Histological Study and some biochemical parameters of Effects of Stevia on Pancreas in White Mice

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Abstract: The goal of this study was to look at the histological and physiological alterations in the pancreas of mice after they were given stevia. The experimental animals were divided into two main groups for each sex of animals. The male experimental groups which included A1 and B1. Group A1 served as a control, whereas Group B1 was given 1 mL of stevia solution orally every day for thirty days. The female experimental animals were divided in the same way as the male groups A2 and B2 each group composed of fifteen mice. All experimental animals housing in Animal House of science college in AL-Muthanna university, all environmental conditions were under control. The histological parameters included tissue sections from Pancreas. Blood samples were taken when the treatment period ended to measure glucose concentrations, and the animals were then killed for histological analysis. The histological result of Pancreas gland in both sex of experimental animals after treated with stevia didn’t showed any prominent changes in cellular structures of islet of Langerhans, the histological changes of pancreas gland after treated with stevia have normal cellular structures in islets of Langerhans without main histological effects. The glucose values didn’t have significant differences in treated groups with stevia (219.53±29.15 mg/dl) in male and (228.93±31.28 mg/dl) in female compared with control groups in both sex.

Keywords: Stevia, Sweeteners, Glucose, Histology, Pancreas.

1. Introduction

Stevia (Stevia rebaudiana) is a perennial herb belonging to the Asteraceae family, known as sweet leaf, sweet herb and honey leaf. This was first discovered in 1887 by M.S.Bertoni. Stevia leaves contain approximately 10 sweetening glycosides, of which Stevioside (3–10%), Rebaudioside A (13%) and Rebaudioside B, C, D are more significant. Stevia is 100% natural sweetener since it is derived from the stevia plant and does not undergo any chemical alteration during manufacturing process. Stevia is widely used in Korea, Brazil, the USA, Japan and China in confectionery, beverage, bakery and home goods reported by various researchers [1]. Currently, there are more than 150 species of stevia, but S.
Stevia rebaudiana Bertoni is the only one that has a sweet nature because of the high content of steviol glycosides in its leaves. Stevia leaves also contain several important phytochemical constituents such as alkaloids, flavonoids, chlorophyll, xanthophyll, oligosaccharides, amino acids, essential oils, lipids, proteins, free sugars, trace elements and hydroxycinnamic acids (chlorogenic acid, caffeic acid) [2].

Stevia, or stevioside, is an extremely sweet substance that is 200–300 times sweeter than sucrose, is not metabolized in the body, and therefore does not generate calories. [3]. Stevia is one of the most recent and readily available sweeteners on the market. Stevia can also be known as PureVia and TruVia as trade name that were patented by Coca Cola and Pepsi [4]. The FDA confirm the safety of rebaudiana for human consumption through published scientific research, public and multi-generational safety studies [1]. With its sweet taste, stevioside may increase insulin response, improve insulin sensitivity and probably decrease blood pressure [3]. Stevia has recently been advertised as a natural, non-caloric table sweetener, as well as being used in soft beverages. [5].

Stevioside can be used as an alternate sweetener by diabetics and obese people with hyperglycemia who are unable to adhere to a rigorous diet. The plant also has anti-inflammatory, antibacterial, antiseptic, hypotensive, anti-fertility, diuretic and cardiotonic qualities in addition to its hypoglycemic characteristics. It has also been shown to be effective in the treatment of skin problems such as eczema, dermatitis and acne. Stevia leaves supplemented with phytoconstituents could be an alternative natural sweetener for adults, children, and the elderly who have a propensity of drinking beverages and eating sweetened food products [6]. Taking Stevia leaf powder with medicine helps to lower blood sugar levels. Type I and type II diabetes can be lessened with the use of stevioside. Stevia will gradually lower the amount of medicine required [7].

The increased acceptance of this inbred sweetener, combined with rising obesity and diabetes rates, has resulted in large-scale commercialized stevia crop production in both developing and industrialized countries [8].

The pancreas is an endocrine organ that produces and secretes hormones in the blood to monitor energy storage and use
in the body. It is a part of the gastrointestinal system that secretes and produces digestive enzymes in the intestine. The region of the pancreas that produces and secretes digestive enzymes to the duodenum is known as the exocrine pancreas. This includes duct cells and acinar, as well as associated arteries, nerves, and connective tissue [9]. Over 95% of the pancreatic mass is made up of exocrine components. The sections of the pancreas (islets) that manufacture and secrete insulin, pancreatic polypeptide, glucagon, and somatostatin into the blood are known as the endocrine pancreas. Islets make about 1-2 percent of the pancreas' bulk [9]. Pancreatic islets, also known as the Langerhans Islets, are a cluster of endocrine cells that create glucose regulatory hormones as well as other vital biological tasks [10]. The islets are made up of 5 different types of cells that produce hormones (β cells secrete insulin, α cells secrete glucagon, ε cells secrete ghrelin, δ cells secrete somatostatin, and PP cells release polypeptide pancreas) Pancreatic islets are a model system for studying insulin secretion (ex vivo) [11], [12].

Humans and mice have similar islet size distributions [13]. The Beta cells, which manufacture and emit insulin, are the most abundant. In human and mouse islets, beta cells constitute up (50–70) % and (60–80) % of the cells, respectively. Glucagon is secreted by alpha cells, which make up (20–40) % and (10–20) % of the total number of cells in humans and mice, respectively. In addition, mice have a larger ratio of Beta to Alpha cells than humans [14]. The pancreas has two primary functions: endocrine cells in the islets of Langerhans organize blood sugar, and exocrine acinar cells produce digestive enzymes, which are produced by exocrine acinar cells and diverted to the gut by a branched ductal network [15]. The pancreas is frequently referred to as two organs in one because of the unique control and function of its endocrine and exocrine components [16].

The exocrine gland of the pancreas secretes enzymes that aid in the digestion of fats, carbs, proteins and acids in the duodenum. These enzymes travel down the pancreatic canal and into the bile duct in an inactive state. As they approach the duodenum, they are activated. In the duodenum, exocrine tissue secretes a bicarbonate to neutralize
gastric acid. It is the first part of the small intestine. Insulin and glucagon are the two primary hormones secreted by the endocrine gland in the pancreas, which control blood glucose levels and somatostatin, preventing insulin and glucagon from releasing. It is a part of the digestive system that produces insulin and other important hormones and enzymes that aid in the digestion of food. The pancreas has an endocrine role because it distributes juices directly into the bloodstream, and it has an exocrine function because it releases juices into ducts [17]. Hyperinsulinemia is caused by a deficiency in insulin activity on its receptor, which impairs glucose uptake by the tissue and contributes to hyperglycemia development. As a result, normal insulin secretion is lost, hepatic glucose synthesis rises, and inflammation cytokines in plasma rise. Hyperglycemia and Hyperinsulinemia are both independent mortality risk factors [18].

Nonnutritive sweeteners bind to unique sweet taste receptors found in non-taste organs such as the pancreas and stomach, which can affect insulin release [19]. Artificial and natural sugars are processed differently by our stomach microorganisms and pancreases, which might cause us to consume more, gain weight, and have a tougher difficulty digesting the natural sugars that our bodies require [20]. Pancreatic cancer has a very poor prognosis, due in large part to poor early detection record [21]. Obesity, diet, and diabetes are all known risk factors for pancreatic cancer, implicating glucose control and ingestion being a crucial part. Therefore, the use of artificial sweeteners can be important to kinetics of diseases. The oncogenic effect of artificial sweeteners is an extremely controversial area. At the other hand, stevia has been the subject of very few researches, so the possible health benefits are based at extrapolation instead of direct testing [22]. Results also demonstrated no impact of dietary stevia on the kinetics of pancreatic acinar carcinoma [22].

2. Methodology (Experimental Procedure)

2.1. Experimental Animals

Sixty matured male and female albino mice with weighing range 27-30gm (three-month-old) were bought from Drug and Healthy center in Baghdad, Iraq. Mice were kept in plastic mice cages, all cages put in the animal house
of the college of science in Al-Muthanna University under controlling of temperature 25-28ºC, controlled humidity situation (65%) with feeding by using standard pellets for about 15 days to adapt to their environment.

2.2. Exposure dose
The dose level is 0.8 g weight of administered commercial stevia that diluted with 1ml of distilled water which orally gavage for a period of 30 days [23].

2.3. Experimental design
Thirty male mice were split into two groups [A1 and B1], with Group A serving as the control group and Group B serving as the treatment group. The female experimental animals were separated into groups [A2 and B2] in the same fashion as the males, with each group consisting of 15 mice.

2.3.1. The control group which consisted of Fifteen mice who ate chow that was devoid of any chemical. A feeding tube was used to provide enough of water.

2.3.2. The treated group consisted of Fifteen mice who were given 1ml of stevia orally every day for thirty days.

2.4. Histological slide preparation:
The animals were sedated with chloroform when the treatment period ended, and then executed by hemorrhaging from the main carotid artery. The pancreas was carefully removed from the mice using a criterion process. To remove any blood contamination, the samples were rinsed with normal saline. Fixation; The tissue samples were fixated with (10%) formalin for 48 hours, then rinsed under running tap water for an hour to remove the majority of the formalin odder from the tissue specimens. Following the washing, the tissues were dehydrated by immersing them in a series of steadily increasing alcohol concentrations [50 percent, 70 percent, 80 percent, 90 percent, and absolute alcohol]. It must be cleared since the dehydrating alcohol employed will not dissolve or combine with liquid paraffin. Blocking refers to the process of embedding specimens in paraffin wax to create blocks. The block was to be cut by dissolving wax from the surface of the block to reveal the tissue, and then cutting the tissue with a microtome. The microtome was pre-set to cut the tissue as thicknesses with 5 μm. Blocks Small ribbons of tissue sections were placed on a microscopic slide with help of warm distil water containing few drops of Mayer’s albumen and deparaffinized with xylene solution, the slide was put on the hot plate with (40°C)
for overnight. Hematoxylin and eosin yellow solution were used to stain the tissue for preparing permanent slide [24]. Histological changes were observed under a light microscope and snaps were taken.

3. Results and Discussion

3.1 Male Control Group: The pancreas histology findings in the control group revealed the masses of serous acini arranged into many small indistinct lobules. The interlobular and conforming ducts of the acini were drained by long narrow intercalated ducts which have a small lumen, the pancreatic islets were spherical structures in shape varying in size but they’re always wider than exocrine portion. The connective tissue capsule surrounded the pancreas gland with progressive of exocrine diameter. The diameter of Beta-cell was (51.51±7.48) μm (Table 1). The Beta cell of pancreas was to be centrally located and in most common in pancreatic islets having granular cytoplasm. The beta cell has prominent nuclei and most of the Beta-cells were found in the middle area fig (1).

The present result showed Alfa-cell was located in the islet of Langerhans mostly in its peripherally location. The Alfa-cell in the endocrine portion which have variation in the diameter of nuclei (46.13±8.15) μm (Table 1). Alfa-cell found in the peripheral area of islet of Langerhans. The current study showed that the Beta-cells were composed of common cellular structures of islet endocrine. The Islets of Langerhans were poised either entirely of Beta-cells, with several cell types, with the majority being Beta-cells. Alfa-cells were the next most populated cell types, and can typically be found forming a layer around a Beta-cell Fig (2).

3.2 Female Control Group: All pancreas tissue sections have histological structures similar to tissue structure of male control group. The present result showed that the diameter of Beta-cells was (51.29±4.22 μm) and diameter of Alfa-cell was (46.00±7.57 μm) as shown in Table (1). In the control group, a tissue section of the female pancreatic revealed islet of Langerhans with normal shape which composed of normal beta-cells, normal Alfa-cells and blood vessels fig (3).

3.3 Male Treated Group with stevia: showed the histological structures of pancreas without prominent histological changes in most tissue section compared
with tissue section of control group. Nonnutritive sweeteners are a widespread sugar alternative, particularly among diabetics according to [18] the results clearly show that the effects of stevia mimic water effects on insulin levels fig (4). The tissue sections of the Pancreas gland after 30 days of oral administrations with 1 ml of stevia solution showed the diameter of Beta-cells was (50.60±6.12 μm) Which compared to the control group, this difference was not significant Table (1). In addition, the diameter of Alfa-cells was (38.49±7.85 μm), which was significantly different from the control group Table (1). The tissue section of treated pancreas gland with stevia have normal shape of islet of Langerhans so the tissue surrounded the islet of Langerhans was normally structures, the cells in the peripheral have oval prominent dark nuclei, the cells that situation in the middle zone of the islet of Langerhans have normal spherical nuclei fig (5). According to [22] their results demonstrated no impact of dietary stevia on the kinetics of pancreatic acinar carcinoma.

3.4 Female Treated Group with Stevia:
Female mice with pancreatic tissue slices revealed some histological changes. The tissue results of the Pancreas gland after 30 days of oral administrations with 1 ml stevia solution showed the diameter of Beta-cells was (49.94±6.75 μm) which are not statistically significant when compared to the control group Table (1). In addition, the diameter of Alfa-cells was (38.92±8.89 μm), which was significantly different from the control group Table (1), Stevia is a promising therapeutic for type 2 diabetes mellitus, according to one author, because it stimulates insulin secretion exclusively in high glucose concentrations, enhances parasympathetic signaling pathways, and inhibits sympathetic signaling pathways in beta cells [25].

The tissue section of female pancreas treated with stevia showed normal shape of islet of Langerhans, normal cellular distribution that composed of islet of Langerhans and showed normal blood vessels fig (6). The tissue section of pancreas in the female mice after treated with stevia showed normal islet of Langerhans and the regions that surrounded the islet of Langerhans is normal, normal connective tissue capsule and normal cellular distribution fig (7), disagreed with the histopathological examination of [26] according to the study, the liver from the control and stevia sweetener treated groups appeared normal,
with regular cell arrangement, mild inflammation, minimal fibrosis and normal cells with well-preserved cytoplasm, nucleolus, nucleus and central vein.

4. The physiological Results of Glucose:
4.1 Control Group: The glucose level in the control group was shown in table (2), which highlighted the normal value of glucose in the serum of female and male control groups. The normal level of glucose in male was (217.26±23.53) mg/dl, while in female was (230.13±1.68) mg/dl, because of the animals did not treated with sweeteners. The blood glucose normal level of white mice is recorded (106–278) mg/dl [27].

4.2 Stevia Treated Group: Table 2 showed the amount of serum glucose in the male group after being treated with stevia was (219.53±29.15) mg/dl, while in female was (228.93±31.28) mg/dl. The result noted the level of serum glucose in treated group with stevia didn’t have significant differences compared with control group in level of glucose. Also, the result showed no significant differences in the level of glucose between male and female treated groups may be due to that stevia have no effect on blood glucose in male and female treated groups. These findings corroborated those of a group of researchers who claimed that Stevia improves glucose homeostasis by enhancing glucose-mediated insulin release while lowering gluconeogenesis without producing hypoglycemia [28].
Fig (1): Tissue section of a male pancreas in the control group that revealed 
A - normal islet of Langerhans, B - normal blood vessel. (H & E) stain 200X

Fig (2): Tissue section of a male pancreas within control group, which disclosed A-normal islet of Langerhans, B- exocrine portion. (H & E) stain 200X

Fig (3): Tissue section of a male pancreas in the treated group (Stevia) that revealed A- nuclei of Beta cell, B- Islet of Langerhans, C- Alfa-cell. (H & E) stain 400X

Fig (4): Tissue section of a male pancreas in the treated group (Stevia) that revealed A- nuclei of Beta cell, B- Islet of Langerhans, C- Alfa-cell. (H & E) stain 400X
4. Conclusion

The Stevia had no significant effect on Pancreas which includes normal cellular arrangement in islet of Langerhans and normal excretory unit.
The effect of stevia had no significant on glucose level of male and female mice compared with control group.

**Table (1): Effect of Stevia on Pancreas gland (mean± standard deviation).**

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<th>Treatments</th>
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**References**


