

## Variegation in the Number of Islets of Langerhans in the Pancreas of Rats Fed on Sodium Cyclamate

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### ABSTRACT

**Objective:** To observe the effects of an artificial sweetener (sodium cyclamate) on a rat's pancreas and glucose levels.

**Study Design:** Laboratory-based experimental study.

**Place and Duration of Study:** Army Medical College Rawalpindi, in association with the National Institute of Health, Islamabad, Pakistan from Jun 2014 to Jan 2015.

**Methodology:** Forty Sprague Dawley rats (male and female housed separately to avoid mating and pregnancy) were involved in the experiment. Twenty rats in each control and experimental group. C served as control and E for the experimental group. A normal laboratory diet was fed to control Group-C for two months, whereas through an oral gavage needle, artificial sweetener 60mg/kg/day (sodium cyclamate) was fed to experimental animals for two months. During the two months after twelve-hour fasting, rats' blood glucose level was recorded weekly. At the commencement and completion of these two months, the weight of the animals was noted.

**Results:** Fasting blood sugars of experimental group was found to be statistically significant from week 3 to 9 with *p*-values of 0.034, <0.001, <0.001, 0.002, 0.001, <0.001, <0.001 respectively. Number of islets of Langerhans was significantly higher  $13.70 \pm 1.87\mu\text{m}$  in experimental group compared with control group with the *p*-value of <0.001.

**Conclusion:** Sodium cyclamate not only affected the glucose metabolism but also disturbed the normal histology of the endocrine pancreas.

**Keywords:** Sodium cyclamate, Fasting blood glucose, Islets of Langerhans.

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### INTRODUCTION

Sugar-free items have gained much attention because of their low-calorie provision property. Therefore, the food industry is more into low-calorie sweeteners instead of high-calorie sugars. United States Food and Drug Administration-approved sweeteners include Aspartame, Acesulfame K, Neotame, Cyclamate, and ALITAME as per ADI value. However, till now, their breakdown products' health hazards and metabolic effects have been controversial.<sup>1</sup>

The food we take plays a vital role in controlling human hunger, craving, and nutritional demand. A sweetener is a flavouring agent or additive which imitates the sugars without affecting caloric intake. Their low caloric value and the same flavour as sugars are the reason for their preference over regular sugars. Less caloric intake may also help control blood sugar levels and obesity.<sup>1</sup> However, these sweeteners may be very intense, and only small quantity may replace the high amount of sugar. Currently, the most common

sweeteners in use are Aspartame, Neotame, Saccharin, Cyclamate, Acesulfame-K, Alitame, and Sucralose, approved by the EU scientific committee on food.<sup>1,2</sup>

Many artificial sweeteners are used in a wide variety of foods, beverages, and medicines. Since their discovery, scientists have been reporting their health hazards and cancer risks, raising a sense of insecurity in consumers' minds. It is observed that every person is a consumer of artificial sweeteners, intentionally or unintentionally. A cancer-producing property of any one of the sweeteners would mean a cancer risk to the entire population.<sup>3</sup>

It has been seen recently that artificial sweetener campaigns are on the rise due to an increase in obesity in all ethnic groups, and due to excessive marketing of these artificial sweeteners, these are now considered important health-ensuring components. These products are marketed as beneficial to health compared to sugar, especially in the diabetic population. Although these campaigns are attractive to the customers, these sweeteners have never been vigorously tested on a large population and for a longer duration to detect their adverse effects and hazards.<sup>4,5</sup>

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Physiological studies carried out on artificial sweeteners showed increased postprandial glucose levels. Some studies also showed increased postprandial insulin levels when compared with natural sugars.<sup>6,7</sup> Therefore, it suggests that artificial sweetener is linked to some metabolic disorders. The rationale of this study is to find out any change in fasting blood glucose levels in rats after artificial sweetener consumption. The number of islets of Langerhans in rat's pancreas was also considered to find any link to metabolic disorders.

## METHODOLOGY

This laboratory-based experimental study was carried out at the Anatomy Department of Army Medical College Rawalpindi Pakistan, with the co-operation of the National Institute of Health Sciences, Islamabad Pakistan (02/CREAM-A/Hina Kundi) from June 2014 to January 2015. Forty rats were used in this study.

**Inclusion Criteria:** Healthy adult male and female (non-pregnant) rats of Sprague Dawley species were included in the study.

**Exclusion Criteria:** Unhealthy and juvenile rats were excluded from the study.

The sampling technique used was non-probability convenient sampling. The weight of the rats was between 175gm-205gms. The experimental animals were kept at NIH animal house in a controlled environment. Division in groups was random. An equal number of males and females (caged separately) were grouped in two. There was the provision of the normal diet with 1ml plain water to control Group-C. whereas experimental Group-E took 60mg/kg body weight sodium cyclamate once daily for two months with the help of a gavage tube. Fasting blood glucose levels were recorded once a week early in the morning by taking blood samples from the rat's lateral tail vein with the help of a glucometer after 12 hours of overnight fasting of all of the animals.<sup>6</sup> Rats were dissected after two months' completion. Their pancreas was dissected, and weight was documented. Three sections (intestine, middle and spleen region),<sup>7</sup> were made from each specimen and were placed in tissue containers. After automated processing, routine haematoxylin and eosin were done, and histology was observed. Several islets of Langerhans were counted per slide (three slides per specimen) at X 10. Islets containing three and more endocrine cells were taken into account, and the whole slide was scanned with the help of pointer.<sup>8,9</sup> Mean was taken for three slides for each specimen.

Data was analyzed on Statistical Package for the social sciences (SPSS) version 20:00. Results representation was in mean  $\pm$  standard deviation (mean  $\pm$  SD). An independent sample t-test was applied for inter-group differences. Results were considered significant when  $p$ -value was  $\leq 0.05$ .

## RESULTS

Forty Sprague Dawley rats divided into two groups (control and experimental) were taken. The animals of the control group remained active for the entire duration.

Detailed histological examination of H& E stained slides revealed a difference in the number of islets of Langerhans when both groups were compared (Figure-1A and Figure-1B).

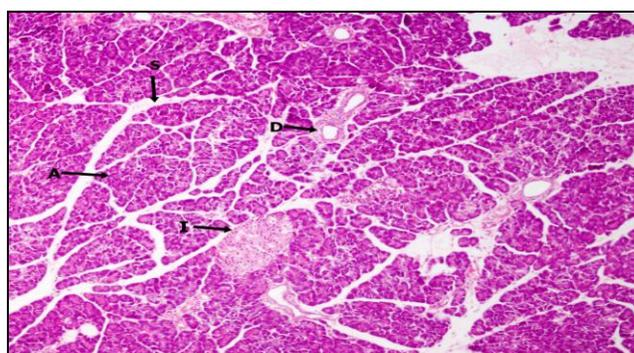


Figure-1: Photomicrograph of histological section of pancreas of an animal from Group C showing pancreatic acinus (A), ducts (D), connective tissue septa (S) and islets of Langerhans (I).

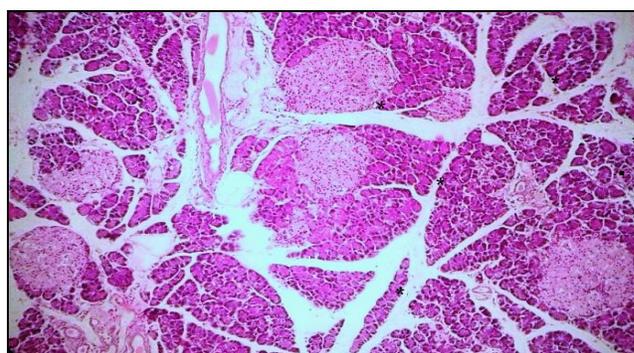


Figure-1B. Photomicrograph of histological section of pancreas of an animal of group E showing increased number of islets of Langerhans (\*).

The mean number of islets of Langerhans in the control group and experimental group were  $11.70 \pm 1.38$  vs  $13.70 \pm 1.87$  with the  $p$ -value of  $< 0.001$  (Table-I).

Fasting blood sugar levels between the control and experimental groups were considered insignificant during the first two weeks showing a  $p$ -value of 0.880 and 0.721, respectively. From 3rd to 9th week fasting

blood sugars of experimental group was found to be statistically significant as they showed *p*-values of 0.034, <0.001, <0.001, 0.002, 0.001, <0.001, <0.001 respectively (Table-II).

**Table-I: Comparison of mean number of islets of Langerhans between the control and experimental group.**

Parameters	Control group C (n = 20)	Experimental group E (n = 20)	<i>p</i> -value
Number of islets of Langerhans (Mean ± SD)	11.70 ± 1.38	13.70 ± 1.87	<0.001*

**Table-II: Comparison of mean fasting blood sugar levels in mg/dl between the control and experimental group.**

Weeks	Control group C (n = 20)	Experimental group E (n = 20)	<i>p</i> -value
First week	102.55 ± 4.56	102.30 ± 5.76	0.880
Second week	106.90 ± 8.07	106.00 ± 7.77	0.721
Third week	98.45 ± 4.51	94.20 ± 7.37	0.034*
Fourth week	91.65 ± 6.47	81.60 ± 8.70	<0.001*
Fifth week	100.10 ± 4.72	85.40 ± 7.55	<0.001*
Sixth week	96.55 ± 4.38	91.20 ± 5.83	0.002*
Seventh week	91.30 ± 11.38	80.80 ± 6.29	0.001*
Eighth week	96.55 ± 4.37	81.60 ± 7.67	<0.001*
Ninth week	101.40 ± 6.85	80.75 ± 3.82	<0.001*

## DISCUSSION

The islet of Langerhans number was significantly higher group treated with sodium cyclamate as compared to the control group (*p*<0.001). It has been seen that the size of the pancreas, number of islets of Langerhans and total area of islets are dependent on insulin levels. Sodium cyclamate has an overall role on the islet of Langerhans by affecting their major, minor and mean diameters, volume, area, perimeter and volume-area ratio in the treated group.<sup>10</sup>

Therefore, any increase in demand for insulin leads to an increase in the number and the size of islets of Langerhans in compensation.<sup>11</sup> In addition, other research has shown a direct relationship between artificial sweeteners and the release of insulin.<sup>12</sup>

Chronic administration of artificial sweeteners has hyperstimulation effects on pancreatic islets and acinar cells.<sup>12,13</sup> Another hypothesis for the increase in the number of islets of Langerhans is that potential endocrine progenitor cells exist in the wall of the ducts of the pancreas. These progenitor cells tend to form new pancreatic cells in the embryonic and adult period.<sup>12</sup>

Any disturbance (pathological or experimental) in the pancreas may result in the formation of new

endocrine tissue within the pancreas by these potential cells, even in adults with significant efficacy.<sup>14</sup>

Mean fasting glucose sugar levels were compared for the first two weeks of control and experimental groups. It was found to be statistically insignificant (*p* = 0.880 and 0.721), but from the third to the ninth week, the mean fasting blood glucose levels were significant when both groups were compared. It was found to be significantly less in rats treated with artificial sweetener (*p* values: 0.034, 0.001, <0.001, 0.002, 0.001, 0.001, 0.001) respectively. Our results agreed with a study conducted by Anton SD in 2010 that showed decreased blood glucose levels in the artificial sweeteners exposed group compared to the sucrose exposed group.<sup>15</sup>

Our findings coincide with previous research conducted by Suez *et al*, (2004) and Alsunni *et al*, (2020) that the use of artificial sweeteners in diet may lead to obesity and glucose intolerance.<sup>16,17</sup> Sweeteners are considered metabolically inactive but may disrupt glucose metabolism in three ways. Firstly, they deregulate the learned response that controls blood glucose levels and maintains homeostasis. Then, in succession, they upset the glucose absorption mechanism and stimulate insulin secretion by interacting with sweet taste receptors. Last of all, they cause glucose intolerance by interfering with gut microbiota.<sup>18</sup> It has also been seen that different artificial sweeteners may influence the gut microbiota and fluctuate the intestinal absorption of glucose and thus disrupting the post-prandial glucose levels.<sup>2</sup> Similarly, it has also been seen that artificial sweeter may disrupt liver enzymes and functioning, affecting glucose metabolism.<sup>19</sup>

Thus, our study results show that artificial sweeteners tend to alter the glucose levels and may affect the endocrine pancreas histological structure, which has not been much explored previously. There is a dire need to explore the metabolic effects of sweeteners on glucose metabolism as artificial sweeteners are added to almost every confectionary.

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## LIMITATIONS OF STUDY

The effect of one particular sweeter's results can be generalized as different sweeteners have a different mechanisms of action. The study duration was a limitation as long-term effects cannot be studied.

## RECOMMENDATIONS

Sodium cyclamate effects can also be seen in other organs, especially the liver.

## CONCLUSION

Sodium cyclamate, an artificial sweetener, decreases blood glucose levels and increases the number of islets of Langerhans in rats' pancreas.

**Conflict of Interest:** None.

## Author's Contribution

HK: Conception of idea & design of study. drafting the article, NW: Data Collection, literature search & interpretation, AQ: Data analysis & microscopic slides evaluation, RB: Interpretation of results, SQ: SPSS statistical analysis, MY: Data collection & drafting the article.

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