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Topiramate induces weight loss and improves insulin sensitivity in dietary obese rats: comparison to sibutramine

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Abstract. – *Background and Objectives:* **Topiramate is newly approved as anticonvulsant that seems to promote body weight loss in humans. The present study was designed to evaluate the weight-controlling properties of topiramate in dietary obese female rats in comparison with sibutramine.**

Materials and Methods: **Fifty rats were assigned as normal, high fat diet (HFD), HFD + sibutramine (7.5 mg/kg, p.o.), HFD + topiramate (25 mg/kg, p.o.) and HFD + topiramate (50 mg/kg, p.o.). Body weight was registered, anxiety was tested in Vogel's test and blood pressure (BP) was measured. In addition, liver index, adipose tissue index, fasting blood glucose and serum lipid profile were measured in all groups. Further, serum insulin, leptin and adiponectin were determined.**

Results: **Feeding with HFD induced a significant increase in body weight of rats as well as insulin resistance and serum lipids as compared to normal group ($p<0.05$). These measurements were suppressed by sibutramine treatment. However, a significant elevation in BP and anxiety behavior were detected as compared with HFD group ($p<0.05$). Topiramate (50 mg/kg, p.o.) group showed weight loss, improved insulin resistance, lessened anxiety behavior without influence on BP.**

Discussion: **Our data ensures the findings that topiramate has a weight controlling properties with no anxiogenic or hypertensive effects. Further investigations are needed to determine the utility of topiramate in the clinical management of obesity.**

Key Words:

Obesity, Topiramate, Weight control, Anxiety, Blood Pressure.

Abbreviations

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BP = Blood pressure
DBP = Diastolic blood pressure
FAs = Fatty acids
FDA = Food and Drugs Administration
HDL-C = High density lipoprotein cholesterol
HFD = High fat diet
HOMA-IR index = Homeostasis model assessment insulin resistance index
LDL-C = Low density lipoprotein cholesterol
NPD = Normal palatable diet
R-QUICKI = Revised quantitative insulin sensitivity check index
SBP = Systolic blood pressure
TAG = Triacylglycerol
TC = Total cholesterol

Introduction

The current obesity pandemic imposes a major global disease burden. The rise in obesity will be accompanied by increases in related disorders such as diabetes, hypertension and heart diseases[1]. Obesity has been traditionally challenged with diets, exercise and behavioral modification. These techniques have still so far failed to halt the obesity pandemic. Given the enormity of the obesity problem, adjunctive pharmacotherapy provides an attractive solution[2]. However, very few drugs have been developed for obesity treatment and those that are approved have only limited success[1].

When sibutramine was received approval from the American Food and Drugs Administration (FDA) in November of 1997, it represented the first unique agent of a new class of medications for the treatment of obesity. Sibutramine is a

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pharmacological agent with selective norepinephrine, serotonin and a lesser extent, dopamine, reuptake inhibitor[3]. Cardiovascular side effects include an increase in systolic and diastolic BP, and an increase in heart rate, tachycardia and palpitations. These adrenergic side effects are a particular concern for patients with hypertension. Additionally, an increase appearance of mood-related disorders has been reported during sibutramine treatment such as insomnia[4] and generalized anxiety and panic disorders[5].

However, in October of 2010, sibutramine was removed from the US market due to an evident increased risk of heart attack and stroke. After withdrawal of sibutramine, only one medication-orlistat-has been approved by the FDA for long-term use in the treatment of obesity[6]. Reported side effects of orlistat include flatus with discharge and oily stool. Severe problems such as fecal urgency, incontinence and abdominal pain can also occur[2]. The adverse effects of these agents highlighted a continued need for safe and effective medications for the treatment of obesity with a positive impact on health related quality of life.

Topiramate is a structurally novel therapeutic agent currently approved for marketing as an antiepileptic drug. Topiramate has been reported to exert multiple pharmacological effects that may determine its broad range of activities including anticonvulsant, analgesic, and mood-stabilizing properties[7]. Topiramate has been reported to cause a decrease in body weight in some epileptic patients during clinical evaluation[8]. These observations prompted studies to elucidate the mechanistic basis of topiramate induced weight loss in animals[9].

Therefore, the present study was designed for evaluation of the weight controlling properties of topiramate in dietary obese rats in comparison with sibutramine. Further, we aimed to explore the role of improving insulin resistance or regulation of adipokines in this pharmacological effect.

Materials and Methods

Animals and Experimental Design

Fifty female albino rats were housed in standard cages and maintained under controlled room temperature (25 ± 3) and normal light-dark cycle with free access to food and water. Rats had initial body weight 150-180 g. Ten rats received NPD for five months; the remaining rats received HFD for three months to establish diet-induced obesity. Table I illustrates the formula of the diet: it provides 17% energy as carbohydrates, 25% as protein, and 58% as fat as a percentage of total kcal/g[10]. Rats receiving HFD were divided equally into four groups. One group returned to normal diet and the other groups returned to normal diet and treated with sibutramine (7.5 mg/kg/day, p.o.)[11] or topiramate (25 mg/kg/day, p.o.) or topiramate (50 mg/kg/day, p.o.)[12] for additional two months. The changes in body weight were monitored every week. The animals were cared for in accordance with the principles and guidelines of the Canadian Council on care and use of experimental animals. All experimental procedures followed were in accordance with guidelines of the Institutional Animal Care and Use Committee.

Drugs

Sibutramine hydrochloride (Medical Union Pharmaceuticals, Ismailia, Egypt) was dissolved in distilled water and administered orally. Topiramate (Delta Pharm, 10th of Ramadan City, Egypt) was dissolved in 2% tween-80 solution.

Testing Anxiety in Vogel's Proconflict Situation Test

Briefly, after 24 hrs of water deprivation, each rat was placed in the cage of the anxiometer. The cage is a Plexiglas box (25×25×25 cm) equipped with a grid floor of stainless steel bars and a drinking bottle containing water (Anxiometer Model, LE 3206, Panlab s.l., Barcelona, Spain) was used for training and testing for animals where it was given an opportunity to consume

Table I. Composition of the high fat diet.

Ingredients	Amount (g/kg)e
Powdered NPD[1]	365
Lard[2]	310
Casein[3]	250
Cholesterol[4]	10
Vitamin and mineral mix[5]	60
DL-Methionine[6]	03
Yeast powder[7]	01
Sodium chloride[8]	01

[1,2,7,8]Purchased from the market; [3]Difco (Becton Dickinson, France), [4]Oxford Lab, Mumbai, India; [5,6]Sigma-Aldrich, MO, USA; [8]ADWIC Co., Cairo, Egypt.

water from a drinking bottle. A sharp decrease of the number of drinking episodes testifies to the existence of anxiety and increased fear in animal[13,14].

Measuring Systemic Arterial Blood Pressure

Rats were deeply anaesthetized with thiopental sodium (40 mg/kg, i.p.)[15] and placed on a temperature-controlled panel. A laparotomy was performed and a non-occlusive, polyvinyl catheter was implanted in the abdominal aorta, caudal to the kidneys, through a puncture wound in the aortic wall made with the tip of an L-shaped 18-gauge needle. The catheter was fixed by tying a ligature. The arterial catheter was filled with heparin solution (1.000 USP U/ml)[16] and connected to digital BP monitor (Columbus Instruments, OHIO, 43204, USA) for BP recording. Mean systolic BP (SBP) and diastolic BP (DBP) were measured for each group.

Blood Sampling and Biochemical Analysis

After measuring the BP, blood samples were collected by cardiac puncture. Serum was separated and kept at –80°C until performing the biochemical measurements. Serum insulin was determined using ultra sensitive rat insulin ELISA kit (Crystal Chem Inc., Downers Grove, IL 60515, USA)[17]. Serum leptin was measured by rat leptin ELISA kit (Crystal Chem Inc., USA)[18]. Serum adiponectin was detected by rat adiponectin ELISA kit (AdipoGen Inc. Korea) according to the manufactures' instructions[19].

Serum glucose level was estimated enzymatically according to the method of Trinder[20] using Spinreact diagnostics kits (Girona, Spain). Total cholesterol (TC), triacyl glycerol (TAG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured colorimetrically using commercial kits from (Stanbio, Boerne, TX, USA)[21]. Additionally, liver index was calculated by the formula: liver index = (liver weight/body weight) × 100, adipose tissue index = (retroperitoneal adipose tissue weight/body weight) × 100 and atherosclerosis index was calculated by the formula: atherosclerosis index = (serum TC – HDL-C) / HDL-C[22].

Finally, two indirect indices were calculated. First, homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated by the following formula: [fasting serum insulin (μU/ml)×fasting serum glucose (mM/L)]/22.5. A high HOMA index denotes low insulin sensitivity[23]. To assess insulin sensitivity, another derived index was suggested, i.e., the revised quantitative insulin sensitivity check index (R-QUICKI) = [1/log fasting insulin (μU/ml) + log fasting glucose (mg/dl)][24].

Statistical Analysis

The results are expressed as mean ± SEM. The data was analyzed using Statistical Package of Social Sciences (SPSS) program version 16, Chicago, IL, USA. One-way analysis of variance, ANOVA, followed by Bonferroni's multiple comparisons test were employed for statistical analysis. A value of $p \leq 0.05$ was considered to be statistically significant.

Results

Body Weight Gain, Liver Index and Adipose Tissue Index

In the current study, HFD group showed a significant increase in the percentage body weight gain as compared to NPD group (53 ± 6 vs. 17 ± 2 g, respectively, $p \leq 0.05$, Table II). Treatment with sibutramine (7.5 mg/kg/day, p.o.) for two months significantly reduced percentage body weight gain to 31 ± 3 g ($p \leq 0.05$). Similarly, treatment with the high dose of topiramate (50 mg/kg/day, p.o.) reduced the percentage weight gain value significantly to 36 ± 5 g as compared to HFD group ($p \leq 0.05$, Table II).

Moreover, liver and adipose tissue indices were significantly higher in HFD group as compared to NPD group ($p \leq 0.05$). Sibutramine (7.5 mg/kg/day, p.o.) and topiramate (50 mg/kg, p.o.) could reduce the high liver index value. However, only sibutramine could reduce the high adipose tissue index ($p \leq 0.05$, Table II).

Serum Lipid Profile and Atherogenic Index

Serum lipid profile was significantly increased by feeding HFD. Table II shows that a significant increase in TC, TG and LDL-C and a decrease in HDL-C were detected in HFD group as compared to NPD group. Sibutramine or topiramate could reduce the serum level of TAG as compared to HFD group. Additionally, the difference in the calculated atherogenic index between the HFD group and the treated groups was not significant ($p \leq 0.05$, Table II).

Table II. Body weight, liver index, lipid index, serum lipid profile and atherogenic index in the experimental groups.

Groups	NPD group	HFD group	Sibutramine (7.5 mg/kg)	Topiramate (25 mg/kg)	Topiramate (50 mg/kg)
Body Weight (baseline)	163.8 ± 3.5	165.7 ± 2.5	165.7 ± 3.5	165.8 ± 5	166 ± 4
Body Weight (final)	192 ± 4.4	250 ± 0.2	216 ± 0.3	238 ± 0.7	224 ± 0.2
% Δ wt (g)	17 ± 2	53 ± 6*	31 ± 3#	42 ± 6*	36 ± 5*#
Liver index	4 ± 0.1	5.8 ± 0.2*	4.7 ± 0.1*#	5.1 ± 0.3*	5 ± 0.2*#
Adipose tissue index	0.7 ± 0.06	1.7 ± 0.1*	1 ± 0.06*#	1.5 ± 0.2*a	1.5 ± 0.1*a
TC (mg/dl)	80 ± 4	122 ± 3*	112 ± 4*	120 ± 5*	119 ± 4*
TAG (mg/dl)	92 ± 4	161 ± 4*	118 ± 4*#	128 ± 5*#	126 ± 5*#
LDL-C (mg/dl)	49 ± 2	60 ± 2*	53 ± 3	61 ± 3*	60 ± 2.5*
HDL-C (mg/dl)	39 ± 2	30 ± 1*	34 ± 2	31 ± 3*	32 ± 2
Atherogenic index	1 ± 0.1	3.2 ± 0.2*	2.4 ± 0.2*	2.8 ± 0.2*	2.8 ± 0.2*

NPD: normal palatable diet, HFD: high fat diet, TC: total cholesterol, TAG: triacyl glycerol, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol. Results are expressed as mean ± SEM (n=10). *Significantly different from NPD group at $p \leq 0.05$. #Significantly different from HFD group at $p \leq 0.05$. aSignificantly different from sibutramine group at $p < 0.05$.

Fasting Blood Glucose, Serum Insulin, HOMA-IR index and R-QUICKI

Table III reveals that feeding with HFD resulted in significant hyperglycemia and hyperinsulinemia in rats as compared to rats fed with NPD ($p \leq 0.05$). Sibutramine and topiramate groups showed significant reductions in serum glucose and insulin levels as compared to HFD group ($p \leq 0.05$, Table III).

HOMA-IR index was significantly increased in HFD group by 302% times as compared with NPD group. Treatment with sibutramine or topiramate could decrease the calculate HOMA-IR index ($p \leq 0.05$, Table III). On the other hand, R-QUICKI calculated in HFD group was significantly lower than the calculated value for NPD group. The lessened insulin sensitivity was improved after treatment with sibutramine or the two dose levels of topiramate ($p \leq 0.05$, Table III).

Serum Leptin and Adiponectin

HFD group showed a significant decrease in serum leptin and adiponectin levels as compared to NPD group ($p \leq 0.05$). Treatment with sibutramine could increase serum leptin without influence on serum adiponectin level. In contrast, topiramate group showed a significant increase in serum adiponectin without influence on leptin as compared to HFD group ($p \leq 0.05$, Table III). Moreover, serum adiponectin level in the topiramate (50 mg/kg) group was significantly higher than the observed level in sibutramine group.

Table III. Fasting serum levels of glucose, insulin, HOMA-IR index, R-QUICKI, leptin and adiponectin in the experimental groups.

Groups	NPD group	HFD group	Sibutramine (7.5 mg/kg)	Topiramate (25 mg/kg)	Topiramate (50 mg/kg)
Serum glucose (mg/dl)	80.6 ± 2.9	134 ± 3*	113 ± 4.2*#	111 ± 5*#	111 ± 4*#
Insulin (ng/ml)	0.95 ± 0.04	2.3 ± 0.12*	1.6 ± 0.1*#	1.4 ± 0.1*#	1.6 ± 0.1*#
HOMA-IR index	4.7 ± 0.2	18.9 ± 0.83*	11.3 ± 0.8*#	10.8 ± 1*#	11 ± 0.8*#
R-QUICKI	$0.3 \pm 2 \times 10^{-3}$	$0.26 \pm 1 \times 10^{-3}$*	0.27 ± 2 × 10-3*#	$0.29 \pm 2 \times 10^{-3}$*#	$0.28 \pm 2 \times 10^{-3}$*#
Leptin (ng/ml)	4.2 ± 0.14	3.6 ± 0.06*	4.17 ± 0.12#	3.7 ± 0.2	3.9 ± 0.1
Adiponectin (ng/ml)	5.9 ± 0.2	3.5 ± 0.1*	3.8 ± 0.2*	4.1 ± 0.5*#	4.3 ± 0.4*#a

NPD: normal palatable diet, HFD: high fat diet, HOMA-IR index: homeostasis model assessment- insulin resistance index, R-QUICKI: revised quantitative insulin sensitivity check index. Results are expressed as mean ± SEM (n=10). *Significantly different from NPD group at $p \leq 0.05$. #Significantly different from HFD group at $p \leq 0.05$. aSignificantly different from sibutramine group at $p \leq 0.05$.

Vogel's Proconflict Situation Test

In the current study, HFD-fed rats showed the same drinking behavior in Vogel test as the NPD-fed rats. Licking is suppressed in sibutramine-treated rats in comparison to HFD-fed rats. However, treatment with topiramate (25 mg/kg) and (50 mg/kg) increased the drinking behavior and the number of shocks received by the animal as compared to HFD group ($p \leq 0.05$, Figure 1a and b).

Arterial Blood Pressure Measurement

Figure 2 illustrates that sibutramine group had a significant elevation in both SBP and DBP as compared with HFD group. Whereas, rats treated with topiramate showed normal BP values ($p \leq 0.05$).

Discussion

The results of the current study showed that over a period of three months, exposure of rats to HFD led to increased weight gain, liver index and adipose tissue index. In addition, hyperlipidemia, hyperinsulinemia and impairment in blood glucose regulation were observed as compared to NPD group. Consistent with our results, HFD has been shown to produce rapid weight gain in rodents, mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia together with reduced glucose disappearance rate[25,26].

In the current study, serum leptin level was decreased in rats fed HFD compared to those under NPD. In agreement, fat feeding has been shown to impair leptin expression and to alter hypothalamic peptide expression in a way that promotes food ingestion and fat accumulation[27,28]. The important physiologic function of leptin is to regulate in nonadipocytes the intracellular homeostasis of FAs and TAG so as to maintain a sufficient supply of FAs for essential cell functions while avoiding TAG overload[29]. Thus, prevention of obesity is not the primary function of leptin.

Leptin also reduces food intake and body weight through interactions with the central neural network, particularly in the hypothalamus[30,31]. In addition, central leptin administration suppressed daily food intake in rats[32]. Leptin resistance has been shown to exist in obese human patients, who are hyperphagic but have very high serum leptin levels. It was suggested that there is

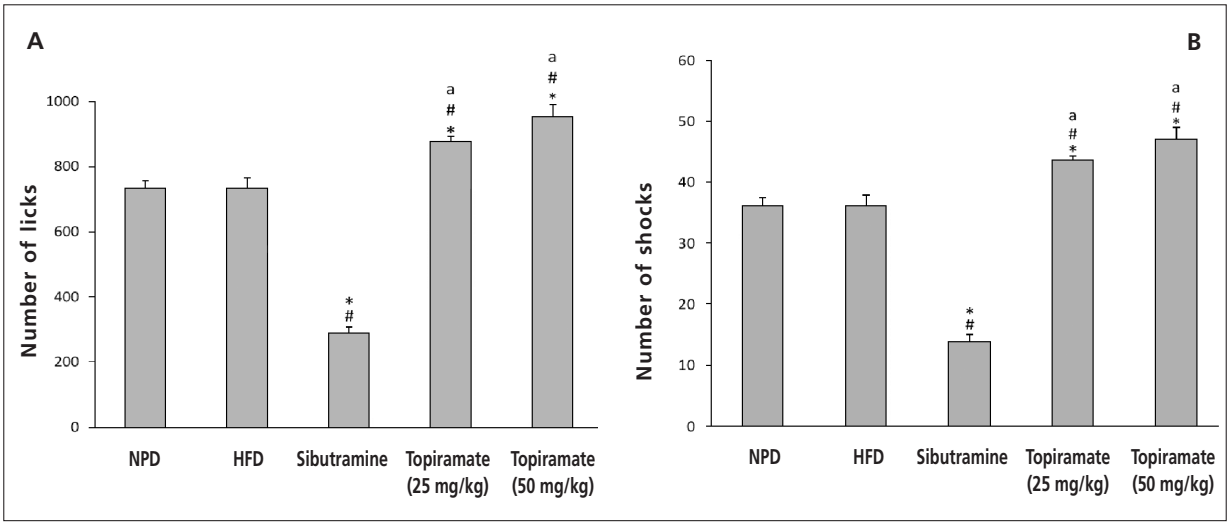

Figure 1. Number of licks and number of shocks received by rats in Vogel test. Rats were fasted overnight and placed individually in the cage of the anxiometer. 20 drinking episodes were allowed (unpunished response) before the timer started followed by a 10 min punished drinking component, during which an electric shock (0.3 mA during 2 sec) was delivered upon every 20 drinking episodes. Number of drinking episodes ***(A)*** and number of shocks ***(B)*** were decreased in sibutramine group (7.5 mg/kg) as compared to HFD group. Topiramate (25 mg/kg) and (50 mg/kg) increased the number of licks and shocks significantly as compared to HFD group or sibutramine group. NPD: normal palatable diet, HFD: high fat diet. Results are expressed as mean ± SEM (n=10). *Significantly different from NPD group at $p \leq 0.05$. #Significantly different from HFD group at $p \leq 0.05$. [a]Significantly different from sibutramine group at $p \leq 0.05$.

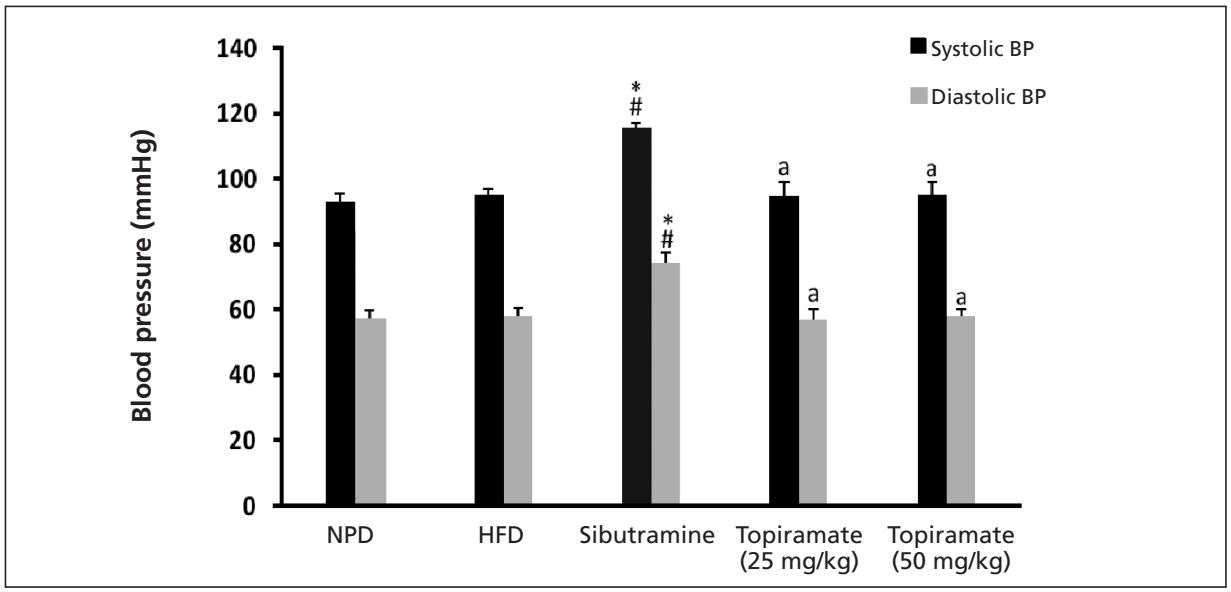

Figure 2. Systolic and diastolic BP of rats. Rats were deeply anaesthetized and BP was measured from the renal artery. Sibutramine group (7.5 mg/kg) showed a significant increase in both systolic and diastolic BP values as compared to HFD group. Topiramate group (25 mg/kg) or (50 mg/kg) showed normal BP values. NPD: normal palatable diet, HFD: high fat diet. Results are expressed as mean ± SEM (n=10). *Significantly different from NPD group at $p \leq 0.05$. #Significantly different from HFD group at $p \leq 0.05$. aSignificantly different from sibutramine group at $p \leq 0.05$.

a leptin-sensitive pathway and a leptin-insensitive (or less sensitive) pathway in the regulation of feeding[33].

A recent hypothesis argued that there is dissociation between the increase in both leptin store and adipose tissue weight, and the decrease in plasma leptin levels initially begin after six weeks after sucrose feeding in rats[34]. Similar to leptin results, the current study revealed that serum adiponectin level was decreased in rats fed HFD compared to those under NPD. In agreement, adiponectin is downregulated in two models of HFD feeding[35,36].

The current results showed that sibutramine mediated body weight loss in obese rats along with favorable effects on biochemical parameters and insulin sensitivity. In accordance, many studies reported that treatment with sibutramine suppressed food intake and weight gain in rats[1,25,37,38] and our findings are compatible with these observations. Additionally, sibutramine administration enhances sympathetic nervous system activation, which in turn reduces plasma adiponectin level[25]. This observation may further support our findings since serum adiponectin level in sibutramine group did not increase in comparison with the HFD group. Clinically, sibutramine reduces food intake and body weight more than placebo and has positive effects on the lipid profile (mainly TAG and HDL-C) and glycemic control in studies for up to one year[39].

In the current investigation, sibutramine group showed high SBP and DBP values as compared to NPD group. Many studies revealed an increase in SBP, DBP and pulse rate in obese patients after sibutramine treatment compared to placebo treatment[40-42]. The Authors highlighted that sibutramine induced more weight loss than placebo which is coupled with a significant tachycardia. These adrenergic side effects are of a particular concern for patients with hypertension. The cardiostimulatory effect represents the most common adverse effect of sibutramine. This may be done by inhibition of dopamine reuptake and of sibutramine's in vivo efficacy to increase extracellular levels of dopamine in nucleus accumbens[43]. Consistently, some Authors suggest that the use of sibutramine could raise BP and induce arrhythmias in some patients[44], may be because of paradoxical effect on the autonomic system[45,46].

Furthermore, our study revealed an apparent interplay between sibutramine medication and behavior; treatment with sibutramine increased the expression of anxiety-related behaviors in the rats submitted to Vogel's proconflict situation

test. In previous reports, sibutramine has been associated with psychosis[47], hypomania and mania[48,49], exacerbations of panic attacks, depression, and suicidal tendencies[50].

Our findings demonstrate that chronic treatment with topiramate (50 mg/kg) produced a significant weight loss in obese rats. Moreover, IR was diminished significantly as indicated by HOMA-IR and R-QUICKI indices. These results seem to be compatible with those obtained by Wilkes et al.[51]. The Authors emphasized that topiramate treatment in obese rats led to a decrease in plasma glucose and an increase in insulin sensitivity. More recently, Jing et al[12] concluded that topiramate decreased serum insulin levels in young and adult rats. Studies with obese (fa/fa) female Zucker rats demonstrated that topiramate treatment led to a reduction in blood glucose and TAG levels[52]. Clinically, some Authors concluded that topiramate can significantly reduce body weight and BP in obese people, with mild to moderate adverse effects[53].

The precise mechanism by which topiramate induces weight loss remains under investigation but appears not to involve noradrenergic or serotonergic mechanism. Topiramate has consistently decreased the efficacy of energy utilization in animal models, and effects on food consumption have varied with the model. These effects on energy efficiency may be mediated by stimulation of lipoprotein lipase in brown adipose tissue and skeletal muscle, thus increasing thermogenesis[9,52]. Topiramate also increases the expression of uncoupling proteins 2 and 3[1,54], thus directly decreasing the efficiency of energy utilization[53]. Another possible mechanism is that topiramate might directly induce weight loss in rodent by stimulation of energy expenditure as well as reduction of energy intake[55].

Additionally, animal studies have shown that stimulation of the lateral hypothalamus by glutamate and glutamate agonist, including kainite/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) agonist, causes an intense, rapid, dose dependent increase in food intake. Topiramate is an antagonist at kainite/AMPA glutamate receptors[56]. Furthermore, diverse pharmacological properties of topiramate have been identified including the following: a positive modulatory effect on the activity of GABA at GABA-A receptors, a negative modulatory effect on voltage-dependent sodium channels, and some negative effect on high voltage-activated calcium channels[55]. These pharmacological properties may explain the anxiolytic effect of topiramate that was observed in the Vogel pro-conflict test.

In conclusion, our data ensures the findings that topiramate possess weight controlling and insulin sensitizing properties with no anxiogenic or hypertensive effects. Therefore, it may be used safely in obese hypertensive patients. Further investigations are needed to confirm our findings and to determine the utility of topiramate in the clinical management of obesity.

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