ComparisonbetweenTheEffectofSucraloseandSodiumSaccharinon Some Physiological Parameters in Male Albino Rats

ABSTRACT

Background: Non-nutritive sweeteners(NNSs) are becomingpopular as sugar substitutes for diabeticpatients or to decrease body weight. **Aim of the work:** This study was aimed to determine the effects of sucralose and sodium saccharin on some physiological parameters in male albino rats. **Materials and methods:** We used thirty male albino rats weighing from 100 to120 gm. The period of the experiment was 30 days. The animals were divided intothree groups; **Group1:** control, **Group2:** ratsreceived sucralose and **group3:** rats received sodium saccharin. The following parameters were processed: serum glucose, ASAT, ALAT, serum creatinine, serum urea, protein and lipid profiles and hormonal levels (insulin, testosterone, serum T3 and T4). **Results:** There was an increase in ASAT and ALAT activities, serum creatinine and serum urea levels in group 2 and group 3, lipid profile in the group received sucralose (TC and HDL) and T3&T4 in the group received saccharin as compared to the control group. Meanwhile, a drop in serum glucose, insulin, total protein, albumin, albumin/globulin ratio and triglycerides in group 2 and group 3, lipid profile in the group received saccharin (TC and HDL) and T3&T4 in the group received sucralose was observed when compared to the control group. **Conclusion:** it could be concluded that sucralose and sodium saccharin must be carefully used because they have very dangerous effects-especially sodium saccharin- and we have to replace them with natural sugar.

Comment [SC1]: It can be mentioned that

Keywords:*sucralose,sodiumsaccharin,ASAT,ALAT,T3,T4,testosterone,insulin.*

Comment [SC2]: PUT IN ITALICS

INTRODUCTION

Artificial sweeteners are used as sugar substitutes called “zero” or “light”- beverages, foodstuffs, pharmaceuticals and personnel care products. They have been used by consumers to acquirea sweet taste, for reasons of economics, blood glucose control, or energy control. The healthrisks of artificial sweeteners consumptionis still a highly controversial topic[(1)]. Artificial sweeteners have allegedly been related to some effects such as cancer, weight gain, metabolic disorders, migraines, type-2 diabetes, vascular events, preterm delivery, kidney functiondisorders, liver antioxidant system, hepatotoxicity, immune system disruptions and alteration of gut microbiota activity. Human studies failed to showa direct connection to cancer risk. However, other studies, , have shown association with kidney function decline and vascular risk factors[(2)].

Sucralose, one of the newest artificial sweeteners has been approved by the Food and Drug Administration in 1998.Sucralose itself contains no calories but due to its very sweet taste (approximately 600 times as sweet as sugar), sucralose in the granulated format is often mixed with other sweetening ingredients such as maltodextrin. This dilutes its intense sweetnessandprovidesvolumeandtexture[(3)].Ithasno adverse effects on the central nervous system, immune

system, reproductive performance, and red blood cells constituents and morphology. On the contrary, some reports suggest sucralose is a possible trigger for some migraine patients[(4)].

Saccharin is a non-nutritive, non-caloric intense artificial sweetener. In the EuropeanUnion, Saccharin is known under the E number E954. It has 300-500 times the sweetness of sucrose, with a slight bitter aftertaste. It is widely used sweeteneras it is heat-stable, so, it is used in hot beverages, canned vegetables, bakery products and reduced sugarjams. It has a longshelf life and it is inexpensive. Saccharin goes directly through the human digestive system without being digested; and can trigger the release of insulin in rats[(5)]. Although 66%–84% of saccharin was excreted in the urine and 10%– 40% of the dose was recoveredfromthefeces afterdosingfor24h, traces of saccharin radioactivity remained in many tissues after 72 h, including liver, heart, adrenals, pancreas, thymus, and testes. These data promote the possibility that saccharin may have specific biological functions when entering into these tissues[(6)]. This chemical is one of the most sweetenersthathasbeenextensivelystudiedand

investigated due to its possible carcinogenic effects[7].

MATERIALSANDMETHODS

Sucralose was obtained as CANDEREL® (Manufactured in EU (Czech Republic) by Merisant Company 2.
Sodium saccharin was obtained as HERMESETAS, Made in Switzerland by Hermes Sweeteners Ltd, Zurich.

Thirty young male albino rats (weighing from 100 to120 g) were used in this study. Animalswere housed in stainless steel cages, fed on rat chew and offered water *ad libidum.* The animals weredividedintothreeequal groups(10ratseach) as follows: **The first group:** the control untreated group, **the second group:** rats received orally sucralose (5 mg/kg b.wt./ day) and **the third group:** rats received orally sodium saccharin (5 mg/kg b.wt./day). Body weights were recorded at the beginning and the end. After 30 days, animals were weighed and then decapitated.
Blood samples were collected for biochemical parameters. Blood samples were centrifuged for15 min. at 5000 rpm and supernatant sera were separated for analysis.

BiochemicalExamination

In the present study total protein (TP) and albumin concentration were estimated, then serum globulin concentrations were calculated according to the formula:
Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)
Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, Creatinine, urea, glucose concentrations as well as lipidprofile that including total cholesterol,triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. All parameters were estimated using **BioMerieux SA kits, France.**
The ratio of serum albumin/ globulin was determined. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low-density lipoprotein cholesterol) using the **Friedwald's** [8]and **Norbert** [9]formulas, respectively as following:
Friedewald's[8]equation:LDL(mg/dl)=TC-{HDL+[TG/5]}.

Norbert[9]equation:VLDL= TG/5

Concentrations of insulin, testosterone and thyroid hormones (T3 and T4) were measured accordingto the following methods:
An insulin ELISA kit (10-1250-01, MercodiaAB, Uppsala, Sweden) was used to measureinsulin levels. **The HOMA-2 IR index** (**Homeostatic Model Assessment of Insulin Resistance**) was calculated by a free online calculator (HOMA Calculator, Version 2.2.3, Diabetes Trail Unit, The University of Oxford, Oxford, UK).

$$\text{HOMA-IR} = \frac{[(\text{Glycaemia}(\text{mg/dl})/18.2) \times \text{Insulin}}{(\text{mU/ml})]_{(10)}22.517}$$

Testosterone,T3 andT4wereestimatedbyusing VIDAS® kits, which is an automated quantitative test.

Statisticalanalysis

The results were expressed as Mean ± SEM of the mean. Data were analyzed by using T-test and were performed using the Statistical Package (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance between groups.

RESULTS

Body weight: no significant change was noticedin the percentage of body weight change in sucralosegroup,whiletherewas highlysignificant decrease ($p<0.01$) in sodium saccharin group as compared to control animals (Table 1).
Glucose level: there was a significant decrease ($p<0.05$) in glucose level in sucralose group and highly significant decrease ($p<0.01$) in sodium saccharin group in contrast to control rats (Table 1).
Insulin level: the present study showed that there was asignificant decrease ($p<0.05$)ininsulinlevel of sucralose group, and highlysignificant decrease ($p<0.01$) in sodium saccharin group comparing to control (Table 1).
HOMA-IR: a significant decrease ($p<0.05$) was observed in the ratio of HOMA-IR in sucralose group, and it was highly significant decrease ($p<0.01$) in sodiumsaccharin group in comparison to normal rats
(Table 1).

Table(1):Percentageofbodyweightchange, glucoselevel andHOMA-IRincontrol,sucraloseand sodium saccharin treated animals.

Groups parameters	Control	Sucralose	Sodiumsaccharin
%ofbodyweight	8.6±0.5	9±0.6	2±0.8**
%ofchange		5%	-77%
Glucose(mg/dl)	109±0.7	102.4±1.9*	99±0.7**
%ofchange		-6%	-9%
Insulin (mU/ml)	0.97±0.007	0.76±0.066*	0.68±0.057**
%ofchange		-21%	-30%
HOMA-IR	0.26±0.003	0.19±0.021*	0.16±0.009**
%ofchange		-26%	-38%

Valuesrepresentmean±SE(standarderror).(*P**<0.05,*P***<0.01ascomparedtocontrolgroup)

Protein profile: animals that received sucralose has significant decrease (p<0.05) in serum total protein, albuminandalbumin/globulinratio,andthosereceivedsodiumsaccharinshowedahighlysignificantdecrease (p<0.01) in the previous parameters as compared to the corresponding control group (Table 2).

Table(2):Serumtotalprotein,albumin,globulinandalbumin/globulinratioincontrol,sucraloseand sodium saccharin treated animals.

Groups parameters	Control	Sucralose	Sodiumsaccharin
TotalProtein(g/dl)	6.63±0.04	5.69±0.28*	4.2±0.07**
%ofchange		-10%	-37%
Albumin(g/dl)	3.43±0.046	2.54±0.27*	1.2±0.07**
%ofchange		-26%	-65%
Globulin(g/dl)	3.2±0.028	3.15±0.08	3±0.09
%ofchange		-3%	-6%
Albumin/Globulin	1.074±0.02	0.8±0.08*	0.4±0.07**
%ofchange		-26%	-63%

Valuesrepresentmean±SE(standarderror). (*P**<0.05,*P***<0.01ascomparedtocontrolgroup) **Liver enzymes:** ASAT and ALAT revealed significant increase (p<0.05) among sucralose group and highly significant increase (p<0.01) among sodium saccharin group in contrast to control rats (Table 3).

Table(3):ASATandALATactivitiesincontrol,sucraloseandsodiumsaccharintreated animals.

Groups parameters	Control	Sucralose	Sodiumsaccharin
ASAT(U/L)	115.88±0.3	122±1.83*	181.6±0.9**
%ofchange		5%	57%
ALAT(U/L)	55.4±0.16	62±1.98*	83.8±0.86**
%ofchange		12%	51%

Valuesrepresentmean±SE(standarderror).(*P**<0.05,*P***<0.01ascomparedtocontrolgroup)

Lipid profile: the present results revealed significant increase (p<0.05) in total cholesterol and HDL-C, significant decrease (p<0.05) in triglycerides, and no significant change in LDL-C, VLDL-C and ratios of TC/HDL-C(riskfactor1)and LDL-C/HDL-C(riskfactor2)inratsreceivedsucraloseascomparedtocontrol group. Meanwhile, there was highly significant decrease (p<0.01) in total cholesterol and triglycerides, significant decrease (p<0.05) in HDL-C and no detectable change in LDL-C, VLDL-C and risk factors in rats received sodium saccharin as compared to the control group (Table 4).

Table(4):Changesintotalcholesterol(TC),triglyceride(TG),HDL-C,LDL-C,VLDL-C,TC/HDL ratio and LDL/HDL ratio in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodiumsaccharin
TotalCholesterol(mg/dl)	121.1±0.38	130±2.66*	105±0.7**
%ofchange		7%	-13%
Triglycerides(mg/dl)	135±0.7	128±2.1*	120±0.8**
%ofchange		-5%	-11%
HDL-C(mg/dl)	59.4±0.48	65±1.7*	51±2.48*
%ofchange		9%	-14%
LDL-C(mg/dl)	34.6±0.48	39.4±2.1	30±2.2
%ofchange		14%	-13%
VLDL(mg/dl)	27±0.1	25.6±0.8	24±1.35
%ofchange		-5%	-11%
TC/HDL	2.04±0.015	2±0.07	2.058±0.067
%ofchange		-2%	0.8%
LDL/HDL	0.6±0.01	0.6±0.05	0.58±0.02
%ofchange		0%	-3%

Valuesrepresentmean±SE(standarderror).(*P**<0.05,*P***<0.01ascomparedtocontrolgroup)

Kidneyfunctions:serumureaandcreatinineshowedsignificantincrease(p<0.05)insucralosegroupand highly significant increase (p<0.01) in sodium saccharin group as compared to control animals (Table 5).

Table(5):Serumureaandcreatininelevelsincontrol,sucraloseorsodiumsaccharintreatedanimals.

Groups / parameters	Control	Sucralose	Sodiumsaccharin
Urea(mg/dl)	37.3±0.66	41.4±1.1*	53.4±0.9**
%ofchange		11%	43%
Creatinine(mg/dl)	1.18±0.025	1.5±0.098*	1.9±0.02**
%ofchange		27%	62%

Valuesrepresentmean±SE(standarderror).(*P**<0.05,*P***<0.01ascomparedtocontrolgroup)

Hormones: sucralose group revealed no detectable change in the level of testosterone hormone, but there was a highlysignificant decrease (p<0.01)found inits level insodiumsaccharin group as comparedtocontrol rats. On the other hand, a significant decline (p<0.05) in the levels of both T3& T4 concentrations in sucralose group,butsodiumsaccharingroupshowedsignificantincrease(p<0.05) intheirconcentrationsascomparedto control values (Table 6).

Table(6):Seruminsulin,testosterone,T3andT4levelsincontrol,sucraloseandsodiumsaccharin treated animals.

Groups / parameters	Control	Sucralose	Sodiumsaccharin
Testosterone(ng/dl)	1.03±0.004	0.99±0.03	0.65±0.05**
%ofchange		-4%	-37%
T3(ng/dl)	47.9±0.6	43.1±1.38*	53±1.5*
%ofchange		-10%	12%
T4(µg/dl)	3.036±0.009	2.5±0.16*	3.5±0.14*
%ofchange		-18%	15%

Valuesrepresentmean±SE(standarderror).(*P**<0.05,*P***<0.01ascomparedtocontrolgroup)

DISCUSSION

The impact of artificial sweeteners on human health is still a matter of doubtful dispute. The present study revealed that body weight wasn't change by using sucralose. Several studies in rodents announced that sucralose modulates physiological processes involved in nutrient absorption and body weight regulation through its interaction with sweet taste receptors (called T1R2/T1R3) located in enteroendocrine cells of the GIT, pancreatic ß cells, and the hypothalamus[11].

The present results showed highly significant reductioninbodyweightpercentinsaccharingroup when compared to the control group. In harmony with this result **Dib *et al.*[12]**reported a significant reduction in body weight of rats (50%) after administration of sodium saccharin for 14 days. They attributed this weigh loss to the reduction of food consumption per day as a result of hypotriglyceridemia and hypocholesterolemiceffect of sodium saccharin [13].

The administration of sucralose reducedglucoselevel, ascomparedtothecontrol.Itappears that the decrease of glucose might be resulted from a reduction in its absorption[13], this result was in parallel with **Abou-Donia *et al.*[14]**who reported that the administration of sucralose at 1.1 - 11 mg/kg to male rats for 12-week interferes with the absorption of nutrients and drugs.

In the present work, there was highlysignificant reduction in blood glucose level of saccharin group as compared to the control group. **Amin*etal.*[5]**have declared thatoraladministration of low and high doses of saccharin to rats for 30 days induced a decrease in blood glucose, which was in parallel with **Jacquille*t et al.*[15]**. The hypoglycemic effect of saccharin in rats is in accordance with **Abdallah[13]**who noticed that consumption of large amounts of saccharin might reduced blood glucose concentration. This may be due to that saccharin can trigger the release of insulin and thereby reduce blood glucose leve[16].

In our study sucralose reduce insulin secretion. There is a study revealed that sucralose has been indicated to create prediabetic state. The study announced that sucralose ingestion causes significant damage to pancreas leading not only to breakdowninitsarchitecture but alsotodestruction of its islets and β cells[17].

The study of **AmornpanLertrit*et al.*[18]**demonstrated reduced acute insulin response (AIR) after a 4-week ingestion of sucralose. This clarify the present results which showed a decrease in HOMA-IR. This may indicate that chronicexposuretosucraloseleadsfirstlytoincreased insulin secretion, and later to reduction of insulin secretion via depletion of insulin secretorygranules, whichleaded to loss of first phaseinsulinsecretion. A defect in early phase insulin secretion is an early predictor of type 2 diabetes mellitus[18].

This study showed a reduction in insulin and HOMA-IR of sodium saccharin group, this is in concomitant with **Bailey *et al.*[19]**who reported a reduction of hyperinsulinemia, decrease the insulin resistance and improve glycemic control during saccharin consumption in hyperglycemic obese mice.

In the present study there was a significant reductioninproteinprofile in sucralose group butit washighlysignificantdecreaseinsodiumsaccharin group. **Hassan and Yousef[20]**announced an inhibitory effect of some food additives on the biosynthesis of protein and albumin, which indicated that the liver is unable to perform its functions. This may be resulted in a reduction of protein synthesis or especially albumin through the effect of sucralose or saccharin on the liver by inhibiting oxidative phosphorylation .

There was significant increase in ASAT and ALAT enzymes to the rats received sucralose , this may be due to hepatotoxicity and liver damage, as the more severe the liver damages the higher the release of the liver enzymes [21].

Also, there was a highly significant increase in ASAT and ALAT enzymes after using sodium saccharin. **Osfor& Elias[22]**reported that saccharin treated rats showed a significant increase in ALAT activity after both 6 and 12 weeks ofadministration. ASAT levels were significantly higher in rats received saccharine, where chronic saccharin intake reflects some abnormal changes in metabolic, hormonal and neural responses in males and female rats[23]. The elevation in serum aminotransferase activities could be due to severe effects caused by free radicals that interact with cellularmembranesorrelatedtobreakdownofliver parenchyma.Thechangesinliverfunctioncouldbe due to hepatocellular impairment which subsequently caused leakage and release of greater than normal levels of intracellular enzymes into the blood[16].

The administration of sucralose reduces the level of triglycerides, while elevates the level of total cholesterol and HDL-C when compared to control rats. The decrease of triglycerides might be attributed to the effect of sucralose on the peroxisome proliferator-activated receptors-alpha (PPAR-α) thus increasing the expression of lipoproteinlipase.Inaddition,activationofPPAR-γ inadiposetissuestimulatestriglyceridestorage[24].

Theincrease of HDL-C might result fromtheeffect of sucralose on PPAR-α and activation of apo A-I and apo A-II. The increased cholesterol following administration of sucralose may be attributed to increased intestinal absorption and/or increased cholesterol synthesis[25].

Our study revealed that saccharin induced hypocholesterolemiaandhypotriglyceridemiawhen compared to control groups which was inagreementwiththeresultsrecordedby**Ashourand Abdelaziz**[26]. The mechanism of hypocholesterolemic and hypolipidemic effect induced bysaccharin maybe duetothesuppressive offect of saccharin on liver enzymatic activity of acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange leading to a reduction of available cytoplasmic acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids[27]. Furthermore, liver acetyl-CoA carboxylase, phosphatidatephosphohydralase, and glycerol-3-phosphate acyl transferase activitieswere reduced by the saccharin analogues. Suppression of these enzymes would lead to a reduction of triglyceride synthesis. The saccharin analogues accelerated bile excretion of cholesterol metabolites and increased the fecal excretion of the cholesterol, triglycerides, neutral lipids, and phospholipids, thus, the liver and plasmalipoprotein lipid contents including, cholesterol, triglycerides, and neutral lipids were markedly reduced by the saccharin[5].

There was a significant increase in creatinine and urea in sucralose treated group, while it was highly significant increase in sodium saccharin group. This may be due to the toxic effects of these artificial sweeteners on the kidney that can lead to disordersintherenal function, duetoareductionin glomerular filtration rate followed by retention of urea and creatinine in the blood[28]. Also, there is a study of **Singh**[29]who reported the ability of saccharin to produce bladder cancer in two generation biassays.

By using sucralose , there was a decrease in thyroid hormones T3 and T4.

Study of **Goz´dzik*et al*.**[30]explained that sucralose intake seems to diminish thyroid axis activity by decreasing TPO activity, TSH, and plasma total TH concentrations, but at the same time, it increases both free T3 and T4 indexes. Those findings confirmed that sucralose is physiologically active and may stimulate disturbances in pituitary-thyroid axis activity, and also regular exposure to sucralose might alters the thyroid axis response to ingested food[31].

Also, in **"Very Well health" magazine**[32]there was a report about artificial sweeteners and theireffectonthyroidactivity: accordingtoa report presented at the 2015 International Thyroid Congress[33], the use of artificial sweeteners may be linked to the development of a type of hypothyroidism called Hashimoto's thyroid disease (HT). The research, conducted by investigators in New YorkCity, who had been positivelydiagnosed with (HT). The use of artificial sweeteners within this population—including aspartame (Equal, NutraSweet) and sucralose (Splenda)—correlated with elevated levels of thyroid stimulatinghormone (TSH). Increased TSH levels are considered indicative of hypothyroidism. In the study, two of every three who had subsequently stopped using artificial sweeteners had a complete reversal oftheir HT. Their thyroid antibodies gradually returned to normal, and they were even able to stop their hormone replacement medication. This response to discontinuing the sweeteners supports the idea that artificial sweeteners may play a role in thyroid disease[32]. On the other hand, there was a significant increase in thyroid hormones when rats received sodium saccharin. These changes in thyroid hormones could also be attributed to the alteration in the pitutary – thyroid axis as a consequence of the stressing effect of sodium saccharin[34].

There was a significant decrease in testosterone hormone in rats received sodium saccharin. Inmice, an earlier study discussed the effects of saccharin on the reproductive functions has shown that saccharin reduced fertility. CAMP and PKAare known to be key regulators of steroidogenesisin Leydig cells[35]. In Leydig cells, higher cAMP levels are essential to produce testosterone in response to LH. In the present study, thedifferential changes found in LH level after saccharin treatment suggest that there are pituitary effectsalteringLH,andthese changes arerelatedto different levels in testosterone production. Also, saccharin leads to attenuated sperm quality (including sperm count, viability, motility, and augmented abnormalities)[34].

CONCLUSION

We recommended to reduce or stop using artificialsweetenersandreplacethemwithnatural products.Othertoxicologicalstudiesmustbedone on them.

Comment [SC3]: Based on animal data evaluation . Human data needs to be extrapolated

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