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DIFFERENTIAL EFFECT OF A POLYHERBAL FORMULATION-OB-200G IN MALE AND FEMALE MICE SUBJECTED TO FORCED SWIM STRESS

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Abstract : The antistress effect of a polyherbal formulation–OB-200G (500 mg/kg. p.o.) was studied in both male and female mice subjected to forced swim stress. Fluoxetine (10 mg/kg, i.p.) was chosen as standard drug for comparison. Exposure of mice to chronic stress regime resulted in decreased body weight in both male and female mice, increased sweetened food intake, anxiety, depression and locomotor activity in stressed female mice as compared to unstressed control (normal) mice. Treatment with OB-200G resulted in a further decrease in body weight, increased food intake and locomotor activity in both stressed male and female mice. It also reduced immobility time, decreased latency to enter and increased number of entries and time spent in mirror chamber in stressed female mice. Administration of fluoxetine (10 mg/kg, i.p.) decreased body weight and food intake in both stressed male and female mice. Fluoxetine treatment also increased time spent in mirror chamber and decreased immobility time in stressed female mice. Thus, like fluoxetine, OB-200G decreased body weight and produced antianxiety and antidepressant effects in stressed female mice and may prove beneficial in obese patients reported to be more susceptible to stress-related psychological disorders.

Key words : OB-200G, antistress, mice, chronic stress, food intake, anxiety, depression, locomotor activity

INTRODUCTION

Although short-term controllable stress constitutes a beneficial stimulus to the growth and development of the organism, loss of control on stressors acutely and on long term entails pathophysiology or 'diseases of adaptation'. A host of chronic disease states like melancholic depression, anorexia nervosa, malnutrition, panic disorders, obsessive compulsive disorder, metabolic syndrome X and diabetes etc. have been reported to involve abnormalities of stress axis (1, 2). Further, the incidence of life stressors-induced eating (3) and emotional disorders (4) is reported to be

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higher in women. The behavioral response to the stressors also varies depending on the nature of stressor (uncontrollability, intensity, frequency and duration of the stimulus), experimental factors (prior exposure to acute stressors) and the organismic variables (gender, strain, age) (5, 6). Thus, either decrease or increase in body weight (7, 8, 9) and food intake (10, 11, 12) follows the exposure to stress. The chronically stressed animals also show increased ingestion of sweetened foods particularly carbohydrates (13). In humans too, sweet snacking is a frequent behavior at times of stress (14). Further, the psychosocial stress has been implicated in affective disorders like anxiety and depression (15, 16). Certain psychotropic drugs, particularly fluoxetine (selective serotonin reuptake inhibitor) is reported to decrease both the food intake and body weight, suppress stress-induced eating (17, 18, 19, 20) and elicit anxiolytic (21) and antidepressant (22) effects is animals and human.

OB-200G is a polyherbal formulation containing aqueous extracts of *Garcinia cambogia, Piper longum, Gymnema sylvestre, Zingiber officinale* and resin from *Commiphora mukul*. The whole aqueous extract of *Piper longum* has been reported to offer protection against physical, chemical and biological stressors (23). Further, *Zingiber officinale* has been reported to remove dullness and inertia and to exert anxiolytic effect comparable to diazepam (24). *Commiphora mukul* possessed rejuvenating, general and nervine tonic property (25). Besides, the ingredients of formulation had overall stimulating and thermogenic property. In the light of above facts, the present study was designed to investigate the effect of OB-200 G on food intake, body weight, anxiety and depression level and locomotor activity in both male and female mice subjected to forced swim stress for 7 days. Fluoxetine was used as a standard reference drug.

METHODS

Animals: Twenty four male and female Laka mice (20–25 g) bred at Central Animal House, Panjab University, Chandigarh were used in this study. They were housed six per cage under standard laboratory conditions at room temperature of 25 ± 2°C with 12 h light/dark cycle. The animals were provided with pellet chow and water *ad libitum*. All the experiments were conducted between 0900 and 1700 h.

Drugs and treatment schedules: The dried powder of the polyherbal preparation, OB-200G was provided by the Himalaya Drug Company, Bangalore. The constituents of OB-200 G included *Garcinia cambogia* (fruit and, aqueous extract–50%), *Commiphora mukul* (gum resin, purified resin–20%), *Zingiber officinale* (rhizome, aqueous extract–5%), *Piper longum* (fruit, aqueous extract, 10%), *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 specification of herbarium specimens lie with the company. OB-200 G was suspended in distilled water and administered orally in dose of 500 mg/kg, p.o. twice a day for 7 days at a constant volume of 1 ml/100 g of body weight. This dose was selected on the basis of our

preliminary studies. The control animals received only the vehicle in the same volume and through the same route. Fluoxetine (Divis Pharma, India) was administered in dose of 10 mg/kg, i.p. The drugs were administered to respective groups 30 min before testing.

Induction of stress: The male and female mice were separately divided into 4 groups (6 animals/group) each of control (normal), control (stressed), fluoxetine (stressed), OB-200 G (stressed). The animals in all the groups except control (normal) were forced to swim in groups of 6 in a plastic container (38×23×16 cm^3) containing water to a height of 7 cm for 1 h everyday for 7 days. The mice were prevented from clinging to each other or to walls of container.

Parameters tested and procedures:

a) Body weight: The initial body weight was recorded on day 1. The final body weight was recorded on day 7 after subjecting the mice to chronic forced swimming stress and then percent increase in body weight in each group was calculated as compared to day 1.

b) Food intake: The food intake studies were carried out on day 1, 3, 5 and 7 without prior fasting of mice. The test food for the feeding experiment was standard mice chow modified for palatability by adding glucose (26). On experimental days, 15 min after drug administration, sweetened food (15 g/group of 10 g glucose + 25 ml water + 100 g feed) was presented to mice in glass petri dishes and the food intake was recorded for 0.5, 1, 2 and 5 h. The cumulated food intake/mouse (g/20 g mouse weight) was calculated (27).

c) Anxiety level: After subjecting mice to forced swimming stress for 1 h on day 8, the anxiety level of various groups of mice was measured using mirror chamber and following parameters were recorded latency to enter the chamber (sec), number of entries and time spent (sec) (28).

d) Depression level: On day 9, the depression level (duration of immobility period) was recorded in all groups of mice. The mice were forced to swim individually for 6 min session in a glass jar (25 × 12 × 25 cm^3) containing water to a height of 15 cm at 25°C (± 3°C) and the duration of immobility period was recorded (29, 30).

e) Locomotor activity: The locomotor activity (horizontal activity) was assessed using computerized animal activity meter (Opto-Varimex Mini, Columbus Instruments, Ohio, USA). The activity meter consisted of an arena (29 cm × 22 cm × 22 cm) and operated on photoelectric cells that were connected in circuit with a counter. When the animal cuts off the beam of light falling on the photoelectric cell, a circuit is recorded. On day 10, after subjecting mice to 1 h of forced swimming stress and 30 min after drug administration, mouse was placed gently in this arena and number of counts (locomotor activity scores), recorded for 5 minutes (31).

Statistical analysis

The results are expressed as mean ± SEM. Comparisons between the treatment groups and control were performed by analysis of variance (ANOVA) followed by Duncan's multiple range test. In all tests the criterion for statistical significance was $P<0.05$.

RESULTS

Effect on body weight: The percent increase in body weight was significantly ($P<0.05$) less in both male and female mice subjected to forced swimming stress i.e., control (stressed) as compared to unstressed control mice. The administration of OB-200 G in stressed male and female mice caused further reduction in the percent increase in body weight as compared to control (stressed) mice. However, fluoxetine administration caused further reduction in the percent increase in body weight in stressed female mice ($P<0.05$) but not in stressed male mice as compared to control (stressed) mice (Fig. 1).

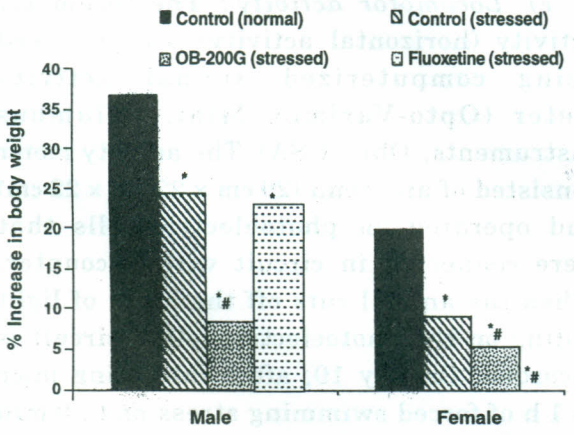

Fig. 1: Effect of OB-200 G and fluoxetine administration on body weight in male and female mice subjected to forced swim stress for 7 days. *$P<0.05$, #$P<0.05$ considered statistically significant as compared to control (normal) and control (stressed) respectively.

Effect on food intake:

Female mice: Exposure of female mice to forced swimming stress (control stressed) caused significant ($P<0.05$) increase in sweetened food intake on day 1, 3, 5 and 7 as compared to control (normal) mice. Administration of OB-200 G in stressed female mice on day 1 showed significant ($P<0.05$) increase in food intake at all time intervals as compared to control (normal) mice and at 1 and 2 h as compared to control (stressed) mice. On day 3, OB-200 G administration significantly ($P<0.05$) decreased food intake at 0.5 and 1 h as compared to control (stressed) mice and control (normal) mice. However, increase at 5 h was observed as compared to control (normal) mice and control (stressed) mice. On day 5, OB-200 G significantly ($P<0.05$) decreased food intake at 0.5, 1, 2 h and increased at 5 h as compared to control (stressed) mice. But on day 7, OB-200 G administration significantly ($P<0.05$) decreased food intake as compared to control (stressed) mice, however, increase at 0.5 and 1 h and decrease at 5 h was observed as compared to control (normal) mice. Administration of fluoxetine resulted in significant ($P<0.05$) decrease in food intake on day 1, 3, 5 and 7 as compared to control (stressed) mice. However, as compared to control (normal) mice, fluoxetine administration produced significant ($P<0.05$) decrease in food intake at all time intervals except at 1 h on day 1, 0.5 h on day 5 and decreased only at 2 h on day 7 (Fig. 2).

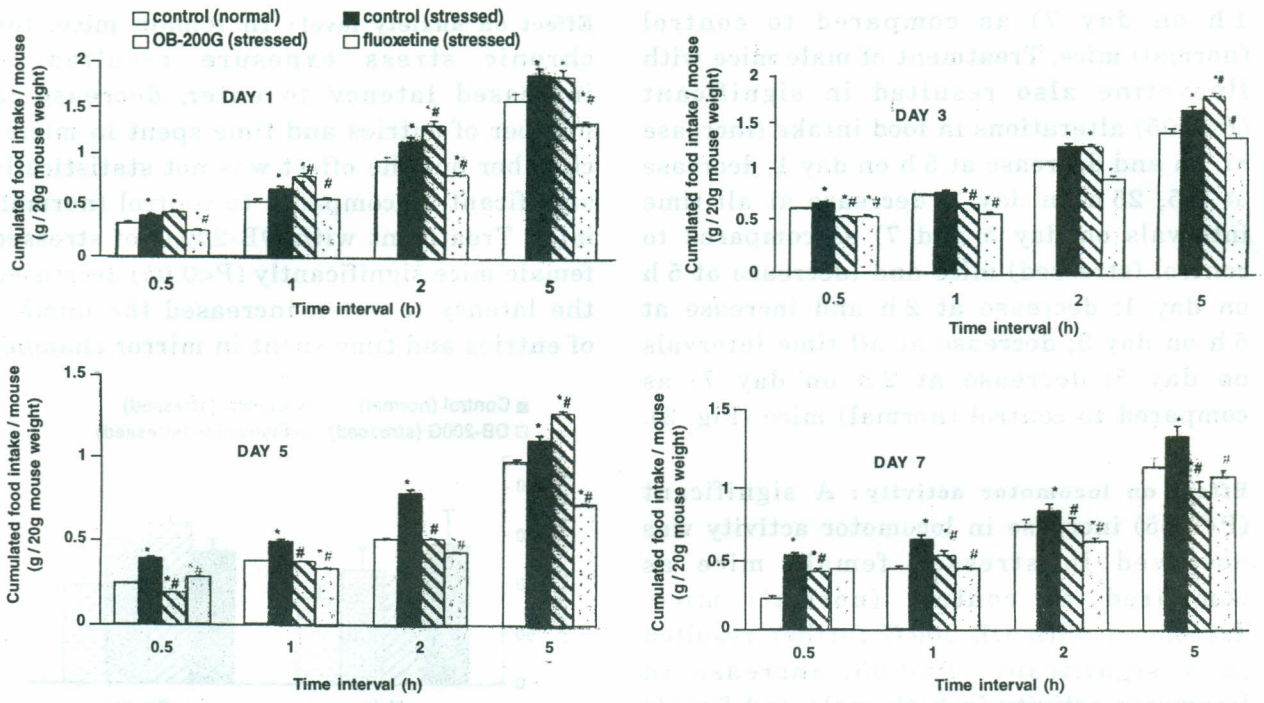

Fig. 2 : Effect of OB-200 G and fluoxetine administration on cumulated food intake/mouse (g/20 g mouse weight) by female mice subjected to forced swim stress for 7 days. *P<0.05, #P<0.05 considered statistically significant as compared to control (normal) and control (stressed) respectively.

Male mice : Exposure of male mice to forced swim stress caused significant (P<0.05) alteration (decrease at 1 h and increase at 2 h on day 1, increase at 0.5, 5 h and decrease at 1 h on day 3, decrease at 1, 2 h on day 5, increase at 0.5 and decrease at 2 h on day 7) in food intake as compared to control (normal) mice. Administration of OB-200 G to stressed male mice also produced significant (P<0.05) alternation in food intake (decrease in food intake at 0.5 h on day 1; increase at 2 h on day 3; increase at 1, 2 and 5 h on day 5, increase at 1, 2 h on day 7) as compared to stressed (control) and (decrease at 1 h and increase at 2 h on day 1; increase at 5 h on day 3; decrease at 1 h and increase at 5 h on day 5; increase in food intake at 0.5 and

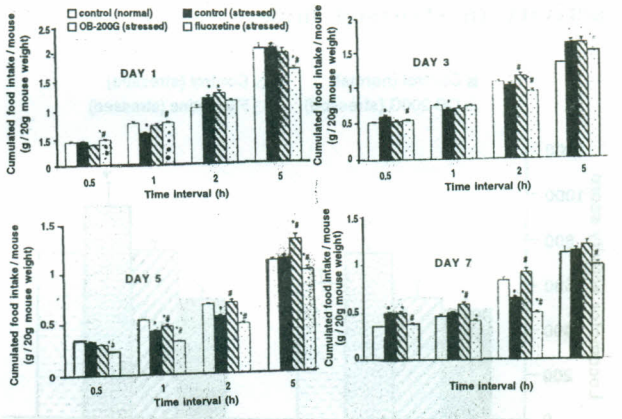

Fig. 3 : Effect of OB-200 G and fluoxetine administration on cumulated food intake/mouse (g/20 g mouse weight) by male mice subjected to forced swim stress for 7 days. *P<0.05, #P<0.05 considered statistically significant as compared to control (normal) and control (stressed) respectively.

1 h on day 7) as compared to control (normal) mice. Treatment of male mice with fluoxetine also resulted in significant ($P<0.05$) alterations in food intake (increase at 1 h and decrease at 5 h on day 1; decrease at 0.5, 25 h on day 3; decrease at all time intervals on day 5 and 7) as compared to control (stressed) mice and (decrease at 5 h on day 1; decrease at 2 h and increase at 5 h on day 3; decrease at all time intervals on day 5; decrease at 2 h on day 7) as compared to control (normal) mice (Fig. 3).

Effect on locomotor activity: A significant ($P<0.05$) increase in locomotor activity was observed in stressed female mice as compared to control (normal) mice. Treatment with OB-200 G further resulted in a significant ($P<0.05$) increase in locomotor activity in both male and female mice as compared to control (normal) and control (stressed) mice respectively (Fig. 4). However, fluoxetine administration did not cause significant change in locomotor activity in stressed mice.

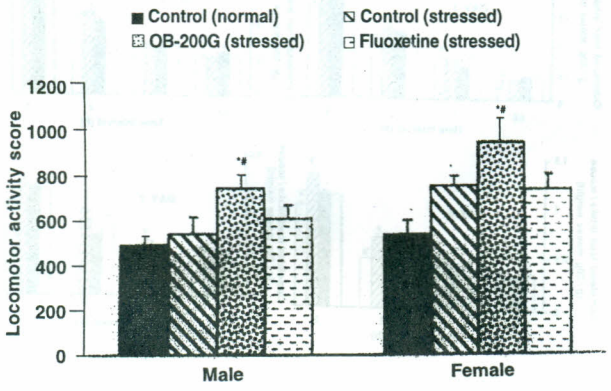

Fig. 4: Effect of OB-200 G and fluoxetine administration on locomotor activity score in male mice subjected to forced swim stress for 7 days. *P<0.05, #P<0.05 considered statistically significant as compared to control (normal) and control (stressed) respectively.

Effect on anxiety level: In female mice, the chronic stress exposure resulted in increased latency to enter, decrease in number of entries and time spent in mirror chamber but the effect was not statistically significant as compared to control (normal) mice. Treatment with OB-200 G of stressed female mice significantly ($P<0.05$) decreased the latency to enter, increased the number of entries and time spent in mirror chamber

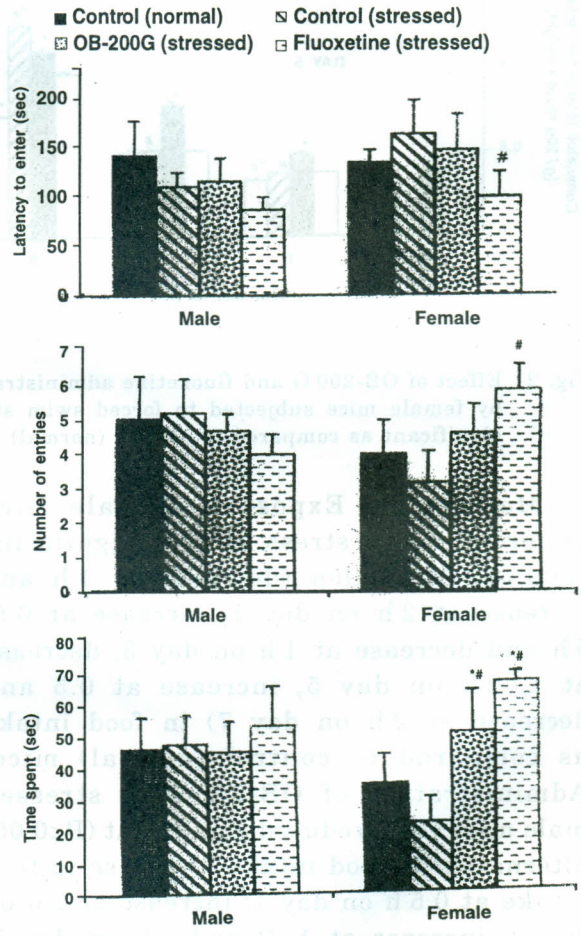

Fig. 5: Effect of OB-200 G and fluoxetine administration on mean immobility time (sec ± SEM) in male mice subjected to forced swim stress for 7 days. *P<0.05, #P<0.05 considered statistically significant as compared to control (normal) and control (stressed) respectively.

as compared to control (stressed) mice. Fluoxetine administration increased the time spent in mirror chamber by stressed female mice as compared to control (normal) and control (stressed) mice. Furthermore, no significant change was observed in these parameters in male mice after exposure to stress or drug treatment as compared to control (normal) mice (Fig. 5).

Effect on immobility time: The exposure to stress for 7 days resulted in increase in despair behaviour (immobility time) in female mice as compared to control (normal) mice and this behavioral despair was significantly ($P<0.05$) reversed after treatment with OB-200 G and fluoxetine in stressed female mice. However, no significant change in immobility time was observed in stressed male mice after exposure to stress or drug treatment as compared to control (normal) mice (Fig. 6).

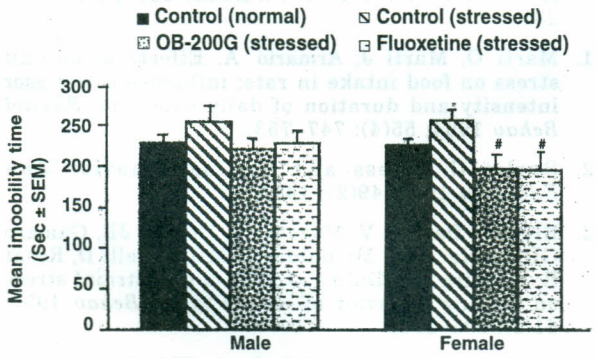

Fig. 6: Effect of OB-200 G and fluoxetine on anxiety level-(latency to enter (sec), number of entries, time spent (sec) by male and female mice subjected to forced swimming stress for 7 days. *$P<0.05$, #$P<0.05$ considered statistically significant as compared to control (normal) and control (stressed) respectively.

DISCUSSION

Exposure to forced swim stress has been previously reported to cause both the physical exercise stress and psychological stress (32, 33, 34). In the present study, the mice were exposed to repeated long-duration inescapable stressful situation by subjecting them to forced swim stress for 1 hour each day for 7 days. Exposure to stress caused significant behavioral changes particularly in female mice. There was significant decrease in body weight in both male and female mice, increased food intake, anxiety, depression and locomotor activity in female mice. Similar gender differences have been reported in response to stress with higher incidence of stress related eating and emotional disorders in female subjects (3, 4, 35).

Fluoxetine, a selective serotonin reuptake inhibitor is reported to be useful for the treatment of eating (17, 36) and psychiatric (21, 22, 37) disorders. In the present study, fluoxetine elicited significant ($P<0.05$) decrease in body weight and hypophagia in both male and female mice, and reduced anxiety and duration of immobility (behavioral depression) in stressed female mice. Various reports also suggest the gender differences in serotonergic control of stress-related disorders like melancholic depression, feeding disorders, the serotonergic receptor densities in various brain regions and enzyme activities (38, 39, 40, 41).

OB-200 G also produced significant behavior alterations in stressed mice. The weight lowering effect of OB-200 G in both

male and female mice may be attributed to the thermogenic property (25, 42, 43, 44) of all the ingredients of the formulation and reported antiobesity effects of *Commiphora mukul* (42) and *Garcinia cambogia* (41). Due to the digestive stimulant effect of *Piper longum, Zingiber officinale* and *Commiphora mukul* (25, 42, 43, 44) substantial increase in food intake was recorded in both male and female mice exposed to stress. The increased locomotor activity could be due to overall stimulant effect of the formulation. OB-200 G exerted antianxiety and antidepressant effect only in female stressed mice, which may be due to reported antistress effects of *Piper longum, Zingiber officinale* and *Commiphora mukul* (23, 24, 25). In conclusion, OB-200 G besides reducing body weight, also decreased stress-induced anxiety and depression in female mice and has shown efficacy comparable to that of fluoxetine. Thus, like fluoxetine, OB-200 G may also prove to be beneficial in obese patients who are more susceptible to stress-related psychological disorders.

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