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Der Pharmacia Lettre, 2018, 10 [7]: 48-64
[http://scholarsresearchlibrary.com/archive.html]

ISSN 0975-5071
USA CODEN: DPLEB4

The Effect of Stevia Supplementation on Sperm Quality, Food Intake and Weight Gain in Obese and Non-Obese Rats

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ABSTRACT

***Background:** Non-nutritive sweeteners (NNSs) are becoming more popular as sugar substitutes for diabetic patients. Stevia is a naturally-occurring NNS that has been reported to have a number of health benefits in both humans and experimental animals. We aimed to assess the impact of stevia supplementation on food intake and weight gain as well as liver tissue histopathology in obese and non-obese rats. Also, we aimed to examine the association between stevia supplementation and sperm quality in obesity.*

Methods: *In this experimental study, forty adult male albino rats which were divided into four groups (n=10 each). Both control and obese groups received oral supplementations of stevioside (25 mg/kg daily) as positive groups and the other obese and control groups received oral supplementations of sucrose 5 g/kg daily as negative group. The amount of food intake, weight of rats and biochemical tests were measured at baseline and at the end of the study (8 weeks), as well as liver tissue histopathology examination was done at end of the study.*

Results: *our study revealed that there were higher levels of total cholesterol, triglycerides, LDL cholesterol, in obese group compared to control group. However, stevia supplementation for 8 weeks to obese group had non-significant changes of these parameters compared to sucrose supplementations. Regarding semen quality, there were non-significant difference between stevioside and sucrose received obese group. Nevertheless, in obese group there were decreased in sperm count and viability compared to control group. Regarding histopathological features of the liver, after induction of obesity there was increase weight of liver as well as severe damage to liver parenchyma such as cellular swelling with degenerative changes, vacuolar changes and foci of hemorrhages compared to the normal control. However stevioside supplementations for 8 weeks led to decrease liver weight and improve its histopathological features.*

Conclusion: *stevia supplementation for 8 weeks to obese rats increased body fat %, epididymal fat pad mass, food intake (g/rat/day) and weight of rats (gm) as well as decreased liver weight and fluid intake. However, stevia supplementation had non-significant changes of rat sperm quality compared to sucrose received obese group, on the other hand, histopathological features of the liver significantly improved after stevia supplementation.*

Keywords: Stevia; Food intake; Obese; Liver; Semen; Weight; Histopathological.

INTRODUCTION

Obesity is a worldwide public health crisis associated with several comorbidities including hypertension, dyslipidemia, type 2 diabetes mellitus (T2DM), coronary heart disease, stroke, osteoarthritis, sleep apnea and respiratory problems, as well as some types of cancers [1]. The prevalence of obesity in Egypt has increased at an alarming rate during the last three decades affecting 22% of adult males and 48% of adult females [2]. As a matter of fact, the consumption of a high sugar/high fat diet is one of the contributing factors attributed to the increase in obesity [3]. Increasing evidence points to critical roles of consumption of nonnutritive sweeteners (NNS) as an alternative sugar intake [4]. There are eight FDA approved NNS; sucralose, aspartame,

saccharin, acesulfame-K, neotame, and advantame. Even more importantly, naturally derived NNS, steviol glycosides and Luo han guo extract, are generally recognized as safe and endorsed for use in food by the US Food Drug Administration (FDA) and the European Food Safety Authority (EFSA) [5,6].

Stevia, the common name for the naturally derived NNS, steviol glycosides which extract from the leaves of *Stevia rebaudiana Bertoni*,. Stevia is a natural, sweet-tasting calorie free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners. There was scattered evidence that stevia improve glycemic control in diabetic patients and increase insulin levels [7] which suggested it may have a role in food intake regulation. In experimental studies, Stevia has been found to increase insulin sensitivity [8]. Regarding safety of stevia consumption, evidence suggested that there were no negative side effects reported. Furthermore, stevia is inexpensive and available to most consumers; thus, it has the potential to be widely used and may assist individuals in regulating their weight if it has a positive effect on caloric substitution [9]. Obese men with type 2 diabetes may have secondary hypogonadism due to central and peripheral insulin resistance and the negative effect of pro-inflammatory cytokines (TNFa and IL-6) on the HPG axis [10].

The pandemic of obesity represents a major public health concern, as this disorder is associated with an increased risk of medical comorbidities contributing to a significant rise in mortality. Despite a wide-range of research being conducted, till now the treatment of obesity is still suboptimal. To our knowledge, no study to date in our region especially our country; Egypt evaluated stevia supplementation on health and disease. Thus the aim of our study was to assess the impact of stevia supplementation on food intake and weight gain as well as liver tissue histopathology in obese and non-obese rats. Also, we aimed to examine the association between stevia supplementation and sperm quality in obesity.

MATERIALS AND METHODS

Animals and experimental design

Experiment 1

In this experimental study, fourty adult male albino rats weighing (190-210 g) were purchased from Veterinary medicine faculty, Zagazig University that used throughout this study. They were housed in stainless steel rodent cages at the animal house, Faculty of Medicine, Zagazig University and allowed one week as adaptation period at room temperature with a 12 hours dark / light cycle before beginning the experimental work. They were kept under environmentally controlled conditions and were fed with standard rodent chow (SCD) (El-Nasr pharmaceuticals and Chemicals Industry, Egypt) and allowed free access of tap water *ad libitum*.

Experiment 2

After that rats divided into two main groups, the first one (20 rats) was control group and the second one (20 rats) was rendered obese by feeding a high fat diet. Water and feed were available all the time during the experimental period. The diet consisted of wheatflour (16.07),meat meal (20),animal fat (57.93),wheat bran (3.0),lysine (0.1),methionine (0.4), Di-calcium phosphate (1.0), sodium chloride (0.5) Vit. & Min. Mix (1.0). Moreover, the calculated nutrient composition; crud protein (12.67),energy (ME / KG) (6239.23),ether extract (60.02).,crude fiber (3.75), ash (5.77),lysine (0.81), methionine (0.58), calcium (2.06), phosphorus (1.12)

Experiment 3

After 1 week of acclimatization, rats were divided into 4 groups (n=10 each). The first group (sucrose received control group) received 5 g/kg sucrose daily and orally the second group (stevioside received control group) received stevioside in a dose level 25 mg/kg daily and orally. The third group (sucrose received obese group) received 5 g/kg sucrose daily and orally the fourth group (stevioside received obese group) received stevioside in a dose level 25 mg/kg daily and orally. Amount of food intake and weight of rats were recorded every week along the period of the experiment (8 weeks). All the experiments and animal handling were approved by the ethical committee of the Faculty of medicine, Zagazig University. Stevioside (SVS) (200 times as sucrose) was obtained from Agricultural Research Center,Giza..

Blood samples

Blood samples were collected from the retro-orbital venous plexus under light ether anesthesia after fasting 12 hours to estimate for serum lipid profiles (serum TC, TG, HDL-c, and LDL-c).

Epididymal sperm preparation

After 35 days (more than one duration of spermatogenesis in rat), a small piece of the cauda epididymis of each animal was dissected and placed in 1 mL of pre-warmed Ham's F10 medium (37°C, 5% CO_2). The tissue was cut quietly to make spermatozoa swim-out into the culture medium and was located in the incubator for 15 min.

Sperm analysis

For 200 spermatozoa of each animal, sperm parameters including count (10^6/mL), motility (%), viability and normal morphology (%) were evaluated. We used Makler chamber (Sefi Medical Co., Haifa, Israel) to access sperm count and motility [11]. Motility was expressed as percentage of progressive (rapid and slow) and non-progressive spermatozoa. Eosin test and Papanicolaou staining for evaluating sperm viability and morphology were used [12].

Tissue sampling

At the end, rats were killed by decapitation; liver was collected from rats and fixed in 10% buffered formalin solution for histological examination.

Assessments of lean mass and fat mass

At the end of 8 weeks study, the lean and fat mass were determined by dual energy x-ray absorptiometry (DEXA) with small animal software (Lunar Prodigy, General Electric). Standard values for animals were used for compliance with the small animal software. Standard DEXA values included height of 10 inches, and weight of 0.3 lbs. Removal of the epididymis fat pads was conducted following the DEXA scan, and was immediately weighted.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed using "t" test. We considered P to be significant at <0.05 with a 95% confidence interval (CI).

RESULTS

Clinical and laboratory characteristics of studied groups at base line

In obese group, we found significant higher levels of total cholesterol, triglycerides and LDL cholesterol compared to control group. However, HDL levels were significantly lower in case group compared to control group. (p >0.05) (Table 1).

Table 1: Clinical and laboratory characteristics of studied groups at base line.

Variables	**Control group (mean ± SD) (n=20)**	**Obese group (mean ± SD) (n=20)**	**P**
Rat weight (g)	196.01 ± 5.16	287.01 ± 61.54	<0.001*
Total cholesterol (mg/dL)	184.4 ± 3.37	212.3 ± 30.4	<0.001*
Triglycerides (mg/dL)	127.36 ± 12.28	168.09 ± 26.3	<0.001*
LDL cholesterol (mg/dL)	85.92 ± 0.168	123.68 ± 24.2	<0.001*
HDL cholesterol (mg/dL)	55.6 ± 2.95	35.09 ± 20.76	<0.001*
Note: *Significant difference from control group			

The impact of stevioside supplementations on clinical and laboratory characteristics of control group

We tested the role of stevioside supplementations as an alternative to sugar sweetener in control group, our results revealed non-significant difference regarding; LDL, HDL, total cholesterol and triglycerides, after 8 weeks of stevioside supplementations compared to sucrose received control group (Table 2).

Table 2: Impact of stevia consumption on lipid profile of control groups

Variables	**Sucrose received control group (mean ± SD), (n=10)**	**Stevioside received control group (mean ± SD), (n=10)**	**P**
Total cholesterol (mg/dL)	185.4 ± 4.37	183.6 ± 3.86	NS
Triglycerides (mg/dL)	125.36 ± 14.28	126.48 ± 33.29	NS
LDL cholesterol (mg/dL)	84.92 ± 14.168	86.38 ± 23.09	NS
HDL cholesterol (mg/dL)	54.6 ± 3.15	54.9 ± 3.381	NS
Note: NS; non-significant, P >0.05			

The impact of stevioside supplementations on clinical and laboratory characteristics of obese group

At the end of the study (8 weeks), our results revealed significantly decreased of total cholesterol,LDL and triglycerides in stevioside received obese group compared to sucrose received obese group (Table 3).

Table 3: Impact of stevia consumption on lipid profile of obese groups

Variables	Sucrose received obese group (mean ± SD), (n=10)	Stevioside received obese group (mean ± SD), (n=10)	p-value
Total cholesterol (mg/dL)	199.95 ± 53.91	185.8 ± 26.97	<0.001*
Triglycerides (mg/dL)	138.02 ± 28.36	133.4 ± 33.29	<0.001*
LDL cholesterol (mg/dL)	123.6 ± 24.20	111.09 ± 60.90	<0.001*
HDL cholesterol (mg/dL)	40.01 ± 13.02	48.9 ± 12.973	<0.001*
Note: * Significant difference from Sucrose treated diabetic group.			

The impact of stevioside supplementations on sperm quality of control groups are shown in Table 4.

Table 4: Effect of stevia on sperm parameters of control groups

Variables	Sucrose received control group (mean ± SD), (n=10)	Stevioside received control group (mean ± SD), (n=10)	P
Count ($\times 10^6$)	21.4 ± 45.12	20.2 ± 5.32	NS
Rapid motility (%)	22.5 ± 5.53	23.4 ± 5.34	NS
Slow motility (%)	23.3 ± 6.40	23.6 ± 3.23	NS
Immotile sperm (%)	33.5 ± 5.56	34.7 ± 4.37	NS
Normal morphology (%)	74 ± 5.66	66 ± 5.44	NS
Viability (%)	70 ± 5.36	68 ± 3.23	NS
Note: NS; non-significant, P >0.05			

In control group, our results revealed there was non-significant difference regarding sperm parameters; count, viability, morphology and motility between stevioside and sucrose received obese group. The impact of stevioside supplementations on sperm quality of obese groups are shown in Table 5.

Table 5: Effect of stevia on sperm parameters of obese groups.

Variables	Sucrose received obese group (mean ± SD), (n=10)	Stevioside received obese group (mean ± SD), (n=10)	p-value
Count ($\times 10^6$)	18.4 ± 5.12	17.2 ± 5.32	NS
Rapid motility (%)	21.5 ± 4.53	22.4 ± 5.34	NS
Slow motility (%)	25.3 ± 6.40	24.6 ± 3.23	NS
Immotile sperm (%)	34.5 ± 5.36	37.7 ± 4.37	NS
Normal morphology (%)	71 ± 5.36	69 ± 3.23	NS
Viability (%)	68.4 ± 5.12	67.2 ± 5.32	NS
Note: NS; non-significant, P >0.05			

Our results found that, there were non-significant difference between stevioside and sucrose received obese group regarding sperm parameters; count, viability, morphology and motility.

Comparison between different groups of the study regarding; fluid intake, food intake and weight of rats

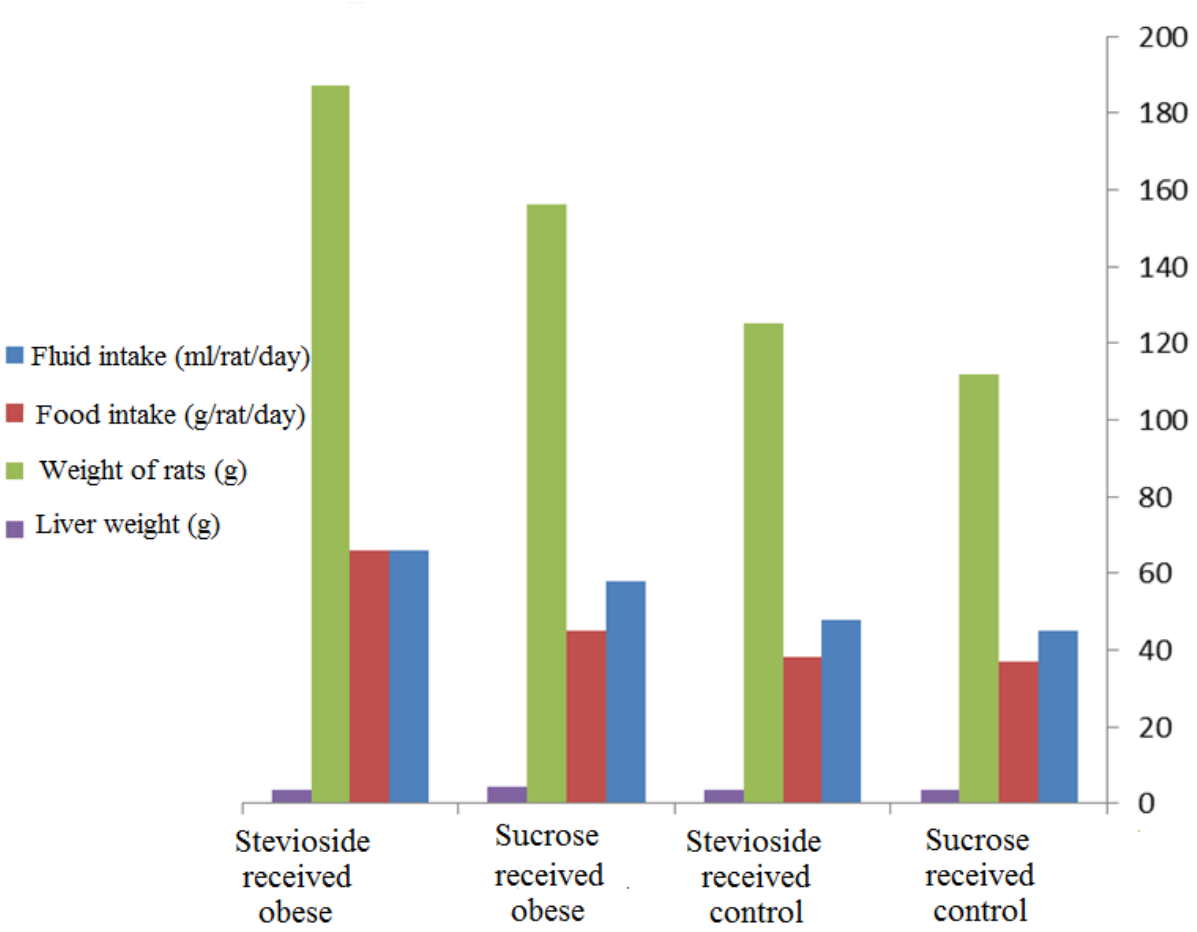

Figure 1: Comparison between different groups of the study regarding; fluid intake, food intake and weight of rats.

In control groups, stevioside supplementations increased food intake and weight of rats. Also, in obese groups, our study revealed that stevioside supplementations increased food intake (g/rat/day), weight of rats and. However, stevioside supplementations for 8 weeks led to decrease fluid intake and liver weight as shown in Figure 1.

The impact of stevioside supplementations on body composition in studied groups

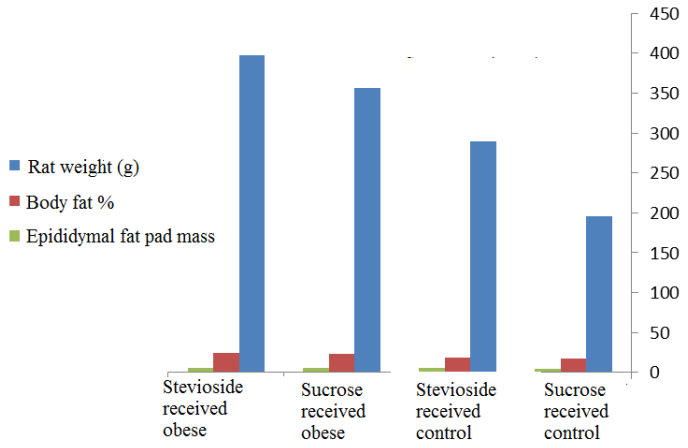

Figure 2: The impact of stevioside supplementations on body composition in studied groups.

Our study shown that, Epididymal fat pad mass and body fat % were significantly higher in obese groups compared to control groups. Conversely, in obese group stevioside supplementations for 8 weeks led to increase body fat % and epididymal fat pad mass compared to sucrose received obese group and decrease liver weight as shown in Figure 2. The impact of stevioside supplementations on histopathological examination of liver in studied groups (Figures 3a, 3b, 3c and 3d).

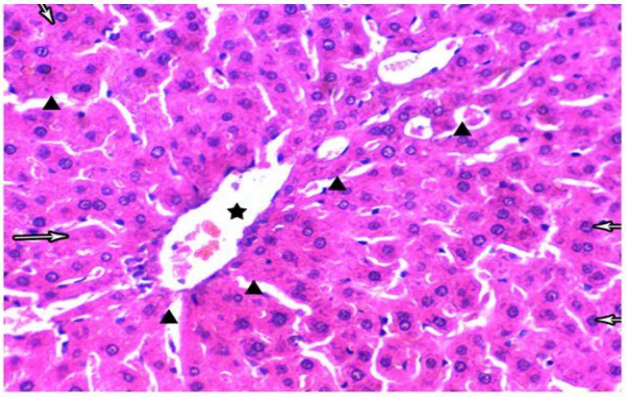

Figure 3a: Photomicrograph of normal liver tissue of a rat received sucrose showing normal seized central vein surrounded by rows and cods of normal hepatocytes with central nuclei and abundant eosinophilic cytoplasm. The hepatocytes are separated by blood sinusoids (Hematoxylin & Eosin 400x)

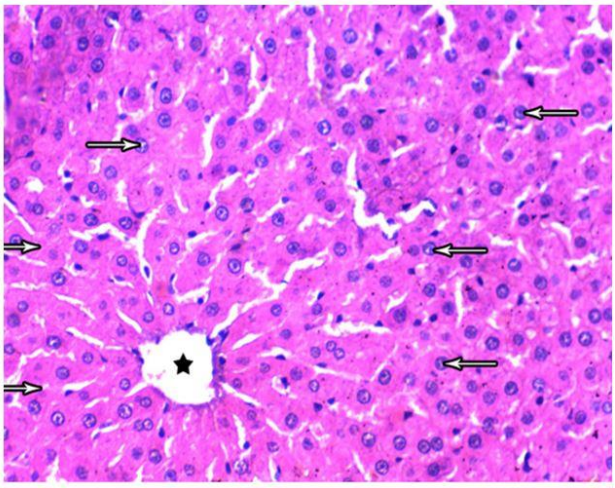

Figure 3b: Photomicrograph of normal liver tissue of a rat received stevioside showing normal side central vein. Surrounded by rows and cods of normal hepatocytes (H&E 400x).

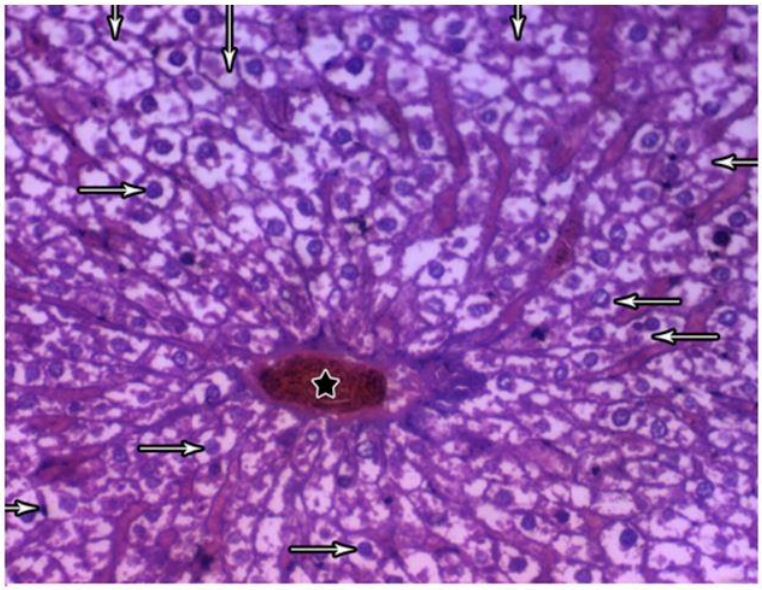

Figure 3c: Photomicrograph of liver tissue of obese rat received sucrose showing central vein surrounded by swollen hepatocytes showing marked fatty change with central nuclei and clear cytoplasm (H&E 8400x).

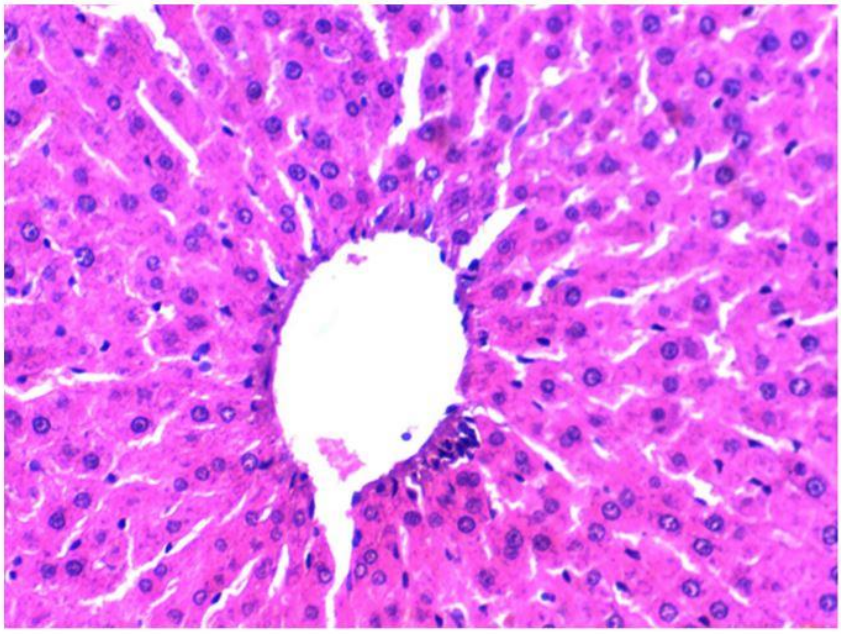

Figure 3d: Photomicrograph of liver tissue of obese rat received stevioside showing return of the liver tissue to its normal state (H&E 400x).

Regarding histopathological features of the liver, after induction of obesity there was increase weight of liver as well as severe damage to liver parenchyma such as cellular swelling with degenerative changes, vacuolar changes and foci of hemorrhages compared to the normal control in. However, stevioside supplementations for 8 weeks led to decrease liver weight and improve histopathological features of the liver.

DISCUSSION

Obesity is a highly prevalent non-communicable disease worldwide. It is a prevalent medical condition characterized by abnormal or excessive fat accumulation leading to negative impact on health, reduction of life expectancy, and/or increased risk for health problems [15]. The 'energy imbalance' is the main cause of obesity. It occurs when energy input exceeds expenditure. The global increase in the prevalence of obesity is contribute to multiple factors including a shift in diet toward high-calorie junk food containing excess fat and sugars with low fiber content and a trend toward decreased physical activity and a sedentary lifestyle. However, several factors may attribute to obesity such as smoking, sleep, drugs, and environmental factors [16].

Experimental and humans studies proposed that interaction between dietary habits, environmental factors, genetic predisposition, and gut microbiota (intestinal commensalism that regulates the rate of digestion, absorption, and metabolism of nutrients) may also contribute to obesity [17]. Obesity is associated with reduced levels of sex hormone binding globulin and testosterone with a concomitant rise in the level of estrogen that leads to alteration of spermatogenesis [18].

Few controlled studies have been conducted on obese patients to evaluate the effect of weight reduction strategies on male reproductive capacity. Despite this lack of definitive evidence, there is a consensus among these studies that men who lost weight through diet control and exercise experienced a high increase in androgen and inhibin B levels as well as an improvement in semen parameters [19].

It is also well-established that the consumption of foods and beverages containing nonnutritive sweeteners has dramatically increased over the past few decades [20,21]. Stevia is a naturally sourced, zero-calorie sweetener that has been used as a natural sugar substitute and flavoring ingredient for hundreds of years [22].

Modern societies have witnessed an alarming increase in cases of obesity, T2D, and other comorbidities associated with poor eating habits. In addition, the incorporation of processed and industrialized food into everyday eating correlates with this phenomenon. In an attempt to attack that threat, much care has been paid to the amount of calories present in food. Specifically, the amount of fat and simple sugar is considered the main villains of modern processed food [23]. We in this study attempted to pierce out the impact of stevia supplementation on food intake and weight gain as well as liver tissue histopathology in obese and non-obese rats'. Also; we aimed to examine the association between stevia supplementation and sperm quality in obesity. At the end of the intervention, the current study revealed that, there was increase in food intake and body weight of rats, weight gain after sativoside supplementations in obese groups. However stevioside supplementations for 8 weeks in control group increased food intake and body weight of rats compared to sucrose supplementations

There is no clear evidence that NNS augment appetite by activating cephalic phase responses, altering osmotic balance, or enhancing food palatability. Indeed, there is emerging evidence that selected NNS may stimulate the release of satiety hormones, although the link between these hormones and energy intake in free-living individuals is also open to debate. With respect to energy intake, there is no substantive evidence that inherent liking for sweetness or NNS activation of reward systems is problematic.

Porikos et al. found that during an 8-week period, the sucrose rats gained considerable weight while the NNS rats showed the same weight gain as controls. When the sweetened solutions were switched, obese sucrose rats lost weight during the next 8 weeks while rats previously on NNS gained weight rapidly. The results show that substitution of artificial sweeteners for sugars prevents weight gain and promotes weight loss in rats [24].

Similar results obtained by previous animal studies, researchers suggested that intake of NNSs may promote weight gain, either by increasing energy intake [25], or by decreasing energy expenditure [26-29]. Previous researches reported that pre-meal consumption of high calories leads to reduce food intake, a process known as caloric compensation [30]. Thus, it is possible that the controls fed high calorie sugars were subjected to caloric compensation and consumed less food and this led to reduced weight gain when compared to treated groups Similarly, Abo Elnaga et al. observed administration of stevia sweetener at doses of 25 mg/kg b. w decreased feed intake as compared to control group [31]. In agreement with our results, Gregersen et al. detected that administration of stevia sweetener decreased body weight gain during growth and maturation in male rats [32]. Results reported by Curry and Roberts found decreased rat weight after supplementation of stevia, they explained these changes in body weight of rats could be due to the absence of quick glucose releasing source or decrease the caloric intake by rats [33].

Previous researchers found that body weight of rats receiving 5.0 mg/kg stevioside was reduced significantly in comparison with the group receiving the diet without stevioside. This was probably due to the poor palatability of the food because of the high amount of stevioside. However, the non-caloric sweetener group had a decrease in body mass index compared to an increase in body mass index in the sucrose group [33]. The main finding of the present study is that, stevia supplementation for 8 weeks to obese group led to significantly decrease of total cholesterol, compared to sucrose supplementations. Cons similarly, reports of Abo Elnaga et al. they found that groups of rats treated with stevia sweetener showed improvement in lipid profile levels comparing with negative or positive control group [31].

Till now no study confirmed the metabolic effects of NNS on energy balance independently of their influence on macronutrient and energy intakes. With respect to the former, if it is assumed that substitution of NNS for NS only results in decreased carbohydrate intake, the fat and protein to carbohydrate ratios of the diet would increase. Although weight loss is achievable with energy-restricted diets of varying macronutrient composition [34], recent evidence supports the efficacy of an unrestricted diet with elevated fat and protein to carbohydrate ratios [35,36]. Excess body fat is associated with many endocrinal disturbances that lead to altered spermatogenesis. The most common mechanism is increased peripheral aromatization of testosterone into estrogen by aromatase enzyme with subsequent inhibition of the hypothalamo-hypophyseal-gonadal (HPG) axis [37].

There are several studies that have investigated the impact of male obesity on the traditional sperm parameters mandated by the World Health Organization (WHO), namely sperm concentration, sperm motility and sperm morphology [38,39]. The effects of obesity on male reproduction are less well documented than in the female. However, several studies indicate that sperm quality and fertility are reduced in overweight and obese men. Male obesity is suspected to cause alterations in semen parameters, especially sperm concentration [40] total sperm count, total motile sperm count, [40] total progressively motile sperm count, [40] sperm morphology, and DNA fragmentation**.**

According to our results, there were non-significant difference regarding sperm parameters; count, viability, morphology and motility between stevioside and sucrose received obese group. Our finding adds to the growing body of evidence implicating that increase weight of liver as well as severe damage and fat accumulation in hepatocytes in comparison to the normal control. However stevioside supplementations for 8 weeks led to decrease liver weight and improve histopathological features of the liver. Similar results obtained by Abo Elnaga et al. they found that groups of rats treated with stevia sweetener showed at doses of 25 mg/kg, 250 mg/kg and 500 mg/kg b. wt/day for 12 weeks had insignificant decreased in liver weight and improvement in hepatic tissues histopathological features [31].

CONCLUSION

Obesity is a widely spreading pandemic worldwide that has a negative impact on multiple organs, liver and male fertility. Stevia supplementation for 8 weeks to obese rat had non- significant changes on rat weight, food intake and sperm quality compared to sucrose received obese group, on the other hand, the weight and histopathological features of the liver significantly improved after stevia supplementation.

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