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*Full Length Research Paper*

# Effect of grapefruit juice and sibutramine on body weight loss in obese rats

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**Grapefruit (Citrus Paradise, family Rutaceae) is a citrus fruit that is low in calories and rich in dietary fibers. Sibutramine (Sibutramine hydrochloride monohydrate) is an anti-obesity drug that enhances satiety. The effect of grapefruit juice and sibutramine on body weight and neurotransmitters controlling appetite was investigated in obese rats. 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 grapefruit juice was studied on obese rats. Statistical difference and interactions were evaluated through one-way analysis of variance test (one-way ANOVA) followed by Dunnett's 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. Grapefruit juice produced its weight reduction effect after 1 week of administration and lasted till the end of the experiment and did not affect brain neurotransmitters. 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 grapefruit juice produced its effect from the first week till the end of the study. It can be concluded that grapefruit juice is better than sibutramine since its effect lasted till the end of the experiment and also did not affect brain noradrenaline.**

**Key words:** Obesity, sibutramine, grapefruit juice, cafeteria diet.

## INTRODUCTION

Obesity represents one of the most serious global health issues that have increased to the extent that it could be considered pandemic (Abolfotouh et al., 2008).

Obesity has reached epidemic proportions globally (WHO, 2003). A recent report from the World Health Organization estimated that approximately 500 million individuals are obese while 1.5 billion are overweight worldwide (WHO, 2011). Obesity occurs through a longstanding imbalance between energy intake and energy expenditure, influenced by a complex biologic system that regulates appetite (Wilding, 2011).

During the past decade, overweight and obesity joined underweight, malnutrition, and infectious diseases as major health problems threatening the developing world (Hossain et al., 2007). At least 2.6 million people each year die as a result of being overweight or obese

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(WHO, 2010).

It seems clear that an energy-dense, high fat diet, palatable foods with high caloric density and increasingly sedentary lifestyles are particularly conducive to the development of obesity (Pi-Sunyer, 2002). Obesity is a risk factor for many chronic conditions including diabetes and cardiovascular diseases (Wang et al., 2014).

Serotonin, particularly via the $5HT_{2c}$ receptor, has also been implicated in satiety signaling. The hypothalamus is densely innervated by serotonin-containing fibres originnating in the mid-brain raphe nucleus. Serotonin is the target of a number of obesity drugs such as the re-uptake inhibitors sibutramine (Jackson et al., 1997).

In the hypothalamus, the paraventricular nucleus (PVN) (the area associated with control of food intake) (Jackson et al., 1997) is innervated by norepinephrine (NE) fibers and is a site at which infusion of exogenous NE elicits eating at low doses. Two subtypes of α-adrenergic receptors within the PVN exert antagonistic actions on eating in the rat: activation of PVN $\alpha_2$-adrenoceptors increases eating, $\alpha_2$-adrenoceptor agonists such as clonidine have been shown to increase food intake in rats (Jackson et al., 1997). On the other hand activation of PVN $\alpha_1$-adrenoceptors suppresses eating (Wellman, 2000).

Grapefruit (Citrus Paradise, family Rutaceae) (Scora et al., 1982) is a citrus fruit that is low in calories and rich in dietary fibers (Stump et al., 2006). It is a commercial crop of great importance, mainly for the juice industry, and also as a source of essential oils and pectin (Hodgson, 1967). It had been shown that grapefruit juice decreased body weight (Fujioka et al., 2006).

Sibutramine is an anti-obesity drug that enhances satiety (suppressing the appetite) (Kim et al., 2003). It acts centrally as an inhibitor of both norepinephrine and serotonin reuptake. Due to inhibition of noradrenaline reuptake, sibutramine is expected to increase systolic and diastolic blood pressure as well as pulse rate with the result that the drug therapy being discontinued in about 5% of patients (Yanovski and Yanovski, 2002).

The aim of this work was to study the effect of grapefruit juice and sibutramine on obesity induced by cafeteria diet in rats

## MATERIALS AND METHODS

### Animals

Thirty female Sprague-Dawley albino rats weighing 120 to 140 g purchased from the animal house colony of the National Research Center (Dokki, Giza, Egypt) were kept in the animal house under hygienic conditions at room temperature with reversed 12 h light, dark cycle. The animals were fed on commercial standard pellets and given water *ad libitum* for an adaptation period of 1 week. Experiments were performed according to the national regulations of animal welfare and institutional animal committee (IAEC).

### Animal grouping

Rats were then divided into two groups. The first group (6 rats) was fed on commercial standard pellets throughout the experiment and served as control group. The second group (24 rats) was fed high calorie highly palatable "cafeteria diet" consisting of chocolate and cookies (Hamilton and Doods, 2002) and served as obese group in addition to standard pellets for 3 weeks for the induction of obesity.

After 3 weeks, the obese group was divided into 3 subgroups: Group I received cafeteria diet for 3 weeks and served as positive control. Group II received grapefruit juice (4 ml, 3 times daily, by gavage) (Fujioka et al., 2006; Paget and Barnes, 1964) together with feeding cafeteria diet for 3 weeks. Group III received sibutramine (3 mg/kg/day, by gavage) (Brown et al., 2001) together with feeding cafeteria diet and standard pellets for 3 weeks.

Body was recorded weekly. Percent change of body weight gain was calculated as:

$$\text{Change of body weight (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

At the end of the experimental period, blood samples were collected, rats were killed by decapitation and brain tissues were isolated.

Blood samples (3 ml) were collected from retro-orbital plexus in dry centrifuge tubes and left to clot at room temperature. Samples were centrifuged at 1500 rpm for 10 min, the clear supernatant was separated and used for determination of serum glucose, triglycerides, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), creatinine and urea.

### Preparation of brain samples

The brain was immediately excised on ice and weighed then homogenized in 75% high performance liquid chromatography (HPLC) methanol (1/10 weight/volume) using a homogenizer (Jake and Kunkle IKA labortechnick, Ultra-turrax T25, Germany) surrounded with an ice jacket and the homogenates were centrifuged in a cooling centrifuge (Sigma and laborzentrifugen, 2K15, Germany) for 15 min at 5000 rpm. The supernatant was used for determination of monoamines' concentration by HPLC according to the method described by Pagel et al. (2000).

### Statistical analysis

Data were expressed as mean ± standard error (SE). Statistical differences and interactions were evaluated using one-way analysis of variance test (One-way ANOVA) followed by Dunnett's test. For all statistical tests done, a 0.05 level of probability was used as the criterion of significance.

## RESULTS

### Effect of feeding cafeteria diet for 3 weeks on body weight gain of rats

Feeding cafeteria diet for 3 weeks significantly increased body weight gain of rats, as compared to normal control values (50.11 ± 2.35 and 21.36 ± 1.92 g at $P<0.05$, respectively) (Figure 1).

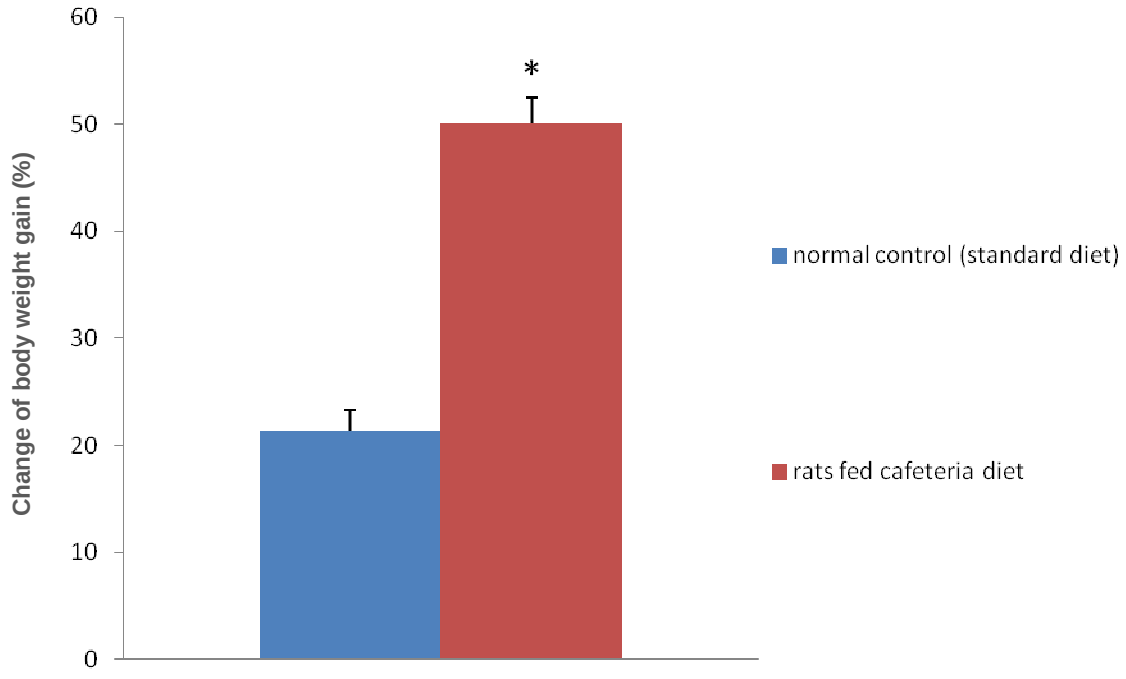


**Figure 1.** Effect of feeding cafeteria diet for 3 weeks on body weight gain of rats.
*Significantly different from normal control at P<0.05.

### Effect of grapefruit juice and sibutramine on food consumption

Grapefruit juice significantly decreased food consumption after the second and the third weeks (323.17 ± 18.53 and 317.39 ± 29.04 Kcal at P<0.05, respectively). Sibutramine significantly decreased food consumption after the second week only (354.25 ± 13.49 Kcal at P<0.05) (Figure 2).

### Effect of grapefruit juice and sibutramine on body weight

Grapefruit juice significantly decreased body weight gain after weeks 1, 2 and 3 (7.1 ±0.26, -3.58 ± 0.31 and 4.42 ± 0.45 g at P<0.05 respectively). Sibutramine significantly decreased body weight gain after week 1 and week 2 (-2 ± 0.25 and 6.2 ±0.65 g at P<0.05 respectively) (Figure 3).

### Effect of grapefruit juice and sibutramine on serum glucose and triglycerides

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 as compared to normal control. Cafeteria diet fed rats (positive control) showed a significant increase in serum triglycerides as compared to normal control (312.44 ± 15.24 mg/dl) at P<0.05. Grapefruit juice did not show any significant effect on serum triglycerides as compared to normal control but it showed significant decrease in serum triglycerides as compared to positive control (141.7 ± 8 mg/dl at P<0.05). Sibutramine showed significant increase in serum triglycerides compared to normal control and did not show any significant effect as compared to positive control (259.12 ± 22.7 mg/dl) (Figure 4).

### Effect of grapefruit juice and sibutramine on serum GPT and GOT

Cafeteria diet fed rats (positive control) showed a significant increase in serum GPT compared to normal control (49.75 ± 2.4 U/ml) at P<0.05. All treated groups showed significant decrease in serum GPT compared to positive control and did not show any significant difference compared to normal control. All treated groups did not show any significant difference in serum GOT compared to control groups (Figure 5).

### Effect of grapefruit juice and sibutramine on serum creatinine and urea

Cafeteria diet fed rats showed significant increase in serum creatinine compared to normal control (0.721 ± 0.022 mg/dl at P<0.05). All treated groups did not show any significant effect compared to positive control. Cafeteria diet fed rats showed significant decrease in

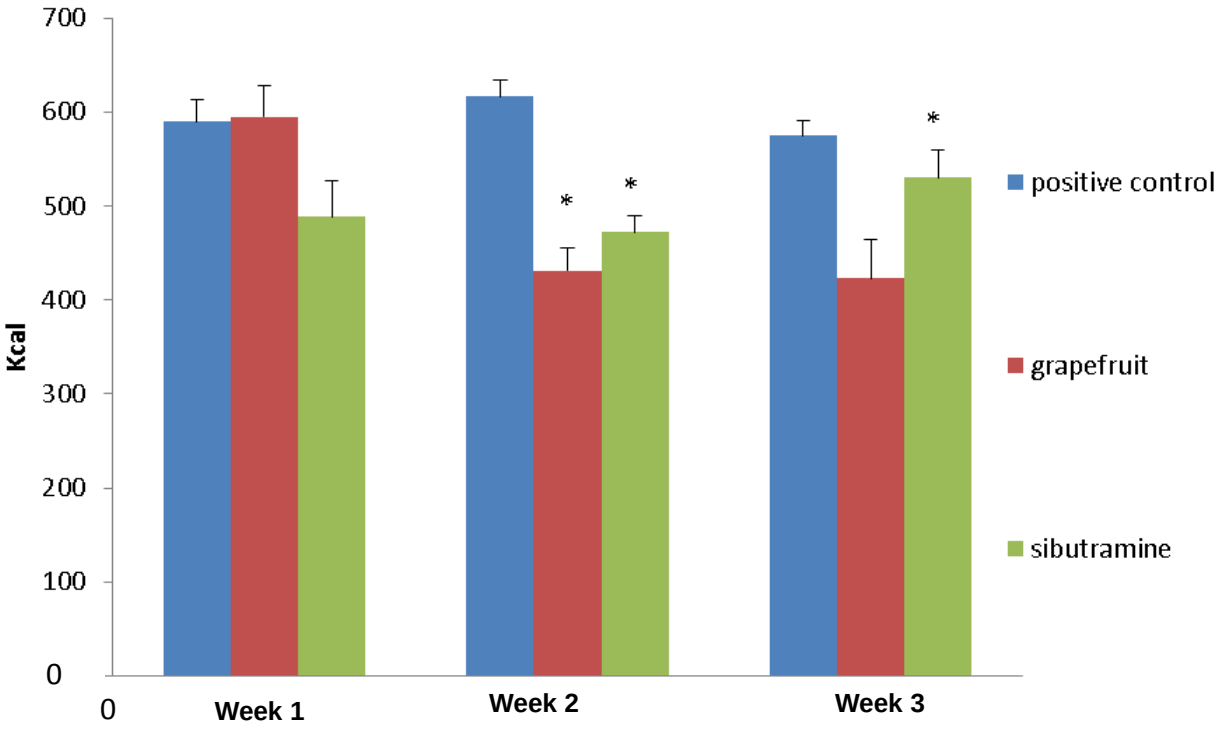


**Figure 2.** Effect of grapefruit juice and sibutramine on food consumption.
*Significantly different from positive control at P<0.05.

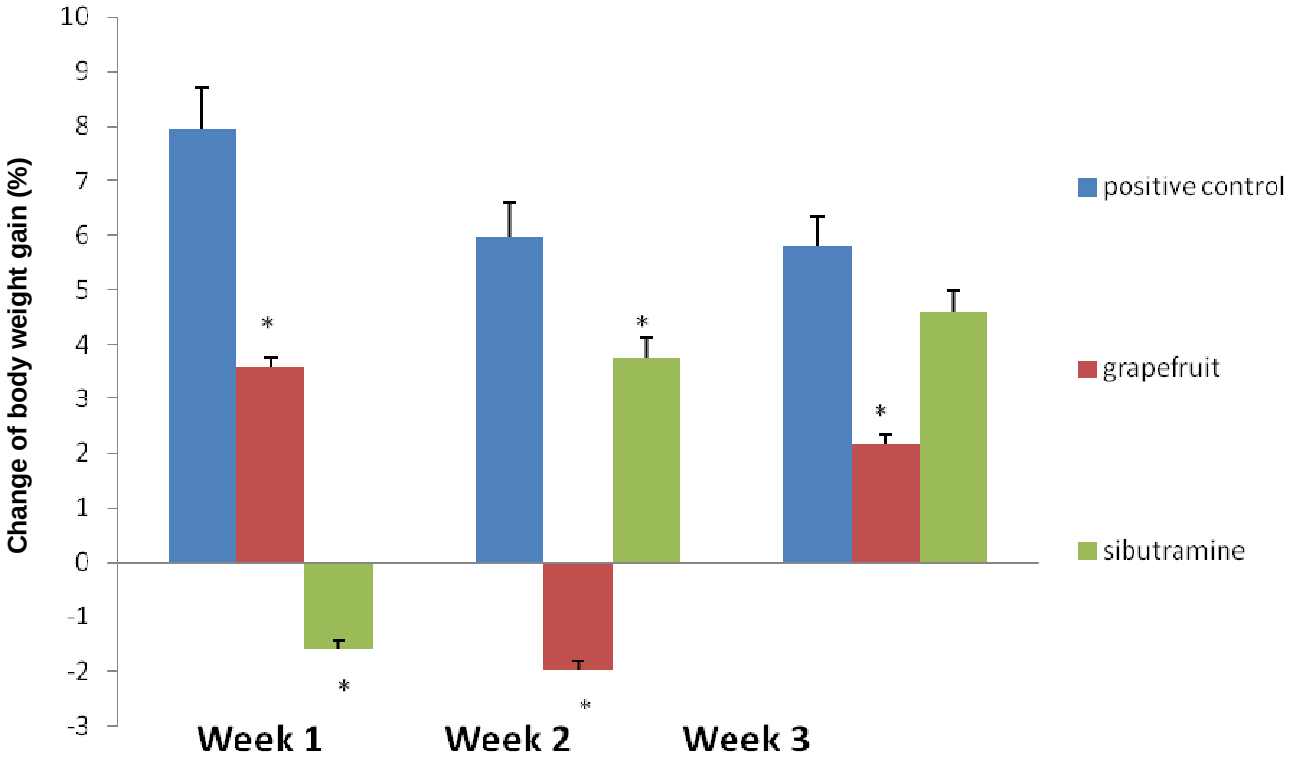


**Figure 3.** Effect of grapefruit and sibutramine on body weight gain in weeks 1, 2 and 3.
*Significantly different from positive control at P<0.05.

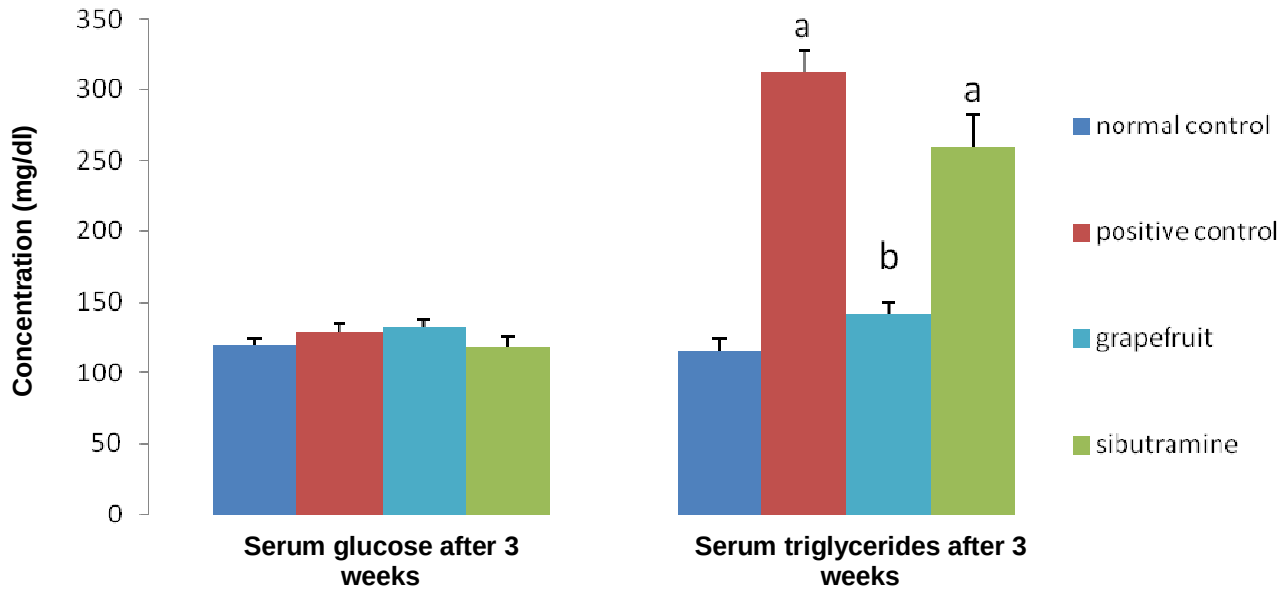


**Figure 4.** Effect of grapefruit and sibutramine on serum glucose and triglycerides after 3 weeks.

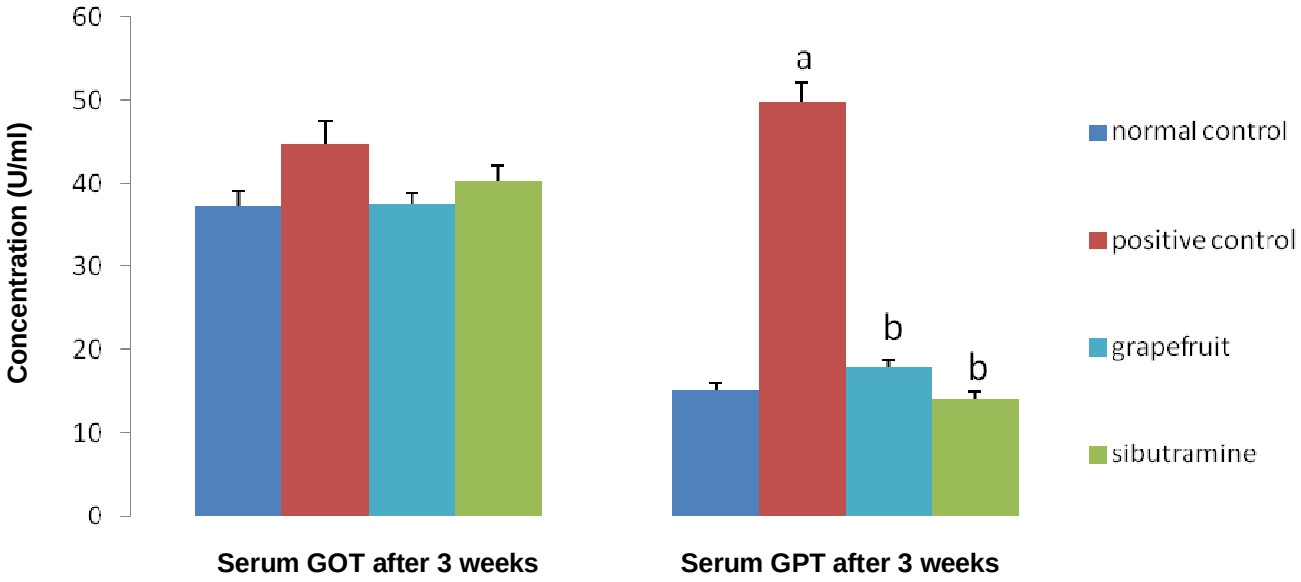


**Figure 5.** Effect of grapefruit and sibutramine on serum GOT and GPT after 3 weeks.

serum urea compared to normal control (23.56 ± 1.53 mg/dl at $P<0.05$). Grapefruit juice did not show any significant effect on serum urea compared to the control groups. Sibutramine showed significant increase in serum urea compared to positive control but did not show any significant effect compared to normal control (Figure 6).

**Effect of grapefruit juice and sibutramine on brain serotonin and noradrenaline**

Cafeteria diet did not show any significant effect on brain serotonin compared to normal control. All treated groups did not show any significant effect on serotonin compared to control groups. Sibutramine showed significant

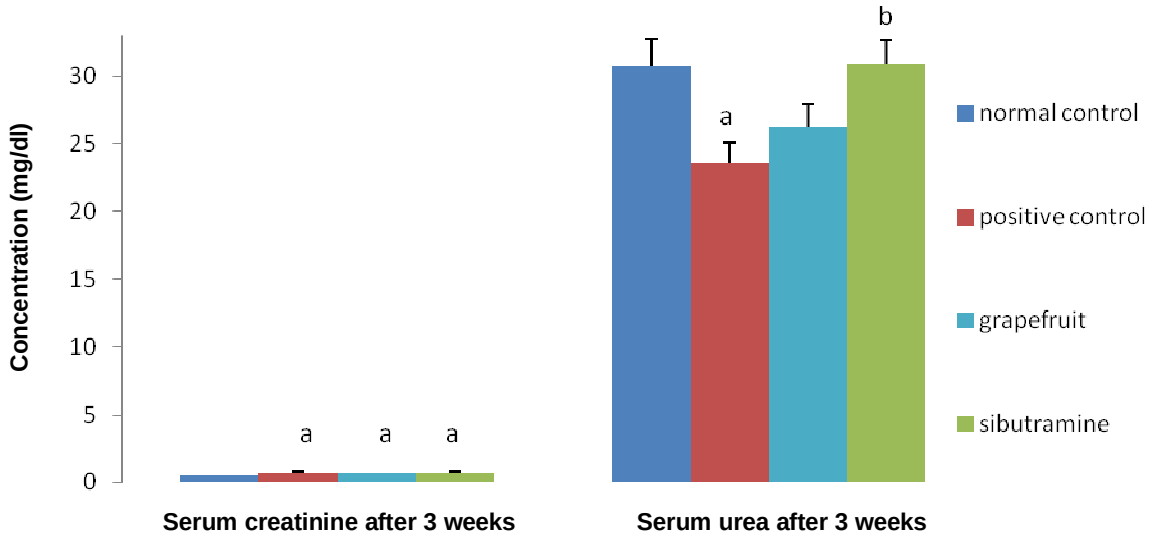


**Figure 6.** Effect of grapefruit and sibutramine on serum creatinine and urea after 3 weeks. [a]Significantly different from normal control at P<0.05. [b]Significantly different from positive control at P<0.05.

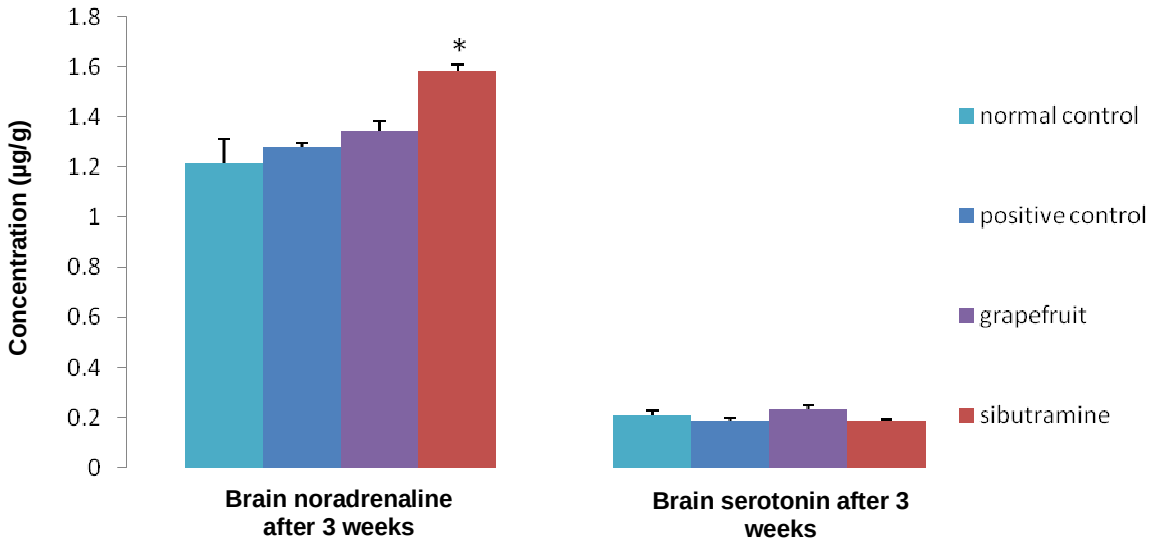


**Figure 7.** Effect of grapefruit and sibutramine on brain noradrenaline and serotonin after 3 weeks. *Significantly different from positive control at P<0.05.

increase in brain noradrenaline concentration compared to control groups (1.583 ± 0.03 µg/g at P<0.05) (Figure 7).

## DISCUSSION

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 (Rodríguez et al., 2001). Exposure to a palatable diet had long-term effects on feeding patterns. Rats became overweight because they initially ate more frequently and ultimately ate more of foods with higher energy density (Martire et al., 2013).

In the present study, cafeteria (CAF) diet was used to induce obesity in rats (Hamilton and Doods, 2002). Such diet increases body weight and adipose mass in rats even after a short period of use (Rodríguez et al., 2004).

In the present study, grapefruit juice significantly decreased body weight gain after weeks 1, 2 and 3 and

after 3 weeks of administration (at the end of the treatment) compared to positive control group. The mechanism of this reduced weight may be due to delayed gastric emptying secondary to grapefruit and its relative acidity (Fujioka et al., 2006). It has been noted in other studies that a decrease in pH of gastric contents can delay gastric emptying which causes gastric distension which contribute to satiety (Chaw et al., 2001) and so decrease in body weight gain.

The decrease in body weight gain may also be attributed to nootkatone, a constituent of grapefruit, which is naturally occurring adenosine monophosphate-activated protein kinase (AMPK) activator. AMPK is a serine/threonine kinase that is implicated in the control of energy metabolism and is considered to be a molecular target for the suppression of obesity (Murase et al., 2010). It consists of three proteins (α, β, and γ subunits) that together make a functional enzyme. Each of these three subunits takes on a specific role in both the stability and activity of AMPK (Stapleton et al., 1996). The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells (Winder and Hardie, 1999).

The energy-sensing capability of AMPK can be attributed to its ability to detect and react to fluctuations in the AMP:ATP ratio that take place during rest and exercise (muscle stimulation) (Winder and Hardie, 1996). During exercise, AMPK activity increases while the muscle cell experiences metabolic stress, and brought about by an extreme cellular demand for ATP. Upon activation, AMPK increases cellular energy levels by inhibiting anabolic energy consuming pathways (such as fatty acid synthesis and protein synthesis) and stimulating energy producing catabolic pathways (such as fatty acid oxidation and glucose transport) (Thomson et al., 2007).

Nootkatone significantly reduced high-fat and high-sucrose diet-induced body weight gain. These findings indicate that nootkatone is beneficial toward preventing obesity due to enhanced energy metabolism through AMPK activation in skeletal muscle and liver (Murase et al., 2010). The present study revealed that sibutramine (3 mg/kg) produced significant reduction of body weight gain after the first and the second weeks only, as compared to obese control values. No significant change of body weight gain was observed after the third week of drug administration, as compared to obese control values.

It has been reported that sibutramine produces significant reduction of body weight when used in conjunction with caloric restriction all over the period of its administration (Florentin et al., 2007). In the present study CAF diet did not produce a significant increase in the level of serum glucose compared to normal control, but it increased the serum triglycerides level (Kuhlmann et al., 2005). These results may be attributed to hyperinsulinemia and hyperlipidemia (Akiyamaab et al., 1996).

Studies have shown that hyperinsulinima is a major cause of obesity because insulin causes the body to store more fats (Reaven et al., 2002). Hyperinsulinemia accelerates hepatic triglyceride (TG) production which leads to elevated plasma TG concentrations (Akiyamaab et al., 1996) and this may explain why TG showed high serum level in cafeteria diet fed rats. Data of the present study showed that grapefruit juice produced a significant reduction in serum triglycerides level compared to positive control group. This is in harmony with a previous study that showed that hesperidin and naringin, bioactive compounds of citrus fruits (grapefruit), are powerful plasma lipid lowering flavonones (Shela et al., 2007). Also a previous work showed that diet supplemented with fresh grapefruit positively influences serum lipid levels of all fractions, especially serum triglycerides. The addition of fresh grapefruit to generally accepted diets could be beneficial for hyperlipidemic, especially hypertriglyceridemic (Gorinstein et al., 2006). While compared to normal control there was no significant difference which suggests that grapefruit may normalize the serum triglycerides level of obese rats as grapefruit is rich in flavonoids. Several studies have shown that flavonoids possess lipolytic activity via inhibition of cAMP-phosphodiesterase and maintain lipolysis-inducing cAMP levels. Lipolysis is a catabolic process leading to the breakdown of TG stored in fat cells (adipocytes) and the release of free fatty acids (FFA) and glycerol. And so grapefruit prevents obesity and helps to decrease body weight and body fat (Dallas et al., 2008).

Results of the current study revealed that CAF-diet-fed rats produced a significant increase in serum GPT level while non significant effect on serum GOT was shown as compared to normal control group. This may be explained by previous studies that showed that in young healthy subjects, a fast food-based diet (CAF diet) dramatically increased serum GPT (Marchesini et al., 2009), as well as that, a study done on human showed the effect of obesity was particularly important in the case of GPT than GOT (Robinson and Whitehead, 1989). Also, several studies showed that hyperlipidemia causes fatty liver disease which increases serum GPT (Kim et al., 2008).

In the present study, grapefruit juice showed an improvement in the level of serum GPT compared to positive control group. This study further shows that CAF diet produced a significant increase in serum creatinine and reduction in serum urea level compared to normal control group. These findings can be explained by the increase in nitrogen retention in CAF diet fed rats. The marked $N_2$ retention was due to a decrease in amino acid catabolism and in urea production by the liver (Sadhu, 2010). Therefore daily $N_2$ retention was greatly enhanced in these animals which is in agreement with the known protein-sparing effect of fat (excess dietary fats spare or retain the body protein) (Estornell et al., 1994).

The decrease in urea production 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 are decreased, the major percentage decreases are those of carbamoylphosphate synthetase and of argininosuccinate synthetase, the enzymes involved in the regulation of the overall rate of the cycle, when rats are switched to normal chow diet, the enzyme activities return to normal values; (iii) ihe uptake of amino acids by liver of cafeteria-diet-fed rats is lower than in controls. These results contrast with those obtained previously by using other models of obesity in rat (that is genetic or hypothalamic), in which N excretion was increased (Barber et al., 1985).

Data of the present study showed that all treated groups showed a significant increase in serum creatinine level compared to normal control, but when compared with positive control they did not show any significant effect. This means that all treatments cannot normalize serum creatinine level in the presence of CAF diet. Sibutramine produced a significant increase in brain noradrenaline level compared to positive control. This is due to the reuptake inhibition effect of sibutramine on brain noradrenaline (Brown et al., 2001).

The present data showed that sibutramine did not produce a significant effect on brain serotonin level after 3 weeks of administration compared to control groups. This may be due to the low dose of sibutramine used in the present study (3 mg/kg) since previous study showed that low doses of sibutramine had no effect on serotonin concentration and only high doses (5 mg/kg) increased serotonin level (Balcioglu and Wurtaman, 2000). This may explain why the effect of sibutramine on body weight did not last till the end of the experiment since there is a synergistic interaction between serotonin and noradrenaline (Jackson et al., 1997).

## Conclusion

Conclusively, grapefruit juice decreased the body weight gain from the first week till the end of the study and also decreased food consumption without affecting brain noradrenaline level. Sibutramine decreased the body weight gain and food consumption only in week 2 (that is, it did not last till the end of the treatment) but it increased the brain noradrenaline level which may lead to an increase in blood pressure.

So from the present study, it was concluded that grapefruit juice is better than sibutramine since its effect lasted till the end of the experiment and also did not affect brain noradrenaline.

## Conflict of interest

The authors declare no conflict of interest.

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