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ORIGINAL ARTICLE

Effects of Artificial Sweeteners Aspartame and Sucralose on the Size of Hepatocytes in Rat Liver

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ABSTRACT

Background: The excessive use of artificial sweeteners now a days just to lose weight and to remain fit and due to other reasons without knowing its effects on liver gave me a thought to conduct this study to see whether they are safe or not for liver health.

Objectives: The objectives of the study were to see the effects of artificial sweeteners Aspartame and Sucralose on the size of rat hepatocytes and to compare their effects to choose relatively safe artificial sweetener for routine use.

Methods: Fifty adult Wistar albino rats used in this study were randomly divided into five groups. Group I (control group) animals were given drinking water by oral gavage. The animals of group II and III were given low (40mg/kg body weight) and high doses (1000mg/kg body weight) of Aspartame respectively. Similarly, animals of group IV and V were given low (5mg/kg body weight) and high doses (1000mg/kg body weight) of Sucralose respectively. Doses were given by oral gavage once daily, six days a week for a total duration of eight weeks. At the end of the experiment histological observation of liver in all animal groups was made.

Results: Microscopic examination revealed that hepatocytes of group III animals (high dose group of Aspartame) had largest size with the mean value of 20.86±2.00µm as compared to the control group hepatocytes having mean value of 16.46±1.74µm.

Conclusion: The increase in the size of hepatocytes was seen in high dose groups of Aspartame and Sucralose whereas changes seen in low dose groups of Aspartame and Sucralose were not significant.

Keywords: Artificial sweeteners, Histology of rat liver, Size of hepatocytes.

INTRODUCTION

Sugars are an essential part of food and drinks and it is added to food during processing to improve the taste. Use of energy and vitamin drinks, iced tea and sugar sweetened beverages has risen across the globe.[1] Diabetes Mellitus becomes easily treatable with artificial sweeteners because they are slowly metabolized allowing blood sugar levels to remain stable over time.[2] Similarly, individuals with reactive hypoglycemia can overcome their hypoglycemia using sugar substitutes. [3] Artificial sweeteners help to control dental problems.[4] Sugar substitutes also increase the flavor of foods and beverages as well as used as an alternative to refined white sugar.

Artificial sweeteners can be nutritive or non-nutritive. Nutritive sweeteners provide about four Calories /gm of food and non-nutritive ones add no energy value to the food. There are five nonnutritive sweeteners approved by FDA; Acesulfame potassium, Saccharin, Neotame, Aspartame and Sucralose.[5]

The use of nonnutritive sweeteners has increased dramatically in the past few decades and approximately 15% of the U.S population is estimated to be using non-nutritive sweeteners among those Aspartame and Sucralose are the most commonly used sweeteners.[6]

Aspartame is a white crystalline powder 180 times sweeter than sugar. It consists of phenylalanine and aspartic acid which are linked together by methanol. Phenylalanine is an essential amino acid which breaks down to fumarate and acetoacetate during energy metabolism whereas aspartic acid is a non-essential amino acid and acts as a brain neurotransmitter. Methanol is broken down into formaldehyde which is oxidized to formate in the liver and results in the formation of oxygen free radicals so that it can be excreted out. If taken in large quantities the metabolic machinery of liver is failed to handle it leading to a state of oxidative stress.[7]

In comparison the bulk of Sucralose that is ingested is excreted out in the faces whereas only 11-27% of ingested is absorbed.[8]

In the past many studies have been conducted to see various effects of artificial sweeteners. This study is designed to see the effects of two most commonly used artificial sweeteners Aspartame and Sucralose on the liver of albino rats which acts as a main center of metabolism. The microscopic anatomy of rat liver shows that it is composed of parenchymal (hepatocytes 80%) and non-parenchymal (endothelial cells, Kupffer cells, Ito cells and Pit cells) cells.[9] Hepatic acinus is a diamond shaped area of liver involving two portal triads and two central veins at its boundary. It is subdivided into periportal (Zone I), transitional (Zone II) and paracentral (Zone III) zones.[10]

Objectives:

1- To see the effects of artificial sweeteners Aspartame and Sucralose on size of hepatocytes in rat liver.

2- To compare their effects to choose relatively safe artificial sweetener for routine use.

METHODS

Study Design: Randomized control trial

Sampling Technique: Sampling was done by using lottery method

Study Settings: The study was conducted in the Anatomy department of King Edward Medical University, Lahore. Departmental ethics committee of King Edward Medical University, Lahore approved this study with reference to the letter number 408/RC/KEMU.

Duration of Study: Three months (March 2018 to May 2018)

Sample Size Calculation: This sample size was calculated using confidence level of 95% and power of test as 90% along with expected hepatic fat aggregation of 100% ±20 in control group and 130% ±20 in the experimental groups.[11] For each variation of sweetener and its dose a group of 10 was added and total sample size of 50 animals was calculated.

Adult Wistar albino rats of both gender (average age between 2 to 3 months) and weight between 175-225gms were kept in the animal house of University of Veterinary and Animal Sciences (UVAS). They were acclimatized for 2 weeks. A 24-hour light and dark cycle was maintained at room temperature between 22-25^{0}C.These animals were provided by food and water ad-libitum. The food was in the form of chick feed.

Grouping of animals: Animals were divided randomly into five groups, one control group and four experimental groups. In each group there were 10 animals. Groups were labeled as I, II, III, IV and V. Similarly cages of experimental animals were also labeled as group I, II, III, IV and V. Animals in each group were numbered 1, 2, 3 to 10 using color.

Group I (Control group): There were 10 animals in this group. Each animal of this group was administered with 3ml of distilled water with oral gavage.

Group II & III (Experimental groups of low and high doses of Aspartame respectively): There were 10 animals in each group.10 animals for low dose and 10 for high dose of Aspartame.

Group IV & V (Experimental groups of low and high doses of Sucralose respectively): There were 10 animals in each group.10 animals of low dose and 10 of high dose of Sucralose.

Preparation of doses: 3ml once daily dose was prepared for each animal.

Preparation of low dose of Aspartame of group II: As ADI (Acceptable Daily Intake) of Aspartame in humans is 40mg/kg body weight so low dose for group II animals was calculated to be 8mg for an average 200gm weight rat and high dose for group III animals was calculated to be 200mg for an average 200gm rat as LD50 of oral Aspartame is>10,000mg/kg in rats.[12]

For preparation of 8mg dose of each rat of this group, 30ml of distilled water was taken and 4.5 tablets (81mg) of Aspartame were dissolved in it, making 3ml of the solution containing 8mg of Aspartame.

Preparation of high dose of Aspartame of group III: For preparation of 200mg dose of each rat of this group, 30ml of distilled water was taken and 112 tablets (2016mg) of Aspartame were dissolved in it, making 3ml of the solution containing 200mg (approximately) of Aspartame.

Preparation of low dose of Sucralose of group IV: As ADI (Acceptable Daily Intake) of Sucralose is 5mg/kg body weight in humans so low dose for group IV animals was calculated to be 1mg for an average 200gm weight rat and high dose for group V animals was calculated to be 200mg for an average 200gm rat as LD50 of oral Sucralose is>10g/kg body weight in rats.[13]

As average weight of each rat was 200gm so 1mg was the low dose of Sucralose for each rat.

For preparation of 1mg dose of each rat of this group, 650ml of distilled water was taken and 650mgs (One teaspoon of Sucralose) were dissolved in it, making 1ml of the solution containing 1mg of Sucralose.

Preparation of high dose of Sucralose of group V: Average weight of each rat was 200gm so 200mg was the high dose of Sucralose for each rat.

For preparation of 200mg dose of each rat of this group, 30ml of distilled water was taken and 2000mgs of Sucralose (approximately 3 teaspoons of Sucralose) were dissolved in it, making 3ml of the solution containing 200mg of Sucralose.

Preservation of doses: Doses were prepared weekly and kept at room temperature (25-30 ^{0}C).

Dose administration to albino rats by oral gavage: For administration of dose, a pediatric nasogastric (NG) tube of number 8 was attached with a 10cc disposable syringe (Figure 4). NG tube with attached syringe was introduced into bottle labeled as group I and 3ml dose (distilled water) was sucked into it. With gloved hands animal 1 of group I (control group) was brought out of its cage by holding the tail and later grasped from its back and neck so that it opened its mouth. At that time NG tube was introduced from the side into its mouth deep up to the pharynx and immediately dose was administered. The grip was loosened and animal was returned to the cage. Animal was also observed for any signs of chocking.

All animals of each group were given the doses in same way.

Doses were administered once daily, six days a week, for total duration of 8 weeks. At the end of 8 weeks all animals in each group were anaesthetized by using chloroform anesthesia.

Procedure of dissection: The rat was stretched out on the dissection board and the limbs were stretched out. A midline skin incision was made, extending from the xiphisternum to the pubic symphysis. Abdominal wall was opened with the help of scissors.

After identifying the liver, falciform and coronary ligaments were cut and liver was removed. The specimens of liver were kept in 10% formalin solution in separate labeled plastic jars.

Tissue processing: The fixed liver specimens were placed in individual plastic cassettes, labelled and were then processed in automatic tissue processor (Histotech III USA). In it the tissue went through dehydration, removal of fixative, clearing and paraffin infiltration. Tissue blocks were prepared, placed in refrigerator to consolidate further and then stored in freezer. Sectioning was done by using Histoline RM 2258 rotary microtome. Every fourth of the serial sections was lifted singly from the ribbon and spread on pre labeled albumin glass slide. Extra water was drained and slides were dried by a slide warmer and then were put in an incubator for 15 minutes at 60^{o}C temperature, dewaxed and stained with Haematoxylin and Eosin[14]. Light microscopy under different magnifications was done.

Measurement of size of hepatocytes in µm: Under light microscopy, micrometry[15] was performed at 400X. In each slide 20 hepatocytes were selected randomly. Size of each hepatocyte was measured in transverse, anteroposterior and oblique dimensions. Mean of these three dimensions was calculated to get average size of a hepatocyte. The size of 20 hepatocytes was calculated in a similar manner and an average of that was taken as a size of hepatocytes in that particular slide.

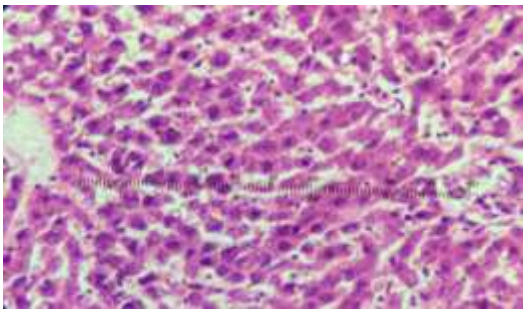

Figure 4a: Measurement of size of hepatocytes by oculometer (visible as graduated scale) in control group (Group I). (H&E) 40X×10X=400X

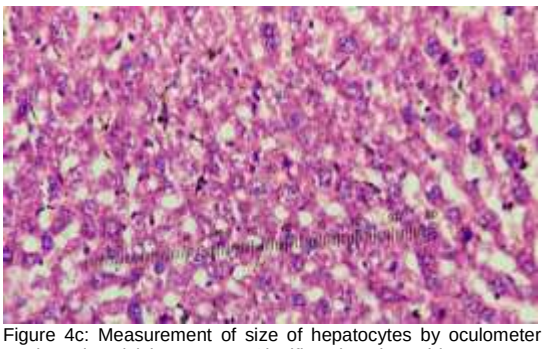

Figure 4c: Measurement of size of hepatocytes by oculometer (visible as graduated scale) in group III. Significantly enlarged hepatocytes are visible. (H&E) Magnification 40X×10X=400

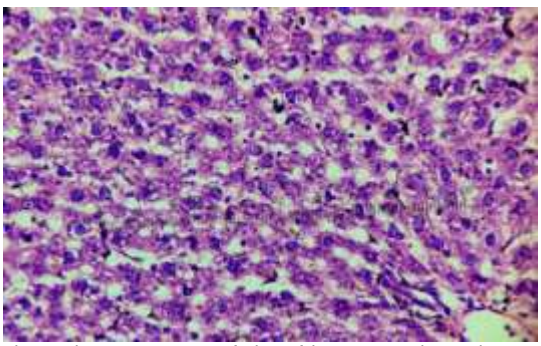

Figure 4b: Measurement of size of hepatocytes by oculometer (visible as graduated scale) in group II. (H&E) Magnification 40X×10X=400X

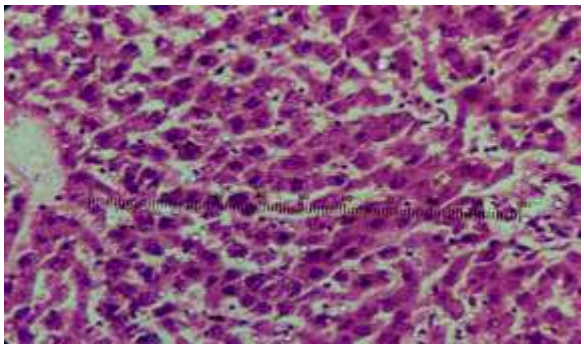

Figure 4d: Measurement of size of hepatocytes by oculometer (visible as graduated scale) in group IV. (H&E) Magnification 40X×10X=400X.

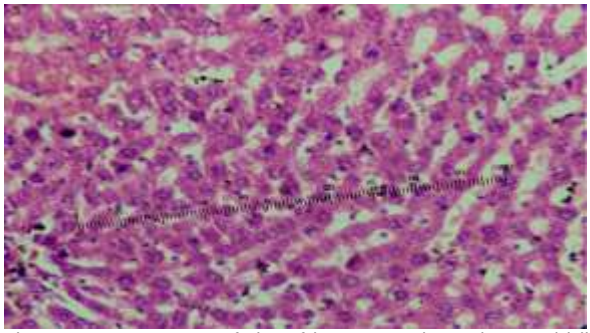

Figure 4e: Measurement of size of hepatocytes by oculometer (visible as graduated scale) in group V. (H&E) Magnification 40X×10X=4

RESULTS

In group I (control group) mean size of hepatocytes was 16.46 ± 1.74 µm which was taken as a standard to compare the size of other groups. In group II (low dose group of Aspartame) mean size of hepatocytes measured was 16.09 ± 0.85µm. It was quite similar to the mean size of hepatocytes in control group with statistically insignificant difference. In group III (high dose group of Aspartame) mean size of hepatocytes measured was 20.86 ± 2µm. This mean size of hepatocytes was significantly increased as compared to all other groups. In group IV (low dose group of Sucralose) size of hepatocytes seemed to be decreased as compared to the other groups. On micrometry mean size of hepatocytes measured was 15.62 ± 1.3 µm (Table 1). This mean size of hepatocytes was significantly decreased as compared to all other groups but it was statistically insignificant (Table 4). In group V (high dose group of Sucralose) mean size of hepatocytes measured was 17.54 ± 2.04µm (Table 1). This mean size of hepatocytes was increased as compared to the control group.

Table 2: Size of hepatocyte of animals in all experimental groups.

	Size of hepatocytes (µm)			
Group	Mean	Standard Deviation	Minimum	Maximum
Group - I	16. 46	1. 74	13. 92	19. 2
Group - II	16. 09	0. 85	14. 64	17. 76
Group - III	20. 86	2	17. 28	24
Group - IV	15. 62	1. 3	13. 92	17. 76
Group - V	17. 54	2. 04	16. 08	23. 04

Key
Group –I Control group
Group –II Experimental group (Low dose of Aspartame)
Group –III Experimental group (High dose of Aspartame)
Group –IV Experimental group (Low dose of Sucralose)
Group –V Experimental group (High dose of Sucralose)

Statistical Analysis: Data was entered and analyzed by using SPSS 21. 0. The description of quantitative variable of size of hepatocytes was done by using mean ± S. D. One way ANOVA was used for the comparison among groups. Post-hoc analysis was done using Tukey's test.

P-value less than 0.05 was taken as statistically significant

Table 3: Pair wise comparison of size of hepatocytes of all experimental groups by using Tukey's test.

Group(I)	Group(J)	Mean Difference (I-J)	Standard Error	Significance
Group – I	Group – II	0. 37	0. 74	0. 986
	Group – III	-4. 40*	0. 74	<0. 001
	Group – IV	0. 84	0. 74	0. 787
	Group – V	-1. 08	0. 74	0. 589
Group – II	Group – III	-4. 77*	0. 74	<0. 001
	Group – IV	0. 46	0. 74	0. 970
	Group – V	-1. 45	0. 74	0. 296
Group – III	Group – IV	5. 24*	0. 74	<0. 001
	Group – V	3. 32*	0. 74	<0. 001
Group – IV	Group – V	-1. 92	0. 74	0. 087

Key
Group –I Control group
Group –II Experimental group (Low dose of Aspartame)
Group –III Experimental group (High dose of Aspartame)
Group –IV Experimental group (Low dose of Sucralose)
Group –V Experimental group (High dose of Sucralose)

Mean size of hepatocytes was observed to be maximally increased in group III (high dose of Aspartame) which was also statistically significant and in group V (high dose of Sucralose) which was statistically insignificant when compared with control group. Significant statistical difference was present between low and high dose groups (group II and III) of Aspartame.

There was also a significant statistical difference present between high dose groups (Group III and V) of Aspartame and Sucralose.

DISCUSSION

In the control group mean size of hepatocytes measured was 16.46 ± 1.74 µm which was taken as a standard for comparison with other groups. In low dose group of Aspartame mean size of hepatocytes was quite similar to the mean size of hepatocytes in control group. In high dose group of Aspartame mean size of hepatocytes measured was significantly increased as compared to all other groups. In low dose group of Sucralose, the mean size of hepatocytes was significantly decreased as compared to all other groups. In high dose group of Sucralose, the mean size of hepatocytes was increased as compared to the control group.

The statistically significant increase in the mean size of hepatocytes was only seen in group III (high dose of Aspartame) whereas increase seen in group V (high dose of Sucralose) was statistically insignificant. Significant statistical difference was also present between low and high dose groups of Aspartame. Similarly, significant statistical difference was also seen between high dose groups of Aspartame and Sucralose.

In present study the hepatocytes were labeled to be enlarged when compared with control group and this fact was confirmed by micrometry and found to be statistically significant. In this study maximum increase in the size of hepatocytes was seen in high dose group of Aspartame (Group III) whereas increase in the size is also observed in high dose group of Sucralose (Group V). The reason for this increase in size could be, cellular edema as a result of cellular injury, hypertrophy as a phase of degenerative process or more convincingly due to cellular infiltration, vacuolation and fatty change.[16]

Finamor I. et al, observed alterations in oxidative defense status of liver after administration of Aspartame to albino rats.[17]Mohamed El-sayed Alkafafy. et al, gave Aspartame in 250 to 1000mg/kg body weight doses to two experimental groups for eight weeks duration and observed degenerative changes of hepatocytes in the form of cellular swelling and other were necrotic. Changes seen were more pronounced in high dose group of rats.[18]

It is suggested by Jiang. et al, that Sucralose can cause

liver damage by enhancing the growth of gut bacteria that are more efficient in getting energy from our food and turning that into stored fat leading to increased risk of liver diseases.[19]

After analyzing the results of this study some question is raised as low dose of Aspartame may be safe to use because it did not cause significant increase in the size of hepatocytes. Similarly, even high dose of Sucralose did not cause any significant increase in the size of hepatocytes so is it a safe sweetener to be used? Decrease in the size of hepatocytes is another important fact seen in this study which needs further exploration of facts.

To solve the above-mentioned queries, this study has some shortcomings which should be overcome in the next studies such as exposure of liver to artificial sweeteners for a longer duration of time. So, this study is opening new gateways for the next researchers.

CONCLUSION

The conclusion drawn is thus that Aspartame and Sucralose are less damaging for liver if taken in low doses but in large doses both are damaging for hepatocytes though Sucralose is a comparatively safe artificial sweetener to be used in large doses.

It is further recommended that the facts about harmful effects of these artificial sweeteners should be brought to the notice of medical personal as well as highlighted to the general population specially diabetics and obese people.

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