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Research Article

COMPARATIVE STUDY OF THE EFFECT OF SUCRALOSE AND SUGAR ON SOME SERUM BIOMARKERS OF RATS

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ABSTRACT
Sucralose (1, 4, 6 Trichlorogalactosucrose) is the only non-caloric sweetener derived from sugar which is 600 times sweeter than sugar. The sweetening effect of a small tablet of sucralose equals with 2.7 g sugar. It readily dissolves in water and can be used in cooking because of its good stability. In addition to sucralose, the tablets contain lactose, L leucine, cross, carmolose sodium, and PVP which produce a low level of calories. So, the present study evaluated the effect of commercial sucralose on serum levels of glucose, total protein, and AST, ALP, ALT enzymatic activity. The study was conducted on 18 male Vistar rats with 250±20 g weight. They were divided into 3 groups each consisted of 6 rats: control, sucralose, and sugar groups. The control group received basal diet and no intervention was done about them. Administered dosage of sucralose in the treatment group was 15 mg/k/day via gavage for one month. Also, considering the sweetening rate of commercial sucralose which was 2.7 g sugar at the producer's brochure, the rate of sugar was calculated and administered via gavage daily and monthly. Finally, all rats were sampled followed by isolating serum by Pars Azmoon kits and evaluating glucose, total protein, and ASP, ALT, ALT enzymatic activity. Obtained results were analyzed by SPSS statistical software (version 18.0) and one-way ANOVA. The results show that there was no significant difference between two groups on serum glucose, total protein, AST, and ALP enzymatic activity but a significant difference about ALT was proved. In conclusion, it can be said that commercial sucralose not only has no adverse effect on the liver but also has a protective effect on the organ so it won't be contraindicated for diabetics and fat people.

Keywords: *Sucralose, Sugar, Serum Biochemical Factor*

INTRODUCTION
For the last many years the development of cardiovascular disease, obesity, diabetes and metabolic disorders caused many changes in lifestyle and eating habits of humans. This led food manufacturers to use artificial sweeteners instead of natural sugar. Most of the sweeteners do not produce much energy in the body and even some of them pass from body unused in the metabolic process. Sucralose was discovered in 1976 by Tate and Lyle Co and was approved in 1998 by FDA. Sucralose, with the chemical formula 1, 4, 6 Trichlorogalactosucrose is the only no-calorie sweetener that is derived from sugar, 600 times sweeter than it. By substituting 3 chlorine molecules in sucralose ensures that, unlike sugar, it is not metabolized and remain unchanged in the body, and rapidly passes from the body; so, it is considered safe and unreactive material. This sweetener sold in pharmacies in commercial varieties. The sweetening effect of a small tablet of commercial package Mardin (sucralose) is equal to 2.7 g sugar. It readily dissolves in water and due to better stability can be used in cooking. This sweetener has been confirmed by various organizations such as the FDA and Europe food safety. Sucralose tastes like sugar and has no unpleasant taste. In studies conducted by researchers for over 20 years, it has been determined that this sweetener is completely safe and healthy and so far no documented adverse side effects such as tooth decay, increased sugar in people with diabetes, genetic changes, cancer, immunological problems, central and peripheral nervous system disorders, as well as fetus defects have been published. This sweetener has no contraindications for pregnant women, breastfeeding mothers and children and its maximum

acceptable daily intake is 15 mg /kgbw. It was determined in a survey conducted by Shastry *et al.*, That consumption of sugary sweeteners of aspartame, acesulfame and sucralose in ADI doses for three weeks had no mutagenic effect in normal and diabetic rats and glucose and serum profiles. Helen and colleagues found in a study that sucralose consumption in normal rats caused a 14% decline in serum glucose, total cholesterol increased by 20 percent, a 25 % increase in HDL and a 32% increase in LDL, and triglyceride levels were lowered by as much as 17%. In the double-blind study conducted by Grots and colleagues it was identified that sucralose at a dose of 7.5 mg / kg / day for one month had no significant effect on serum glucose and HbA1c levels in humans. According to a company brochure, each sucralose pill of Mardin brand has lactose, L-Leucine, Cross, Karmulose soda and PVP compounds in addition to sucralose and creates low-calorie. Therefore, in this research the effect of artificial sweetener, sucralose with the brand name of Mardin, on serum glucose, total protein, and enzymatic activity of AST, ALP and ALT was evaluated.

MATERIALS AND METHODS

The study included 18 male Wistar rats in the weight range 20 ± 250 g which were randomly divided into 3 groups each consisted of 6 rats. The groups were control, sucralose and sugar groups. Controls have basal diet and no intervention was performed on them. According to a survey conducted by Shastry and colleagues as well as the standards of the Food and Drug Administration (FDA) which has mentioned the amount of maximum acceptable daily intake (ADI) of sucralose 15 mg /kgbw, consumption dose of sucralose in the present study was determined 15 mg/k that was administered orally in the treatment group daily for one month. Also according to the sweetening rate of sucralose in commercial tablets with 2.7 grams of sugar listed company brochure and based on the Sweetening power, the amount of sugar was calculated for sugar treatment group and was administered in gavage method daily for one month. It should be noted that during the period of study, the rats were exposed to 12 hours light and 12 hours dark and they had no restrictions on access to food and water. Blood samples were taken from all animals at the end of the period. The sera were isolated followed by evaluating serum glucose, total protein, and enzymatic activity of AST, ALP and ALT using Pars Azmoon diagnostic kits. Obtained results were used for statistical analysis by SPSS package (version 18.0). The results of the study were compared using one-way ANOVA at 95% probability level. $P<0.05$ was considered as significant, and $p<0.01$ was considered very significant. If there was a significant difference, Tukey test was used to determine differences between the groups.

RESULTS AND DISCUSSION

Comparing the amount of glucose in the groups:

In order to check glucose levels in the study groups, the results were examined using ANOVA and Tukey test which are shown in Table 1.

Table 1: mean, standard error, standard deviation, significance of glucose mean (mg/dl)

Group	Mean ± SE	SD	Sig (P Value)
Control	106.00 ±7.06	17.30	0.879
Sugar	107.338.87	21.73	0.879
Sucralose	101.66 ±8.60	21.07	0.879

The results of ANOVA showed that the mean glucose levels were not significantly different between groups ($p> 0.05$).

Comparing the amount of total protein in the groups:

In order to assess the total protein in the study groups, the results were evaluated using ANOVA and Tukey test which is given in Table 2.

Table 2: mean, standard error, standard deviation, significance of total protein mean (g/dl)

Group	Mean ± SE	SD	Sig (P Value)
Control	8.06 ±0.51	1.25	0.967
Sugar	8.25± 0.48	1.19	0.967
Sucralose	8.21 ± 0.58	1.43	0.967

The results of ANOVA showed that the mean total protein levels were not significantly different between groups (p> 0.05). Furthermore, the results of the study showed that the lowest mean total protein was in the control group, 51/0 ± 06/8 and its highest mean was in sugar group, 48/0 ± 25/8.

Comparing the amount of ALP activity in the groups:

In order to assess the amount of ALP in the study groups, the results were evaluated using ANOVA and Tukey test which is given in Table 3.

Table 3: mean, standard error, standard deviation, significance of ALP mean (IU/I)

Group	Mean ± SE	SD	Sig (P Value)
Control	399.16 ± 52.46	128.50	0.375
Sugar	271.33 ± 24.40	59.79	0.375
Sucralose	358.50 ± 51.50	126.14	0.375

The results of ANOVA showed that the mean ALP activity levels were not significantly different between groups (p> 0.05). The results of the study showed that the lowest ALP was in sugar group, 271.33 ± 24.40 and its highest mean was in sucralose group, 50/51 ± 50/358.

Comparing the amount of AST activity in the groups:

In order to assess the amount of AST in the study groups, the results were evaluated using ANOVA and Tukey test which is given in Table 4.

Table 4: mean, standard error, standard deviation, significance of AST mean (IU/I)

Group	Mean ± SE	SD	Sig (P Value)
Control	130.66±19.55	47.89	0.976
Sugar	134.00±28.49	69.79	0.976
Sucralose	126.66±22.32	54.68	0.976

The results of ANOVA showed that the mean ALP activity levels were not significantly different between groups (p> 0.05). The results of the study showed that the lowest ALP was in sugar group, 126.66±22.32 and its highest mean was in sucralose group, 134.00±28.49.

Comparing the amount of ALT activity in the groups:

In order to assess the amount of AST in the study groups, the results were evaluated using ANOVA and Tukey test which is given in Table 5.

Table5: mean, standard error, standard deviation, significance of ALT mean (IU/I)

Group	Mean ± SE	SD	Sig (P Value)
Control	82.66±13.48	33.02^{*b}	0.027
Sugar	53.33±8.17	20.02^{ab}	0.027
Sucralose	43.66±4.30	10.53^{a}	0.027

Different letters at each week show a significant statistical difference.

Based on the results of the Tukey test, the lowest ALT mean was in tablet group, 43.66 ± 4.30 and its highest mean was in the control group, 82.66 ±13. 48. Furthermore, there was a significant difference in the control group with tablet group (p<0.05).

Table 6: comparing the mean ALT activity (IU/I) among different groups using a Tukey test

	Group	SE	Sig (P Value)
Control	Sugar	13.34	0.104
	Sucralose	13.34	0.027
Sugar	Control	13.34	0.104
	Sucralose	13.34	0.753
Sucralose	Control	13.34	0.027
	Sugar	13.34	0.753

Discussion

Based on the hypothesis that hydrolysis may converts sucralose to the toxic metabolites, many studies were conducted on this issue and it was determined that sucralose transforms to 4-CG and 1,6 DCF; which are stable to more hydrolysis and degradation because chlorinated sucrose and its conversion into sucralose cause some changes in molecule conformation and makes it resistant to glycoside enzymes of digestive tract, which normally causes the breakdown of carbohydrates (SCF/CS/ADDS/EDUL/190 Final 2000). In one study (Shastry *et al.*, 2012) which was conducted on rats by oral administration of sucralose with ADI dose equal to 15 mg / kg over 3 timing phases of 0-3 weeks at a dose of 1×ADI, 3-7 weeks at a dose of 2×ADI, and 7-13 weeks at a dose of 4×ADI, the reduction of serum glucose was reported in all of three phases with the difference that in phase one the reduction was very small but in phases 2 and 3 the glucose reduction was noticeable but not significant. Also in the present study the amount of serum lipid profiles has been reported in phase 1 similar to controls, whereas a significant increase in lipid profiles in phases 2 and 3 was proved that conformed to the findings of the present study. Sucralose metabolites are excreted from the body in two ways: A) Reclamation to a substance called 1.6-Di chloroaminitol and disposal through urine flow and B) conjugated with glutathione (Ademir, 2009). In a research on the effect of 2-20 mg / kg sucralose on mice it was found that 80% of sucralose is excreted through the urine and 9-165 by the feces. Also sucralose is absorbed very poorly by the renal tubules and its value is below 5% within 24 hours (Simes *et al.*, 2000). It was found in sucralose a pharmaco-kinetic study that over 85 percent of sucralose is excreted in the faeces without absorption by the gastrointestinal tract and only 15% of the consumed sucralose is absorbed from the gastrointestinal tract as passive diffusion (Ademir, 2009). Because sucralose has no additive effect on glucose and other carbohydrates; hence, is considered as a safe sweetener for diabetics (Campose 2000). In the study conducted by Helen *et al.*, (2013), in which the impact of Splenda commercial tablets contained sucralose in normal and diabetic rats was studied, it was found that sucralose decreases serum glucose level to 14% in normal rats compared with control group which was significant. Also it was found that there was a 150 % increase of glucose and 22% reduction of insulin in diabetes–sucralose group which was conformed to the present study findings that demonstrated about 5% serum glucose reduction in sucralose-fed normal rats. Sucralose suppressive effect on serum glucose may be related to the decreased absorption in the gastrointestinal tract and/or is associated with increased insulin secretion, since in vitro studies have shown that sucralose induces insulin secretion by calcium and CAMP-dependent mechanisms (Nakagava, 2009). In another study (Mezitis, 1996) it was found that 1000 mg sucralose has no additive effect on blood glucose levels that is consistent with the findings of this research.

According to conducted studies (Grotez, 2003) sucralose consumption at a dose of 7.5 mg / kg had no significant effect on blood glucose in type 2 diabetics. The function of the liver in the metabolism of carbohydrates, lipids and proteins has been demonstrated (Giannini *et al.*, 2005). Changes in the levels of liver enzymes are considered as one of the indicators of liver injury (Giannini *et al.*, 2005). AST and ALT are intracellular liver enzymes that their increase in serum confirms that liver parenchymal injury while the origin of the ALP is epithelial cells of the gallbladder and bile duct cells of the liver, which has increased activity in internal and external obstruction of the liver bile ducts. The ALP has various Iso-enzymes but increased serum activities have linked to liver (Field *et al.*, 2008). In a study with oral administration of sugar cane molasses (containing 95% sugar) was performed compared to control mice, no significant change in the activity of serum enzymes AST, ALP and total protein was observed that are

quite consistent with the results obtained in this study (Rahiman, 2011). The relationship between ALT Alanine amino transferase and clinical signs of liver failure is poor. Although the enzyme is very valuable in diagnosis of liver diseases of species such as cats and dogs, in animal species such as horses, cattle, sheep, and goat have no diagnostic value. In acute liver diseases that lead to membrane damage or cell necrosis, significant increases in serum ALT enzyme activity are observed. In this study, sucralose reduces serum activities of AST, ALT, which was a non-significant about AST and significant about ALT that indicated the absence of liver damage, probably due to lack of complete hydrolysis of sucralose and its excreted from the body. Similarly, in this study, non-significant increase in ALP enzyme activity was observed in the sucralose group which may be related to cellular damage of liver internal and external bile ducts, although different types of ALP isoenzymes have been found that their origin may be bone, steroidal, intestinal and placental tissues. In conclusion it can be said that Mardin commercial sweetener containing sucralose, not only has no negative effect on the liver, but also has a protective effect on it as well as it wouldn't be contraindicated for people with diabetes and obesity due to hypoglycemic effect.
In conclusion, it is proposed:1- In future studies the effect of different doses of sucralose on other factors such tissue hematological, biochemical and histopathological factors, especially the liver and kidneys.2- With high doses of sucralose (more than the allowable daily intake of 15 mg / kg) the toxic effects of sucralose on the liver and kidneys are examined. 3- It is proposed that a Comparative study is conducted on serum hematological and biochemical changes with consuming of other non-sugar sweeteners such as aspartame, acesulfame and Saccharin.4- It is recommended that the relative activity of ALP isoenzymes in serum of rats receiving different types of sweeteners is determined in future studies.

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