie intake and physical activity. The present finding indicate both cafeteria diet and MSG increases body weight of animals but an increase of body weight was highest in MSG group whereas increase in triglycerides and VLDL was highest in

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**Table 1 — Effect of cafeteria diet and monosodium glutamate on body weight, food & water intake and blood sugar**

<table>
<thead>
<tr>
<th>S N</th>
<th>Groups</th>
<th>Initial weight</th>
<th>Weight after 14 days</th>
<th>Water intake</th>
<th>Food intake</th>
<th>Blood sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>152.5 ± 8.95</td>
<td>156.00 ± 9.04</td>
<td>250 ± 2.81</td>
<td>144 ± 6.04</td>
<td>73.03 ± 6.91</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>145.16 ± 7.74</td>
<td>164.66 ± 6.89</td>
<td>320 ± 1.68</td>
<td>156 ± 5.19</td>
<td>83.41 ± 6.11</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>143.5 ± 3.87</td>
<td>169.66 ± 8.72</td>
<td>260 ± 2.141</td>
<td>186 ± 4.12</td>
<td>81.98 ± 7.08</td>
</tr>
</tbody>
</table>

n: six animals in each group, values are mean ± SD, * p < 0.05 as compare to control

**Table 2 — Effect of cafeteria diet and monosodium glutamate on lipid profile**

<table>
<thead>
<tr>
<th>S N</th>
<th>Group</th>
<th>Cholesterol</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>34.43 ± 3.66</td>
<td>6.96 ± 1.67</td>
<td>10.83 ± 2.48</td>
<td>17.91 ± 6.10</td>
<td>91.66 ± 15.18</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>43.65 ± 6.32</td>
<td>8.1 ± 3.12</td>
<td>11.6 ± 3.47</td>
<td>23.46 ± 6.67</td>
<td>107.28 ± 5.55</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>48.13 ± 7.61</td>
<td>7.01 ± 1.92</td>
<td>20.26 ± 6.35</td>
<td>20.25 ± 4.33</td>
<td>94.11 ± 9.38</td>
</tr>
</tbody>
</table>

n: six animals in each group, values are mean ± SD, MSG- Monosodium Glutamate, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, and VLDL-Very low Density Lipoprotein, * p < 0.05 as compare to control, # p < 0.05 as compare to cafeteria group.
To make sure that information gained through dose finding studies can be put in a series of studies that build over a longer dosing period are usually required in order to select compounds that have a suitable profile for chronic testing (e.g. 28 days) because effects of a drug typically undertaken in animals to obtain information in regard to the potency, efficacy, and potentially the side effect profile of a compound. Such models can be used to determine the optimum dose is selected on the basis of the highest therapeutic index (the maximal separation between risk and benefit). So Sahaj Vati at the dose of 200 mg/kg body weight is appropriate for reduction of body weight.

**Conclusion**

Monosodium glutamate is more appropriate for induction of obesity in our laboratory condition and Sahaj Vati in the dose of 200 mg/kg body weight is an appropriate dose for anti-obesity activity in experimental animals.

**References**


