Differential effect of polyherbal, antiobesity preparation, OB-200G in male and female mice and monosodium glutamate-treated rats

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Effect of administration of different doses (0.25, 0.5, 1 and 2 g/kg, twice daily, po) of a poly herbal preparation, OB-200G and fluoxetine (10 mg/kg, ip) for 21 days was studied on food intake and body weight in male and female Laka mice. The study further investigated the effect of administration of 0.5 g/kg dose of OB-200G for 40 days on body weight, fat pad weights, locomotor activity and biochemical parameters in monosodium glutamate (MSG)-treated male and female Wistar rat pups. Administration of OB-200G produced dose dependent decrease in body weight in both male and female mice. On the other hand, fluoxetine decreased body weight only in female mice. The food intake was significantly ($P < 0.05$) increased in both fasted male and female mice after treatment with the lower dose (0.25 g/kg, po) of OB-200G. However, significant ($P < 0.05$) decrease in food intake was recorded with the administration of higher doses (0.5, 1 and 2 g/kg, po) of OB-200G and fluoxetine in fasted female mice on day 1, 7, 14 and 21. But in male mice differential effect on food intake was recorded at different doses on day 1, 7, 14 and 21. Further, OB-200G administration significantly ($P < 0.05$) decreased body weight and fat pad weights, increased serum glucose levels and ambulatory activity in MSG-treated female rats but not in MSG-treated male rats. The results suggest that OB-200G involves gender differences in mediating its antiobesity effect and may supplement the current armamentarium for the treatment of obesity.

Obesity is a serious health problem. Its incidence is on the rise in all the age groups and in all the countries of the world. The multiple causative factors, like genetic, environmental, nutritional, physiological, psychological, social and cultural have been linked to its development and progression\textsuperscript{4.5}. Besides, gender differences have also been reported in the control of eating patterns and development of eating disorders in humans and animals with predominant occurrence in female subjects especially during the adolescent years\textsuperscript{3 - 5}.

The neonatal administration of monosodium glutamate (MSG) has been reported to produce destructive lesions of the arcuate nucleus of the hypothalamus followed by hypoplastic-hypertrophic obesity despite normophagia\textsuperscript{6}. Obese rats treated this way have been shown to exhibit diminished growth, reduced body and muscle mass, decreased organ weights but an extraordinary increase in fat tissue stores as compared to lean control rats. The MSG-treated rats have also been reported to elicit decreased locomotor activity\textsuperscript{7} and thermogenesis\textsuperscript{6}.

OB-200G (Himalaya Drug Company) is a polyherbal formulation containing aqueous extracts of Garcinia cambogia, Zingiber officinale, Piper longum, Gymnema sylvestre and resin of Commiphora mukul. The ingredients in the formulation have been reported to possess thermogenic, hypocholesterolemic, body weight lowering, antidiabetic and digestive stimulant properties. Thus, the present study has been designed with an aim to investigate the effect of subacute treatment with different doses of OB-200G and fluoxetine on body weight and food intake in male and female mice. Fluoxetine, a selective serotonin reuptake inhibitor has been chosen as a standard drug for comparison as it has been reported to reduce hunger and food intake in humans and produce hypophagia in rats\textsuperscript{9 - 11}. The study, further investigated the effect of OB-200G on body weight, fat pads, biochemical parameters and locomotor activity in MSG-treated male and female rats.

**Materials and Methods**

**Animals**—Thirty male and female Laka mice (18-25 g) each and 1 day old neonatal Wistar rat pups, bred at Central Animal House, Panjab University, Chandigarh were used. The mice were housed five per cage under standard laboratory conditions at room temperature (25°C ± 2°C) with 12 hr-light/dark cycle. The animals were provided with pellet chow and water *ad libitum*, except during experimentation. The pups were kept under similar conditions with the
mother rats. All the experiments were conducted between 0900 and 1700 h.

Drug treatment—The dried powder of the polyherbal preparation, OB-200G was provided by the Himalaya Drug Company, Bangalore. The constituents of OB-200G included *Garcinia cambogia* (fruit rind, aqueous extract-50%), *Commiphora mukul* (purified gum resin-20%), *Zingiber officinale* (rhizome, aqueous extract-5%), *Piper longum* (fruit, aqueous extract-10%) and *Gymnema sylvestre* (leaves, aqueous extract-15%). The ingredients as well as their compositions were identified and confirmed with the in-house authentic specimens of Himalaya Drug Company and the voucher deposition specifications of herbarium specimens lie with the company.

OB-200G was suspended in distilled water and administered orally in a dose of 0.25, 0.5, 1 and 2 g/kg, po, twice a day for 21 days to both male and female mice. Fluoxetine HCl (Divis Pharma, India) was dissolved in distilled water and administered intraperitoneally (ip) at a dose of 10 mg/kg to both male and female mice for 21 days. The control group mice received the vehicle in the same volume and through the same route. The drugs were given at a constant volume of 1 ml/100 g body wt in mice and 0.5 ml/100 g body weight in rats.

The neonatal pups were injected monosodium glutamate (SRL Pvt. Ltd., Bombay) 4 mg/g body weight subcutaneously (sc) on day 2, 3, 5 and 6 of life. After sixty days, MSG treated male and female rats, 10 each were divided into 2 groups — MSG-control and MSG-OB-200G. The treatment groups were administered OB-200G in a dose of 0.5 g/kg, po twice a day for 40 days and the control group received the vehicle.

Biochemical estimation—The biochemical parameters were analyzed using kits. The serum glucose was measured using direct sugar (glucose) reagent (End-point O-toluidine method, J. Mitra and Co. Ltd., New Delhi), and serum total cholesterol (one step method of Wybenga and Pilleggi, Span Diagnostics Ltd., Surat) and triglycerides (Enzymatic, GPO/Trinder, End-point colorimetry, Span Diagnostics Ltd., Surat).

Experimental Procedures

Recording of body weight and food intake in mice—The test food for the feeding experiments was standard mice chow modified for palatability by adding glucose. The food intake studies were carried out on day 1, 7, 14 and 21 after fasting the mice for 20 hr. On the same days the body weights of the fasted mice were also recorded. On the day of the experiment, 15 min after drug administration to the different groups of mice housed in separate cages, test food was presented in petridishes and thereafter the cumulated food intake was recorded at 0.5, 1, 2 and 4 hr time intervals.

Recording of different parameters in MSG-treated Wistar rat pups

Body weight—The body weight of the rats was recorded twice a week.

Open field behavior—At the end of the study, on

<table>
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<tr>
<th>Treatment</th>
<th>Ambulation</th>
<th>Rearing</th>
<th>Grooming</th>
</tr>
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<tr>
<td>MSG-control (A)</td>
<td>115.8 ± 10.37</td>
<td>29.3 ± 4.89</td>
<td>7.17 ± 1.11</td>
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<tr>
<td>MSG-control (B)</td>
<td>98.0 ± 12.05</td>
<td>41.7 ± 2.18</td>
<td>6.7 ± 1.66</td>
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<tr>
<td>MSG-OB-200G (A)</td>
<td>134.0 ± 11.16</td>
<td>37.2 ± 5.96</td>
<td>5.0 ± 1.08</td>
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<tr>
<td>MSG-OB-200G (B)</td>
<td>181.8 ± 34.45*</td>
<td>45.5 ± 3.48</td>
<td>3.75 ± 1.93</td>
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Table 1—Effect of OB-200G on open field behavior of MSG-treated male (A) and Female (B) Wistar rats

[Values are mean ± SE]
Fig. 2—Effect of administration of different doses of OB-200G (0.25, 0.5, 1 and 2 g/kg x 2, po) and fluoxetine (10 mg/kg, ip) for 21 days on food intake in female mice. *P<0.05 considered statistically significant as compared to control group.
Fig. 3—Effect of administration of different doses of OB-200G (0.25, 0.5, 1 and 2 g/kg × 2, po) and fluoxetine (10 mg/kg, ip) for 21 days on food intake in male mice. *P<0.05 considered statistically significant as compared to control group.
day 101, the changes in ambulatory, rearing and grooming activity in different groups was noted using open field behavior test apparatus, 30 min after OB-200G administration to treatment groups. The apparatus consisted of a circular wooden arena of 75 cm diameter and wall with a height of 25 cm. The open field test consisted of placing the rat in the center circle and visually monitoring its movement for 5 min. The ambulatory activity, in terms of the number of partitions crossed, and the frequency of rearing and grooming during the 5 min test period were recorded.

Biochemical parameters—The biochemical changes in serum glucose and triglyceride levels were analyzed on day 102. The blood samples were collected by retroorbital puncture under light ether anaesthesia. The blood was allowed to clot and immediately pretreated to obtain serum. The serum samples were refrigerated till analysis.

Fat pad weights—The animals were sacrificed on day 102 and the different fat pads (mesenteric, left and right perirenal and gonadal fat pads) were removed and weighed.

Statistical analysis—Results were expressed as mean ± SE Comparisons between the treatment groups and control were performed using analysis of variance (ANOVA) followed by Dunnett’s test. In MSG-study, comparisons between control and treatment groups were performed using unpaired Student’s t test. In all tests, the criterion for statistical significance was P<0.05.

Results

Effect of OB-200G and fluoxetine on body weight in mice—A subacute 21 days treatment with OB-200G (0.25, 0.5, 1 and 2 g/kg X 2, po) produced dose dependent decrease in body weight in both male and female mice on day 7, 14 and 21 with more marked effect in female mice. On the contrary, after treatment with fluoxetine (10 mg/kg, ip) a significant (P<0.05) decrease in body weight was observed only in female mice (Fig. 1).

Effect of OB-200G and fluoxetine on food intake in mice

Female mice—The administration of fluoxetine (10 mg/kg, ip) for 21 days significantly (P<0.05) decreased food intake as recorded on day 1, 7, 14 and 21. The lower dose of OB-200G (0.25 g/kg) significantly (P<0.05) increased food intake on day 1, 7 and 21. However, treatment with higher doses of OB-200G (0.5, 1 and 2 g/kg, po) significantly (P<0.05) decreased food intake on day 1, 7 and 14. But on day 21, significant (P<0.05) decrease in food intake was observed with 0.5 g/kg (at 0.5 and 4 hr) and 1 g/kg (at

<table>
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<tr>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
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<tr>
<td>MSG-Control (A)</td>
<td>83.7 ± 7.67</td>
<td>95.4 ± 7.74</td>
<td>102.2 ± 5.89</td>
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<td>MSG-Control (B)</td>
<td>68.1 ± 0.70</td>
<td>90.1 ± 5.32</td>
<td>113.9 ± 0.99</td>
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<td>MSG-OB-200G (A)</td>
<td>88.1 ± 4.21</td>
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<td>101.9 ± 3.41</td>
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<td>MSG-OB-200G (B)</td>
<td>88.7 ± 7.66*</td>
<td>80.2 ± 1.79*</td>
<td>109.0 ± 4.39</td>
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<tr>
<th>Treatment</th>
<th>Mesenteric (g)</th>
<th>Gonadal (g)</th>
<th>Perirenal (g)</th>
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<tr>
<td>MSG-Control (A)</td>
<td>0.896 ± 0.13</td>
<td>0.785 ± 0.21</td>
<td>0.156 ± 0.18</td>
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<tr>
<td>MSG-Control (B)</td>
<td>1.369 ± 0.36</td>
<td>1.100 ± 0.16</td>
<td>0.800 ± 0.03</td>
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<tr>
<td>MSG-OB-200G (A)</td>
<td>1.573 ± 0.24*</td>
<td>1.000 ± 0.25</td>
<td>0.883 ± 0.32*</td>
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<tr>
<td>MSG-OB-200G (B)</td>
<td>0.917 ± 0.16</td>
<td>0.473 ± 0.27*</td>
<td>0.436 ± 0.12*</td>
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</tbody>
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Fig. 4—Effect of administration of 0.5 g/kg × 2, po dose of OB-200G on body weight in monosodium glutamate-treated male (M) and female (F) rats (for treatment schedule see methods). *P<0.05 considered statistically significant as compared to control group.
0.5, 1 and 4 hr) dose of OB-200G but 2g/kg dose of OB-200G increased food intake at 2hr as compared to control (Fig. 2).

Male mice—Administration of fluoxetine (10 mg/kg) produced significant decrease in food intake on day 1, 7, 14 (at 0.5 and 1 hr), and 21 (at 0.5 hr). On the other hand, treatment with 0.25 g/kg dose of OB-200G significantly increased food intake on day 1, 7 (at 0.5 and 2 hr) and 21 but decreased the food intake on day 14. Treatment with 0.5 g/kg dose of OB-200G increased food intake on day 1 (at 0.5, 1 and 2 hr) and 21 but decreased the food intake on day 7 at all time points, whereas decreased the same at 0.5 hr and increased at 2 and 4 hr on day 14. Administration of 1 g/kg dose of OB-200G significantly increased food intake on day 1 (at 0.5 and 1 hr) and decreased on day 7 (at 1 and 4 hr) and 14 (at 0.5 and 1 hr). Treatment with the highest dose, 2 g/kg significantly decreased food intake at all time intervals on day 1, 7 and 14 (Fig. 5).

Effect of OB-200G on body weight in MSG-treated rats—Administration of OB-200G (0.5 g/kg X 2, po for 40 days) significantly (P < 0.05) decreased body weight in MSG-treated female rats after day 84 onwards. However, there was no significant difference in body weights between MSG-control and MSG-OB-200G treated male rats (Fig. 4).

Effect of OB-200G on open field behavior in MSG-treated rats—In female rats, OB-200G administration significantly (P < 0.05) increased ambulatory activity with little change in rearing or grooming activity. No significant alteration was recorded in these activities in MSG-treated male rats as compared to MSG-controls (Table 1).

Effect of OB-200G on biochemical parameters in MSG-treated rats—OB-200G treatment significantly increased the serum blood glucose but decreased the total serum cholesterol levels in MSG-treated female rats. However, no significant change was recorded in serum triglyceride levels in MSG-treated male and female rats (Table 2).

Effect of OB-200G on fat pad weight in MSG-treated rats—Administration of OB-200G to female rats produced significant decrease in mesenteric, perirenal and uterine fat pad weights. On the other hand, significant increase in these fat pad weights was observed in MSG-treated male rats (Table 3).

Discussion
In the present study, higher weight gain was observed in MSG-treated male rats as compared to female rats corroborating Bunyan et al. Similar effect was also observed in control rats (data not shown). Further evidence is available suggesting that male animals grow faster and for an extended period as compared to females. Treatment with OB-200G reduced body weight and fat pad weights in MSG-treated female rats. However, the failure of OB-200G to act as an antiobesity in MSG-treated male rats may be due to its inability to reverse the hypothalamic damage and related physiological changes caused by MSG treatment. Further, a 21-days treatment with OB-200G lowered body weight in both male and female mice. This weight lowering effect of OB-200G may be attributed to the thermogenic property of all the ingredients of the formulation and the reported antiobesity effects of Commiphora mukul and Garcinia cambogia. The latter has been known to contain hydroxy citric acid and is reported to inhibit lipogenesis. The increase in food intake reported with OB-200G may be due to the reported digestive stimulant effect of Piper longum, Zingiber officinale and Commiphora mukul. The increased locomotor activity could be due to the overall stimulant effect of the formulation. The underlying mechanism by which OB-200G increased blood glucose levels as compared to controls could not be defined.

Results of the study also suggest gender differences in the control of food intake and body weight. Fluoxetine, a selective serotonin reuptake inhibitor produced significant decrease in food intake and body weight in female mice only. This observation is supported by findings indicating sex differences in control of serotonergic function. According to Dalta & Curzon and Haleem et al., female rats exhibit greater responsiveness to serotonergic agents and also show higher 5-HT turnover rates. The findings of Leibowitz et al. suggest that female rats exhibit a higher density of medial hypothalamic 5-HT1A and 5-HT1B receptors as compared to male rats. Besides, females are reported to be more vulnerable to disorders of feeding which may well involve 5-HT abnormalities. Treatment with OB-200G also produced more pronounced reduction in food intake and body weight in female mice as compared to male mice.

Thus, in conclusion OB-200G involves gender differences in mediating its antiobesity effect and may supplement the current armamentarium for the treatment of obesity.
Acknowledgement
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References