

## VARIATION IN THE AREA OF ISLETS OF LANGERHANS IN SODIUM CYCLAMATE TREATED RATS

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### ABSTRACT

**Objective:** To observe the effects of sodium cyclamate on islets of langerhans in rats pancreas.

**Study Design:** Laboratory based randomized control trial.

**Duration of study:** Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from March to May 2014.

**Material and Methods:** Twenty male and twenty female Sprague dawley rats weighing 175-205 gms were used in the experiment. Half male and half female rats were randomly divided in two groups (control group C and experimental group E, n=20 animals in each group). Group C served as control group in which rats were given normal diet. Group E served as experimental group and was given sodium cyclamate 60mg/kg/day through oral gavage tube for two months. Animals were dissected. Pancreas was examined and weighed. Slides were made after processing the organ for histological study. Area of islets of langerhans was calculated by image j software. Results were analyzed on SPSS version 20.

**Results:** The mean weight of pancreas in control and experimental group was 0.75 gm (SD  $\pm$  0.094) and 0.805 gm (SD  $\pm$  0.068) respectively. It was significantly higher ( $p = 0.043$ ) in experimental group. The area of islet of langerhans in control and experimental group was 15285.40  $\mu\text{m}^2$  (IQR: 9881.08 – 23001.35) and 33213.50  $\mu\text{m}^2$  (IQR: 21258.05–45879.18) respectively. There was an increase in area in experimental group ( $p = 0.014$ ).

**Conclusion:** Sodium cyclamate affects the histomorphology of endocrine pancreas by increasing the area of islets of langerhans in treated group.

**Keywords:** Islets of langerhans, Pancreatic lesions, Sodium cyclamate.

### INTRODUCTION

Alternative sweeteners tend to copy the sweetness of sugar (sucrose) with least amount of calories. Sense of sweetness is transferred through receptors on specialized taste cells. Sweeteners bind themselves to these receptors. Artificial sweeteners bind themselves tighter or longer to cause increased sweetness<sup>1</sup>. Most of these sweeteners are synthetic and require approval of relevant regulatory authorities. There is a controversy that whether the use of these sweeteners is harmful for health and it is often claimed that their use might induce toxicity<sup>2</sup>. The major reasons for the need of these sweeteners are to assist process of weight loss, minimize risk of dental problems, provide sweetness for diabetics and to produce cheaper food items<sup>2</sup>.

Sodium cyclamate is used as an artificial sweetener in many foods, beverages as well as medicines due to its odorless nature and solubility in water, propylene glycol and alcohol. It is far more stable than aspartame and saccharine. It is derived from N-cyclo-hexyl-sulfamic acid, which was first discovered accidentally by Michael Sveda, who found it to be 30 times sweeter than saccharine with no bitterness<sup>3</sup>.

Cyclamate sweeteners are attention grabbing for many reasons. They are permitted in the European Union by European Parliament and Council and other countries. They exhibit good synergy, are relatively cheaper to produce and have a lot of other qualities<sup>4</sup>. Cyclamate metabolizes into two main byproducts cyclohexylamine and dicyclohexylamine. These are considered very toxic. In a study conducted on primates which showed highest testicular and urinary cyclohexylamine levels causing focal germ aplasia<sup>5</sup>. Sodium cyclamate can also cross blood placental barrier suggesting retardation in development of placenta and restriction in intrauterine fetal growth<sup>6</sup>. It also

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brings histomorphological changes in fetal liver and exocrine pancreas<sup>3</sup>.

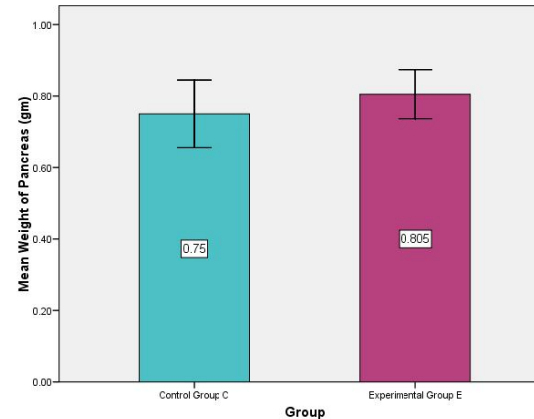
Not much work has been done upon the effects of sodium cyclamate on pancreas. A study done on artificial sweetener showed the unfavorable metabolic effects which include increase in weight, diabetes mellitus and other metabolic disorders<sup>7</sup>. A long term study on primates showed that it altered the blood glucose levels as well as the histology of pancreas showing hyalinization of pancreatic islets of langerhans<sup>8</sup>.

In the light of previous studies the rationale of current study was to access the effects of sodium cyclamate on histomorphology of endocrine pancreas and to observe any change in the area of islets of langerhans.

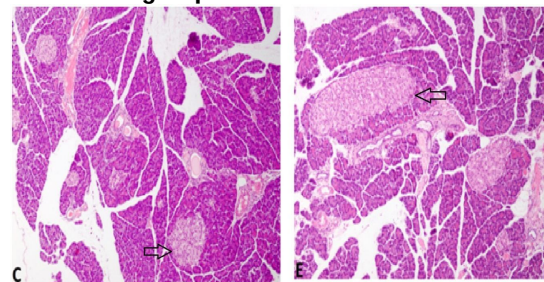
## MATERIAL AND METHODS

The study was performed at the department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from 11<sup>th</sup> March to 11<sup>th</sup> May 2014. It was a laboratory based randomized control trial. Twenty male and twenty female Sprague dawley rats weighing 175-205gms were used in the experiment and were kept in Animal house of NIH, Islamabad. The room was well ventilated and cycles of 12 hours light and 12 hours dark were maintained under a constant temperature range of 20-26°C to provide controlled environment. Rats were fed with NIH laboratory diet for two months. Water was provided ad libitum. Half males and half females rats were randomly divided into two groups, C control and E experimental group (n = 20 animals in each group). Animals in control Group "C" were given normal diet where as the animals in experimental Group "E" were given Sodium cyclamate at a dose of 60mg/kg by oral gavage tube for two months. Sodium cyclamate was purchased from Zhejiang Chemicals Import And Export Corporation (product no CP95). At the end of 60 days the animals were dissected. Pancreas were weighed and studied for any gross abnormality. After proper fixation of specimens, three sections were cut from each pancreas (intestine, middle and spleen region)<sup>9</sup> and placed in tissue cassettes for automated

processing. 5µm thin sections were cut and stained in autostainer by routine haematoxylin and eosin (H&E) for histological study of pancreas. Islets of langerhans shape was



**Figure-1: Comparison of weight of pancreas between the groups.**



**Figure-2: Photomicrographs, an arrow head showing islet of langerhans in pancreas of control group C, an arrow head showing change in shape and an increase in area of islets of langerhans in experimental group E (H&Ex100).**

observed and size was measured. Images were taken at X10 for each slide by taking four fields per slide. Olympus digital camera stylus 1010 (10mega pixel) was used through the ocular of light microscope Olympus DP21. Area of islets was measured using image j<sup>10</sup>. Three slides per specimen were taken. The final reading was recorded as the average area of islets of three slides per specimen. Data was analyzed on statistical package for data analysis SPSS version 20. Independent samples't-test/Mann-whitney U test was applied (where applicable) to find out any intergroup differences. *p* value < 0.05 was considered significant. Results were represented as mean ± standard deviation (mean ± SD), median and interquartile range (IQR).

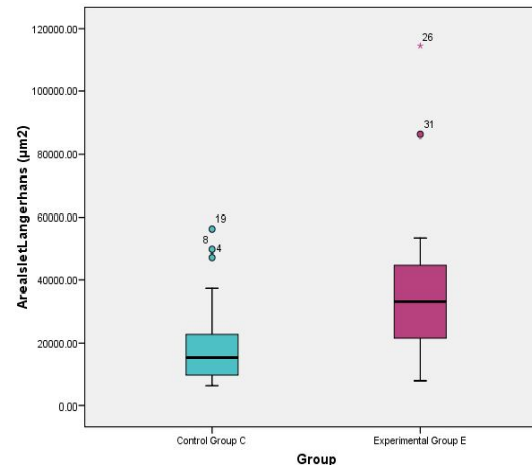
## RESULTS

The pancreas of control group C was pinkish in color and soft in consistency. When compared with experimental group E, the pancreases were slightly darker in color showing congested appearance. Mean weight of pancreas was 0.75 gm (SD  $\pm$  0.094) in control group C while it was 0.805 gm (SD  $\pm$  0.068) in experimental group E. Weight of pancreas was significantly higher in experimental group E as compared to control group C ( $p=0.043$ ) (fig-1). The histological section of control group showed a thin capsule made up of loose connective tissue all around the gland. The connective tissue septa were dividing the gland into irregular lobules. Between each lobule large amount of connective tissue was present that contains arteries, veins and nerves which are supplying the gland. The large ducts were also present in these connective tissue septa. The pancreatic acinar cells were present in the lobules in tubuloacinar shape which is forming the secretory unit of pancreas. The small clusters of endocrine cells forming the islets of langerhans were present in the parenchyma of the gland. These groups of variable size were scattered throughout the glandular portion of pancreas. The islets were regular round to oval in shape and median area of islet of langerhans in control group C was 15285.40  $\mu\text{m}^2$  (IQR: 9881.08 – 23001.35) (fig-2c). In experimental group E the islets of langerhans showed changes in their shape from round and oval to irregular in shape along with increase in size. The median area was 33213.50  $\mu\text{m}^2$  (IQR: 21258.05 – 45879.18) (fig-2E). Area of islet of langerhans was significantly more in experimental group E as compared to control group C ( $p=0.014$ ) (Figure-3). Sodium cyclamate affects the endocrine pancreas by increasing the area of islets of langerhans.

## DISCUSSION

The endocrine component of pancreas plays an important role in maintaining the body's glucose and insulin levels. Artificial sweeteners tend to decrease the total calories intake and indirectly decrease blood glucose levels. The objective of this study was to see the effects of sodium cyclamate on islets of

langerhans. It has been proposed that artificial sweeteners affect the glucose metabolism of the body as well as insulin secretion. A number of studies support the fact that artificial sweetener cause an increase in insulin release<sup>11,12</sup>. Previous



**Figure-3: Comparison of area of islets of langerhans between the groups.**

studies mentioned that sodium cyclamate altered the blood glucose levels as well as the histology of pancreas showing hyalinization of pancreatic islets of langerhans<sup>8</sup>. In this study it was seen that weight of pancreas as well as the area of pancreatic islets of langerhans was increased in experimental group when compared with control group. The possible explanation for increase in size of langerhans is that sodium cyclamate increases the insulin release which resulted in compensatory increase in area of langrehans. This demand for increase in insulin release is compensated by increase in the size of islet of langerhans of pancreas. It has been seen that diet containing artificial sweeteners tend to increase sugar absorption by stimulating the taste bud as well as intestinal taste receptors. This indirectly lead to increase in insulin secretion which affects the appetite, weight and glucose levels<sup>13</sup>. Receptors for sweet taste are present on taste buds as well as enteroendocrine cells of gastrointestinal tract. These receptors act as sugar sensors. The mechanism for eliciting insulin secretion by pancreatic beta cells is that the sweet receptor proteins and mRNA is expressed on pancreatic beta cells similar to those present in taste buds.

Artificial sweeteners are found to be sweet receptor agonist present on endocrine cells of pancreas. These sweeteners bind with the receptors and stimulate secretion of insulin from pancreatic islets<sup>14</sup>. There are studies which report that artificial sweeteners may cause an increase in hunger, yearning and food intake. So there is great risk of weight gain by using artificial sweeteners in diet<sup>15</sup>. It has also been said that these products hamper the efforts to lose weight by encouraging the food intake<sup>16</sup>.

Artificial sweetener disrupts the body's physiological and homeostatic mechanisms by affecting the pancreas endocrine component<sup>17</sup>. Martin et al showed hypertrophy of acinar cells in rats fetal pancreas when sodium cyclamate was given intraperitoneally from the 10<sup>th</sup> to the 14<sup>th</sup> day of pregnancy<sup>3</sup>. In current study it is seen that sodium cyclamate induces histomorphological changes in endocrine pancreas. The pancreas of sodium cyclamate treated group was congested and the islets of langerhans showed change in shape and increase in area which led to increase in weight of pancreas.

## CONCLUSION

The current study proved a statistical significant affects on endocrine pancreas by showing an increase in area of islets of langerhans.

## Future Recommendations

A comparison of different sweeteners can be done with regard to its effects on exocrine/endocrine pancreas.

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## CONFLICT OF INTEREST

This research was funded by National University of Sciences and Technology (NUST) has no conflict of interest to declare by any author.

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