

# COMPARATIVE EVALUATION OF DIABETOGENIC AND MUTAGENIC POTENTIAL OF ARTIFICIAL SWEETENERS - ASPARTAME, ACESULFAME-K AND SUCRALOSE

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## Abstract :

**Objectives:** Artificial sweeteners provide the sweetness of sugar without calories. Since from discovery, safety of artificial sweeteners has been controversial as they directly or indirectly link to induce carcinogenic and genotoxic risks. Hence the present study was undertaken to compare the diabetogenic and mutagenic potential of most widely using artificial sweeteners; aspartame, acesulfame-K, and sucralose.

**Methods:** Diabetic potential is assessed by ascending repeated dose study in which acceptable daily intake (ADI) dose of artificial sweeteners after converting human dose to animal dose using a standard reference table and administered up to 13 weeks with 3 different phases in an ascending manner on experimental rats. Mutagenic potential was accessed by Ames test with and without metabolic activation using *Salmonella typhimurium* strains TA 97 and TA1535.

**Results:** At ADI doses between 0-3 weeks, no significant changes but after 13 weeks significant increase was observed in the levels of fasting blood glucose, glycated haemoglobin, total cholesterol, triglyceride, LDL and VLDL in all artificial sweetener groups. Sucralose showed comparatively less increase which was supported by histology reports. In Ames mutagenic assay aspartame, acesulfame-K and sucralose gave negative results.

**Conclusion:** Aspartame, acesulfame-K and sucralose were found to exhibit diabetogenic effect at higher dose levels but they were safer to use at ADI doses and non mutagenic compounds. Comparatively sucralose is safer than aspartame and acesulfame-K. Hence these artificial sweeteners should be used with caution and over usage is not appreciated.

**Keywords :** Artificial sweeteners, aspartame, acesulfame-K, sucralose, diabetogenic, mutagenic.

## Introduction :

Artificial sweeteners include substances from several different chemical classes that interact with taste receptors and typically exceed the sweetness of sucrose by a factor of 30 to 13,000 times but have no or low calories. They provide only sweetness but not the daily calorie needs [1].

Due to their intense sweetness they are needed in small quantity and hence are economical. Currently FDA approved artificial sweeteners for consumption are acesulfame-K, aspartame, neotame, saccharin, and sucralose, out of which

most extensively used sweeteners are aspartame, sucralose and acesulfame-K [2]. As the artificial sweeteners provide the sweetness of sugar without calories, public health attention has turned to reversing the obesity epidemics in the individuals of all ages by choosing to use the products containing artificial sweeteners. Hence the use of low-calorie, sugar-free products tripled in the last two decades of the 20th century. In the United States alone, more than 150 million people use these products regularly. However safety of these artificial sweeteners is unresolved and controversial.

Diabetes mellitus has now assumed epidemic proportions in many countries of the world. With the present population of 19.4 million diabetics, and approximately 60

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million by the year 2025, India would rank first in its share of the global burden of diabetes. When a portion of the population suffers from a disease in which sucrose is the initiating culprit, the treatment choices are to either eliminate the source of glucose or add/regulate the amount of insulin available to the bloodstream [3]. So, in lieu of ridding the diet of sweet, science went looking for a sweet replacement by the artificial sweeteners. But, however recent epidemiologic studies showed the association between diet soda consumption (which contains artificial sweeteners) and the risk of development of obesity, metabolic syndrome and Type 2 diabetes [4,5]. The earlier studies linked artificial sweeteners to carcinogenic and genotoxic risk. Aspartame exhibited carcinogenicity on prolonged use, sucralose in mouse lymphoma assay showed positive mutation frequency at higher doses and acesulfame-K caused slight chromosomal aberration indicating that these artificial sweeteners not entirely safe even though they are FDA approved [6-9].

Aspartame, acesulfame-K and sucralose are not entirely safe as they were artificially synthesized and their metabolites may yield to toxic chemicals. Most importantly, the risk-benefit ratio of artificial sweeteners is unclear. Recent study also shows health risk even below the acceptable daily intake (ADI) doses after the long term consumption. So, further studies are essential to assess the safety of these three artificial sweeteners. Hence present study was undertaken to assess the diabetogenic and mutagenic potentials of the artificial sweeteners- aspartame, acesulfame-K and sucralose.

#### Materials and Methods :

##### Chemicals

Aspartame, acesulfame-K and sucralose were procured by Highmedia Bombay, India. HbA1C (glycated haemoglobin) kit, total cholesterol kit, triglyceride kit, etc, were procured from Agappe diagnostics Ltd, Kerala. And all other chemicals were of analytical grade and used as received.

##### Animals

All the experiments were carried out with Sprague-Dawley rats weighing 150-200g. Animals were kept in the animal

house of NGSM Institute of Pharmaceutical Sciences, Mangalore under controlled conditions of temperature ( $23\pm 2^{\circ}\text{C}$ ), humidity ( $50\pm 5\%$ ) and 12 h light-dark cycle. Animals were fed pellet diet (Venkateshwara enterprises, Bangalore) and water ad libitum. All the animals were acclimatized for seven days before the study. The experimental protocol was approved by institutional animal ethical committee (approval number: Reg.No.KSHEMA/AEC/39/2010)

##### Selection of Drug Doses

The human ADI of aspartame, sucralose and acesulfame-K was 50 mg/kg, 15 mg/kg and 15 mg/kg respectively. The ADI doses were converted to animal doses as per the conversion chart and used for the study [10].

##### Assessment of diabetogenic potential [11,12]

##### Experimental design:

Diabetic potential assessed by ascending repeated dose study up to 13 week in 3 phases on rats. Study involved 4 groups with 6 animals each. Drugs were administered orally through oral gavage.

- ā Control group
- ā Aspartame treated group
- ā Acesulfame-K treated group
- ā Sucralose treated group

##### Phase 1(0-3 weeks- ADI dose):

- Control group: administered with distilled water for 0-3 weeks.
- Aspartame treated group: administered with 315 mg/kg rat
- Acesulfame-K treated group: administered with 94.5 mg/kg rat
- Sucralose treated group: administered with 94.5 mg/kg rat

##### Phase 2 (3-7 weeks- 2 x ADI dose):

- Control group: administered with distilled water
- Aspartame treated group: administered with 630 mg/kg rat
- Acesulfame-K treated group: administered with 75.6

mg/kg rat

- Sucralose treated group: administered with 75.6 mg/kg rat

Phase (7-13 weeks- 4 x ADI dose):

- Control group: administered with distilled water
- Aspartame treated group: administered with 1260 mg/kg rat
- Acesulfame-K treated group: administered with 151.2 mg/kg rat
- Sucralose treated group: administered with 151.2 mg/kg rat

At the end of 3rd, 7th, 13th week rats were fasted for 18 hour and the fasting blood glucose (FBG) levels were measured. Blood was collected by retro orbital sinus method [13] and centrifuged at 2500 rpm. Serum was separated and lipid profiles like total cholesterol, triglyceride, LDL and VLDL was measured along with the HbA1C levels which are measured only at the end of the 13th week.

Assessment of mutagenic potential by Ames test [14,15]  
For comparative evaluation of mutagenic potential, samples of aspartame, acesulfame-K, and sucralose were sent to Shree Dhanwantary Pharmaceutical Analysis and Research Centre (SDPARC) at Kim, Surat, Gujrat. The potential of mutagenic effects of aspartame, acesulfame-k and sucralose were evaluated on two Salmonella typhimurium strains TA97 (Detects frame shift mutations) and TA1535. (Detects base pair substitution mutations), with and without metabolic activation. Salmonella/microsome reversion assay was conducted using the plate incorporation procedure described by Maron and Ames and as per 471-OECD guidelines for testing of chemicals.

Statistical Analysis:

The data were expressed as mean  $\pm$  standard error of the mean (S.E.M.) of 6 animals per group. Parametric one way analysis of variance (ANOVA) followed by Dunnett's test. Statistical analysis was performed using Graph pad prism 5.0. The minimal level of significance was identified at  $P < 0.05$ .

Results:

Assessment of a diabetogenic potential

Fasting blood glucose level

In Phase I, at ADI doses of aspartame, acesulfame-K and sucralose, FBG level were found to be similar/slightly lower than control group values in all the drug treated groups. In Phase II (at 2 x ADI doses) and phase III (at 4 x ADI doses) of aspartame, acesulfame-K and sucralose, the FBG level was significantly raised ( $p < 0.001$ ) in all the drug treated groups compared control group indicating induction of diabetes. In comparison with aspartame and acesulfame-K, sucralose shows less increase in FBG levels. (Table 1)

HbA1c Level

After the 13 week, HbA1C levels were raised significantly ( $p < 0.001$ ) in all three drug treated groups (aspartame, acesulfame-K and sucralose) compared to control group. However the sucralose shows less increase compared to other two artificial sweetener groups. (Table 2)

Lipid profile

In Phase I, lipid profiles like Total cholesterol, Triglyceride level, LDL and VLDL levels were normal as control group. But at phase II and phase III there was significant ( $p < 0.001$ ) increase in lipid profile of all artificial sweetener treated groups compared to control group. (Table 3)

Assessment of mutagenic potential by Ames test

Ames test with and without metabolic activation results revealed Aspartame, Acesulfame-K and Sucralose were non mutagenic.

Histology

Histology of normal rat pancreas showed no architectural changes. Aspartame treated rat pancreas showed diffused necrotic changes. Acesulfame-K treated rat pancreas showed focal lymphocytic aggregation/focal chronic infiltrate in the form of lymphocyte indicating autoimmune response against cells. Histology of sucralose treated rat pancreas showed no significant architectural changes which is comparable with normal rat pancreas. (Figure 1)

Table 1: Effect of aspartame, acesulfame-K and sucralose on FBG level

Group	Fasting Glucose Level (mg/dl)		
	0-3 weeks	3-7 weeks	7-13 weeks
Control group	73.00±1.78	78.21±4.18	78.33±1.96
Aspartame treated	73.17±2.13	86.24±5.45 <sup>a</sup>	120.50±1.37 <sup>a</sup>
Acesulfame-K treated	72.67±1.03	93.20±5.65 <sup>a</sup>	124.00±3.03 <sup>a</sup>
Sucralose treated	73.83±1.72	87.69±5.60 <sup>a</sup>	118.80±3.37 <sup>a</sup>

The Values are expressed as Mean ± SEM, n=6 rats in one group. <sup>a</sup> significant compared with control group (p<0.001).

Table 2: Effect of Aspartame, Acesulfame-K and Sucralose on HbA1c level

Group	HbA1c level (%)
Control group	5.83±0.73
Aspartame treated	7.05±0.26 <sup>a</sup>
Acesulfame-K treated	6.68±0.02 <sup>a</sup>
Sucralose treated	6.52±0.08 <sup>a</sup>

The Values are expressed as Mean ± SEM, n=6 rats in one group. <sup>a</sup> significant compared with control group (p<0.001).

Table 3: Effect of aspartame, acesulfame-K and sucralose on Lipid profiles

Group	Total cholesterol (mg/dl)			Triglyceride level (mg/dl)		
	0-3 weeks	3-7 weeks	7-13 weeks	0-3 weeks	3-7 weeks	7-13 weeks
Control group	67.50±2.07	68.67±2.16	71.67±4.63	67.83±4.83	72.00±2.00	74.17±2.56
Aspartame treated	69.50±4.23	81.50±3.39 <sup>a</sup>	82.50±2.66 <sup>a</sup>	72.50±2.66	78.00±2.19 <sup>a</sup>	90.17±6.49 <sup>a</sup>
Acesulfame-K treated	68.83±2.85	76.83±1.47 <sup>a</sup>	84.50±2.34 <sup>a</sup>	71.33±1.50	77.67±1.50 <sup>a</sup>	95.67±11.60 <sup>a</sup>
Sucralose treated	69.17±3.31	74.00±2.36 <sup>a</sup>	81.67±1.36 <sup>a</sup>	71.67±3.55	77.50±2.42 <sup>a</sup>	87.83±6.49 <sup>a</sup>

Group	LDL cholesterol (mg/dl)			VLDL-C (mg/dl)		
	0-3 weeks	3-7 weeks	7-13 weeks	0-3 weeks	3-7 weeks	7-13 weeks
Control group	17.27±3.13	18.93±3.74	20.82±3.27	13.57±0.96	14.40±0.40	14.83±0.51
Aspartame treated	20.42±2.00	32.97±2.50 <sup>a</sup>	32.64±6.98 <sup>a</sup>	14.08±0.90	15.51±0.43 <sup>a</sup>	18.03±1.29 <sup>a</sup>
Acesulfame-K treated	20.73±3.25	31.13±2.59 <sup>a</sup>	40.80±7.25 <sup>a</sup>	14.27±0.30	15.53±0.30 <sup>a</sup>	19.13±2.32 <sup>a</sup>
Sucralose treated	21.07±4.63	27.67±5.59 <sup>a</sup>	35.03±1.95 <sup>a</sup>	14.33±0.711	15.50±0.48 <sup>a</sup>	17.77±1.09 <sup>a</sup>

The Values are expressed as Mean ± SEM, n=6 rats in one group. <sup>a</sup> significant compared with control group (p<0.001).

#### Discussion :

In the present diabetogenic study, ascending repeated dose 13 weeks with three phases gave the clear indication of effect of these artificial sweeteners at various dose levels. The doses started at ADI doses since recent long term studies indicated aspartame not safe even at daily acceptable doses [7]. Mutagenic potential was accessed by Ames test with and without metabolic activation using Salmonella typhimurium strains TA 97 and TA1535.

Present study revealed that the artificial sweeteners cause significant increase in FBG levels, HbA1C levels and lipid profile. The sucralose group showed lesser increase compared to other two artificial sweeteners. The pancreatic histology report supports the same. The Ames test was negative for all three artificial sweeteners.

#### Conclusion :

From the present study it was confirmed that aspartame, acesulfame-K and sucralose exhibit diabetogenic effect at higher dose levels but they were safer to use at ADI doses.

They were non mutagenic compounds, confirmed from the Ames mutagenic test with and without metabolic activation. So study indicated these artificial sweeteners can be used with caution to limited extent. Over usage above the ADI doses and long term usage of these artificial sweeteners is not advisable.

Overall comparative evaluation showed that sucralose found to be safer than aspartame and acesulfame-K. The study also appreciates the carrying a similar long term study for these sweeteners for further safety assessment on health risks.

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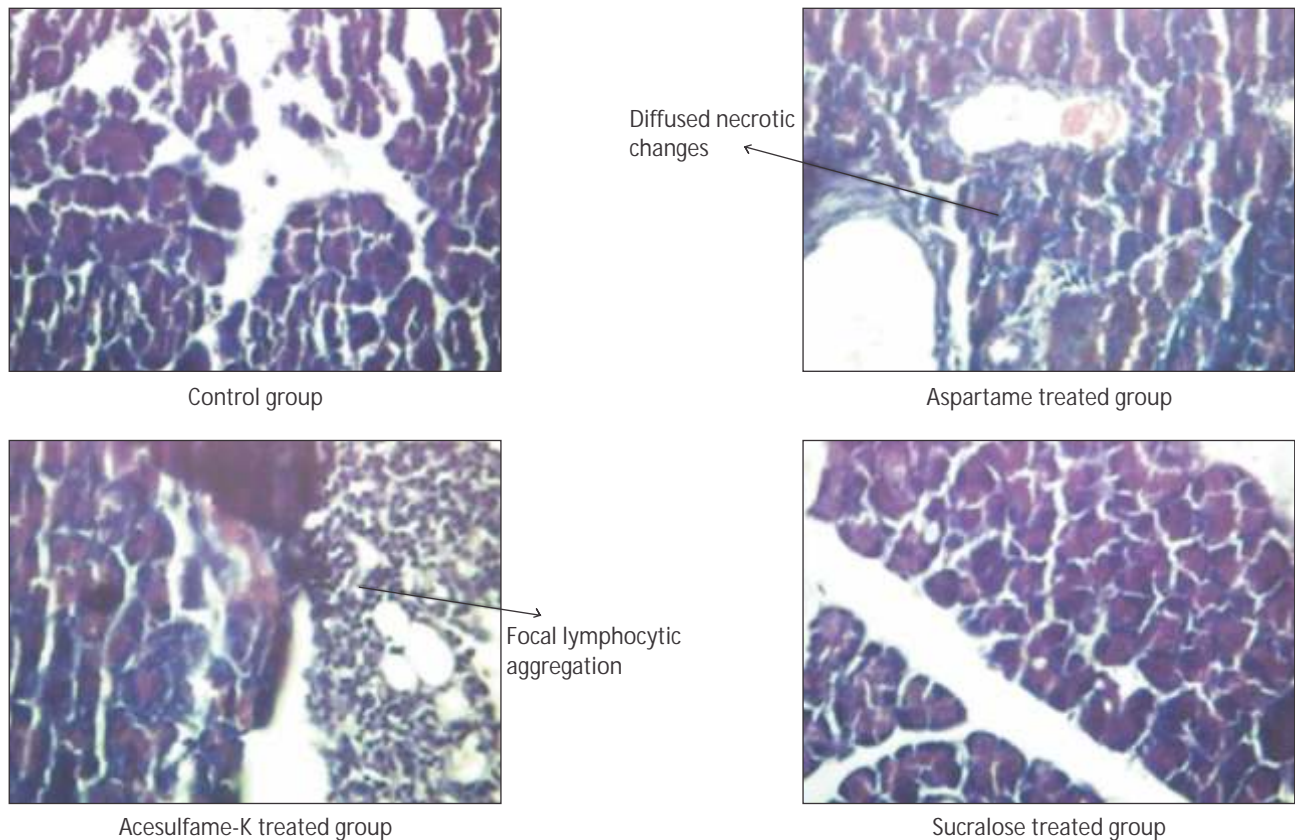
**Conflict of interest**

The authors declare that they have no conflicts of interest to disclose.

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Figure 1 : Histology report



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