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EFFECTS OF NON-CALORIC ARTIFICIAL SWEETENERS (NAS) ON GLYCAEMIC STATUS IN MALE SWISS ALBINO MICE

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Keywords:

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ABSTRACT: Objective: The objective of the study was to estimate the effects of Non-caloric Artificial Sweeteners (NAS) on glycaemic status in male Swiss albino mice. **Methods:** 36 male Swiss albino mice were selected and divided into six groups of 6 animals each. Group 1 served as control and received a normal pellet diet with drinking water. Groups 2, 3, 4, 5 and 6 received normal pellet diet and drinking water along with oral administration of Glucose, Saccharin, Sucralose, Aspartame, and commercial Aspartame, respectively for 8 weeks. Bodyweight of the animals was measured every week from baseline until the end of the experiment. Fasting blood glucose, serum glycated hemoglobin (HbA1c), serum insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), renal function tests, liver enzymes, fecal culture, and lipid profile were assessed during baseline and end of the study. **Results:** The bodyweight of the animals was increased in all groups. Fasting blood glucose and HbA1c were significantly increased in NAS groups. Saccharin group showed increase in serum insulin, HOMA-IR, liver enzymes, urea, and creatinine. Aspartame group had dyslipidemic changes with deranged renal function tests. The fecal microbial culture showed *Escherichia coli* and *Citrobacter koseri* during baseline and *Escherichia coli* at the end of experiment. **Conclusion:** This study provides evidence that long term consumption of NAS can lead to significant increase in fasting blood glucose and HbA1c. The treating physicians, diabetic patients, and the general population may have to consider the metabolic problems associated with NAS before advising and/or choosing NAS for regular consumption.

INTRODUCTION: Non-caloric Artificial sweeteners (NAS) are known as "low-calorie sweeteners", "non-nutritive sweeteners", "high-intensity sweeteners", "non-sucrose sweeteners," "Intense sweeteners", "sugar substitutes" and "sugar-free sweeteners".

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They are used as food additives to provide the taste of sucrose (sugar) as they offer very intense sweet taste with less or zero calories.

According to US FDA, sweeteners that contain zero or less (<5) calories per serving (the recommended portion of food to be eaten) are said to be Non-caloric [1]. Currently, there are six artificial sweeteners approved by US FDA in the market. They are

1. Saccharin
2. Aspartame
3. Sucralose
4. Acesulfame-K

5. Neotame
6. Advantame

Saccharin is 200-700 times sweeter than conventional sugar, but it has an unpleasant bitter or metallic aftertaste especially at higher concentrations [2]. Often it is blended with other sweeteners like aspartame to mask the bitter taste so as to balance the sweet taste. The acceptable daily intake (ADI) of saccharin is 15 mg/kg in humans. Saccharin was linked to the development of bladder cancer based on animal study outcomes in rats during the 1960s. However, later investigations revealed that the mechanism by which carcinogenesis occurs is not relevant in humans and it happens only in animals. Hence WHO reclassified saccharin from the previous "possibly carcinogenic to humans" to "not classifiable as carcinogenic to humans". The USA also removed saccharin from the list of chemicals with cancer potential in May 2000 [3].

The other popular NAS is Aspartame. The ADI is 50 mg/kg in humans. Aspartame is 200 times sweeter than sucrose and hence the quantity needed to achieve the level of sweetness is less than 1% of the amount of sugar required. The sweetness has a slower onset and longer duration than sugar.

With regard to the safety of aspartame, it is considered to be safe in humans at the doses recommended for daily consumption, though there are publications addressing the safety issues of aspartame. The reports implicate the safety concerns of aspartame to three of its metabolites, methanol, phenylalanine and aspartic acid. A large intake of aspartame can increase the phenylalanine and lead to its accumulation in brain that can predispose individuals to get seizures [4]. Studies have also found association between aspartame and allergic manifestations including type IV delayed hypersensitivity reactions [3, 5]. Aspartame contains phenylalanine; hence, it is contraindicated in patients with phenylketonuria (PKU).

Sucralose is one of the popular NAS used in foods and beverages. The ADI of sucralose is 5 mg/kg in humans. It is 600 times sweeter than sucrose, and a very small amount is only needed to achieve sweetness as that of sucrose. Sucralose is considered to be safe among all the populations including people with diabetes mellitus and other chronic health problems. Grotz VL *et al*., did a study in 128 diabetic people by administering 3 times the maximum recommended dose of sucralose to investigate the safety and observed no adverse events and changes in glucose levels [6].

Acesulfame potassium (Acesulfame-K) was approved as a flavour enhancer in food and as a non-nutritive sweetener in carbonated drinks, baking products, frozen desserts, candies. Neotame and Advantame are the agents approved after the year 2000. Neotame is the N-alkylated product of "Aspartame". It was approved by the USFDA as a general-purpose sweetener in July 2002. Advantame was recently approved by US FDA as a general-purpose sweetener and flavor enhancer in 2014. If Advantame is used in chewing gums, it substantially enhances the duration of chewing compared to other sweeteners. It is also used in milk products, frozen dairy, and non-alcoholic beverages.

Health Implications of NAS: Non-caloric artificial sweeteners came into use for two important reasons. One is that they did not seem to be associated with the health hazards of sugar and the other reason is that they are cheaper when compared to the costlier sugar. But there are reports implicating NAS with many health problems.

DeNoon and Daniel *et al*., (2005) investigated the influence of beverages on body weight. They analyzed the bodyweight of subjects who regularly had "diet drinks" that contained NAS and who had naturally sweetened drinks. It was reported by the authors that those who had NAS containing beverages had more weight gain and obesity than the other people [7].

The hypothesis attributed to weight gain seen with the use of NAS is that these agents do not increase blood sugar, may cause hypoglycemia, which will, in turn, result in more food intake and weight gain. This was also supported by preclinical experiments conducted in rats. Those rats that were fed with NAS had more food intake and body weight [8, 9].

There are reports implicating the use of aspartame and saccharin with carcinogenicity in humans and animals. Aspartame is also associated with allergic

reaction including type IV hypersensitivity reactions and seizures [3, 4, 5].

Suez *et al*., (2014), reported that chronic administration of saccharin, sucralose, and aspartame for a period of 11 weeks in mice resulted in glucose intolerance. They analyzed gut microbial flora and commented that NAS agents changed the gut microbial flora, which could be the reason for glucose intolerance. They also did an extended study in humans and reported that people who had the habit of consuming NAS had increased body weight, high fasting blood glucose and elevated HbA1c [10]. In fact, the study of Suez *et al.,* [10] was one of the triggering factors to undertake the present study that was planned to investigate the effects of long term consumption of NAS on the glycemic status in male Swiss albino mice.

The objective of the study was to evaluate the effect of chronic administration of Non-caloric Artificial Sweeteners (Saccharin, Sucralose, and Aspartame) on fasting blood glucose, glycated hemoglobin (HbA1c), serum insulin, insulin resistance, renal function tests, liver enzymes, fecal culture, and lipid profile.

MATERIALS AND METHODS: The study was initiated after getting approval from the Institutional Animal Ethics Committee (IAEC) at Chettinad Hospital and Research Institute (CHRI). The approval number was IAEC 4/ALr.No.17/ Dt.12.12.17. The study was conducted according to the CPCSEA guidelines.

The study was planned to evaluate the glycemic effects of the raw materials of three artificial sweeteners namely, Saccharin, Sucralose, and Aspartame. The members of the Institutional Animal Ethics Committee (IAEC) during the review of the proposal, recommended including at least one more group to investigate any one of the commercially available NAS. Hence, the fourth NAS group was allocated to sugar-free gold, a commercial formulation of Aspartame.

A total of 36 male Swiss albino mice of 10-12 weeks old, weighing about 25-30 g, were selected and used in the study. They were obtained from the central animal house of Chettinad Hospital and Research Institute (CHRI).

The animals were housed in suitable cages in the animal house and acclimatized for a period of 7 days before the onset of the experiment. They were maintained at 24 ± 2 °C with good ventilation, and 12:12 h light and dark cycle with free access to food and water before and throughout the experimental period. The mice were divided into 6 groups of 6 animals each, with one control group and five experimental groups.

Study interventions used in the experiment were

- Glucose monohydrate at a dose of 50 mg/d [11]
- NAS used in the study were Saccharin sodium dihydrate, Sucralose, Aspartame, and commercial Aspartame. Sugar-free gold which contains 4% aspartame, 96% dextrose and maltodextrin were used for commercial Aspartame. Except the commercial Aspartame, all the other three NAS were pure forms of the respective active ingredients.

The animal dose of NAS was calculated based on the Acceptable daily intake (ADI) in humans. The animal equivalent dose of NAS in mg/kg was derived and corrected according to the body surface area of mice by using the following formula [12].

Animal dose (mg/kg) = Human equivalent dose (mg/kg)/0.08

The study interventions were dissolved in 0.5 ml of distilled water and administered orally for 8 weeks [13]. The various groups and their respective interventions are as follows in **Table 1**.

TABLE 1: STUDY INTERVENTIONS USED IN THE EXPERIMENT

Groups	Interventions
Group 1 (C- Control)	Pellet diet + water
Group 2 (GLU-Glucose)	Pellet diet + water + Glucose (50 mg/d)
Group 3 (SAC- Saccharin)	Pellet diet + water + Saccharin (6 mg/d)
Group 4 (SUC-Sucralose)	Pellet diet + water + Sucralose (2 mg/d)
Group 5 (ASP- Aspartame)	Pellet diet + water + Aspartame (20 mg/d)
Group 6 (SFG-Sugar free gold)	Pellet diet + water + Commercial Aspartame (20 mg/d)

The animals were weighed every week for the entire duration of the study. The doses administered were titrated based on the bodyweight

measurements every week until the last week of the study. The following parameters were analyzed at baseline (before the initiation of administration of the interventions) and end of the study.

- Fasting blood glucose
- Serum glycated hemoglobin (HbA1c)
- Serum Insulin
- Homeostatic model assessment of Insulin resistance (HOMA-IR)
- Lipid profile – Total cholesterol (TC), Triglycerides (TGL), High-density lipoprotein (HDL), Low-density lipoprotein (LDL)
- Renal function tests – urea and creatinine
- Liver function tests – Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP) and Gamma-GlutamylTranspeptidase (GGT)
- Fecal culture using Blood agar, Mac Conkey agar and Xylose-lysine-deoxycholate agar (XLD)

Blood was collected through retro-orbital venous puncture for analysis and the animals were anesthetized using halothane during the blood collection. The blood parameters were measured as per the standard operating procedures using auto analyzers. Serum Insulin and HbA1c were measured using ELISA method [14, 15].

The insulin resistance score (HOMA-IR) was estimated by using the formula described by Mathews *et al*. [16]

HOMA-IR = Fasting blood glucose (mmol/L) X Fasting serum insulin (mU/L) / 22.5

Statistical Analysis: Descriptive analysis was carried out to all the data variables measured in the study. Mean and Standard Deviation (SD) were derived for the results of blood sugar, HbA1c, serum insulin, HOMA-IR, renal function tests, liver enzymes, and lipid profile.

The difference between the data obtained during baseline and at the end of the study was analyzed using paired-t-test within the groups. Comparison of data between the groups was done by using multiple comparison Analysis of Variance (ANOVA) followed by Bonferroni post hoc analysis.

RESULTS: The study has investigated the long-term effects of (NAS) in male Swiss albino mice. There were 36 animals included in this and all the animals had completed the study.

The body weight measurements **Table 2** showed an increase in the bodyweight of the animals in all the groups. However, the weight gain observed in animals treated with saccharin, sucralose, aspartame, and commercial aspartame was significantly less compared to control group.

Fasting Blood Glucose (Table 3 and Fig. 1), HbA1C (Table 4 and Fig. 2): There were no significant changes in group 1 (C - control group) and group 2 (GLU – glucose). But, the values were significantly increased in the groups that were treated with non-caloric Artificial Sweeteners groups 3, 4, 5 and 6.

Fasting Serum Insulin (Table 5 and Fig. 3): There were no significant changes observed in groups 1, 2, 4, 5 and 6. But, it was significantly increased in group 3 (SAC-saccharin).

HOMA-IR index (Table 6 and Fig. 4): There were no significant changes observed in groups 1, 2, 4 and 6. But, it was significantly increased in group 3 (SAC-saccharin) and group 5 (ASP-aspartame).

Total Cholesterol (Table 7 and Fig. 5): There were no significant changes observed in groups 1, 2 and 4. But, it was significantly decreased in group 3 (SAC - saccharin) and increased in group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

Triglycerides (Table 7 and Fig. 6): There were no significant changes observed in groups 1, 2 and 3. But, it was significantly reduced in group 4 (SUC - sucralose) and increased in group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

HDL (High-Density Lipoprotein) (Table 7 and Fig. 7): There were no significant changes observed in groups 1, 2 and 3. But, it was significantly increased in group 4 (SUC - sucralose) and decreased in group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

LDL (Low Density Lipoprotein) (Table 7 and Fig. 8): There were no significant changes observed in groups 1, 2, 3 and 4. But, it was significantly increased in group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

Urea and Creatinine: There were no significant changes observed in groups 1, 2 and 4. But, the values were significantly increased in group 3 (SAC - saccharin), group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Gamma-Glutamyl Transpeptidase (GGT): There were no significant changes observed in groups 1, 2 and 4. But, their levels were significantly increased in group 3 (SAC - saccharin), group 5 (ASP - aspartame) and group 6 (SFG – sugar-free gold).

Alkaline Phosphatase (ALP): There were no significant changes observed in groups 1, 2, 4 and 6. But, it was significantly increased in group 3 (SAC - saccharin) and decreased in group 5 (ASP - aspartame).

Fecal Microbial Culture: The microbial culture during the baseline yielded Escherichia coli and Citrobacter koseri. 3 animals in group 1 (C - control), 1 in group 2 (GLU – glucose), 3 in group 3 (SAC - saccharin), 1 each in group 4 (SUC - sucralose) & 5 (ASP - aspartame) and 3 animals in group 6 (SFG - sugar free gold) had *Citrobacter koseri* in their fecal culture and the remaining animals showed *Escherichia coli*.

After completion of the study, all the animals showed only *Escherichia coli* in their fecal samples. These bacteria are gram-negative in nature and usually found in the intestine and only sometimes cause significant gastrointestinal problems. *Citrobacter koseri* is a motile, gram-negative, opportunistic bacterium that rarely causes clinical infection in immunocompetent individuals.

TABLE 2: CHANGES IN BODY WEIGHT

Groups	Baseline (mean ± SD) (g)	End (mean ± SD) (g)	Difference (mean ± SD) (g)	Paired t-test (p-value)
Group 1 (C- Control)	27.72 ± 2.65	43.25 ± 1.93	15.53 ± 2.57	Significant (<0.0001)
Group 2 (GLU-Glucose)	27.15 ± 2.10	41.57 ± 1.90	14.42 ± 3.07 $	Significant (<0.0001)
Group 3 (SAC- Saccharin)	27.85 ± 2.89	34.80 ± 1.12	6.95 ± 2.39 #	Significant (<0.0009)
Group 4 (SUC-Sucralose)	26.50 ± 3.12	37.53 ± 2.03	11.03 ± 3.65 #	Significant (<0.0007)
Group 5 (ASP- Aspartame)	28.48 ± 1.78	35.82 ± 1.78	7.33 ± 1.38 #	Significant (<0.0001)
Group 6 (SFG-Sugar free gold)	26.08 ± 2.79	37.57 ± 3.62	11.48 ± 3.78 #	Significant (<0.0007)

When compared to control group, # - Significantly decreased**,** $ - No significant changes

TABLE 3: MEAN FASTING BLOOD GLUCOSE

Groups	Baseline (mean ± SD) (mg/dl)	End (mean ± SD) (mg/dl)	Difference (mean ± SD) (mg/dl)	Paired t-test (p-value)
Group 1 (C- Control)	101.17 ± 8.61	100.00 ± 11.17	- 1.17 ± 10.65	Not significant (<0.7991)
Group 2 (GLU-Glucose)	105.83 ± 10.80	104.17 ± 8.77	- 1.67 ± 12.09 $	Not significant (<0.7494)
Group 3 (SAC- Saccharin)	101.00 ± 12.23	142.33 ± 8.38	41.33 ± 11.02 *	Significant (<0.0003)
Group 4 (SUC-Sucralose)	107.50 ± 14.21	137.17 ± 9.87	29.67 ± 19.99 *	Significant (<0.0427)
Group 5 (ASP- Aspartame)	108.33 ± 13.87	156.33 ± 11.91	48.00 ± 20.01 *	Significant (<0.0020)
Group 6 (SFG-Sugar free gold)	106.00 ± 12.20	149.67 ± 11.22	43.67 ± 14.95 *	Significant (<0.0008)

When compared to control group, * - Increased significantly, $ - No significant changes

TABLE 4: MEAN HBA1C

Groups	Baseline (mean ± SD) (%)	End (mean ± SD) (%)	Difference (mean ± SD) (%)	Paired t-test (p-value)
Group 1 (C- Control)	5.15 ± 0.27	5.1 ± 0.40	- 0.05 ± 0.38	Not significant (<0.7623)
Group 2 (GLU-Glucose)	5.32 ± 0.39	5.25 ± 0.31	- 0.07 ± 0.45$	Not significant (<0.7291)
Group 3 (SAC- Saccharin)	5.28 ± 0.50	6.58 ± 0.29	1.30 ± 0.32 *	Significant (<0.002)
Group 4 (SUC-Sucralose)	5.35 ± 0.50	6.43 ± 0.32	1.08 ± 0.69 *	Significant (<0.0121)
Group 5 (ASP- Aspartame)	5.40 ± 0.47	7.02 ± 0.53	1.62 ± 0.79 *	Significant (<0.0040)
Group 6 (SFG-Sugar free gold)	5.55 ± 0.37	6.87 ± 0.39	1.32 ± 0.62 *	Significant (<0.0034)

When compared to the control group, * - Increased significantly, $ - No significant changes

TABLE 5: MEAN FASTING SERUM INSULIN

Groups	Baseline (mean ± SD) (mU/L)	End (mean ± SD) (mU/L)	Difference (mean ± SD) (mU/L)	Paired t-test (p-value)
Group 1 (C- Control)	7.56 ± 1.17	5.83 ± 2.16	-1.73 ± 1.82	Not significant (<0.0666)
Group 2 (GLU-Glucose)	7.82 ± 0.83	7.58 ± 2.67	-0.23 ± 3.27 $	Not significant (<0.8680)
Group 3 (SAC- Saccharin)	7.60 ± 0.93	11.30 ±2.63	3.70 ± 3.37 *	Significant (<0.0432)
Group 4 (SUC-Sucralose)	7.43 ± 1.36	7.08 ± 2.41	-0.36 ± 2.91 $	Not significant (<0.7770)
Group 5 (ASP- Aspartame)	7.75 ± 0.89	8.82 ± 4.34	1.07 ± 4.98 $	Not significant (<0.6211)
Group 6 (SFG-Sugar free gold)	7.84 ± 0.98	6.99 ± 1.35	-0.86 ± 1.87 $	Not significant (<0.3142)

When compared to the control group, * - Increased significantly, $ - No significant changes

TABLE 6: MEAN HOMA-IR INDEX

Groups	Baseline (mean ± SD)	End (mean ± SD)	Difference (mean ± SD)	Paired t-test (p-value)
Group 1 (C- Control)	1.89 ± 0.32	1.44 ± 0.58	- 0.44 ± 0.52	Not significant (<0.0926)
Group 2 (GLU-Glucose)	2.05 ± 0.37	1.97 ± 0.78	- 0.08 ± 0.94 $	Not significant (<0.8404)
Group 3 (SAC- Saccharin)	1.90 ± 0.39	3.39 ± 1.01	2.07 ± 1.24 *	Significant (<0.0095)
Group 4 (SUC-Sucralose)	1.99 ± 0.53	2.38 ± 0.81	1.45 ± 1.12 $	Not significant (<0.4304)
Group 5 (ASP- Aspartame)	1.84 ± 0.33	3.38 ± 1.65	1.94 ± 1.12 $	Significant (<0.0450)
Group 6 (SFG-Sugar free gold)	2.05 ± 0.33	2.59 ± 0.54	1.32 ± 0.48 $	Not significant (<0.0626)

When compared to control group, * - Significantly increased, $ - No significant changes

TABLE 7: MEAN SERUM LIPID PROFILE

Groups	Baseline (mean ± SD) (mg/dl)	End (mean ± SD) (mg/dl)	Difference (mean ± SD) (mg/dl)	Paired t-test (p-value)
Group 1	TC – 96.67 ± 16.08	TC – 85.17 ± 11.44	-11.50 ± 16.11	NS (<0.1408)
(C- Control)	TGL – 95.83 ± 31.67	TGL –105.33 ± 17.74	9.50 ± 22.24	NS (<0.3434)
	HDL – 35.33 ± 7.15	HDL – 38.50 ± 3.94	3.17 ± 5.00	NS (<0.1813)
	LDL – 70.00 ± 14.99	LDL – 72.17 ± 14.44	2.17 ± 7.17	NS (<0.4923)
Group 2	TC – 128.67 ± 6.80	TC – 125.50 ± 14.90	-3.17 ± 19.29 *	NS (<0.7042)
(GLU- glucose)	TGL – 103.17 ± 20.64	TGL – 93.00 ± 11.01	-10.17 ± 5.83 $	NS (<0.3792)
	HDL – 40.00 ± 12.36	HDL – 35.83 ± 4.54	-4.17 ± 13.75 $	NS (<0.4912)
	LDL – 72.57 ± 14.36	LDL – 88.33 ± 16.33	15.77 ± 28.01 $	NS (<0.1563)
Group 3	TC – 124.67 ± 13.65	TC – 99.33 ± 8.04	-25.33 ± 18.28 #	Sig (<0.0483)
(SAC- saccharin)	TGL – 97.83 ± 20.60	TGL – 78.00 ± 22.58	-19.83 ± 28.94 $	NS (<0.1541)
	HDL – 39.67 ± 10.39	HDL – 31.67 ± 4.13	-8.00 ± 12.15 $	NS (<0.1677)
	LDL – 82.17 ± 12.42	LDL – 75.83 ± 15.87	-6.33 ± 17.75 $	NS (<0.4221)
Group 4	TC – 129.83 ± 14.84	TC – 147.50 ± 19.58	17.67 ± 27.38 $	NS (<0.1748)
(SUC- sucralose)	TGL – 103.50 ± 14.36	TGL – 89.83 ± 11.02	-13.67 ± 11.62 $	Sig (<0.0346)
	HDL – 42.00 ± 12.70	HDL – 60.50 ± 8.53	18.50 ± 16.16 $	Sig (<0.0378)
	LDL – 72.07 ± 16.56	LDL – 91.33 ± 7.87	19.27 ± 20.07 $	NS (<0.0654)
Group 5	TC – 126.67 ± 15.51	TC – 173.33 ± 16.79	46.67 ± 24.89 *	Sig (<0.0059)
(ASP- aspartame)	TGL – 109.67 ± 6.22	TGL –154.33 ± 12.82	44.67 ± 14.28 $	Sig (<0.0006)
	HDL – 41.00 ± 8.29	HDL – 31.17 ± 3.66	-9.83 ± 5.46 $	Sig (<0.0069)
	LDL – 67.67 ± 13.76	LDL – 89.33 ± 16.15	21.67 ± 23.21 $	Sig (<0.0098)
Group 6	TC – 129.33 ± 12.04	TC – 155.50 ± 19.53	26.17 ± 21.59 *	Sig (<0.0059)
(SFG- sugar free gold)	TGL – 96.83 ± 25.74	TGL –131.17 ± 18.32	34.33 ± 28.91 $	Sig (<0.0334)
	HDL – 43.83 ± 6.18	HDL – 30.33 ± 5.85	-13.50 ± 10.43 $	Sig (<0.0248)
	LDL – 75.50 ± 13.87	LDL –102.50 ± 21.80	27.00 ± 19.96 $	Sig (<0.0212)

When compared to the control group, * - Significantly increased, # - Significantly decreased, $ - No significant changes, NS – Not significant; Sig – Significant

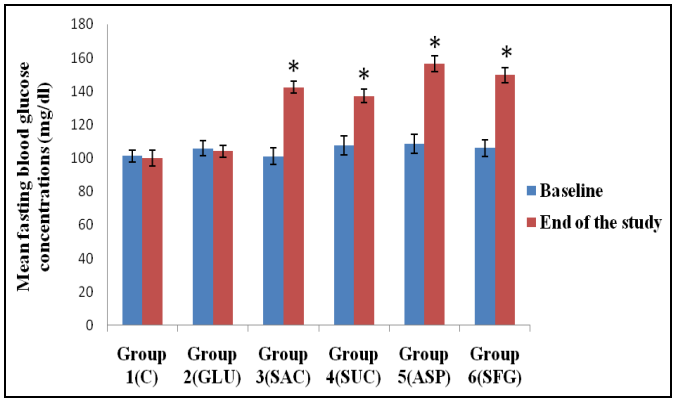

FIG. 1: MEAN FASTING BLOOD GLUCOSE

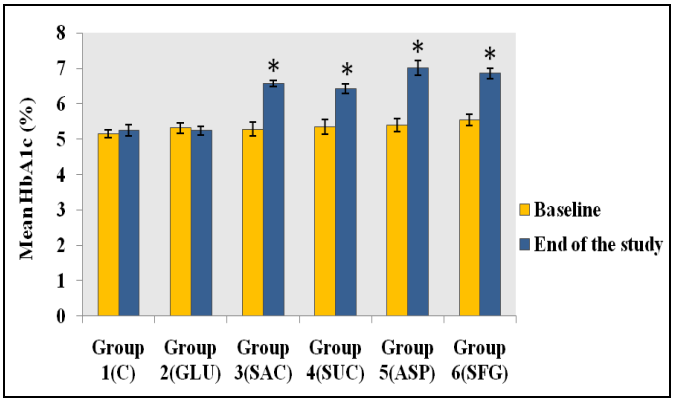

FIG. 2: MEAN HBA1C

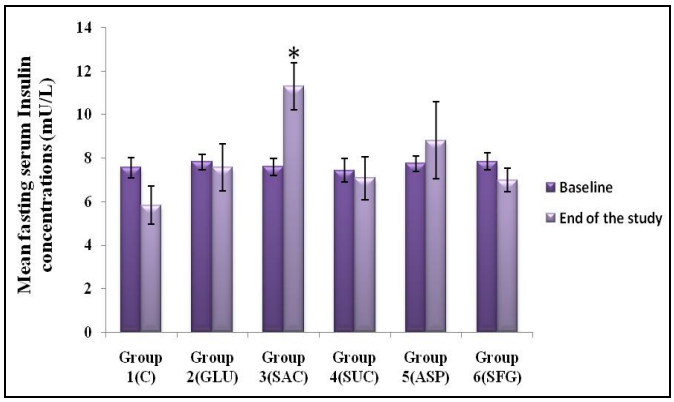

FIG. 3: MEAN FASTING SERUM INSULIN

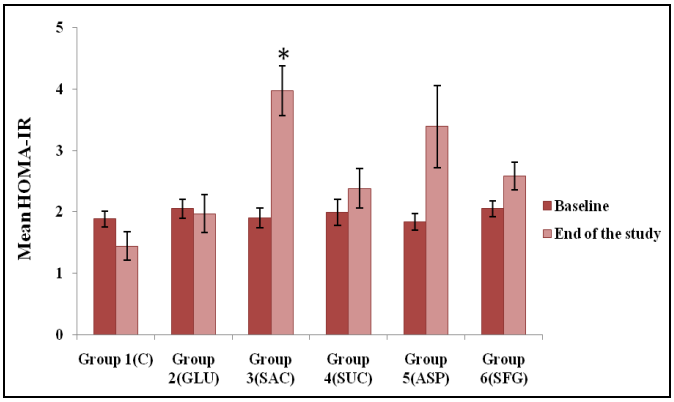

FIG. 4: MEAN HOMA-IR

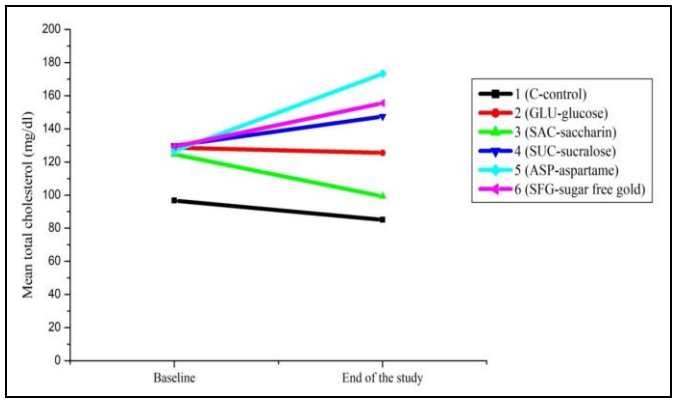

FIG. 5: MEAN TOTAL CHOLESTEROL

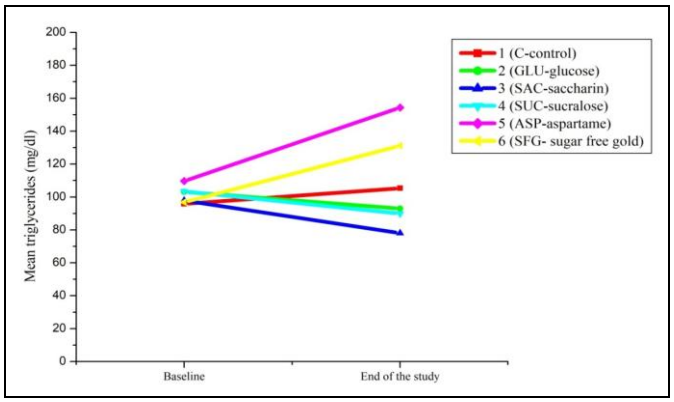

FIG. 6: MEAN TRIGLYCERIDES

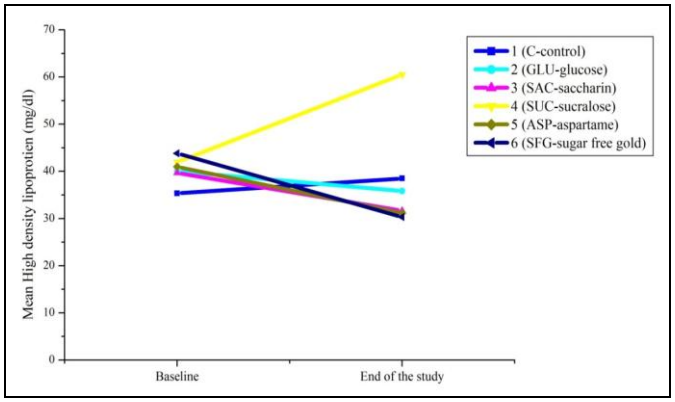

FIG. 7: MEAN HDL

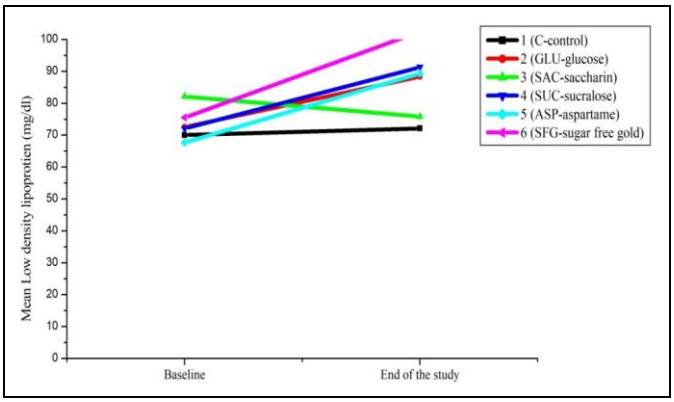

FIG. 8: MEAN LDL

DISCUSSION: The present study has found that the administration of NAS, such as Saccharin, Sucralose, and Aspartame, including the commercial formulation for 8 weeks, could result in significant increases in fasting blood sugar and HbA1c in mice. These changes indicate that NAS had indeed adversely affected the glycemic status compared to those animals in groups 1 and 2 that received water and glucose. Importantly, these changes were observed at the animal equivalent doses of 6 mg/day for saccharin, 2 mg/day for sucralose, and 20 mg/day for aspartame and commercial aspartame with an average weight of 30 g of mice. (The dose of NAS was altered according to the weight of the animals every week).

In this study, the mean fasting blood glucose was between 101 and 108 mg/dl in all the animals during baseline. At the end of the study, in groups 1 and 2 it was between 100 and 104 mg/dl, whereas it was significantly increased in the groups that received NAS. It was between 137, and 156 mg/dl in the NAS treated animals after 8 weeks, and these values were within diabetic range.

The baseline HbA1c was between 5.15 and 5.55% in all the animals that were significantly increased to 6.43 - 7.02% in the animals treated with NAS (group 3 to 6) after 8 weeks. This increase was also within the diabetic range, like fasting blood sugar. In the control and glucose treated groups, the mean

HbA1c was between 5.1 and 5.25% at the end of the study and compared to the baseline values, it was not significantly changed.

Shastry CS *et al*., (2012) investigated the diabetogenic potential of aspartame, acesulfame-K, and sucralose in Sprague-Dawley rats. They administered NAS for 13 weeks at normally used doses (315 mg/kg for aspartame, 94.5 mg/kg for acesulfame-K, 94.5 mg/kg for sucralose) and also at higher doses (1260 mg/kg for aspartame, 151.2 mg/kg for acesulfame-K, 151.2 mg/kg for sucralose). They found that fasting blood glucose values and HbA1c values were significantly increased at higher NAS dose levels.

The baseline fasting blood glucose was around 73 mg/dl that was increased to 120 mg/dl with a high dose of NAS. Similarly HbA1c was increased to 7% compared to 5.83% in control group. It was concluded that NAS could be safe at the usual ADI doses while higher doses could exhibit diabetogenic potential [17].

Najafipour *et al*., (2018) reported that regular consumption of NAS for more than 3 months resulted in a worsening of diabetic status in diabetic patients. They observed that fasting blood glucose and postprandial blood glucose was increased from 121.03 mg/dl to 152 mg/dl and 164.4 to 222 mg/dl respectively. HbA1c levels were increased from 6.89 to 7.40% [18].

In the present study, the animals treated with saccharin had significant increases in serum Insulin and HOMA-IR after 8 weeks. Serum Insulin was 7.60 mU/L at baseline that was increased to 11.30 mU/L. HOMA-IR was 1.90 at baseline and it was increased to 3.39 at the end. These parameters were not significantly altered in sucralose, aspartame and glucose treated animals and control animals. Saccharin could have caused Insulin resistance as indicated by elevated HOMA-IR which could be the reason for increased serum Insulin. It is generally a natural response to compensate for insulin resistance, more insulin is secreted.

Suez *et al*., [10] conducted various experiments in mice models and expressed that NAS compounds could cause glucose intolerance by changing the microbial flora. This was substantiated by their experiment in which the animals that had become glucose intolerant followed by the administration of NAS, were treated with suitable antibiotics. After antibiotic treatment, their glucose intolerance was normalized. They were of the opinion that NAS caused alterations in the commensal microbiota, that adversely influenced glucose tolerance.

Suez *et al*., [10] further conducted fecal transplantation studies in germ-free, normal chow consuming mice. The fecal microbiota configuration was obtained from the feces of the mice consuming normal chow with a) saccharin mixed drinking water and b) glucose mixed drinking water. The germ-free mice transplanted with the fecal microbiota of saccharin fed animals developed glucose intolerance within 6 days of transplantation. But the germ-free mice that received fecal microbiota of glucose fed animals did not develop glucose intolerance. Hence, they concluded that the metabolic abnormalities detected in NAS fed animals were due to alterations in the intestinal microbial flora.

The changes observed in lipid profile parameters in the present study were suggestive of dyslipidemic findings with aspartame. Groups 5 (ASP - aspartame) animals that were treated with aspartame raw material showed significant increase in total cholesterol (126.67 to 173.33 mg/dl), triglycerides (109.67 to 154.33 mg/dl), LDL (67.67 to 89.33 mg/dl) and decrease in HDL (41.00 to 31.17 mg/dl). Commercial aspartame (sugar free gold) treated animals (group 6) had also shown significant increases in total cholesterol (129.33 to 155.50 mg/dl), triglycerides (96.83 to 131.17 mg/dl), LDL (75.50 to 102.5 mg/dl) and decreases in HDL (43.83 to 30.33 mg/dl).

The control and glucose treated animals did not have significant changes in the lipid profile. Saccharin and sucralose treated animals did not have clinically significant changes in the lipid profile though saccharin had produced a statistically significant decrease in total cholesterol and sucralose producing statistically significant decrease in triglycerides and increase in HDL, without affecting the other lipid parameters.

Bodyweight of the animals in all the groups was significantly increased when the baseline body weight as compared to body weight after 8 weeks. The increase in the body weight was more than 14

g in control and glucose treated animals, but it was approximately 7 g in saccharin and aspartame (raw materials) treated animals (groups 3 and 5). In animals treated with commercial aspartame and sucralose (groups 4 and 6), the increase was 11g. The less weight gain observed in NAS treated animals were statistically significant when compared to control and glucose treated animals.

Alkafafy *et al*., (2015) in their study of aspartame and saccharin on hepatic biochemical, molecular and histological parameters in Wistar albino rats observed that NAS increased the liver enzymes and at the same time reduced the body weight and antioxidant parameters [19].

Polyak *et al*., [20] and Fernanda de Matos Feijo *et al*., [21] in their animal experiments observed that NAS increased the bodyweight of the animals and the weight gain was more than the control animals that did not receive NAS.

In the present study, Saccharin, Sucralose, and Aspartame, though increased the body weight, the increase was less than the control animals that received glucose or only water.

The results obtained in this study provide evidence that long term consumption of NAS can lead to glucose intolerance. Diabetic patients and the public at large need to be aware of the fact that NAS consumption may be associated with metabolic derangement.

CONCLUSION: The results showed that NAS agents used in the study, saccharin, sucralose, and aspartame were associated with significant glucose intolerance as shown by elevated fasting blood glucose and elevated HbA1c. In addition, saccharin was associated with elevated liver enzymes and renal parameters. Aspartame was associated with deranged lipid profile in addition to elevated liver enzymes and renal parameters.

Bodyweight was increased in all the animals that received NAS, but the increase was less compared to control and glucose treated animals. Sucralose was associated with only glucose intolerance and it did not produce any significant change in liver enzymes, renal parameters, and lipid profile. The faecal culture showed bacteria belonging to Entero-bacteriacae family during baseline (*Escherichia coli* and *Citrobacter koseri*) and after 8 weeks (*Escherichia coli*). The treating physicians, diabetic patients, and general population may have to consider the metabolic problems, particularly glucose intolerance, associated with NAS before advising and / or choosing them for regular consumption.

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CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest.

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