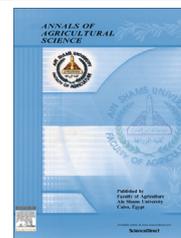




Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

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Effect of stevia sweetener consumption as non-caloric sweetening on body weight gain and biochemical's parameters in overweight female rats



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Received 16 November 2015; accepted 30 November 2015

Available online 9 February 2016

KEYWORDS

Sugar substitutes;
Healthful food;
Stevia sweetener;
Serum biochemical
parameters

Abstract Recently, non nutritive sweeteners that can substitute for sucrose (high in calorie) cause increased the prevalence of overweight children and adults. Non-caloric or low caloric sweeteners as tools for making healthful food choices have been introduced to satisfy consumer demand. The aim of this study was to evaluate the effect of stevia sweetener as a substitute sucrose at different doses (25, 250, 500 and 1000 mg/kg b. wt/day) for twelve weeks on the weight management and on several hematological and biochemical parameters of female rats. The results showed significant improvement and ameliorated reduction in final bodyweight, body weights gain (%) (BWG) and feed efficiency ratio (FER) in the stevia sweetener groups compared with the control. Stevia sweetener at 500 mg/kg b. wt/day helps in weight loss of rats, decrement in the total cholesterol, triglycerides and low-density lipoprotein concentration, increment in the high-density lipoprotein and no significant differences in mean serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), or acid phosphatase (ACP) levels as compared to the control rats, which may be considered as therapeutic beneficial. Also, this dose may be considered as a safe dose for people with diabetes, especially the many individuals who need to lose weight to help control their blood glucose levels.

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Introduction

Overweight and obesity have been a major health concern in the worldwide and as a risk factor of heart disease, diabetes, several types of cancer, hypertension, arthritis, and other musculoskeletal problems (NCHS; 2003). The World Health Organization estimated the adult prevalence of overweight (BMI 25–29.9) at 1.5 billion globally in 2008, of which obese adults (BMI ≥ 30) numbered 500 million; by 2015, these figures are projected to

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Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

rise to 2.3 billion overweight and 700 million obese adults (WHO, 2011). Although diet (van Dam et al., 2002), physical activity (Grilo, 1994 and Tanasescu et al., 2000), and genetic factors (Yamada et al., 2006 and Scott et al., 2008) contribute to obesity in children and adults, dietary behaviors such as a dietary intake pattern (Regev-Tobias et al., 2012), including sugar consumption, have been proved to have a potential impact on this rapid weight gain (Bray et al., 2004).

In addition, new health-related concerns associated with the consumption of sugar substitutes especially low-calorie sweeteners may not stimulate appetite, thereby not increasing calorie intake and not promoting weight gain. Increasing consumer demands for healthy, natural food products has spurred the food industry's interests in non- or low-caloric sweeteners of natural rather than synthetic origin for example, stevia-derived sweeteners (Kim and Kinghorn, 2002). Stevia sweeteners are a natural, functional sweetener and medical supplementary material that have received increasing industry and scientific attention in recent years. Stevia sweeteners (steviosides) are diterpene glycosides obtained from the leaves of *Stevia rebaudiana* Bertoni (family Asteraceae) commonly called as 'sweet herb' and these glycosides are also known as 'sweeteners of the future' (Brahmachari et al., 2011; Lemus-Mondaca et al., 2012). Steviosides, a natural non-caloric sweetener are 100–300 times sweeter than sucrose and contain a complex mixture of sweet diterpene glycosides, including stevioside, steviolbioside, rebaudiosides (A, B, C, D, E) and dulcoside A but the major sweet constituents are stevioside and rebaudioside A. Due to the sweetening property, steviosides have been widely used as a non-caloric sugar substitute in many kinds of foods, beverage, medicine, wine making, cosmetics, household chemical industry and other food industries (Massoud et al., 2005a,b; Wolwer-Rieck et al., 2010; Stoyanova et al., 2011). It is used for the treatment of various conditions such as cancer (Takasaki et al., 2009), diabetes (Lailerd et al., 2004), obesity, cavities, hypertension (Dyrskog et al., 2005), fatigue, depression, and in cosmetic and dental preparations. It possesses hypoglycemic, hypotensive, vasodilating, taste improving, sweetening, antifungal, antiviral, anti-inflammatory, antibacterial (Ghosh et al., 2008) properties and increases urination function of the body. However no significant toxicity has been reported with either stevioside or stevia extract. The Joint Food and Agriculture Organization/World Organization Expert Committee on Food Additives (JECFA) in 2008, established an "acceptable daily intake (ADI) of steviol up to 4 mg/kg body weight (b. wt), which is equivalent to 10 mg/kg b. wt stevioside.

Replacing sugar with low-calorie sweeteners is a common strategy for facilitating weight control. By providing sweet taste without calories, intense sweeteners help lower energy density of beverages and some foods.

The aim of this study was to investigate the effects and evaluate the best clinical amount consumption of stevia sweeteners as a substitute for sucrose on weight gain or the weight loss and weight management of female rats using different doses (25, 250, 500 and 1000 mg/kg b. wt/day).

Materials and methods

Chemicals

The pure stevia sweetener was purchased from AWA for food additives Co. Alexandria, Egypt. Sucrose (S) was supplied by

EL-Gomhouria Co. Alexandria, Egypt. Commercial kits were purchased from Bio-Diagnostic Co. Cairo, Egypt, except Lactate dehydrogenase (LDH) was purchased from Bio-systems Co. Alexandria, Egypt. All other chemicals used in this experiment were of analytical grade.

Experimental design

Sixty adult female Wistar strain rats (average weight 203 ± 6 g) were used in the present experiment. Animals were obtained from faculty of Medicine, Alexandria University, Egypt. The local committee approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of 6 and given distilled water and a standard diet that meets their requirements for growing ad libitum. The diet consisted of 44% soybean cake; 12% berseem clover hay, 13.5% fat, 9.8% yellow maize, 13.2% starch, 5% minerals; 2% lime stone and 0.5% vitamins mixture. After two weeks of acclimatization, animals were divided into six equal groups. The first group was drank distilled water (Negative control), and positive control was given a dose of sucrose dissolved in drinking water at 500 mg/kg/day. This dose of sucrose used in this experiment was predicted to dose of stevia sweeteners equivalent concentration estimated by JECFA as control. On the other hand, groups 3, 4, 5 and 6 were given a different doses of stevia sweeteners which were dissolved in drinking water at a dose level of 25 mg/kg/day according to JECFA (G1), 250 mg/kg/day (G2), 500 mg/kg/day (G3) and 1000 mg/kg/day (G4), respectively.

Measured parameters

Fluid, food intake, Body weight, Feed efficiency ratio and relative weight of organs

Fluid intake was recorded daily, and the intake of the substance being tested (mg/kg/day) was calculated from the mean amount of fluid consumed (ml/kg/day) and the concentration of the tested substance in the solution. Solution concentrations were adjusted weekly based on the average weight of the animals and their current fluid consumption. At the end of the experimental period (12 weeks), body weights of animals were recorded and calculated of body weights gain (%) and feed efficiency ratio (FER) according to the method of Chapman et al. (1959).

Animals were sacrificed by exposure to an atmosphere of 100% diethyl ether and killed by decapitation. The brain, liver, kidney and lung, heart, pancreas and spleen were immediately removed and weighed then the organs weight ratio was calculated. The relative weight of organs (%) was calculated as g/100 g body weight.

Blood hematological parameters

Rats of each group were euthanized at the end of treatment period. Trunk blood samples were collected from the sacrificed animals and blood samples were collected from vein plexus in dry clean tubes with heparin (anti-coagulant). The non-coagulated blood was used to determine red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean platelet volume (MPV) and platelet count (PLT).

Blood biochemical parameters and enzyme activities

Plasma samples were obtained by centrifugation at 860×g for 20 min and the clear serum obtained was stored at -80 °C till measurements; serum was assayed for total lipids (TL) by the method of Zollner and Kirsch (1962) and for cholesterol, by the method of Allain et al. (1974). Triglycerides were determined by the methods of Fossati and Prenape (1982). High density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined according to the methods of Lopes-Virella et al. (1977), and Wieland and Seidel (1983), respectively. Calculation of LDL/HDL ratio was done. Also, the concentrations of glucose were determined with enzymatic GOD/POD kits from Bio-Diagnostic Co according to (Trinder, 1969) whereas urea and creatinine levels were determined by the methods of Fawcett and Soctt (1960) and Larsen (1972), respectively. Serum total bilirubin was measured using the method of Young (2001). Protein estimation was carried out according to the method of Gornal et al. (1949). Albumin concentration in the plasma was determined by the method of Doumas et al. (1971). The serum globulin fraction was calculated by subtracting the value of the albumin fraction from the total serum protein Doumas et al. (1971). Alanine transaminase (ALT; EC 2.6.1.2) and aspartate transaminase (AST; EC 2.6.1.1) activities in the serum were determined according to the protocol by Reitman and Frankel (1957). Colorimetric determination of alkaline phosphatase activity was measured according to the method of Belfield and Goldberg (1971). Serum acid phosphatase (ACP; EC 3.1.3.2) activity was measured as following the method of Kind and King (1954). The activity of plasma lactate dehydrogenase (LDH; EC 1.1.1.27) was determined by the method of Scientific Committee (1989). Thiobarbituric acid reactive substances (TBARS) were measured in plasma according to the method of Tappel and Zalkin (1959).

Tissue enzyme activities and thiobarbituric acid reactive substances

Liver was immediately removed at the end of the experiment weight and washed using chilled saline solution. Tissue were minced and homogenized (10% w/v) in ice-cold sucrose buffer (2.25 mol) in homogenizer is Wise Tis HG-15D. The homogenate was centrifuged at 10 000×g for 30 min at 4 °C. The resultant supernatant of the organ was used for AST, ALT, ALP, ACP, LDH and TBARS determination according to previous methods.

Histological examination

Livers were obtained from rats, and immediately fixed in 10% formalin, and then treated with conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4–6 m thickness. The sections were stained with Hematoxylin and Eosin (H&E) stain for studying the histopathological changes.

The statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA). *P* values <0.05 were considered significant.

Results and discussion

First of all there were no deaths and no signs of adverse reactions to the treatment

Effect of administration of stevia sweetener on and sucrose (S) on the weight gain, food consumption and feed efficiency ratio (FER) in rats

Administration of stevia sweetener decreased feed intake as compared to control group. Stevia sweetener at doses of 25 mg/kg b. wt showed the largest amounts of feed intake (13.85 g/day), followed by 250 mg/kg b. wt (12.86 g/day), then 500 mg/kg b. wt (8.51 g/day) and finally the lowest amounts of feed intake were found to be 1000 mg/kg b. wt stevia sweetener (7.86 g/day). The average body weight gain was slightly increased in all experimental groups during the first two weeks. During the second weeks of the study, this average was reduced in groups given stevia sweetener compared to control groups (Fig. 1). Results revealed that the body weight gain BWG% of rats decreased by -40.29%, -41.38%, -44.98% and -48.29% when rats were given stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt respectively after 12 weeks. The decrease in body weight of rats might be due to the absence of quick glucose releasing source or decrease the caloric intake by rats.

Sucrose treated group (positive control) significantly (*P* < 0.05) increased body weight and feed intake by 4.72% and 14.22% respectively compared to negative control group. Oral administration of stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt significantly (*P* < 0.05) decreased the BWG % when compared to positive control group, by 68.17%, 69.26%, 72.86% and 76.17% respectively, as shown in Table 1. Sucrose-sweetened food and beverages resulted in a 1.6 kg gain in overweight individuals (Raben et al., 2002). These results support previous studies in which significant reductions in body weight gain during growth and maturation were observed in male rats receiving stevioside (Gregersen et al., 2004) or 25 000 ppm rebaudioside A (Curry and Roberts, 2008) or 150 022 mg/kg/day (Awney et al., 2010).

This may be due to increased lipolysis or the absence of quick glucose releasing source. Moreover, Chang et al. (2005) 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 (Raben et al., 2002 and Wiebe et al., 2011).

Feed efficiency ratio (FER) revealed that stevia sweetener at dose 1000 mg/kg b. wt showed the lowest FER value (-6.14), whereas rats given 500 mg/kg b. wt showed - 5.21 FER, those given 250 mg/kg b. wt showed - 3.22 FER and those given 25 mg/kg b. wt showed - 2.91 FER. These results supported the previous finding that the decrease in BWG was related to stevia sweetener which might cause in loss of appetite of rats Feed intake and FER value were significantly (*P* < 0.05) increased in positive control group compared to negative control group by 14.22%. These results supported the previous finding that the increase in BWG was related to the daily feed intake occurred in the animals (Fig. 2).

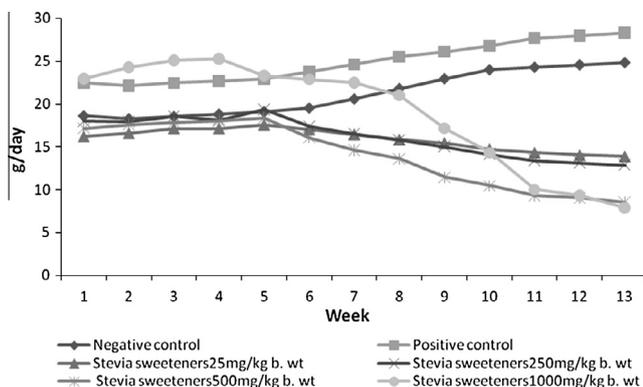


Fig. 1 Body weight of rats treated with administration of sucrose (S) and stevia sweetener compared with control.

Table 1 Means of initial body weight, final body weight and body weight gain % in control female rats and rats treated with different levels of stevia sweetener after 12 weeks.

| Experimental groups | Body weight (gm) | | Body weight gain (%) |
|-------------------------------------|---------------------|-------------------|----------------------|
| | Initial body weight | Final body weight | |
| Negative control | 203 ^a | 254 ^b | 25.12 ^a |
| Positive control | 208 ^a | 266 ^a | 27.88 ^a |
| Stevia sweetener (25 mg/kg b. wt) | 206 ^a | 123 ^c | -40.29 ^b |
| Stevia sweetener (250 mg/kg b. wt) | 203 ^a | 119 ^c | -41.38 ^{bc} |
| Stevia sweetener (500 mg/kg b. wt) | 209 ^a | 115 ^c | -44.98 ^{cd} |
| Stevia sweetener (1000 mg/kg b. wt) | 205 ^a | 106 ^d | -48.29 ^d |

Significant difference between control and all other groups by one-way ANOVA ($P < 0.05$).

The values with different superscripts within each column are significantly different at $P < 0.05$.

Low-caloric sweeteners may not stimulate appetite, thereby not increasing calorie intake and not promoting weight gain (Robarts and Wright, 2010). This finding agreed with the previous work of Awney et al. (2010), Bernal et al. (2011) and Abd El-Razek and Massoud (2012) which showed that there was a positive correlation between the decrease of body weight gain percent and the reduction in feed intake and the dose of stevioside administrated to rats.

Effect of administration of stevia sweetener on absolute and relative organs weight

Treated rats with stevia sweetener at doses of 25, 250 and 500 mg/kg b. wt/day for 12 weeks had insignificant decreased in relative to liver, heart, kidney, lung, pancreas and spleen weight and had insignificant increased in relative to brain weight when compared with positive or negative control groups. Moreover, administration of stevia sweetener at dose 1000 mg/kg b. wt/day showed significant decrease in kidney relative weight compared to the other treatment (Fig. 3). Nikiforov and Eapen (2008) demonstrated that in a 90-day

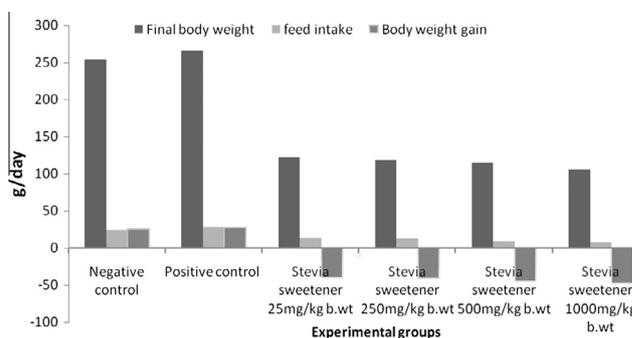


Fig. 2 Final body weight, feed intake and body weight gain % in rats treated with administration of stevia sweetener after 12 weeks.

toxicological study of rebaudioside A, some relative (to body weight) organ weights were statistically significantly different from the control group, but with the exception of reduced absolute liver weight in male rats treated with 2000 mg/kg/day of rebaudioside A, and there were no differences in mean absolute brain weight. Also, Awney et al. (2010) found that the liver weight to body weight ratio was significantly lower in the high dose of stevioside (1500 mg/kg/day) group than in the control group, while significant increases in brain were observed in the low dose of stevioside (15 mg/kg/day) group when compared with the control group.

Effect of administration of stevia sweetener on blood hematological parameters of rats

Results showed that there was no significant change in WBC, RBC, HGB, MCH and PLT between all the groups of rats. While stevia sweetener groups showed a significant decrease in MCHC and MPV when compared to negative control group (Table 2).

Serum biochemical parameters and liver enzymes activities in female rats treated with stevia sweetener

Groups of rats treated with stevia sweetener showed improvement in lipid profile levels comparing with negative or positive control group (Table 3).

It can be seen that the level of TL was decreased by 11.96%, 19.89%, 25.03% and 37.07% when rats were given stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt, respectively compared to negative control. Nevertheless Serum lipids of rats given stevia sweetener at dose 1000 mg/kg showed the lowest total cholesterol (TC) (168.12 mg/dl) followed by that given the dose 500 mg/kg (175.38 mg/dl), dose 250 mg/kg (180.25 mg/dl) and then finally dose 25 mg/kg (185.88 mg/dl). Administration of stevia sweetener in a dose of 25 mg/kg b. wt to female rats for 12 weeks showed no significant change in serum TG, HDL and VLDL when compared to the positive and negative control groups. The LDL values in rat serum lipids decreased with increasing the doses of stevia sweetener. Rats given stevia sweetener at dose 1000 mg/kg b. wt showed the highest decrease in the LDL (26.50%) followed by those given dose 500 mg/kg (24.36%), dose 250 mg/kg (19.90%) and finally dose 25 mg/kg (15.01%). In contrast to LDL, level of high density lipoprotein (HDL) increased with increasing the doses of stevia sweetener. Results revealed that treatment with stevia sweetener decreased serum levels of VLDL as

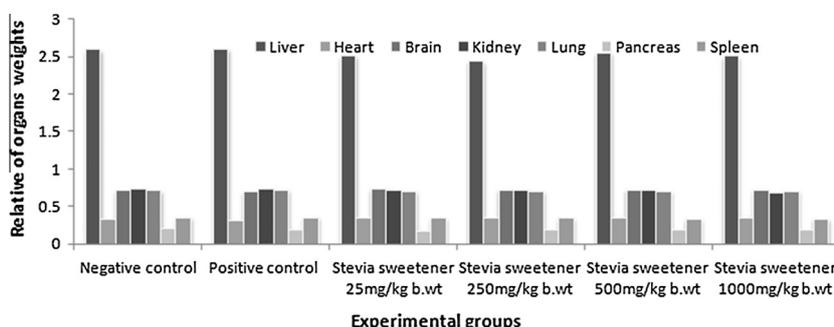


Fig. 3 Organ weights relative to body weight of female rats treated with stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt and sucrose compared with control.

Table 2 Means of WBC, HGB, RBC, HCT, MCV, MCH, MCHC, MPV and PLT in control female rats and rats treated with different levels of stevia sweetener after 12 weeks.

| Parameter | Experimental groups | | | | | |
|---------------------------|---------------------|--------------------|-----------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| | Negative control | Positive control | Stevia sweetener (25 mg/kg b. wt) | Stevia sweetener (250 mg/kg b. wt) | Stevia sweetener (500 mg/kg b. wt) | Stevia sweetener (1000 mg/kg b. wt) |
| WBC (10 ³ /UL) | 10.1 ^{ab} | 8.94 ^{ab} | 9.13 ^{ab} | 9.26 ^{ab} | 10.52 ^{ab} | 8.79 ^b |
| HGB (g/dl) | 11.75 ^a | 12.96 ^a | 12.48 ^a | 12.43 ^a | 13.03 ^a | 12.65 ^a |
| RBC (10 ⁶ /ml) | 5.32 ^a | 5.96 ^a | 5.77 ^a | 5.79 ^a | 5.85 ^a | 5.82 ^a |
| HCT (%) | 31.47 ^b | 34.36 ^a | 33.40 ^{ab} | 33.6 ^{ab} | 34.56 ^a | 34.58 ^a |
| MCV (fl) | 58.3 ^c | 62.22 ^a | 60.3 ^b | 60.62 ^b | 61.5 ^{ab} | 61.78 ^{ab} |
| MCH (P g) | 21.9 ^a | 21.52 ^a | 21.85 ^a | 21.85 ^a | 21.60 ^a | 21.80 ^a |
| MCHC (P g) | 36.8 ^a | 35.02 ^b | 34.93 ^b | 35.13 ^b | 34.58 ^b | 35.4 ^{ab} |
| PLT (10 ³ /UL) | 587 ^a | 529.5 ^a | 578 ^a | 573.5 ^a | 531.2 ^a | 521.25 ^a |
| MPV (fl) | 6.66 ^a | 6.72 ^a | 6.28 ^b | 6.14 ^b | 6.22 ^b | 6.32 ^b |

WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume and PLT = platelets count. Significant difference between control and all other groups by one-way ANOVA ($P < 0.05$). The values with different superscripts within each column are significantly different at $P < 0.05$.

Table 3 Means of serum lipid profile levels (mg/dl) in control female rats and rats treated with different levels of stevia sweetener after 12 weeks.

| Parameter | Experimental groups | | | | | |
|-----------|---------------------|---------------------|-----------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| | Negative control | Positive control | Stevia sweetener (25 mg/kg b. wt) | Stevia sweetener (250 mg/kg b. wt) | Stevia sweetener (500 mg/kg b. wt) | Stevia sweetener (1000 mg/kg b. wt) |
| TL | 448.5 ^a | 455.13 ^a | 394.88 ^b | 359.25 ^{bc} | 336.25 ^c | 282.25 ^d |
| TC | 203 ^a | 206.63 ^a | 185.88 ^b | 180.25 ^{bc} | 175.38 ^{cd} | 1.69 ^d |
| T.G | 101.13 ^a | 105.38 ^a | 102.38 ^a | 90 ^b | 81.88 ^c | 80.25 ^c |
| HDL | 37.88 ^b | 37.25 ^b | 41.13 ^{ab} | 45.13 ^a | 46 ^a | 47.25 ^a |
| LDL | 130 ^a | 140.13 ^a | 119.1 ^b | 112.25 ^{bc} | 106 ^c | 103.13 ^c |
| LDL/HDL | 3.43 ^{ab} | 3.76 ^a | 2.90 ^{bc} | 2.49 ^{bc} | 2.30 ^c | 2.18 ^c |
| VLDL | 20.13 ^{ab} | 21.25 ^a | 19.5 ^{ab} | 17.88 ^{bc} | 16.13 ^{cd} | 1.28 ^d |

TL: total Lipid, TC: Total Cholesterol, TG: Triglyceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein and VLDL: Very Low Density Lipoprotein, Significant difference between control and all other groups by one-way ANOVA ($P < 0.05$). The values with different superscripts within each column are significantly different at $P < 0.05$.

compared to the negative or positive control group. The VLDL levels were decreased 3.13%, 11.18%, 19.87% and 26.08% in rats given stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt respectively when compared to the negative control group. The LDL/HDL ratio was further reduced to the lowest value in groups given stevia sweetener as compared to the negative and positive control groups.

It could be suggested that the reduction of lipid profile may be due to the reduction of blood glucose levels after treatment with different doses of stevia sweetener because it possesses insulinotropic, glucagonostatic, antihyperglycemic, and blood-pressure-lowering effects (Gregersen et al., 2004 and Hony et al., 2006). These results are conforming to the results of Curry and Roberts (2008) and Rajesh et al. (2012) who

Table 4 Means of serum biochemical parameters and liver enzymes activities in female rats treated with stevia sweeteners.

| Parameter | Experimental groups | | | | | |
|-----------------------|-----------------------------|----------------------------|-----------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| | Negative control | Positive control | Stevia sweetener (25 mg/kg b. wt) | Stevia sweetener (250 mg/kg b. wt) | Stevia sweetener (500 mg/kg b. wt) | Stevia sweetener (1000 mg/kg b. wt) |
| Glucose (mg/dl) | 101.34 ± 1.61 ^b | 113.59 ± 1.84 ^a | 90.65 ± 2.92 ^c | 86.85 ± 2.61 ^{cd} | 85.29 ± 2.9 ^{cd} | 83.30 ± 2.91 ^d |
| Urea (mg/dl) | 35.47 ± 0.98 ^a | 36.61 ± 0.97 ^a | 35.37 ± 0.15 ^a | 35.33 ± 0.15 ^a | 35.15 ± 0.19 ^a | 35.13 ± 0.05 ^a |
| Creatinine (mg/dl) | 1.18 ± 0.06 ^a | 1.19 ± 0.085 ^a | 1 ± 0.027 ^b | 0.90 ± 0.038 ^b | 0.90 ± 0.057 ^b | 0.95 ± 0.029 ^b |
| Bilirubin (mg/dl) | 2.65 ± 0.089 ^a | 2.69 ± 0.07 ^a | 2.35 ± 0.087 ^b | 2.33 ± 0.117 ^b | 2.32 ± 0.129 ^b | 2.31 ± 0.086 ^b |
| Total Protein (mg/dl) | 7.02 ± 0.189 ^b | 7.25 ± 0.016 ^a | 7.01 ± 0.017 ^b | 7.01 ± 0.026 ^b | 7.01 ± 0.016 ^b | 7 ± 0.013 ^b |
| Albumin (mg/dl) | 3.51 ± 0.14 ^a | 3.60 ± 0.08 ^a | 3.46 ± 0.07 ^a | 3.45 ± 0.09 ^a | 3.46 ± 0.11 ^a | 3.40 ± 0.09 ^a |
| Globulin (mg/dl) | 3.51 ± 0.238 ^a | 3.61 ± 0.081 ^a | 3.65 ± 0.071 ^a | 3.56 ± 0.096 ^a | 3.59 ± 0.091 ^a | 3.61 ± 0.091 ^a |
| AST (U/L) | 109.53 ± 2.07 ^a | 110.39 ± 2.25 ^a | 107.76 ± 3.25 ^a | 106.4 ± 4.14 ^a | 104.63 ± 2.47 ^a | 102.43 ± 3.95 ^a |
| ALT (U/L) | 82.54 ± 0.96 ^a | 82.66 ± 0.57 ^a | 81.51 ± 2.80 ^{ab} | 78.38 ± 3.09 ^{ab} | 77.09 ± 2.6 ^{ab} | 75.5 ± 2.31 ^b |
| AIP (U/L) | 73.66 ± 1.14 ^a | 74.92 ± 1.02 ^a | 73.54 ± 1.88 ^a | 70.48 ± 4.14 ^a | 70.59 ± 1.73 ^a | 70.3 ± 1.53 ^a |
| ACP (U/L) | 16.83 ± 0.67 ^a | 17.14 ± 0.14 ^a | 16.66 ± 0.54 ^a | 16.66 ± 0.54 ^a | 16.47 ± 0.72 ^a | 16.29 ± 0.31 ^a |
| LDH (U/L) | 202.38 ± 8.53 ^{ab} | 217.41 ± 5.60 ^a | 196.3 ± 7.76 ^{bc} | 194.28 ± 2.65 ^{bc} | 192.26 ± 4.77 ^{bc} | 184.02 ± 4.01 ^c |
| TBARS (U/L) | 2.28 ± 0.195 ^a | 2.34 ± 0.049 ^a | 2.15 ± 0.033 ^a | 1.88 ± 0.012 ^b | 1.85 ± 0.051 ^b | 1.77 ± 0.05 ^b |

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, ACP: acid phosphatase, LDH: Lactate dehydrogenase and TBARS: thiobarbituric acid reactive substances. Significant difference between control and all other groups by one-way ANOVA ($P < 0.05$).

The values with different superscripts within each column are significantly different at $P < 0.05$.

revealed that stevioside significantly lowers in total cholesterol, triglyceride, LDL-C and VLDL and improved HDL-C level as compared with untreated rats. However, [Park and Cha \(2010\)](#) found that total cholesterol concentrations were lower in rats fed stevioside 1 ml kg per day with high fat diet compared to rats fed high fat diet with water.

The plasma glucose concentrations were significantly reduced after 12 weeks of the treatment by 25, 250, 500 and 1000 mg stevia sweetener/kg b. wt by 20.2%, 23.54%, 24.91% and 26.67% respectively as compared to the glucose value of rats given sucrose ([Table 4](#)). Previous study showed that stevioside was able to regulate blood glucose levels by enhancing not only insulin secretion and sensitivity but also insulin utilization in insulin deficient rats which was due to decreased PEPCK gene expression in rat liver, and in addition, no effect on blood glucose in healthy human subjects was observed after treatment with 250 mg stevioside 3 times daily for 1 year ([Geuns et al., 2007](#)). These results are in agreement with [Youssef et al. \(2007\)](#), who found that administration of stevia leaves or stevioside decreased glucose in serum diabetic rats. Also, [Awney et al. \(2010\)](#) reported that administration of stevioside at dose 1500 mg/kg/day caused significant reduction (41%) on glucose level of rats, whereas no significant effects were detected in low dose of stevioside (15 mg/kg). In the same respect [Table 4](#) shows, that the rats treated with sucrose (positive control group) had significant ($P < 0.05$) increase in glucose level compared to normal rats (negative control group). The present results revealed that stevia sweetener decreased creatinine and bilirubin levels as compared to control, while there was no significant change in serum urea in female rats as compared to control ([Table 4](#)). This reduction was associated with decrease in kidney relative weight when compared

with control groups. Contrary to our result, in previous study by [Toskulkao et al., 1995a,b](#) when stevioside is given subcutaneously to rats (1.5 g/kg BW) and via intragastric tube to hamsters (4.1 g/kg BW), it caused nephrotoxicity as indicated by an increase in blood urea nitrogen and plasma creatinine levels. [Curry and Roberts \(2008\)](#) found that there was a tendency in both males and females for the plasma urea and creatinine results to be higher and the urine volume to be lower, especially in the higher dose of rebaudioside A orally treated rats. They suggested that increases in urea and creatinine were due to physiological rather than a toxicological effect as the increases in mean urea and creatinine were relatively small (1.1- and 1.2-fold, respectively), mean values all remained within the laboratory's reference ranges and no histological evidence of kidney toxicity was observed. This result is in accordance with the findings that no significant change in urea in plasma of rats were observed when given stevia sweetener at 25 mg/kg/day as compared to control ([Abd El-Razek and Massoud, 2012](#)).

Groups of rats treated with stevia sweetener had no significant increase in the level of globulin and significantly ($P < 0.05$) decreased the serum levels of albumin (ALB) and total protein (TP) compared to the negative control group. AST and ALT are considered as a specific indicator for hepatic dysfunction and damage ([Osuna et al., 1977](#); [Shakoori et al., 1994](#)). These enzymes are involved in the breakdown of amino acids into α Keto acid which are routed for complete metabolism through the Krebs's cycle and electron transport chain. Acid (ACP) and alkaline (ALP) phosphatase enzymes have very similar catalytic functions and structures, but act within different ranges of pH, and ALP is a widely used marker for both skeletal and hepatobiliary disorders.

Table 5 Means of TBARS and the activities of AST, ALT, ALP, ACP and LDH in liver of female rats treated with stevia sweetener at different doses after 12 weeks.

| Parameter | Experimental groups | | | | | |
|-----------|----------------------|---------------------|-----------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| | Negative control | Positive control | Stevia sweetener (25 mg/kg b. wt) | Stevia sweetener (250 mg/kg b. wt) | Stevia sweetener (500 mg/kg b. wt) | Stevia sweetener (1000 mg/kg b. wt) |
| AST | 141.67 ^a | 143.33 ^a | 141.29 ^{ab} | 140 ^b | 136.67 ^c | 135 ^c |
| ALT | 115.33 ^{ab} | 116.71 ^a | 114.07 ^{ab} | 113.43 ^{ab} | 112.8 ^b | 112.80 ^b |
| ALP | 72.25 ^a | 73.39 ^a | 71.78 ^{ab} | 71.74 ^b | 71.66 ^b | 71.07 ^b |
| ACP | 17.97 ^a | 18.09 ^a | 17.84 ^a | 17.74 ^a | 17.68 ^a | 17.39 ^a |
| LDH | 137.62 ^b | 145.71 ^a | 132.22 ^{bc} | 129.52 ^{cd} | 124.12 ^{dc} | 121.43 ^c |
| TBARS | 63.63 ^a | 65.39 ^a | 62.85 ^a | 57.49 ^b | 51.7 ^c | 48.63 ^c |

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, ACP: acid phosphatase, LDH: Lactate dehydrogenase and TBARS: thiobarbituric acid reactive substances.

Significant difference between control and all other groups by one-way ANOVA ($P < 0.05$).

The values with different superscripts within each column are significantly different at $P < 0.05$.

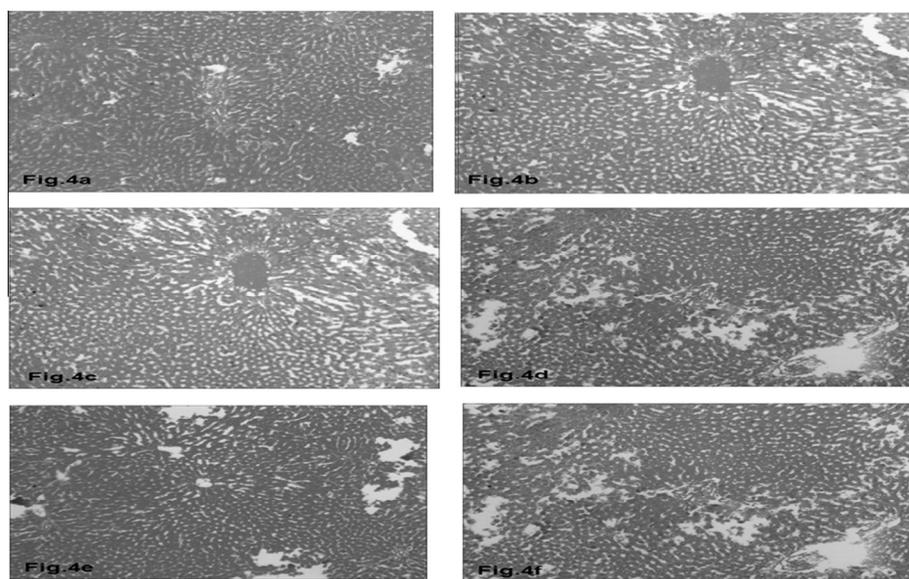


Fig. 4 Light microscopic photograph of rat liver tissues drinking on (a) distilled water; negative control, (b) sucrose dissolved in drinking water, (c, d, e, and f) stevia sweeteners dissolved in water at a doses of 25, 250, 500 and 1000 mg/kg b. wt, respectively after 12 weeks.

Our data indicated that treatment with stevia sweetener at four dosage levels showed remarkably amelioration of the elevation of enzymes level and the reduction in AST, ALP and ACP enzymes activity compared with untreated group indicating that stevia sweetener tended to prevent damage and suppressed the leakage of enzymes through cellular membranes. Treatment with 1000 mg stevia sweetener/kg b. wt/day to rats caused significant decrease in the levels of ALT and LDH enzymes by 8.53% and 9.07% respectively, when compared to the negative control group at the end of 12 week.

Our study showed that treatment of rats with stevia sweetener at doses of 250, 500 and 1000 mg/kg b. wt/day significantly decreased serum thiobarbituric acid reactive substances (TBARS) by 17.54%, 18.86% and 22.37% respectively, when compared to negative control group while stevia sweetener at dose 25 mg/kg b. wt/day showed no significant change in TBARS (Table 4).

This result was in agreement with Williams (1995) who found that the diets given to rats containing stevia extract at

levels two 1% and 2% and stevioside at levels 1%, 2% and 3% led to gradual reduction in plasma AST and ALT levels. Other study with Youssef et al. (2007) stated that diets containing *S. rebaudiana* leaves at three levels 1%, 2% and 3% and stevioside administrated at two levels 0.5% and 1% decrease levels of AST and ALT. Agamy et al. (2008) found that treating rats with different doses (100 or 200 mg stevioside/kg b. wt/day) induced insignificant change in the plasma liver function enzymes levels in reference to control level. Parrimalavalli and Radhai (2010) suggested that rats treated with *S. rebaudiana* extract at doses of 500, 1000 and 2000 mg/kg b. wt/day caused decrease in AST, ALT and ALP values.

Effect of stevia sweetener on TBARS and the activities of AST, ALT, ALP, ACP and LDH changes in liver of female rats

Results indicated that TBARS level was significantly ($P < 0.05$) decreased in liver of rats treated with stevia

sweetener at doses of 250, 500 and 1000 mg/kg/day (Table 5). Treatment with stevia sweetener at doses of 500 and 1000 mg caused significant ($P < 0.05$) decrease in the activities of AST and ALT enzymes compared to both negative and positive controls. In addition, treatment with stevia sweetener in low dose (25 mg/kg b. wt/day) reduced ALP and TBARS levels compared to positive control group. Results revealed that LDH value in liver of rats decreased by 9.26%, 11.11%, 14.82% and 16.66% when rats were given stevia sweetener at doses of 25, 250, 500 and 1000 mg/kg b. wt, respectively after 12 weeks.

Histological examination of liver tissues

Histopathological examination of the liver taken from control group (Fig. 4a) and stevia sweetener treated groups (Fig. 2c–f) showed normal appearance with regular arrangement of hepatocyte cell, no fibrosis, mild inflammation and normal hepatic cells with well preserved cytoplasm, nucleus and nucleolus and central vein.

In conclusion, stevia sweetener treated groups showed significantly improvement and ameliorated reduction in body-weight, BWG % and lesser intake of feed. In addition stevia sweetener proved to be beneficial in decreasing the levels of blood glucose, total lipids, total cholesterol, triglycerides and low-density lipoprotein concentrations, and increasing the high-density lipoprotein.

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