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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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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.

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 $\geqslant$ 30) numbered 500 million; by 2015, these figures are projected to

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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).