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ORIGINAL ARTICLE

Effect of Artificial Sweeteners on the Histological Structure of the Pancreas of Adult Male Albino Rats and Its Possible Recovering withdrawal Effect

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Abstract

Background: Aspartame is a low-calorie artificial non-sugar sweetener and is commonly used as a sugar substitute in foods and beverages. Pancreas has a vital role in carbohydrate metabolism. The aim of the study was to study the effect of aspartame on the structure of pancreas. **Methods:** Eighteen albino Wistar rats (200-250gm) were divided into three groups; control group, aspartame-treated group, was treated with aspartame (250 mg/kg/day) for six months and withdrawal group: was treated with aspartame (250 mg/kg/day) for six months then left without treatment for another month. Serum amylase and lipase were measured, and histological study of the pancreas was conducted. **Results:** histopathological changes were detected in both pancreatic acini and islets of Langerhans, increased collagen deposition around the acini and blood vessels. Additionally, aspartame supplementation resulted in elevated levels of serum amylase and lipase. Upon withdrawal of aspartame, an obvious improvement was noticed in the withdrawal group in comparison with the aspartame-treated group. **Conclusions:** Aspartame produces degenerative changes in the pancreas of the rats and causes uprising of serum pancreatic enzymes. Withdrawal of aspartame results in partial restoration of the histological structure of the pancreas. From these results, it is recommended to spot a light on the dangerous impacts of the usage of aspartame in clinical studies.

Keywords: Aspartame; Pancreas; Amylase; Lipase; Histology; Insulin

INTRODUCTION

Currently, the world is more concerned with physical fitness, via choosing low-fat, non-weight-bearing diet like aspartame [1]. Aspartame is as artificial non-saccharide sweetener that replace the commonly used sucrose. It was unintentionally discovered in 1965, by James M. Schlatter at the G.D. Searle Company. Aspartame is one of the most strictly tested food ingredients. The Federal Drug Administration (FDA) approved it as a non-nutritive sweetener in 1981 and for use in carbonated beverages in 1983. Only a little amount of aspartame is needed to attain sweetness, as it is 200 times sweeter than sucrose. Administration of aspartame is expected to reduce obesity rates in developing countries and help those struggling with diabetes. Chemically, aspartame is a methyl ester of the aspartic acid/phenylalanine dipeptide. Pharmacodynamically, aspartame is completely hydrolyzed in the gastrointestinal tract to 10% methanol, 40% aspartic acid, and 50% phenylalanine [2].

The pancreas is a key player in the metabolism of carbohydrates. It contains about one million islets of Langerhans that are highly condensed particularly in its tail. Each islet contains a variety of cell types, such α-cells, β-cells, D-cells, and PP-cells. The β-

cells secreting insulin hormone make up between 70 and 75 percent of the cell population [3].

Physiologically, sweet substances in the oral cavity activate sweet taste receptors that are expressed in the cells of the taste buds of the tongue, these receptors are also expressed in the gastrointestinal tract, pancreatic β-cells. In the intestine, the sweet taste receptors facilitate glucose absorption. The glucose stimulates the sweet taste receptors in the β-cells leading to direct stimulation of insulin secretion and also indirect release of insulin through Ca+2 pathway. Also, glucose stimulates mitosis of β cells; this was demonstrated by measuring insulin immunofluorescence and performing BrdU or TUNEL on pancreatic graft recipients following glucose ingestion [3].

Artificial sweeteners can bind to sweet taste receptors present not only in the oral cavity, but also on enteroendocrine and pancreatic islet cells. Thus, these sweeteners may have biological activity by eliciting or inhibiting hormone secretion like insulin. Although non-nutritive sweeteners have generally been considered metabolically inert, recent data suggest that these sweeteners may have physiological effects that alter appetite and/or glucose metabolism. Artificial sweetener consumption has been associated in epidemiological studies with numerous adverse metabolic outcomes, including weight gain, the metabolic syndrome, and diabetes [4].

These artificial sweeteners-induced diseases pay attention in this study to explore the impact of one of the most famous non-nutritive sweeteners "aspartame" on the architecture of pancreas in male albino rats. Aspartame has been the subject of very few researches and its health benefits is appoint of debate so far.

METHODS

Chemicals:

Aspartame: from Alfa Aesar, Thermo Fisher Scientific, Germany, at a dose of (250 mg/kg/day) once daily dissolved in water for 6 months [4].

Experimental animals:

This study was performed according to the Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC). The study was approved by the ZU-IACUC with approval number (ZU-IACUC/3/F/364/2023). The experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

A total of 18 albino Wistar rats (200-250gm) were obtained from the animal house, Faculty of Medicine, Zagazig University. The animals were kept in plastic cages at normal room temperature which is 20-24 C^{o}. They had free access to commercial food pellets and water. Throughout the whole experiment, the animals were kept under 12 hr. light/dark cycles.

Experimental design:

The rats were divided into three groups (6 animals for each group).

1-**Control groups:** the rats were kept without any treatment along the experimental period, which is 6 months,

2-**Aspartame-treated group:** Each rat were administered aspartame at a dose of (250 mg/kg/day) once daily dissolved in water for 6 months [4] .

3-**Withdrawal group:** Each rat was administered aspartame at a dose of (250 mg/kg/day) once daily dissolved in water for 6 months then left without treatment for another one month.

Experimental procedures:

By the end of the experiment, all animals were anesthetized by an intra-peritoneal injection of thiopental 50mg/kg body weight then sacrificed. Blood samples were prepared to measure serum lipase and amylase. Pancreatic specimens were obtained for histopathological and Immunohistochemical studies. In addition statistical analysis was performed.

Measurement of Serum amylase and lipase:

Blood samples from all groups were centrifuged for 10 minutes at 12,000 rpm to obtain serum, which was then used to measure the levels of serum lipase and amylase using an autoanalyzer (Hitachi 912 auto-analyser; Germany) in the biochemistry department.[6].

Hematoxylin & Eosin and Masson trichrome Staining

The Pancreatic specimens were fixed in Bouin's solution. The tissues were dehydrated in ascending grades of alcohol, and finally embedded in paraffin wax. After wax blocks were ready, a microtome was used to cut 5 µm thin serial sections to be stained with Hematoxylin and Eosin for assessing the histological architecture [7] and Masson trichrome stain for detection of collagen [8].

Photomicrographs were captured using a light microscope (Leica DM 500) having a digital camera (Lecia ICC50 W) at the Department of Human Anatomy and Embryology, Faculty of Medicine, Zagazig University.

Immunohistochemical study:

Anti-Insulin and anti-glucagon immunostaining; Using the avidin-biotin peroxidase method. Paraffin sections were mounted on positively charged slides. The slides were deparaffinized in xylene, rehydrated in decreasing alcohol grades, then submerged in 0.3% hydrogen peroxide for 30 minutes to inhibit the endogenous peroxidase activity. The slides were treated with monoclonal mouse antibody, dilution of 1:100, against insulin protein and monoclonal mouse antibody against glucagon protein. After rinsing the slides in PBS, they were incubated for 1 hour at room temperature with the secondary antibody (biotinylated anti-mouse IgG, DAKO LSAB 2 Kit; Dako, Denmark). Afterward, they were rinsed once more in PBS. Using 0.05% diaminobenzidine (DAB), the immunoreactivity was stained. Ultimately, hematoxylin was used as a counterstain on each section, followed by dehydration and DPX mounting. The reaction with brown cytoplasmic staining was graded as positive [5].

Morphometric analysis

By utilizing image analyzer system (Image J 1.47v national institute of health, USA) the area percent of collagen deposition was assessed, intensity of the brown color of anti-insulin immune expression and glucagon +ve area /pancreatic area at 400×magnification of 5 non-overlapping randomly selected fields from immune-stained slides of each group.

Statistical analysis

The student's t-test was used to examine and compare the data that were gathered. The significance of changes in each parameter relative to the control group was tested using the p-value. The statistical program for the Social Science Software (SPSS) (version 17.0 for Windows; SPSS Inc., Chicago, Illinois, USA) was used to analyze the data, which were reported as mean ± SD. P value was highly significant if P<0.001, While P<0.05 was significant and was non-significant if P>0.05.

RESULTS

Biochemical results (Levels of serum lipase and amylase)

Serum lipase and amylase, two indicators of pancreatic damage, increased significantly (P<0.001) in the Aspartame group relative to the control group. In contrast to the Aspartame group, the withdrawal group showed a highly significant drop (P<0.001) of both enzymes (**Table 1S**)

Morphometric results:

By morphometric analysis of area percent of collagen deposition, there was a highly significant increase in the area percent of collagen deposition in aspartame treated group(P<0.00) as compared to control group, but there was no significant difference in area percent of collagen deposition between control and withdrawal group (P>0.05).

The means of intensity of insulin immune-expression by morphometric analysis, there was a highly significant decrease in the intensity of insulin expression in aspartame-treated group as compared to control group (P<0.00), but there was no significant difference in intensity of insulin immune-expression between control and withdrawal group (P>0.05)..

The analysis of glucagon +ve area/pancreatic area morphometrically, there was a highly significant decrease in glucagon +ve area/pancreatic area in aspartame treated group as compared to control group (P<0.00), while there was no significant difference in glucagon +ve area/pancreatic area in withdrawal group as compared to control group (P>0.05).

Histological results:

Hematoxylin and eosin stain (H&E):

In the control group sections stained with (H&E) demonstrated intact pancreatic tissue, which is made up of pancreatic lobules divided by tiny septa of connective tissue. Little pancreatic acini with pale islets of Langerhans positioned in between the acini make up each pancreatic lobule. Pyramidal cells with an apical eosinophilic granular cytoplasm and a basal dark nucleus lining the rounded to oval acinus. The Islet of Langerhans is made up of trabeculae of core β-cells with a pale nucleus and lots of cytoplasm that are spaced apart by tiny capillary channels. The outer border of the islet is lined by peripheral α-cells, which have a black nucleus and sparse cytoplasm. (**fig 3 A&B**).

Sections of the Aspartame treated group stained with (H&E) showed significant reduction in acinar content which appeared dispersed and separated by wide septa. The enlarged pyramidal cells of the

pancreatic acini have a basal black nucleus and an abundance of eosinophilic apical cytoplasm. Acinar cells exhibit binucleation in certain acini. Islets of Langerhans were seen, with peripheral α-cells and core β-cells displaying blood capillaries in-between. Dilated pancreatic duct encircled by pale whorls of fibrosis rich in inflammatory cell infiltration and containing large hyaline secretion. Dilated congested blood vessels were also seen. (**fig 4 A2&B2**).

Sections from the withdrawal group stained with (H&E) revealed several pancreatic acini with reduced acinar cell swelling. Eosinophilic cytoplasm and nuclei positioned basally in the acinar cells indicate abundant mitochondria. The islet of Langerhans is encircled by peripheral α-cells and central core of β-cells. (**fig5 A3&B3**)

Masson's trichrome stain:

Sections of the control group stained with Masson's trichrome displayed fine collagen fibers deposition in between the acini and around the islets, blood vessels and duct. (**fig 6 A**).

Aspartame treated group stained with Masson's trichrome displayed thick collagen fibers deposition in between the acini and around the islets, blood vessels and duct. (**fig 6 B**).

Sections from the withdrawal group stained with Masson's trichrome stain displayed slight collagen fibers deposition in between the acini and around the islets, blood vessels and duct. (**fig 6 C**).

Immunohistochemical stain:

Insulin immunohistochemical result:

Immunohistochemical stained pancreatic sections for insulin in the control group demonstrate the islet of Langerhans with central β-cells exhibiting strong positive insulin immune staining. (**Fig. 7A**).

Immunohistochemical stained pancreatic sections for insulin in aspartame treated group displaying the islet of Langerhans with central β-cells displaying significant weak positive insulin cytoplasmic immune staining (**Fig. 7B**).

Immunohistochemical stained pancreatic sections for insulin in withdrawal group demonstrating the islet of Langerhans with mild positive cytoplasmic insulin immunostaining in central β-cells (**Fig. 7C**).

Glucagon immunohistochemical results:

Immunohistochemical stained pancreatic sections for glucagon in the control group, the islet of Langerhans was seen to have strong positive glucagon cytoplasmic immune staining in peripheral α-cells (**Fig. 8A**).

Immunohistochemical stained pancreatic sections for glucagon in aspartame treated group showed islet of Langerhans with very weak cytoplasmic glucagon immunostaining staining in peripheral α-cells (**Fig 8B**).

Immunohistochemical stained pancreatic sections for glucagon in withdrawal group showed a moderate cytoplasmic glucagon immunostaining in peripheral α- cells (**Fig. 8C**).

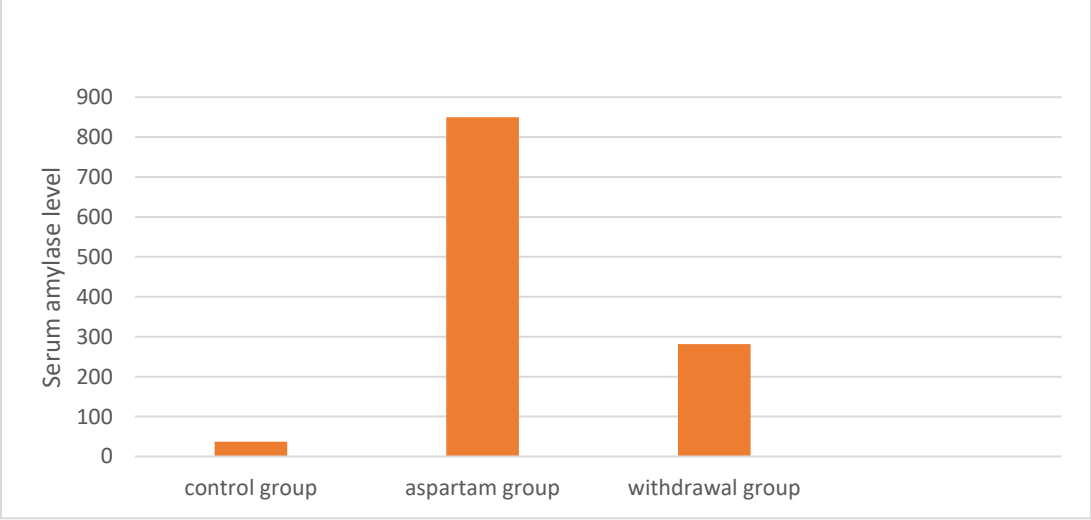

Fig 1: A Bar graph shows serum amylase levels in the different studied groups.

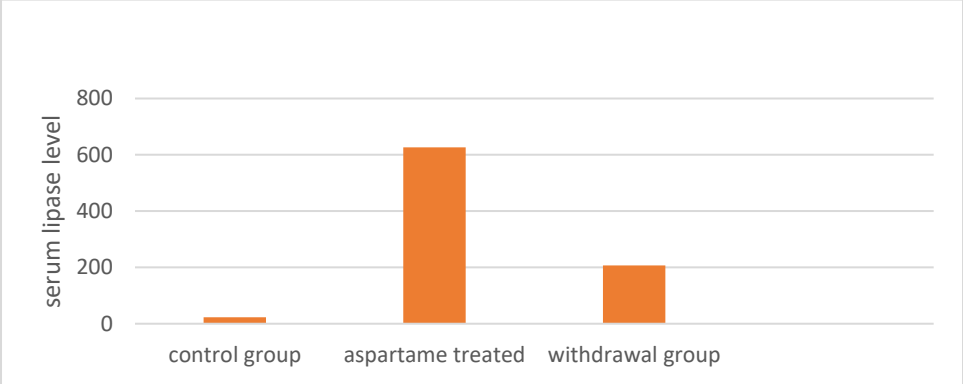

Fig 2: A Bar graph shows serum lipase levels in the different studied groups

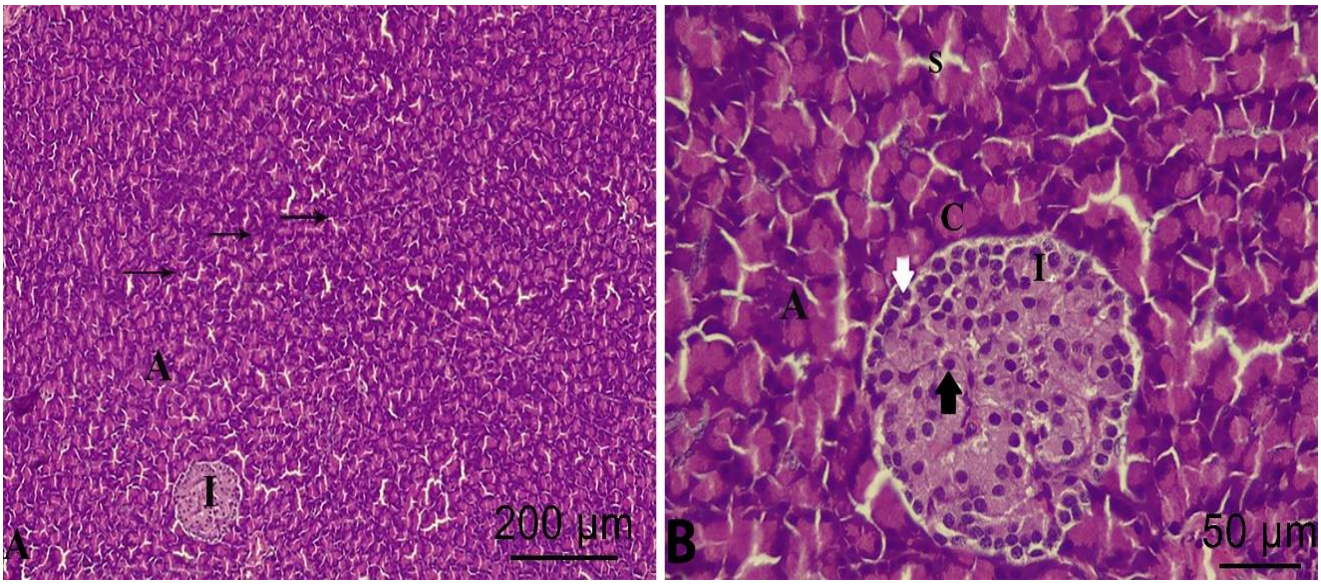

Fig 3: Photomicrographs of pancreatic H&E-stained sections of the control group, ***(A):*** shows *normal pancreatic* rat tissue formed of pancreatic lobules divided by thin connective tissue septa (**arrows**). Every pancreatic lobule is formed of smaller pancreatic acini (**A**) with pale islets of Langerhans (**I**) located between the acini (**H&Ex100**).

(B): Higher magnification of (A)**:** shows the pancreatic lobule having smaller pancreatic acini (**A**) with narrow interacinar spaces (**s**) and pale islets of Langerhans (**I**) in between the acini. Each acinus is rounded to oval and lined with pyramidal cells containing basal dark nuclei and apical eosinophilic cytoplasm (**C**). The Islet of Langerhans is located in between the acini and formed of trabeculae of central β- cells (**black arrow**) and the peripheral α-cells (**white arrow**) line the outer border of the islet (**H&Ex400**).

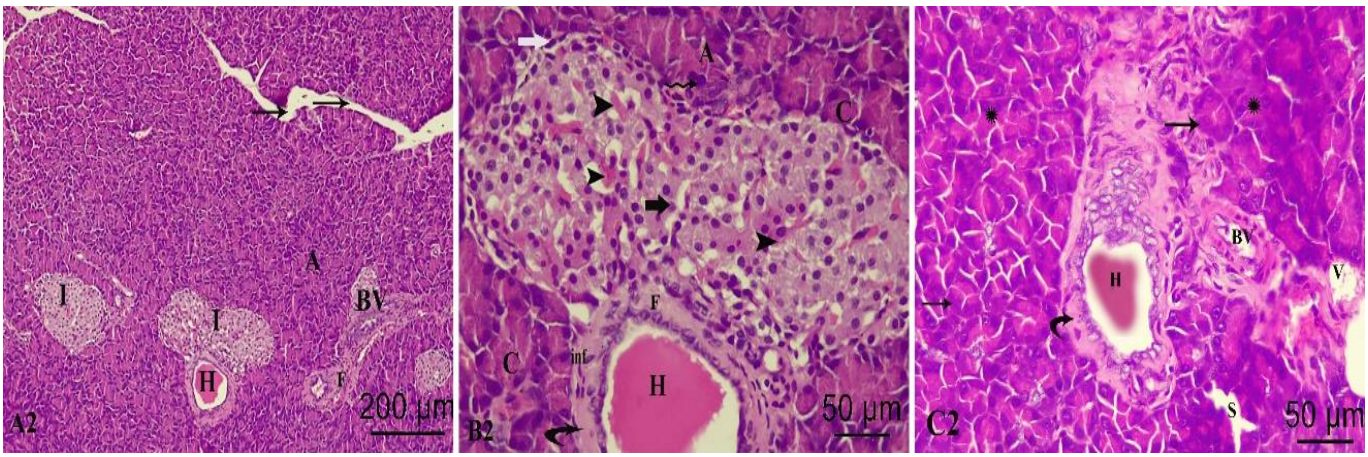

Fig 4: *Photomicrographs of pancreatic H&E -stained sections of aspartame treated group,*

(**A2**): shows reduction of the acinar content of the pancreatic tissue (**A**) with prominent islets of Langerhans (**I**). Pancreatic acini were separated by wider septa (**arrows**) with prominent vascular channels (**BV**) showing abundant fibrosis (**F**) and one dilated pancreatic duct containing hyaline thrombi (**H**) **(H&Ex100**).

(**B2**): Higher magnification of (**A2**) shows prominent islets of Langerhans with central β- cells (**thick black arrow**) with congested blood capillaries (**arrow heads**) and peripheral α- cells (**thick white arrow**). Pancreatic acini (**A**) formed of swollen pyramidal cells with basal dark nucleus and abundant eosinophilic apical cytoplasm (**C**). Some of the acinar cells show binucleation (**wavy arrow**). Large, dilated pancreatic duct (**curved arrow**) is seen and surrounded by pale whorls of fibrosis (**F**) rich in inflammatory cell infiltrate (**inf**) and containing large hyaline secretion (**H**).

(**C2**): shows distorted acini (*) with basal faint basophilic cytoplasm (**arrows**),some vacuolations (**V**) and wide septa (**S**) , dilated thick walled blood vessel (**BV**) is seen between acini. Large, dilated pancreatic duct (**curved arrow**) is seen containing large hyaline secretion (**H**). (**H&Ex400**).

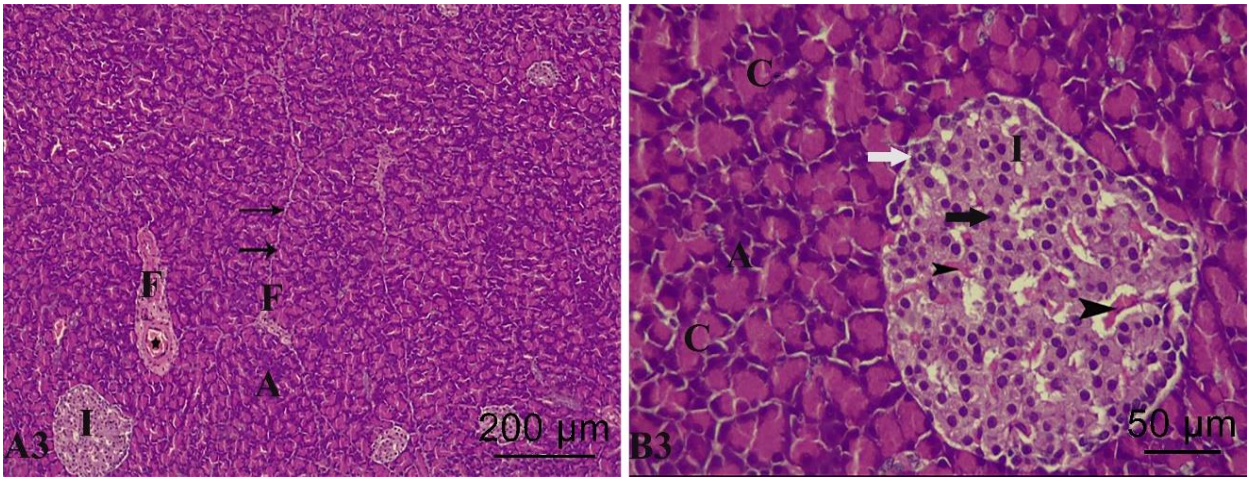

Fig 5: *Photomicrographs of pancreatic H&E-stained sections of withdrawal group,* (**A3**): shows an increase of the acinar content (**A**) of the pancreatic tissue arranged in lobules separated by thin septa (**arrows**) with areas of the septa showing pale fibrous tissue (**F**). The islets of Langerhans were located in between the acini with pale

staining (**I**). Prominent vascular channels surrounded by thick fibrosis (**F**) and contained small hyaline thrombi (**star**) (**H&Ex100**).

(**B3**): Higher magnification of (**A3**) shows multiple pancreatic acini (**A**), the acinar cells show basally located nuclei and eosinophilic cytoplasm (**C**). The islet of Langerhans (**I**) is located among acini with central β- cells (**thick black arrow**), less congested capillaries (**arrow heads**) surrounded by peripheral α- cells (**thick white arrow**) (**H&Ex400**)

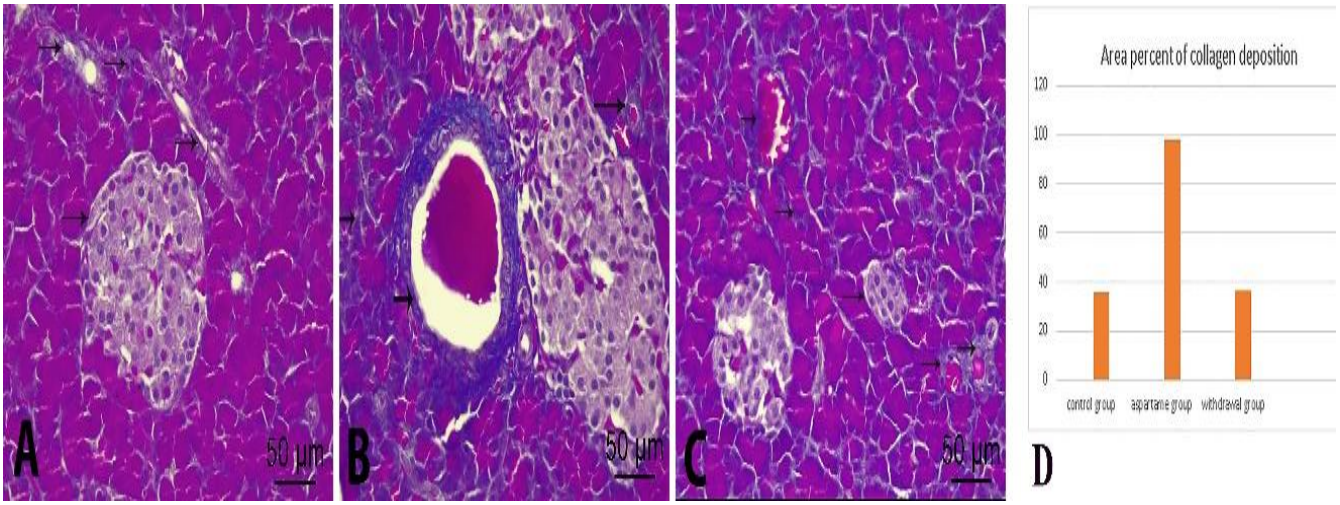

Fig 6: (**A**): A photomicrograph of Masson Trichrome stained sections of pancreas of control group shows fine collagen fibers (**arrows**) around blood vessel, islets, acini and ducts. (**B**): A photomicrograph of Masson Trichrome stained sections of pancreas of aspartame treated group shows thick collagen fibers (**arrows**) surrounding pancreatic acini, islets, ducts and blood vessels. **(C):** A photomicrograph of Masson Trichrome stained sections of pancreas of withdrawal group shows some collagen fibers (**arrows**) deposition around islets, acini, ducts and blood vessels of pancreas. (**Masson Trichrome x400**). (**D**): A Bar graph for area percent of collagen fibers deposition shows a highly significant (P<0.001) increase in area percent of collagen deposition in aspartame treated group as compared to control group, but there was no significant difference in area percent of collagen deposition between control and withdrawal group.

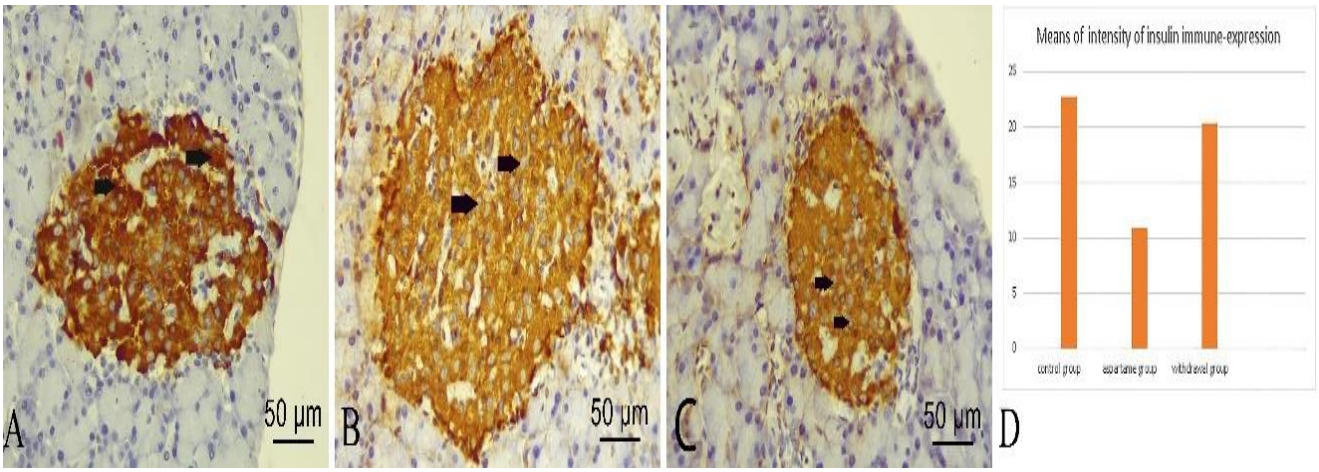

Fig 7: Photomicrographs for Immunohistochemical stained pancreatic sections for insulin in control (**A**), aspartame treated (B) and withdrawal groups (C). **(A):** shows islet of Langerhans with strong positive insulin

cytoplasmic immune staining in central β- cells (**arrows**) **(B):** shows islet of Langerhans with weak positive insulin cytoplasmic immune staining in central β- cells (**arrows**). **(C):** shows islet of Langerhans with moderate cytoplasmic insulin immunostaining in central β- cells (**arrows**). (**Anti Insulin immune-histochemical stain x400**). (**D**): A Bar graph for means of intensity of insulin immune-expression shows a highly significant reduction in intensity of insulin expression in aspartame treated group as compared to control group (P<0.001), while there was no significant difference in intensity of insulin expression between control and withdrawal groups.

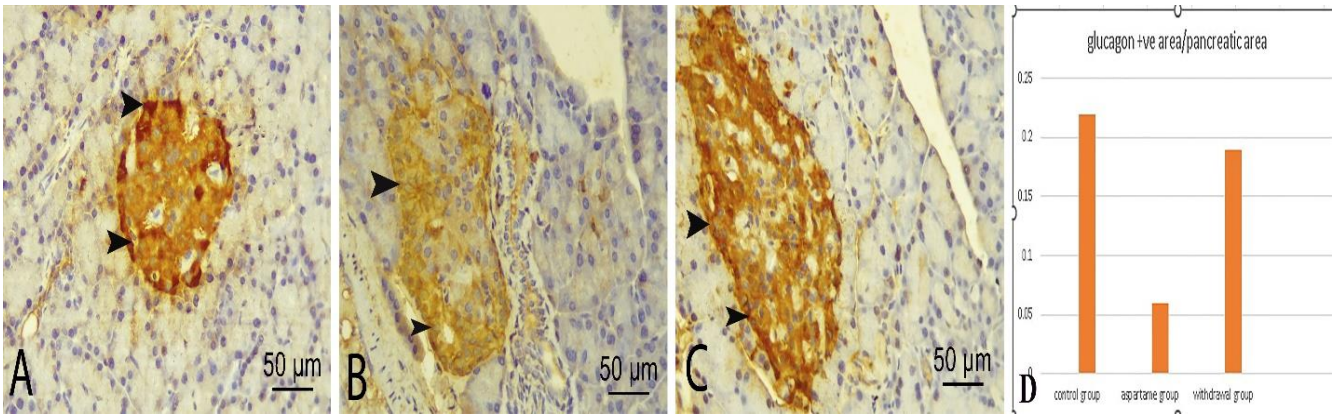

Fig 8: Photomicrographs for Immunohistochemical stained pancreatic sections for glucagon in control (A), Aspartame treated (B) and withdrawal groups (C). (**A**): shows islet of Langerhans with strong positive glucagon cytoplasmic immune staining in peripheral α- cells (**arrow heads**). (**B**): shows islet of Langerhans with very weak cytoplasmic glucagon immunostaining staining in peripheral α- cells (**arrow heads**). (**C**): shows moderate cytoplasmic glucagon immunostaining staining in peripheral α- cells (**arrow heads**). (**Anti-Glucagon immune-histochemical stain x400**). (**D**): A Bar graph for glucagon +ve area/pancreatic area shows a highly significant reduction in glucagon +ve area in aspartame treated group as compared to control group (P<0.001), while there was no significant difference in glucagon +ve area between control and withdrawal groups.

DISCUSSION

A high sugar diet could concern our health with much prevalence of obesity, diabetes, hypertension, and other cardiovascular illnesses [6]. Beverages with added sugar are thought to have a detrimental effect on one's weight and general health. More focus is being paid to non-caloric sweeteners in an effort to reduce the likelihood of these effects[7].

Currently, there is a widespread use of non-nutritive sweeteners with a negligible caloric value to replace the sweetness of a much larger amount of sugar or sucrose. A lot of artificial sweeteners are utilized by the food business in place of sugars.

Evidence has shown that those who regularly use these non-caloric sweeteners may also be more susceptible to conditions such as heart disease, obesity, diabetes mellitus, and metabolic syndrome [7].

Therefore, many concerns have recently been raised about the safe use of these non-caloric sweeteners. One of the common non-caloric sweeteners is aspartame. The effects of prolonged aspartame ingestion upon the function and structure pancreas is scarcely delt in the previous literature Thus, in this study, we tried to explore how aspartame affected the islets of Langerhans ß-cells and the architecture of the exocrine pancreas in adult male rats. In order to replicate the real-world application of sweeteners, the long-term, six-month experimental model of administration was chosen.

In this study, the control animals' pancreas (H&E) stained sections revealed that its normal architecture of lobules divided by tiny septa made of connective tissue. The lobules involved the pancreatic acini, the exocrine component, and the islets of Langerhans, the endocrine component. The acinar cells are pyramid-shaped and had rounded

basal nuclei, basal acidophilic cytoplasm, and apical basophilic cytoplasm. Langerhans islets appeared as Pale oval patches between the acini. They were made up of cell clusters divided by blood vessels. Pale nuclei made up each of their cells. These normal pancreatic structure was tightly confined with those declared by Hulail et al. [8] and Youssef [9].

In the aspartame-treated group, (H&E) stained sections revealed Langerhans islets with peripheral α-cells and core β-cells displaying many blood capillaries in-between the β-cells . The enlarged pyramidal cells that make up pancreatic acini have a basal black nucleus and an abundance of eosinophilic granular apical cytoplasm, which indicates cytoplasm rich in mitochondria. Acinar cells exhibit binucleation in certain of them. enormous, dilated blood vessels encircled by pale whorls of fibrosis rich in inflammatory cell infiltration and containing large hyaline thrombi and these findings were in harmony with El-Gamal et al. [4].

Other studies have also noted that pancreatic injury causes disarray of the acinar polarization, as seen in this study of Kern et al. [10]. They discovered that the content of the zymogen granules was released into the interstitial space when they merged with the lateral plasma membrane rather than the apical one. Interstitial pancreatitis was caused by the blocking of apical exocytosis and the redirection of exocytosis toward the basolateral plasma membrane of acini of pancreas exposed to metabolites of ethanol Likewise, aspartame, which contains methanol, may eventually have the same effect as ethanol.[11].

Collagen was found to be deposited in a noticeable way around clogged blood arteries and ducts using Masson trichrome stain in aspartame-treated group. The presence of stellate cells in the pancreas and their crucial role in the formation of fibrosis may account for this. Stimulating the dormant stellate cells causes them to proliferate into fibroblast-like cells, which are capable of producing fibronectin, types I and II of collagen. In reaction to oxidative stress or the production of various cytokines, such as transforming growth factor, interleukin-1, and interleukin-6, stellate cells make collagen [12].

The current study found that following administration of aspartame, insulin-secreting β cells had weak positive immunoreactivity, displaying a brown core and these finding were declared by [13] who have documented that there was low expression of anti-insulin monoclonal antibody in orlistat treated group and declared this result to orlistat down regulate insulin production.

Strong insulin immunoreactivity in senescent Sprague-Dawley male Rats was previously linked to an enlarged ß cell population. It was reported that the rise in weight and aging-related insulin demand led to compensating increases in ß cell populations [14]. To ascertain whether elevated secretory function or strong insulin immunoreactivity is the reason of the detected β cell surface area/islet in the current investigation. Actually, there was no discernible difference between the two groups according to morphometric analysis, which supports our findings of higher secretory activity.

In aspartame-treated group the islet of Langerhans showed very weak cytoplasmic glucagon immunostaining staining in peripheral α-cells and negative staining of central β- cells and these results were proved by Cao et al. [15]who said that light cola consumption decreased the immunolabeling of glucagon to levels below those of the Water and Cola groups.

In experimental animals, hyperglycemia and hypertriglyceridemia are linked to the activation or inhibition of glucagon release, respectively. According to certain sources, blood glucose concentration of 7 to 8 mmol/L, maximally inhibit glucagon secretion, this phenomenon was linked to changes in Ca2+ levels in the cytosol. Moreover, in guinea pigs, rats, and dogs, hypertriglyceridemia and elevated fatty acid blood levels inhibit release of glucagon [16].

Lipase and amylase are two digestive enzymes secreted by pancreatic acinar cells. Their serum levels are typically modest and they are primarily eliminated through the gastrointestinal tract [17]. In aspartame treated group there is a significant elevation of serum amylase and lipase levels as compared with control and withdrawal group and these results were in harmony with the results of other researchers [18].

Amylase has a 20–60% specificity for pathogenic alterations in the pancreas. However, lipase exceeds 80% [19]. Furthermore, the specificity of

diagnosing pancreatitis is increased to 97-98% by testing two enzymes. Thus, we can conclude that pancreatitis caused by aspartame treatment is confirmed by the increases in serum levels of amylase and lipase in this research. This discovery aligned with Chintanaboina and Gopavaram, [20].

Comparing the aspartame-treated-group to the control group, there was a highly significant rise in the pancreatic damage markers (serum lipase and amylase). In contrast to the Aspartame group, the withdrawal group's serum levels of lipase and amylase showed a highly significant drop and these results were in harmony with those of [4] who has revealed that aspartame caused pancreatic inflammation, which cause injury to islet cells of pancreas, by overstimulating the exocrine pancreas. Aspartic acid, a known stimulative amino acid which opens calcium channels and causes calcium to flood the cell, and activates the secretagogue receptors on acinar cells, is another method that aspartame can stimulate the pancreas to secrete its secretions [21].

In the withdrawal group, there were some improvement but some acinar cells had pyknotic nuclei that were darkly pigmented, giving them an uneven appearance. With the exception of a few empty areas between cell cords and clogged blood capillaries, the Langerhans islets appeared almost normal. These results were augmented by [22].

CONCLUSIONS

Finally, from the aforementioned findings we can conclude that Aspartame promotes enzymes levels changes and degenerative alterations in the pancreas. When aspartame intake is stopped, the pancreas' normal histological structure partially returns which might point to attempts at gland regeneration following aspartame cessation**.** Thus, it is advised that in future research, to decrease the dose and or stop using aspartame or add antioxidants to be used in conjunction with aspartame to counteracts its deleterious hazards.

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Supplementary Materials:

Table 1S: Serum lipase and amylase levels in the studied groups

	Control group	Aspartame treated group	Withdrawal group	Aspartame vs control	Withdrawal vs control	Aspartame vs Withdrawal
Serum amylase (IU/L)	37.17±6.40	849.83±87.67	281.33±33.18	p≤0.0001	p≤0.0001	p≤0.0001
serum lipase (IU/L)	23.25±3.52	626.83±34.78	207.5±24.25	p≤0.0001	p≤0.0001	p≤0.0001

Values are represented as Mean±SD of 6 rats for each group. Highly significant*** (P<0.001)

Citation

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