Effect of Olfactory Stimulation with Grapefruit Oil and Sibutramine in Obese Rats

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ABSTRACT

BACKGROUND: Sibutramine (Sibutramine hydrochloride monohydrate) is an anti-obesity drug that enhances satiety. Olfactory stimulation with grapefruit oil decreases the body weight. In this study, we examined the effects of sibutramine and olfactory stimulation with grapefruit oil on body weight, food consumption and neurotransmitters controlling appetite in obese rats.

METHODS: Rats were assigned to two dietary groups for 3 weeks; control group (n=6) was fed commercial standard pellets diet and obese group (n=24) was fed cafeteria diet (hypercaloric diet consisting of highly palatable food). The effect of sibutramine and olfactory stimulation with grapefruit oil was studied on obese rats. Statistical difference and interactions were evaluated through one-way analysis of variance test (one-way ANOVA) followed by Dunnett test was used for means of different groups. For all statistical tests done, a 0.05 level of probability was used as the criterion for significance.

RESULTS: Sibutramine produced its weight reduction effect after 1 week of administration and lasted for only 2 weeks and produced an increase in brain noradrenaline while olfactory stimulation with grapefruit oil produced its weight reduction effect after 2 weeks and did not affect brain noradrenaline.

CONCLUSION: Olfactory stimulation with grapefruit oil produces a weight reduction effect on obese rats after a lag period but it does not affect brain noradrenaline level, while sibutramine produces a weight reduction effect that started from the first week and lasted for 2 weeks only but it increases brain noradrenaline level which may affect blood pressure.

Key Words: Obesity; Sibutramine; Grapefruit oil; Cafeteria diet
Our aims in the current study were 1) to compare the effects of olfactory stimulation with grapefruit oil and sibutramine on cafeteria diet-induced obesity in rats, 2) to study their combined effect on obesity induced by cafeteria diet in rats, and 3) to examine the effects of sibutramine and grapefruit oil on brain noradrenaline.

MATERIALS AND METHODS

Animals: Thirty female Sprague-Dawley albino rats weighing 120-140g were purchased from the animal house colony of the National Research Center (Dokki, Giza, Egypt) and were kept in the animal house under hygienic conditions. All experiments were performed according to the national regulations of animal welfare and institutional animal committee (IAEC). Animals were housed four per cage in a temperature-controlled room (20-22°C) with reversed 12-hour light-dark cycle. Rats were fed commercial standard pellets and were given water ad libitum for an adaptation period of 1 week.

Animal grouping: After the adaptation period, rats were assigned to two dietary groups. The control group (n=6) was fed commercial standard pellets throughout the experiment. The obese group (n=24) was fed high calorie (rich in fats and carbohydrates) highly palatable “cafeteria diet” consisting of chocolate and cookies weighing approximately 20 g per rat for 3 weeks [9].

After 3 weeks, the obese group was further divided into 4 groups and each was fed cafeteria diet for additional three weeks: Group I received no treatment and served as positive control. Group II rats were subjected to olfactory stimulation with grapefruit oil 3 times weekly [10]. Group III rats received sibutramine by mouth (3 mg/kg/day) [11]. Group IV rats were subjected to olfactory stimulation with grapefruit oil and received sibutramine by mouth (1.5 mg/kg/day).

Olfactory stimulation was achieved by exposing the rats to gauze soaked in grapefruit oil suspended in 10,000 volumes of water. The gauze was placed above the animal cage for 15 min 3 times per week for 3 weeks. Body weight was recorded weekly. Percent change of body weight gain was calculated as

\[
\% \text{ change} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100 \%
\]

Food consumption was measured by calculating the amount of calories (Kcal) consumed every day for each group. The mean of daily food consumption was calculated weekly. At the end of the experiment, blood samples were collected and rats were killed by decapitation and brain tissues were collected.

Preparation of brain samples: The brain was immediately excised on ice and weighed.

It was then homogenized using the homogenizer (Jake and Kunkle IKA Labortechnick, Ultra-turrax T25, Germany) in 75% high performance liquid chromatography (HPLC) methanol (1/10 weight/volume). The homogenizer was surrounded with an ice jacket and the homogenates were centrifuged in a cooling centrifuge for 15 min at 5000 rpm. The supernatant was used to determine monoamines neurotransmitters by HPLC according to the method described by Pagelet al. [12]

Statistical analysis: Data were expressed as mean ± standard error (SE). Means of different groups were compared by one-way analysis of variance test (one-way ANOVA) followed by Dunnett test. The significant threshold for all tests was set at P<0.05.

RESULTS

Effect on body weight: (Figure 1 & 2)
Compared to positive control, olfactory stimulation with grapefruit oil significantly decreased percent change in body weight after week 2 (2.16 ± 0.21 %) at P<0.05. Sibutramine significantly decreased percent change in body weight in week 1 (-1.58 ± 0.15%) and week 2 (3.74 ± 0.37%) at P<0.05. The combination of olfactory stimulation and sibutramine significantly decreased percent change in body weight in week 1 (1.84 ± 0.18%) and 3 (3 ± 0.3%) at P<0.05. At the end of 3 weeks, compared to positive control, only sibutramine and the combination of olfactory stimulation and sibutramine significantly decreased percent change in body weight (7.19 ± 0.45% and 8.0 ± 0.72%, respectively).

Effect on food consumption: (Figure 3)
Compared to positive control, sibutramine significantly decreased food consumption in week 2 only (354.25 ±13.49 Kcal) at P<0.05.

Effect on serum glucose and triglycerides: (Table 1)
Cafeteria diet fed rats (positive control) did not show any significant effect on serum glucose compared to normal control. All treated groups did not show any significant effect on serum glucose compared to normal control. Cafeteria diet fed rats (positive control) showed a significant increase in serum triglycerides compared to normal control (312.44 ± 15.24 mg/dl) at P<0.05. All treated groups showed significant increase in serum triglycerides compared to normal control and did not show any significant effect compared to positive control (257.41 ± 24.59 mg/dl, 259.11 ± 22.70 mg/dl and 275.27 ± 17.19 mg/dl) at P<0.05.

Effect on serum GPT and GOT: (Table 1)
Cafeteria diet fed rats (positive control) showed a significant increase in serum glutamic pyruvate transaminase (GPT) compared to normal control (49.75 ±
Figure 1: Effect of olfactory stimulation with grapefruit oil, sibutramine and their combination on percent change in body weight at weeks 1, 2 and 3
* Significantly different from positive control at P<0.05

Figure 2: Effect of olfactory stimulation with grapefruit oil, sibutramine and their combination on percent change in body weight in at the end of the 3 weeks
* Significantly different from positive control at P<0.05
Sibutramine and their combination showed significant decrease in serum GPT compared to positive control and did not show any significant difference compared to normal control (15.25 ± 1.38 U/ml, 14.00 ± 0.94 U/ml and 19.00 ± 1.46 U/ml, respectively) at P<0.05. All treated groups did not show any significant difference in serum glutamic oxaloacetic transaminase (GOT) compared to control groups at P<0.05.

**Effect on serum urea and creatinine:** (Table 1)
Cafeteria diet fed rats showed significant decrease in serum urea compared to normal control (23.56 ± 1.53 mg/dl) at P<0.05. Cafeteria diet fed rats showed significant increase in serum creatinine compared to normal control (0.721 ± 0.022 mg/dl) at P<0.05.

Olfactory stimulation with grapefruit oil, sibutramine and their combination showed significant increase in serum creatinine compared to normal control and did not show any significant effect compared to positive control (0.770 ± 0.030 mg/dl, 0.715 ± 0.067 mg/dl and 0.800 ± 0.021 mg/dl, respectively) (P<0.05).

**Effect on brain serotonin and noradrenaline:** (Table 1)
Olfactory stimulation with grapefruit oil, sibutramine and their combination did not show any significant effect on serotonin compared to control groups. Sibutramine and the combination of olfactory stimulation with sibutramine showed significant increase in brain noradrenaline concentration (1.583 ± 0.027 μg/g and 1.508 ± 0.075 μg/g, respectively) (P<0.05).

**DISCUSSION**
In the present study, olfactory stimulation with grapefruit oil produced its effect on body weight after a lag period of 2 weeks without affecting food consumption or brain noradrenaline. Sibutramine, on the other hand, produced its effect on body weight from the first week but did not last till the end of the study, affecting food consumption and brain noradrenaline.

In this study, cafeteria diet was used to induce obesity in rats. Previous studies proved that cafeteria diet produce obesity in rats [9]. Feeding rats with a cafeteria diet resulted in increases in total body weight and adipose tissue in a short duration of time [13]. Cafeteria diet fed rats represent a useful model for human obesity studies because the cafeteria diet is a palatable hypercaloric and hyperlipidic diet that induces voluntary hyperphagia and fast body weight gain [14, 15].

In the present study, olfactory stimulation with grapefruit oil produced a significant decrease in percent change of body weight after 2 weeks of exposure to grapefruit oil compared to positive control at P<0.05. This result is agreed with a previous study that showed that grapefruit oil requires a lag period to produce its effect [10].

In the present investigation, sibutramine produced significant reduction in percent body weight in week 1 and week 2 only compared to positive control while in week 3 it does not produce a significant reduction in body weight. In a previous study done on human when weight loss is induced with a very low calorie diet (VLCDL), patients treated with sibutramine continued to lose weight over a 1-year period [16]. This is because sibutramine is effectively used in conjunction with caloric restriction [17]. This may describe why in the present study weight loss did not last till the end of the experiment since rats were offered the same diet during the treatment.

In this study, sibutramine decreased the percent change...
in body weight at the end of the 3 weeks at P<0.05. This is in harmony with a previous study by Brown and his co-workers, which showed that sibutramine (3 mg/kg, 3 weeks) decreases the body weight of obese rats [11].

It was also found that administration of half the dose of sibutramine with the exposure to grapefruit oil significantly decreased percent change of body weight compared to positive control in week 1 and 3 at P<0.05. This may be due to the effect of sibutramine and olfactory stimulation with grapefruit oil.

In a previous study, it was found that after an initial reduction in food intake observed with sibutramine, the average food intake over the course of the study did not differ significantly between the sibutramine treatment group and the untreated control group [2]. Also, in a previous study done on rats, when sibutramine was taken chronically for 10 weeks in a dose of 5 mg/kg/day I.P., it transiently reduced food intake (3-4 weeks) [18]. In the present study, sibutramine reduced food only in week 2. This may be due to the different dose and/or route used in this study (3 mg/kg/day P.O.).

In our study, cafeteria diet did not produce a significant increase in the level of serum glucose compared to normal control at P<0.05. This is in agreement with previous studies that showed that cafeteria diet-fed rats displayed normal blood glucose values [19, 20]. On the other hand, while previous literature has shown an increase in serum glucose levels after olfactory stimulation with grapefruit oil, we did not find such increase. [10]. These discordant results may be due to the shorter duration of the current study.

In contrast to two clinical studies in which sibutramine treatment resulted in lower serum glucose levels, we did not find any such effect in the current study. The reported clinical studies were of longer duration (8 and 52 weeks respectively), which may explain the discordant results [21, 22].

The combination of sibutramine with olfactory stimulation with grapefruit oil did not significantly decrease serum glucose level at the end of our study at P<0.05. Previous studies showed that sibutramine decrease serum glucose level but after a longer duration than the present study (8 weeks) [21] or 52 weeks [22]. Cafeteria diet-fed rats in this study showed a significant increase in serum triglycerides level compared to normal control at P<0.05, which is in harmony with previous studies [20, 23].

Previous study done on humans reported that sibutramine was associated with significant improvement in triglycerides level compared to placebo after 52 weeks [22]. However, in the present study no significant difference was seen in the triglycerides level at P<0.05, which may be due to the shorter duration of treatment in this study (3 weeks). This also may be due to the effect of cafeteria diet on serum triglycerides since in the present study rats continue to feed on cafeteria diet till the end of the experiment.

In a previous study done on humans, sibutramine was associated with significant improvement in triglycerides level compared to placebo after 52 weeks [22]. While in the present study the combination of sibutramine with olfactory stimulation with grapefruit oil did not show a significant effect on serum triglycerides level at P<0.05 which may be due to the shorter duration of treatment in this study (3 weeks) and may suggest that grapefruit oil did not improve the effect of sibutramine on serum triglycerides.

We found that cafeteria diet-fed rats had a significant increase in serum GPT level while there was no change in serum GOT levels when compared to normal control group. This observation is consistent with a previous study that showed that in young healthy subjects, a fast food-based diet (cafeteria diet) dramatically increased serum GPT [24]. The present data is also in agreement with a previous study done on humans where the effect of obesity was particularly important in the case of GPT than other liver enzymes where the prevalence of increased GPT values in obese subjects was more than that of normal weight subjects [25]. Also, a previous study done on humans showed that the effect of obesity on liver enzymes is more on GPT than GOT [26].

We found that while cafeteria-fed rats have increased serum GPT levels than normal controls, olfactory stimulation of cafeteria-fed rats with grapefruit oil can negate the dietary effect and bring down serum GPT levels to the levels in normal controls. This effect may be due to the reduction in body weight achieved by grapefruit oil because previous studies showed that in obese patients reduction in body weight is accompanied by improvement in GPT since increased GPT activity was the most frequent hepatic enzyme abnormality in this population [27, 28].

Moreover, a study was done to find if weight reduction alone can improve liver function in obese patients and it was found that these patients would indeed benefit by weight reduction [29].

In the present work, sibutramine produced an improvement in the level of GPT compared to positive control rats at P<0.05. This observation is consistent with a previous study that showed that sibutramine-induced weight loss result in improvements in biochemical markers of non alcohol fatty liver (NAFLD), one of which is GPT [30]. Nonalcoholic fatty liver disease (NAFLD) is a common liver disease characterized by elevated serum aminotransferase levels [30]. Furthermore, a previous study showed that weight loss following treatment with sibutramine is useful in patients with non-alcoholic fatty liver disease (NAFLD) [31]. This effect may be also due to the reduction in body weight achieved by sibutramine because previous studies showed that in obese patients reduction inbody weight is accompanied by improvement in GPT since increasedGPT activity was the most frequent hepatic enzyme abnormality in this population [27, 28].
In the present work, the combination of sibutramine with olfactory stimulation with grapefruit oil showed an improvement in the level of serum GPT compared to positive control group at P<0.05. This may be due to the effect of sibutramine on serum GPT [30]. This may also be due to the weight reduction effect of sibutramine since a previous study showed that weight reduction alone can improve liver functions in obese patients [29] since fast food-based diet (cafeteria diet) dramatically increase serum GPT [24]. Compared to normal control, this combination did not show significant difference in serum GPT which may suggest that grapefruit normalizes the level of GPT in obese rats.

In the present investigation, cafeteria diet produced a significant increase in serum creatinine level compared to normal control group at P<0.05. These findings are in agreement with a previous study [32]. The present data showed that olfactory stimulation with grapefruit oil did not show any significant effect on serum creatinine level compared to positive control at P<0.05. This may be because the rats continued feeding on cafeteria diet till the end of the experiment. This may indicate that grapefruit oil does not affect serum creatinine level of obese rats.

Data of the present study showed that sibutramine treated group showed a significant increase in serum creatinine level compared to normal control at P<0.05, while compared to positive control, it did not show any significant effect at P<0.05. This may be due to the effect of cafeteria diet since the rats continued to feed on this diet till the end of the experiment [32]. This suggests that sibutramine might not reverse the effect of cafeteria diet on serum creatinine level.

The present study showed that the combination of sibutramine with olfactory stimulation with grapefruit oil did not show any significant effect on serum creatinine level compared to positive control at P<0.05. While compared to normal control, it showed a significant increase in serum creatinine level. This may be due to the effect of cafeteria diet and suggest that this combination might not affect serum creatinine level of obese rats.

In the present study, cafeteria diet group produced a significant reduction in serum urea level compared to normal control at P<0.05. This is in agreement with a study that showed that cafeteria diet-fed rats excreted significantly less N2 than did controls [13]. This may be due to the following facts: (i) The rate of synthesis of urea from precursors by isolated hepatocytes from cafeteria diet-fed rats was lower than in controls. (ii) In cafeteria diet-fed rats the activities of all the enzymes of the urea cycle were decreased. The major percentage decrements were in those of carbamoylphosphatesynthetase and of argininosuccinatesynthetase, the enzymes which are probably the rate limiting enzymes.

When rats were switched to normal chow diet, the enzyme activities return to normal values. (iii) The uptake of amino acids by liver of cafeteria diet-fed rats was lower than in controls. While other studies showed that other models of obesity in rat (i.e. genetic or hypothalamic) increases N2 excretion which is opposite to cafeteria diet model which decreases N2 excretion [13].

In the present study, sibutramine produced a significant increase in brain noradrenaline level compared to control groups at P<0.05. This is in agreement with a previous study that showed that sibutramine induced an increase in brain noradrenaline [33]. It is also in harmony with a previous study done by Brown and his team [11]. The present data showed that sibutramine did not produce a significant effect on brain serotonin level after 3 weeks of administration compared to positive control at P<0.05, while previous studies showed that sibutramine raises the brain serotonin level [11]. This may explain why the effect of sibutramine did not last more than 2 weeks.

Data obtained from the present study revealed that the combination of sibutramine and grapefruit oil significantly increased the brain noradrenaline level compared to normal control at P<0.05. This may be due to the effect of sibutramine on brain noradrenaline.

**CONCLUSION**

In conclusion, olfactory stimulation with grapefruit oil produces a weight reduction effect on obese rats after a lag period but it does not affect the brain noradrenaline level. One the other hand, sibutramine produces a weight reduction effect that started from the first week and lasted for 2 weeks only but it increases brain noradrenaline level, which may increase blood pressure. The combination also increased brain noradrenaline but its effect lasted till the end of the study i.e. it did not stop at week 2 like sibutramine alone.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Positive control</th>
<th>Olfactory stimulation with grapefruit oil</th>
<th>Sibutramine (3mg/kg)</th>
<th>Olfactory stimulation + sibutramine (1.5 mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>119.529 ± 4.701</td>
<td>128.499 ± 6.111</td>
<td>126.887 ± 8.134</td>
<td>118.702 ± 7.074</td>
<td>130.958 ± 12.654</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>115.373 ± 8.560</td>
<td>312.438±15.239</td>
<td>257.413±24.588</td>
<td>259.105±22.701</td>
<td>275.274±17.191</td>
</tr>
<tr>
<td>GOT (U/ml)</td>
<td>37.167 ± 1.905</td>
<td>44.750 ± 2.834</td>
<td>41.667 ± 3.007</td>
<td>40.333 ± 1.838</td>
<td>43.000 ± 1.008</td>
</tr>
<tr>
<td>GPT (U/ml)</td>
<td>15.00 ± 1.095</td>
<td>49.75 ± 2.414</td>
<td>15.25 ± 1.376</td>
<td>14.00 ± 0.940</td>
<td>19.00 ± 1.461</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>30.764 ± 1.984</td>
<td>23.556 ± 1.526</td>
<td>28.536 ± 0.549</td>
<td>30.903 ± 1.723</td>
<td>23.556 ± 1.608</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.509 ± 0.016</td>
<td>0.721 ± 0.022</td>
<td>0.770 ± 0.030</td>
<td>0.715 ± 0.067</td>
<td>0.800 ± 0.021</td>
</tr>
<tr>
<td>Noradrenaline(μg/g)</td>
<td>1.217 ± 0.095</td>
<td>1.282 ± 0.026</td>
<td>1.444 ± 0.074</td>
<td>1.583 ± 0.027</td>
<td>1.508 ± 0.075</td>
</tr>
<tr>
<td>Serotonin(μg/g)</td>
<td>0.21 ± 0.018</td>
<td>0.186 ± 0.013</td>
<td>0.213 ± 0.017</td>
<td>0.186 ± 0.009</td>
<td>0.190 ± 0.018</td>
</tr>
</tbody>
</table>

Table 1: Measurements were carried out at 3 weeks of drugs’ administration. Data was expressed as mean ± S.E. (n=6). Statistical analysis was carried out by one-way ANOVA followed by Dunnett test.

a Significantly different from normal control at P<0.05, b Significantly different from positive control at P<0.05

REFERENCES


