Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 1-10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

ASPARTAME AND DIET SODA IMPACT ON BLOOD SUGAR AND INSULIN IN WISTAR RAT

[1]Eiya, B.O. and [2]Osunbor, J.O.
[1]Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences
[2]Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences

Received: 19-04-2023
Accepted: 16-05-2023

https://dx.doi.org/10.4314/sa.v22i3.1

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: Faculty of Science, University of Port Harcourt.

ABSTRACT

Research reports on the safety of Aspartame as a sugar substitute which was intended to reduce the prevalence of metabolic disorders has been inconclusive. Thus, this study is aimed at investigating the effects of aspartame and diet soda on body weight, blood sugar and insulin levels. Thirty seven Wistar rats were divided into: Group 1 (5): control group and administered 5ml of distilled water daily. Groups 2 and 3 (8 each) received high and low doses of diet soda respectively. Groups 4 and 5 (8 each) received high (45 mg/ kg b.w) and low (22.5 mg/kg b.w) doses of aspartame respectively. After 10 weeks, the rats were subjected to an overnight fast for the determination of fasting blood sugar test after which they were sacrificed. Blood samples were collected and the pancreas harvested and put in formal saline for histological analysis. Insulin level was measured using ELISA technique. Results show various morphological changes in the pancreas of all experimental groups, including hypoplastic islet, vascular congestion and ulceration, nerve hypertrophy, amongst others. Body weight was significantly increased in groups given high diet soda (114 ± 0.12g) and aspartame (121 ± 0.17g) when compared with the control (85 ± 0.21g). However, there was no significant difference in fasting blood sugar and insulin levels in all treated groups compared to control. In conclusion, this study has shown that chronic consumption of aspartame and diet soda increased body weight, but no effect on blood sugar and insulin levels. The adverse effects on pancreas morphology suggest impending health implications.

Keywords: Aspartame, diet soda, pancreas morphology

INTRODUCTION

Aspartame is an artificial non-saccharide sweetener introduced to replace the commonly used sucrose. It was inadvertently discovered in 1965, by James M. Schlatter at the G.D. Searle Company, when he was studying new treatments for gastric ulcers. Aspartame is one of the most rigorously tested food ingredients. The Federal Drug Administration approved it as a non-nutritive sweetener in 1981 and for use in carbonated beverages in 1983. Being 200 times sweeter than sucrose, only little quantity is required to attain sweetness, thus, its intake was expected to reduce obesity rates in developing countries and help those struggling with diabetes. The sweetener is a methyl ester of the aspartic acid/phenylalanine dipeptide, completely hydrolyzed in the gastrointestinal tract to 10% methanol, 40% aspartic acid, and 50% phenylalanine (Ranney et al, 1976). It is available in the trade names NutraSweet,

Equal, and Canderel. The taste of aspartame and other artificial sweeteners differ from that of table sugar in the times of onset and how long the sweetness lasts, though aspartame comes closest to sugar's taste profile among approved artificial sweeteners (O'Donnell, 2006). The sweetness of aspartame lasts longer than that of sucrose, so it is often blended with other artificial sweeteners such as acesulfame potassium to produce an overall taste more like that of sugar. One of the readily products that contain aspartame is the diet sodas. To achieve this taste, the artificial sweeteners, aspartame and acesulfame potassium were used to replace sugar in this beverage. The acceptable daily intake (ADI) of aspartame is currently 50 mg/kg body weight (b.w) in the United States and 40 mg/kg b.w in the European Union for both children and adults. Daily consumption of artificial sweeteners by women of childbearing age and by children has been estimated at 2.5–5.0 mg/kgbw (Butchko et al, 2002).

Over the years, there has been repeated incidence of metabolic disorders all over the world. In 2016, there were more than 1.9 billion overweight adults and 650 million obese adults, representing a global prevalence of 13% (WHO, 2018). Besides adults, over 340 million children and adolescents (aged 5-19 years) were overweight or obese in 2016. Such metabolic disorders increase the risk of diabetes mellitus, which is believed to result from resistance to insulin by the body. The strategy of sugar replacement with aspartame and acesulfame potassium in diet sodas was employed to reduce the prevalence of diabetes mellitus, as well as cardiovascular diseases and cancer (Khan and Sievenpiper, 2016). However, despite being recognized as safe and well-tolerated, a lot of controversies about the effects of these artificial sweeteners on human health still exist (Tandel, 2011). Studies show that aspartame has zero grams of sugar and won't spike insulin levels after it is consumed. However, some studies in mice have shown that aspartame affects gut bacteria in ways that could lead to insulin resistance, especially with frequent and repeated use (Suez et al, 2014). Therefore, this work is aimed to determine the influence of aspartame consumption and intake of diet soda on insulin and blood sugar levels.

MATERIALS AND METHODS

Experimental animals: Thirty seven (37) albino Wistar rats with an average weight of 110g were used for this study. The animals were purchased and cared for in the animal house of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. They were kept in clean cages in a well-ventilated environment. They had access to clean water and sufficient feed ad libitum, in accordance with the guidelines of National Research Council Guide for the care of laboratory animals as described by (NRC, 1996) and revised by (Mansour et al, 2017). The study was conducted for a period of twelve (12) weeks including a two-week acclimatization period.

Experimental design: Thirty seven rats were divided into five groups. Group 1 (n= 5) served as the control group, and was administered 5ml of distilled water daily. Groups 2 and 3 (n= 8) were administered 5ml of the undiluted diet soda and diluted diet soda (1:1 dilution) respectively. Groups 4 and 5 (n=8) were administered 5ml of high dose aspartame (45 mg/kg b.w) and low dose aspartame (22.5mg/kg b. w) respectively. All administration was orally given using a clean gavage. All rats were sacrificed after ten weeks of administration.

Collection of samples: At the end of the experimental period, the animals were observed for general physical characteristics, and were weighed. The rats were subjected to a Fasting Blood Sugar test, which was taken after a 12-hour fast. The rats were anesthetized by exposure to 5% chloroform, after which cardiac puncture was carried out and blood was collected and introduced into plain sample bottles. The blood samples were allowed to clot, after which the serum was

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 1-10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

obtained by centrifugation. Insulin concentrations were measured and quantified using enzyme-linked immunosorbent assay (ELISA) technique.

Histological slide preparation: The pancreas tissue was carefully harvested using a criterion process. To remove any blood contamination, the samples were rinsed and tissue samples were fixated with (10%) formal saline for 48 hours, and then rinsed under running tap water for an hour to rid the tissue of formalin odour. Following the washing, the tissues were dehydrated by immersing them in a series of steadily increasing alcohol concentrations (50%, 70%, 80%, 90% and absolute alcohol). It must be cleared since the dehydrating alcohol will not dissolve in liquid paraffin. Then, the specimens were embedded in paraffin wax, to create blocks. The blocks were cut by dissolving wax from the surface of the block to reveal the tissue, and the tissues were cut with a microtome. Blocks of tissue sections were placed on a microscopic slide with help of warm distilled water containing a few drops of Mayer's albumin and deparaffinized with xylene solutions, the slide was put on hot plate (40°C) and left overnight. The tissue sections were then stained with hematoxylin and eosin (Luna, 1968). Histological changes were observed under a light microscope and photomicrographs were obtained.

Statistical analysis: All data obtained during the experiment were analyzed using Graphpad Prism 8.0 software and one-way analysis of variance (ANOVA). Tukey post-hoc test was used to compare results among groups. Results were expressed as Mean ± SEM and p values of ($p \leq 0.05$) were considered statistically significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

RESULTS

Micrograph of the pancreas stained with hematoxylin and eosin stains for control showed a normal architecture: A. exocrine acini, B. interlobar septum,, C. pancreatic duct, D. islet of Langerhans. Groups treated with different doses of aspartame and zero sugar coke showed various morphological changes such as hypoplastic islet, vascular stenosis and nerve hypertrophy (plates 2 and 3), as well as vascular ulcerations and perivascular infiltrates of inflammatory cells (plates 4 and 5).

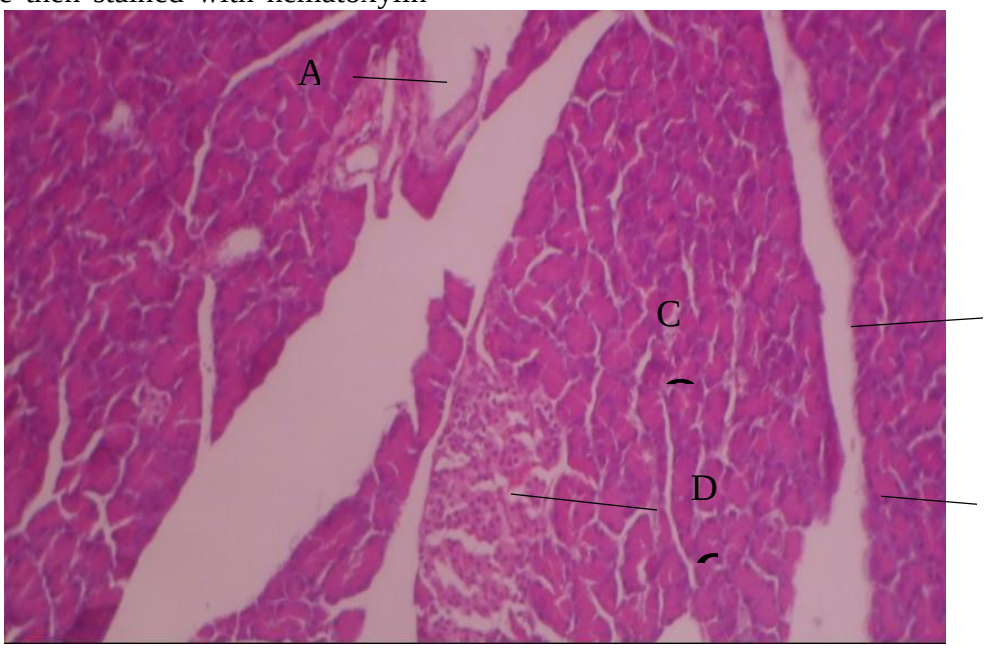

Plate 1: Control: Composed of normal architecture: A. exocrine acini, B. interlobar septum, C. pancreatic duct, D. islet of Langerhans (H&E × 400)

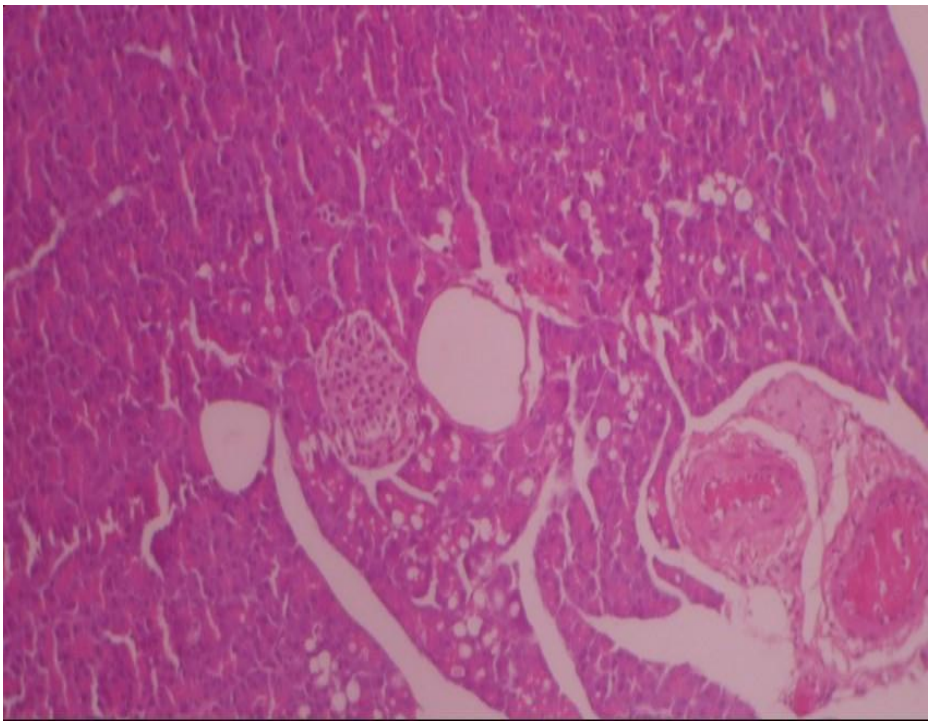

Plate 2: Rat pancreas given 5ml undiluted diet soda showing: A. hypoplastic Islet, B. vascular stenosis and C. nerve hypertrophy (H&E x 400)

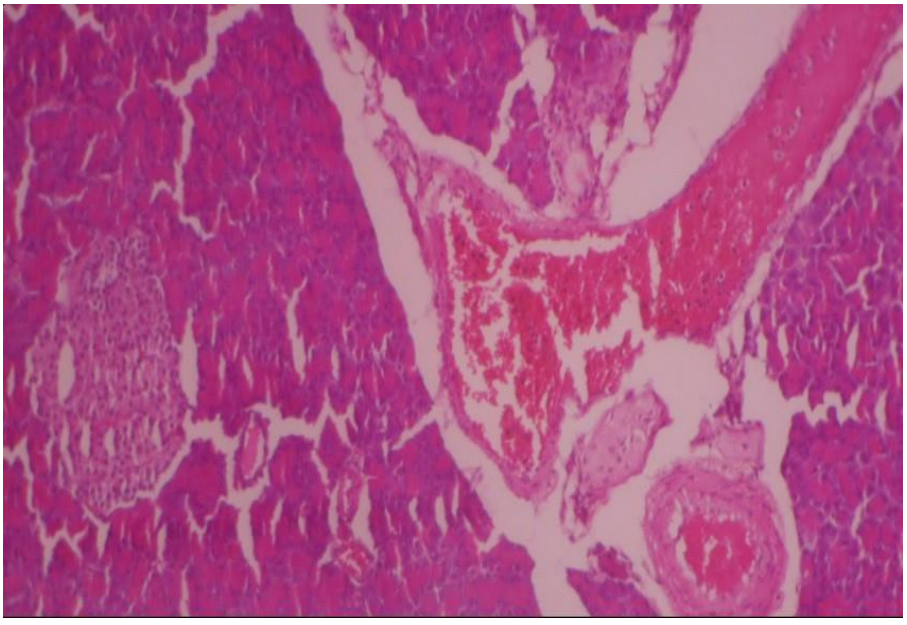

Plate 3: Rat pancreas given 5ml diluted diet soda (1:1) showing: A. severe vascular ulceration and B. vascular congestion, C. nerve hypertrophy (H&E x 400)

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 1-10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

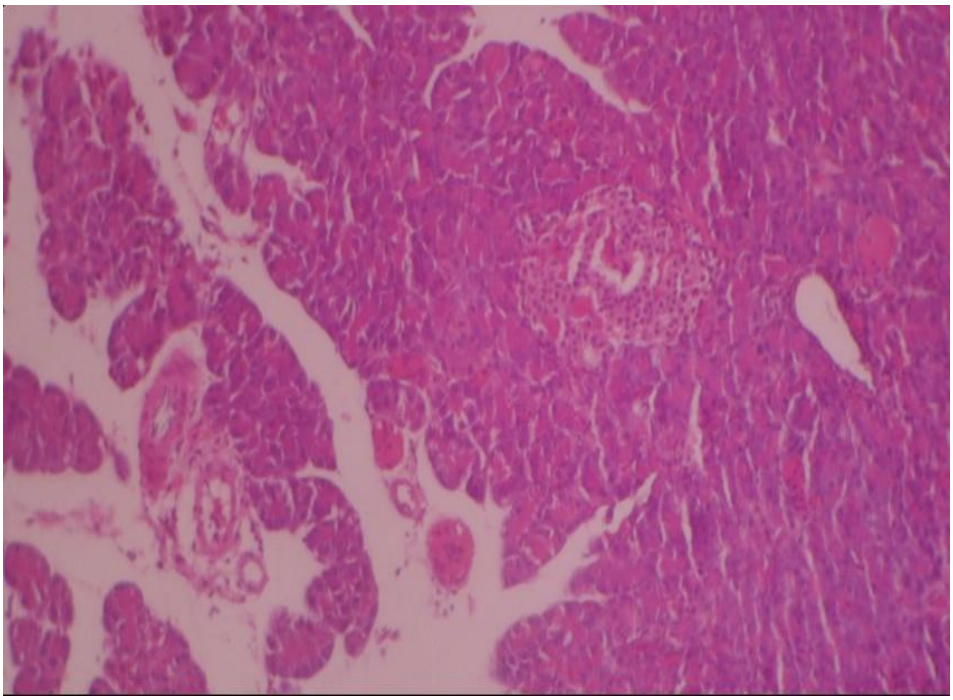

Plate 4: Rat pancreas given 5ml high dose (45 mg/kg b.w) Aspartame showing: A. vascular ulceration and hypertrophy, B. perivascular infiltrates of inflammatory cells C. hypoplastic Islet (H&E x 400)

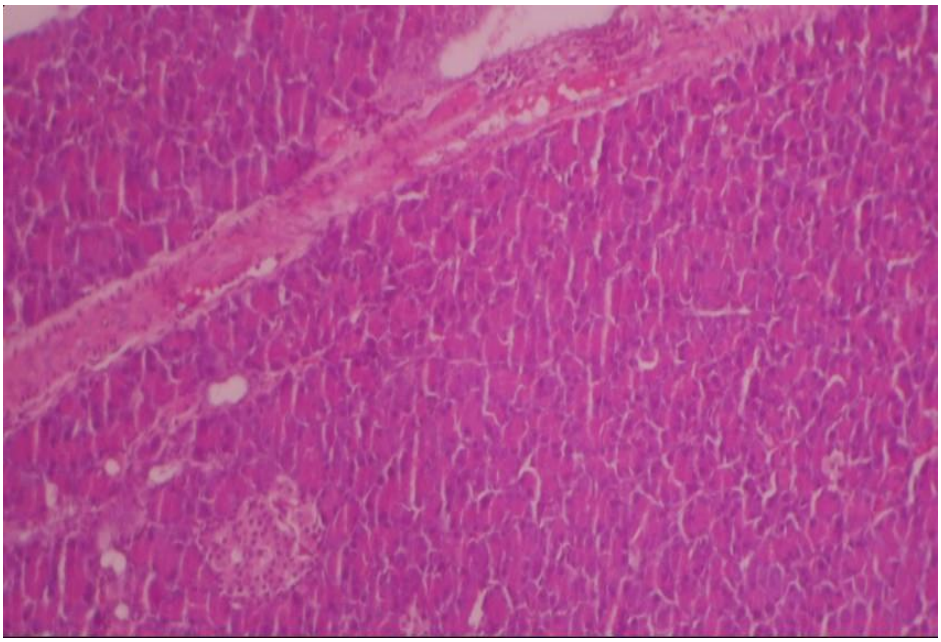

Plate 5: Rat pancreas given 5ml low dose (22.5 mg/kg b.w) Aspartame showing: A. vascular stenosis and ulceration, B. perivascular infiltrates of inflammatory cells, C. hypoplastic Islet (H&E x 400)

Table 1: Mean values of different concentrations of diet soda and aspartame administration in Wistar rats.

Biomarkers	**Control**	**Undiluted Diet Soda**	**Diluted Diet Soda**	**High Dose Aspartame (45g/kgbwt)**	**Low Dose Aspartame (22.5g/kgbwt)**
Body weight (Kg)	85.20 ± 0.21	114 ± 0.12**	94.20 ± 0.24	121 ± 0.17***	90.15 ± 0.24
FBS (Mg/dl)	61.60 ± 0.24	61.90 ± 0.41	64.20 ± 0.22	71.80 ± 0.42	63.40 ± 0.24
Insulin conc. ($\mu IU.ml^{-1}$)	1.20 ± 0.21	0.68 ± 0.11	1.15 ± 0.28	1.48 ± 0.12***	1.04 ± 0.18

Value asterisked (*) are statistically significant from the control group (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) are Mean ± SEM compared to control, (n=3)

DISCUSSION

Histological assessment of the pancreas of the control group and the experimental groups showed contrasting results. While the control group depicted normal architecture of endocrine and exocrine components. This histopathological observation revealed that the pancreatic islets of all experimental groups were degenerated, with their cell size being shrunken and hypoplastic islets of pancreas observed in all groups. The islet cells are formed by β cells which are responsible for producing insulin. A depletion of these cells results in insulin deficiency, which in turn leads to a disorder in carbohydrate metabolism. This is usually seen in Type 1 diabetes mellitus. In groups administered diet soda, there were vascular congestions in the parenchyma of the pancreas, with the destruction of many endocrine cells in the Islets of Langerhans and the loss of their nuclei, as well as a loss of cell communication with each other, otherwise known as endocrine cell dispersal. This agrees with the study by Morovvati et al., (2019) where aspartame intake caused negative effects on histomorphometric parameters and tissue damage in the adrenal gland. This study showed that diet soda did not adversely affect the exocrine pancreatic functions. This result is in line with study by Brown and Rother, (2012). The latter proposed that the intake of diet soda did not affect the secretion of pancreatic enzymes.

Perivascular infiltrates of inflammatory cells were evident in groups 4 and 5 (high dose of aspartame and low dose of aspartame). This inflammation often results in autoimmune responses. It is characterized by lymphocyte and plasma cell infiltration from the venous wall to the lumen, culminating in venous obliteration. The blood vessels were also stenosed as a result of fatty infiltrates, inducing a higher pancreatic duct pressure, which causes pancreatic juice regurgitation and auto digestion. Vascular ulceration and hypertrophy were also evident here, causing an increase in the size of the organ due to swelling of individual cells. This result is in agreement with the findings of Gaujoux et al, (2010) which reported that increase in the cells led to increase in weight of abdominal organs. The changes seen in the aspartame groups may be attributed to incidence of acute pancreatitis, caused by an acute inflammatory response resulting from unregulated activation of pancreatic enzymes. Its severity ranges from its mildest form, which resolves quickly with few complications, to its more severe form necrotizing pancreatitis, which is associated with an increased risk for developing multiple system organ failure and mortality. However, this study does not correspond with that of Dooley et al, (2017), which proposed there

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 1-10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

was no effect of dietary aspartame on pancreatic acinar. Furthermore, the degeneration of islets seen in groups administered aspartame may be attributed to focal lesions of acute pancreatitis. During this, an injurious inflammatory adverse event (ulcerations) occur within the periductal area, and may have negative implications for islet neogenesis, dependent on stem cells residing within or adjacent to the ductal epithelium. This result corresponds with the islet morphology study of Mezza et al, (2014), which stated that the use of aspartame caused alterations in the islets morphology.

A cohort study by Otero-Losada et al, (2011) showed that aspartame is rich in advanced glycation end products that potentially increase insulin resistance and inflammation. During regular consumption of aspartame-sweetened drinks, fat accumulates in the liver by the primary effect of fructose which increases lipogenesis. Studies by Brown et al, (2009) also showed that aspartame augments glucagon-like peptide-1 secretion and can contribute to obesity, insulin resistance and in turn, type 2 diabetes. This may not have been evident in the biochemical report since most pathological conditions arise from the organs involved before they are detected in blood.

In a nut-shell, the pancreases of all treatment groups showed morphological changes, varying according to the substance administered and the concentration given. Undiluted diet soda and diluted diet soda had more effects on the endocrine pancreas, whereas concentrated aspartame and diluted aspartame had more effect on the exocrine acini. However, studies have shown that a maintained pancreatitis can subsequently affect the endocrine function of the pancreas.

According to the findings of this study, there were significant increases in body weights of groups administered undiluted diet soda (group 2) and high dose aspartame (group 4) compared to other groups. It was found to be significantly higher in groups administered high dose aspartame than that of diet soda. This result suggests that aspartame ingestion for 10 weeks was able to increase body weight indicating that a prolonged consumption of beverage containing acesulfame-K has led to an increase in food intake, body weight and body fat accumulation in rats. This notion is supported by a rodent study of Ragi et al, (2020) showing a marked increase in body weights and fat gain after consumption of acesulfame-K. A review on aspartame suggested that the sweetener intensifies the appetite, and this could be attributed to its non-caloric value and inability to provide energy to the body leading to increased hunger and appetite. To satisfy this hunger, one eats more than usual to provide the necessary energy, thereby increasing weight gain. Since the increase in body weights varied based on the concentrations of the substance administered, it can be said that the weight gain was positively associated with the amount of ingested aspartame.

It should be noted that the changes in weight gain were manifested from the 4th week of administration, and this implies that the impact of aspartame is not acute. These findings are in accordance with numerous studies associating long-term consumption of aspartame with weight gain (Almiron-Roig &Drewnowski 2003 and Black & Anderson 1993). Excessive weight gain is characterized with abnormal fat accumulations, which subsequently leads to obesity. Obesity is a risk factor for metabolic syndromes which including, type 2 diabetes, high blood pressure, coronary heart diseases and even certain mental illnesses (Luppino et al., 2010).

From the result of the biochemical analysis, there was no significant difference in fasting blood sugar levels and insulin concentration in all treatment groups when compared with the control group. Therefore, this suggests that the use of aspartame and consumption of diet soda has little or no impact on blood glucose and insulin levels. This result is consistent with studies of Hall et al., (2003),

which showed no significant effect on glucose levels were found after acute or long-term aspartame consumption. A recent study on healthy individuals by Daher et al., (2019) also showed no significant effect of aspartame on glucose homeostasis. However, this result does not correspond with studies by Sylvetsky et al., (2012) which proposed that there were increased insulin levels following a long-term consumption of the sweeteners, aspartame and acesulfame potassium.

CONCLUSION

It was concluded that the consumption of aspartame and diet soda resulted in an increase in the body weights of albino Wistar rats, and the increase was dependent on the concentration of the substance given. The consumption of aspartame and diet soda did not increase blood glucose and insulin levels. However, their adverse effect on the morphology of the pancreas suggests impending health implications. It is therefore recommended that caution should be taken in the intake of aspartame and aspartame sweetened diet sodas.

REFERENCES

Almiron-Roig E., Drewnowski A. (2003). Hunger, thirst, and energy intakes following consumption of caloric beverages. *Physiol. Behav.* ; 79, 212-9.

Black R.M., Leiter L.A., Anderson G.H. (1993). Consuming aspartame with and without taste: Differential effects on appetite and food intake of young adult males. *Physiol. Behav*. ;**53** 139-7.

Brown RJ, Mary W, and K I. Rother, (2012). Effects of Diet Soda on Gut Hormones in Youths with Diabetes, Diabetes Care 35(**5**): 959–964.

Brown, R. J., de Banate, M. A. and Rother, K. I. (2009). Artificial sweeteners: a systematic review of metabolic effects in youth. International Journal of Pediatrics and Obesity. **5**(4):305-12.

Butchko HH, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL (2002). Intake of aspartame vs. the acceptable daily intake. Regul *Toxicol Pharmacol* **35**:S13–S16.

Daher, M. I., Mattar, J. M. and Nour, A. A. (2019). Non-nutritive sweeteners and type 2 diabetes. *Diabetes Research and Clinical Practice.* **155**:107786.

Dooley, M. D., Moultrie, N., Elsbeth, B. S. Patricia B, Crawford, R. D. (2017). Primary Care Interventions to reduce childhood obesity and sugar- sweetened beverage consumption. *Journal of Public Health Dentistry.* **77**(1): 104 - 127.

Gaujoux, S., Cortes, A., Couvelard, A., Noullet, S., Clavel, L. and Rebours, V. (2010). Fatty pancreas and increased body mass index are risk factors of pancreatic fistula after pancreaticoduodenectomy. *Surgery.* **148**(1): 15 - 23.

H Morovvati , H Anbara, MT Sheibani , MK Koohi , A Hasanzadeh (2019). The Effect of Long-term Exposure to Aspartame on Histomorphometric and Histochemical Adrenal Gland in Adult NMRI Mice *Armaghane Danesh* , 24(**2**): 150-169

Hall, W., Millward, D., Rogers, P. and Morgan, L. (2003). Physiological mechanisms mediating aspartame-induced satiety. *Physiological Behaviour*.**78**: 557 – 562.

Khan, T. and Sievenpiper, J. (2016). Controversies about sugar: results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. *European Journal of Nutrition.* **55**(2): 25 – 43.

Luna, L. G. (1968). Manual of Histologic staining methods of the Armed forces Institution of pathology. 3rd Edition, McGraw-Hill, New York.

Luppino, F. S., de Wit, L. M., Bouvy, P. F., Stijnen, T. Cuijpers, P. Penninx, B. W. and Zitman, F. G. (2010). Overweight, obesity and depression: a systematic review and meta-analysis of longitudinal

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 1-10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

studies. *National Library of Medicine*. **67**(3): 220 - 229.

Mansour, A. A., Mohammed, A., Nassan, Osama, M. S. and Mohammed, M. S. (2017). Biochemical, Molecular and Immunohistochemical Study. *Complement Alternative Medicine*. **14**(4): 108 – 119.

Mezza, T., Muscogiuri, G. and Sorice, G. P. (2014). Insulin resistance alters islet morphology in non-diabetic humans. *Diabete.* **63**: 994 – 1007.

National Research Council (1996). Guide for the care and use of laboratory animals. National Academy Press, Washington DC. http://www.nap.edu/readingroom/books/labrats/ chaps.html#anim.

O'Donnell, K. (2006). Aspartame and Neotame: Sweeteners and sugar alternatives in food technology. *Blackwell*. **19**:86 – 95.

Otero-Losada, M., Grana, D. R., Muller, A., Ottaviano, G., Ambrosio, G. and Milei, J. (2011). Lipid profile and plasma antioxidant status in sweet carbonated beverage-induced metabolic syndrome in rat. *International Journal of Cardiology*. **146**: 106 – 109.

Ragi, M. E., El Haber, R., Fidele, E. and Obeid, O. A. (2020). The effect of aspartame and sucralose intake on body weight measures and blood metabolites: role of their form (solid and/or liquid) of ingestion. *The British Journal of Nutrition.* **128**(2): 352 - 360.

Ranney, R. E., Oppermann, J. A., Muldoon, E. and McMahon, F. G. (1976). Comparative metabolism of aspartame in experimental animal and humans. *Journal of Toxicology and Environmental Health:* **2**(10): 65 – 67.

Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C. A. and Mazza, O. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature.* **514**: 181 – 186.

Sylvetsky, A. C., Brown, R. J., Blau, J. E., Walter, M. and Rother, K. I. (2012). Hormonal responses to non-nutritive sweeteners in water and diet soda. *Nutritional Metabolism.* **13**:71 – 75.

Tandel, K. R. (2011). Sugar substitutes: health controversy over perceived benefits. *Journal of Pharmacology Pharmacother.***2**(4): 236 – 24

World Health Organization (2018). Non-communicable Diseases Country Profiles. Geneva.