The Journal of Anatomical Sciences

Email: **journalofanatomicalsciences@gmail.com**

J. Anat Sci 15(1)

Antidiabetic and histomorphological evaluation of composite teas on pancreas following Streptozotocin (STZ)-induced hyperglycemia in adult female Wistar rats

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Abstract

A cure to type 1 diabetes without undesirable side effects still remains a formidable challenge in drug research and development. Many traditional plants and herbal medicines have been discovered to have favourable and safe anti-diabetic activity in comparison to synthetic drugs. This study was aimed at investigating the protective effect of composite teas on pancreas following streptozotocin-induced hyperglycaemia in female Wistar rats. Thirty normoglycemic adult female Wistar rats of an average body weight of 200 g were divided randomly into six groups and the composite teas administered after induction of hyperglycaemia. Fasting Blood Sugar (FBS) and body weights were taken. At the end of the experiment, the rats were fasted overnight and sacrificed and the pancreas was excised. Control group showed decrease in blood glucose levels with significant increase in body weight while the treated groups showed improvement in the blood glucose level with significant increase in their body weights but there were alterations in blood glucose levels of the untreated group with significant decrease in their weights. The control group showed normal pancreatic cells histology; the architecture of pancreatic cells in streptozotocin (STZ) group were distorted but the groups treated with composite teas showed improvement in their micro-architecture. In conclusion, composite teas have protective role on blood sugar level and also restores distortions of pancreatic beta-cells in streptozotocin-induced hyperglycaemia.

Keywords: diabetes mellitus, streptozotocin, composite teas, pancreas

Introduction

Uncontrolled hyperglycemia induced by insulin resistance, inadequate insulin synthesis or excessive glucagon secretion characterizes diabetes mellitus[1]. Chronic hyperglycemia, in combination with other metabolic abnormalities, can damage various organ systems in diabetic patients, leading to the development of disabling and life-threatening health complications, the most prominent of which are microvascular (retinopathy, nephropathy and neuropathy) and macrovascular complications, which lead to a 2-fold or 4-fold increased risk of heart disease[2]. Type 1 Diabetes Mellitus (DM) is a multisystem disease with biochemical, anatomical, and structural consequences that can occur at any age[3]. It is a chronic disease that affects glucose, lipid, and protein metabolism due to a lack of insulin. It is caused by the pancreas' substantial and rising inability to secrete insulin as a result of autoimmune death of pancreatic beta cells. People with type 1 Diabetes Mellitus are typically not fat and initially exhibit with diabetic ketoacidosis (DKA). A defining characteristic of a patient with type 1 DM is that if insulin is removed, ketosis and, eventually, ketoacidosis occur, necessitating exogenous insulin[3].

Although other ideas exist, the current dominant paradigm on the genesis of type 1 diabetes hypothesizes that environmentally induced autoimmune death of pancreatic beta cells occurs against a background of hereditary risk. As a result, global variance in the incidence, prevalence, and temporal trends in type 1 diabetes are documented[4]. According to statistics from extensive epidemiologic studies done across the world, the incidence of type 1 Diabetes Mellitus has increased by 2–5%[4].

As a result of World Health Organization's recommendation that traditional plant therapies for diabetes be examined further, researchers are searching for acceptable traditional plants having anti-diabetic compounds that are less harmful than pharmaceuticals[5]. A popular beverage across the world, tea, offers numerous bioactivities and health benefits, including antioxidant, anticancer, hepatoprotective, cardioprotective, anti-obesity, gut flora-enhancing and anti-diabetic properties, among others[6]. Furthermore, tea includes several bioactive

components, including polyphenols like catechins, flavonols, theaflavins, and thearubigins, which have the ability to reduce the risk of diabetes and its consequences[6].

Recent epidemiological research found that tea consumption was negatively associated with the risk of diabetes mellitus and its complications; additionally, recent *in vitro*, *in vivo*, and clinical trials supported the effects of tea on the prevention and treatment of diabetes mellitus and its complications[6].

Materials and Methods

Streptozotocin was bought from Pascal Pharmacy (Sigma-Aldrich), Akure.

Preparation of Extracts

Fresh *moringa* leaves, mango bark, sunflower leaves and turmeric root were harvested at Oluwatedo quarters, Ipinsa, Akure. The dried seeds of pepper fruit were obtained from Relief Market, Oyingbo, Lagos State. The samples were sorted in air tight glass bottles to protect them from contaminants. The samples were identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Federal University of Technology, Akure.

All plant materials were carefully inspected and all foreign materials removed. The samples were then gently rinsed in tap water. All samples were air dried at 35^0C inside a house, after drying, the samples were milled using an electric Binatone Blender (China model BLG401). The milled material was sieved through an aluminium sieve (425 μm), stored in a container with tight lids and labeled. Composite teas (2 g) were prepared and bagged, a summary of the preparation procedure is shown in Table 1.

Table 1: Formulations of different herbal tea bags

Tea Name	Formulations (%)				
	Peppermint seeds	*Moringa* leaf	Matcha green leaf	Sunflower leaf	Mango bark
Pep Tea	30	25	20	15	10
Mango Tea	25	20	15	10	30
Sunflower tea	20	15	10	30	25
Green tea	15	10	30	25	20
Moringa tea	10	30	25	20	15

Two tea bags each (pep tea, sunflower tea, green tea & mango tea formulations) were soaked in separate air tight containers and kept in an enclosed space for a period of 24 hours with warm water to get a concentrated solution (containers were correctly labelled with their respective extract). After 24 hours, they were then kept in a moderately cold environment and dosage commenced.

4 g teabags (2) → 400 ml (24 hours)

Dosage was given individually according to each rat's weight at time of induction

$$\frac{\text{Mass per rat(g)} \times 5 \text{ ml concentrated extract}}{1000(g)}$$

∴ an average of 1.0 ml of solution was given to each rat

Breeding of the Animals

Thirty presumably healthy, normoglycemic adult female wistar rats having fasting blood glucose level of 70 to 80 mg/dl and average weight of 200g were used for this study. The animals were purchased from a disease free stock of Salmonda farms Akure, Ondo State Nigeria. They were taken to the animal house of the Department of Human Anatomy, School of Basic Medical Sciences, Federal University of Technology, Akure, Ondo. The rats were kept in plastic cages at controlled room temperature of about 30°C and photo-periodicity of 12 hours a day (light) cycle. The rats were randomly assigned into six experimental groups of 6 rats in each group. They were acclimatized for a period of two weeks and were fed with rat pellets made of food and water made available *ad libitum*. All animals were handled properly following the guidelines for animal research as detailed in the guidelines for the care and use of laboratory animals.

Experimental Design

Thirty rats were used for the study; then divided into six groups, each group contained five rats: one control group, another group diabetic and four treatment groups. The groups are as follows:
Group I: (Control) fed with rat pellets and water only

Group II: induced with a single dose of STZ (100 mg/kg body weight) only
Group III: (Treatment group 1) induced with STZ and treated with 1 ml/body weight/day of Pep tea formulation
Group IV: (Treatment group 2) fed and induced with STZ and treated with 1 ml/body weight/day of (green tea) formulation
Group V: (Treatment group 3) fed and induced with STZ and treated with 1 ml/body weight/day of mango tea formulation
Group VI: (Treatment group 4) fed and induced with STZ and treated with 1 ml/body weight/day of sunflower tea formulation.
The body weight measurements were taken every three-day interval and recorded, using a weighing balance. Treatment lasted for four weeks after which sacrifice of rats was done. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals and were approved by the Departmental Committee on the use and care of animals.

Animal Sacrifice and Sample Collection

At the end of the experimental period, animals were euthanized by using diethyl-ether as sedative and by abdomino-pelvic incision the pancreas were excised and processed for histological and biochemical evaluation. The pancreas was removed and fixed in 10% paraformaldehyde.

Histological Analysis

Histological staining (H & E and Masson trichrome) were carried out at the Department of Human Anatomy and Cell Biology, College of Medicine, Obafemi Awolowo University, Ile-Ife. The principle behind Hematoxylin and eosin stain is the chemical attraction between tissue and dye[7]. Hematoxylin, a basic dye imparts blue-purple contrast on basophilic structures, primarily those containing nucleic acid moeties such as chromatin, ribosomes and cytoplasmic regions rich in RNA while an acidic eosin counterstained the basic elements such as red blood cells, cytoplasm, muscle and collagen in varying intensities of pink, orange and red[7].

As the name implies in Masson's trichrome staining, three dyes are employed selectively staining muscle, collagen fibers, fibrin, and erythrocytes[8]. The general rule in trichrome staining is that the less porous tissues are colored by the smallest dye molecule; whenever a dye of large molecular size is able to penetrate, it will always do so at the expense of the smaller molecule[8].

Photomicrographs

All micrographs were obtained using a light microscope with the aid of digital eyepiece (Moticam DCM400) attached to a light microscope (Leica, Germany) which conveyed the images to a personal computer (PC).

Data Presentation and Statistical Analysis

The data obtained were analyzed statistically using one-way and two-way ANOVA, followed by Tukey's comparison test. Data were expressed as mean ± SEM. The level of significance was at $p < 0.05$ except where stated otherwise. Data were analyzed using GraphPad Prism 8 Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Histopathological Findings: As shown in figure 1, the pancreas of the control group given pellets and water only presented a normal histoarchitecture of the pancreas; Haematoxylin and eosin stain revealed intralobular ducts, interlobular ducts, Islet of Langerhans, blood vessels while Masson trichrome stain revealed interlobular ducts, collagen surrounding the Islet cells. As shown in figure 2, the pancreas of the STZ group given pellets and only induced with 100 mg/kg body weight of streptozotocin presented with pathological histoarchitecture: Haematoxylin and eosin stain revealed distended interlobular ducts with hemorrhages, vacuolations and absence of the islet of Langerhans while in Masson trichome stain revealed intralobular ducts, interlobular ducts surrounded by collagen. As shown in figure 3, the pancreas of the peppermint tea group induced with STZ presented with regenerating islets, Haematoxylin and eosin stain revealed interlobular ducts, regenerating islets of Langerhans, intralobular ducts, while Masson trichome stain showed intralobulars duct, regenerating islets of Langerhans, interlobular ducts surrounded by collagen, intercalated ducts. In figure 4, the pancreas of the green tea group induced with STZ presented with regenerating islet cells Haematoxylin and eosin stain revealed intercalated ducts, acini cells, intercalated ducts, while Masson trichome stain revealed regenerating Islet cell of Langerhans, acini cells, intralobular ducts, interlobular ducts and dense collagen.

As shown in figure 5, the pancreas of mango tea group induced with STZ presented with regenerating islet cells; Hematoxylin and eosin stain revealed interlobular ducts, acini cells, blood vessels, intralobular ducts, regenerating islets of Langerhans while Masson trichome revealed intralobular ducts, regenerating islets of Langerhans, interlobular ducts surrounded by collagen acini cells.

As shown in figure 6, the pancreas of the sunflower tea group induced with STZ presented with regenerating islets of Langerhans. Hematoxylin and eosin stain revealed regenerating islet cells, intralobular ducts, while masson trichome revealed intercalated ducts, acini cells, blood vessels**.**

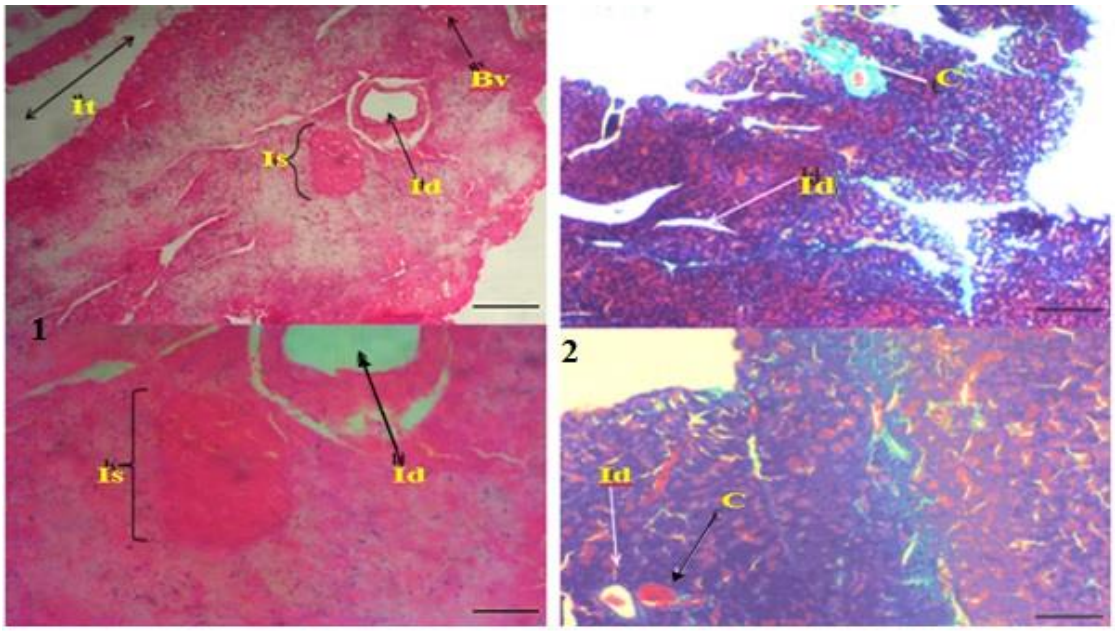


Figure 1: Photomicrograph of pancreatic tissue of experimental rats (Control) Intralobular ducts (It), interlobular ducts (Id), Islet of Langerhans (Is), blood vessels (Bv), collagen surrounding the Islet cells (C). Staining: (1) H & E; (2) Masson Trichrome, Magnifications X40 and X100

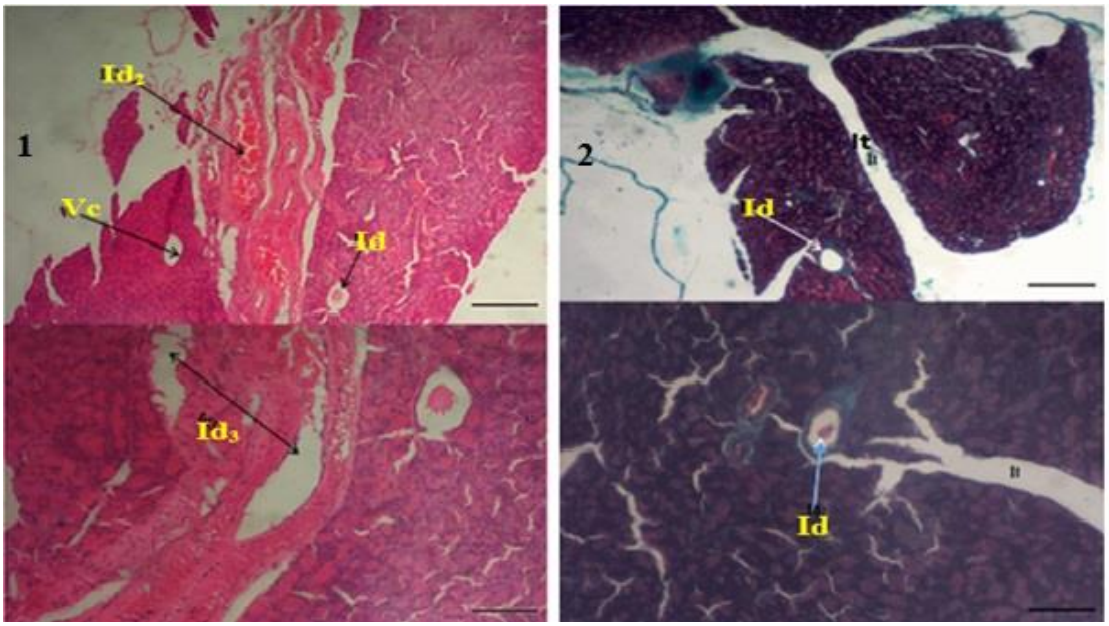
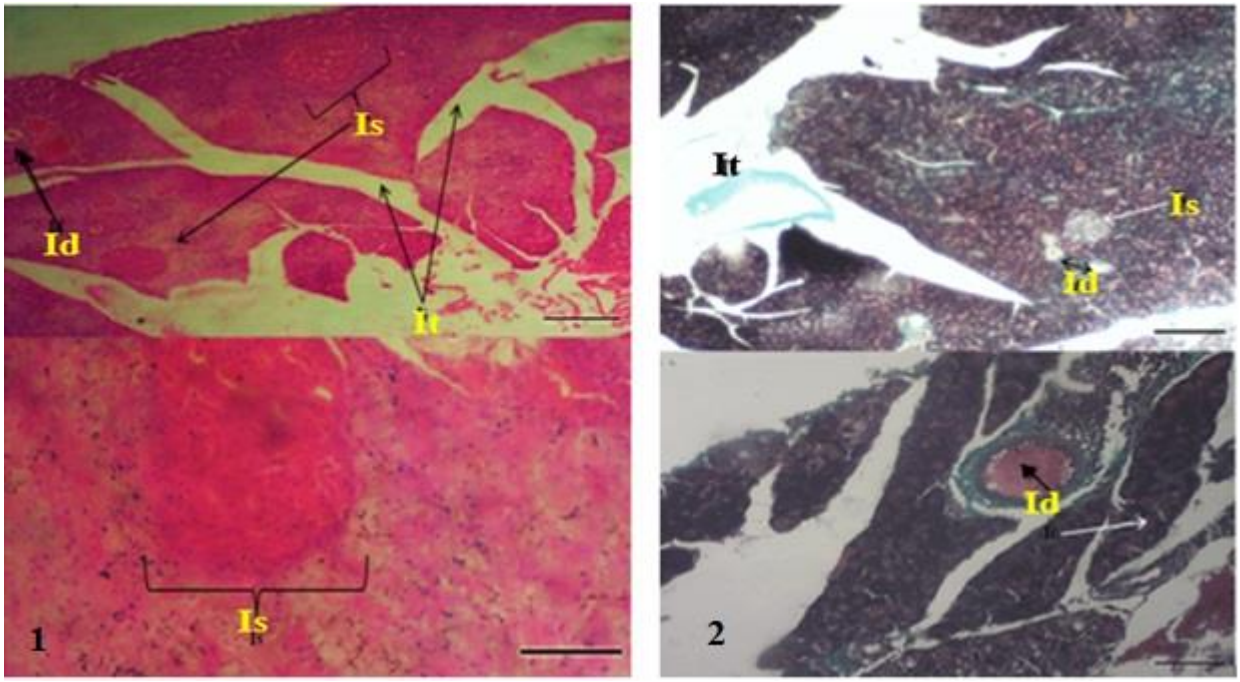


Figure 2: Photomicrograph of pancreatic tissue of experimental rats (STZ) Staining: (1) H & E (2) Masson Trichrome, Magnifications X40 and X100; Distended interlobular ducts with hemorrhages (Id_2 & Id_3), vacuolations and absence of the islet of Langerhans (Vc), intralobular ducts (It), interlobular ducts surrounded by collagen(Id).



Figures 3: Photomicrograph of pancreatic tissue of experimental rats (Peppermint tea) Staining: (1) H & E; (2) Masson Trichrome; Interlobular ducts (Id), regenerating islets of Langerhans (Is), intralobular ducts (Itinterlobular ducts, intercalated ducts (Ic). Magnifications X40 and X100

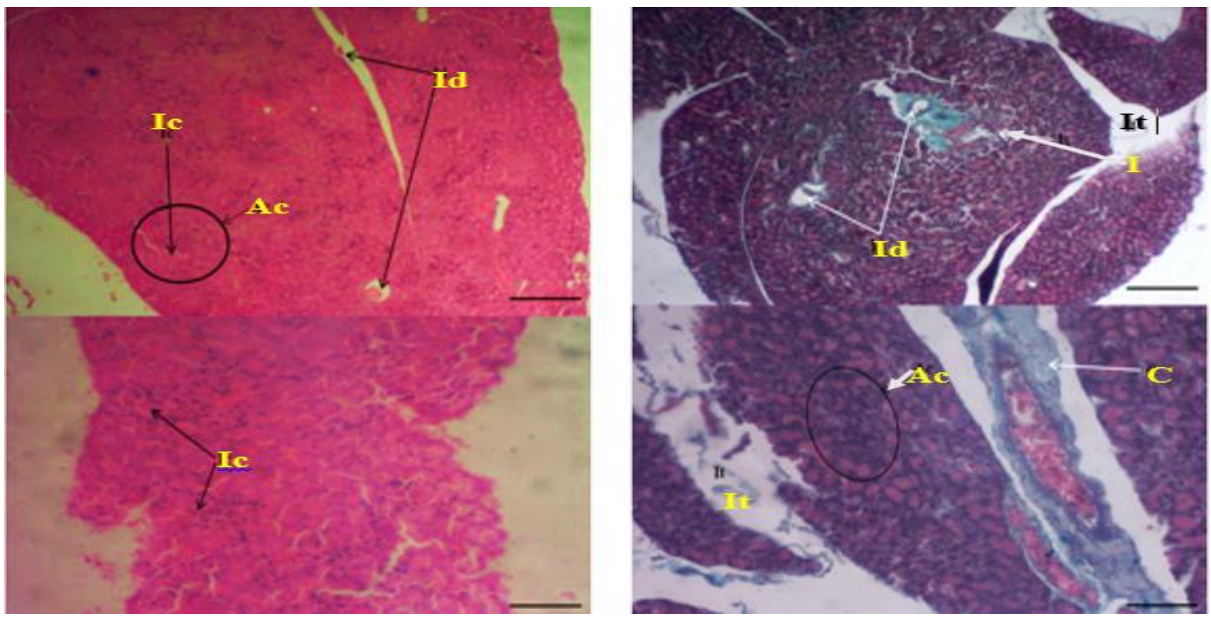


Figure 4: Photomicrograph of pancreatic tissue of experimental rats (Green tea) Staining: (1) H & E; (2) Masson Trichrome, Magnifications X40 and X100; Intercalated ducts (Ic), acini cells (Ac), ntercalated ducts (Id), regenerating Islet cell of Langerhans (Is), acini cells (Ac), intralobular ducts (It), interlobular ducts (Id), dense collagen (C).

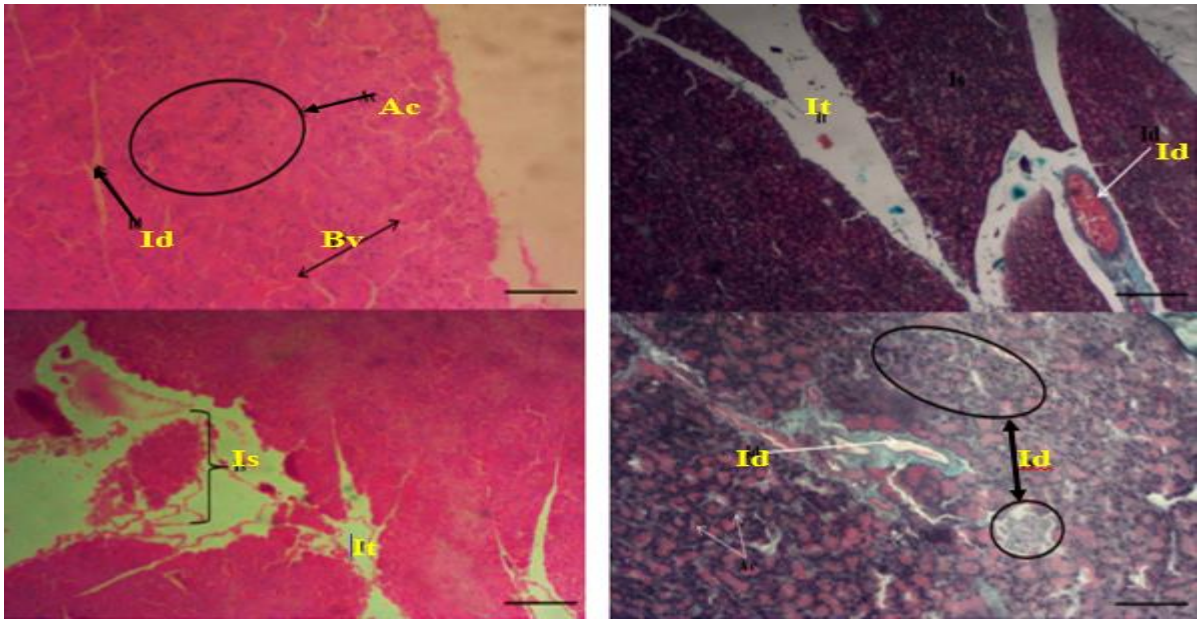


Figure 5: Photomicrograph of pancreatic tissue of experimental animals (Mango tea) Staining: (1) H & E; (2) Masson Trichrome Magnifications X40 and X100; Regenerating islet cells, interlobular ducts (Id), acini cells (Ac), blood vessels (Bv), intralobular ducts (It), regenerating islets of Langerhans (Is), regenerating islets of Langerhans (Is), Interlobular ducts surrounded by collagen (Id) acini cells (Ac).

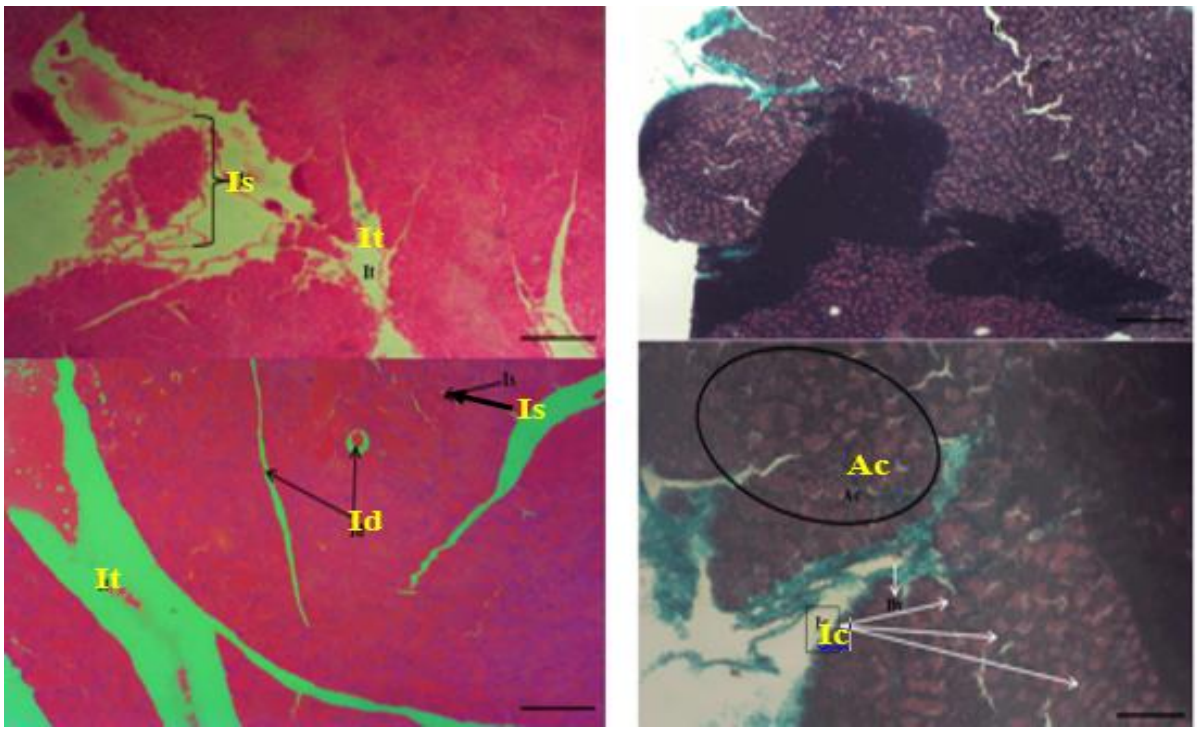


Figure 6: Photomicrograph of pancreatic tissue of experimental animals (Sunflower tea) Staining: (1) H & E; (2) Masson Trichrome Magnifications X40 and X100; Regenerating islet cells (Is), intercalated ducts (Id), acini cells (ac), blood vessels (Bv)**.**

Blood Glucose Levels

Data on blood glucose result are expressed in Figure 7. The five groups except control showed high levels of blood glucose levels at day 1 but at subsequent days, a reduction in blood glucose levels were observed. However, pep tea and sunflower tea group expressed a final blood glucose level not significantly

different from the normal control group ($p < 0.05$). Data in figure 8 showed the different significant changes in blood sugar levels at day 1, 4, 9 & 15. At day 1, pep tea had a high level of blood glucose level showing no significant difference with the STZ group, day 4 and 9 showed a notable drop in blood glucose level in pep tea and a maintained increased level of blood glucose level in Stz and finally day 15 showed no significant difference in the blood glucose level between the control and pep tea group ($p < 0.05$). Data in figure 9 showed the different significant changes in blood sugar levels at day 1, 4, 9 & 15. At day 1, green tea had a high level of blood glucose level showing no significant difference with the STZ group, day showed a small drop in blood glucose level in green tea and a maintained increased level of blood glucose level in STZ and finally day 15 showed no significant difference in the blood glucose level between the control and green tea group although this significance cannot be compared to that of peppermint tea ($p < 0.05$). Data in figure 10 showed the different significant changes in blood sugar levels at day 1, 4, 9 & 15. At day 1, mango tea had a high level of blood glucose level showing no significant difference with the STZ group, day 4 and 9 showed a small drop in blood glucose level in mango tea and a maintained increased level of blood glucose level in STZ and finally day 15 showed a significant difference in the blood glucose level between the control and mango tea group ($p < 0.05$). Data in figure 11 showed the different significant changes in blood sugar levels at day 1, 4, 9 & 15. At day 1, sunflower tea had a high level of blood glucose level showing no significant difference with the stz group, day 4 showed a small drop in blood glucose level in sunflower tea. Although, day 9 showed an decreased level of blood glucose level in sunflower, day 15 proved to show no significant difference with the control group ($p < 0.05$). Data in figure 12 showed the different significant changes in blood sugar levels at day 1, 4, 9 & 15 across different treatment groups. At day 1, blood glucose levels increased in the ratio mango tea > pep tea > green tea > sunflower tea, at day 4 & 9, there were notable drops in glucose levels across all treatment groups. At day 15, pep tea and sunflower tea showed highest significant drop in glucose levels compared to green tea and mango tea with green tea possessing highest final blood glucose level ($p < 0.05$).

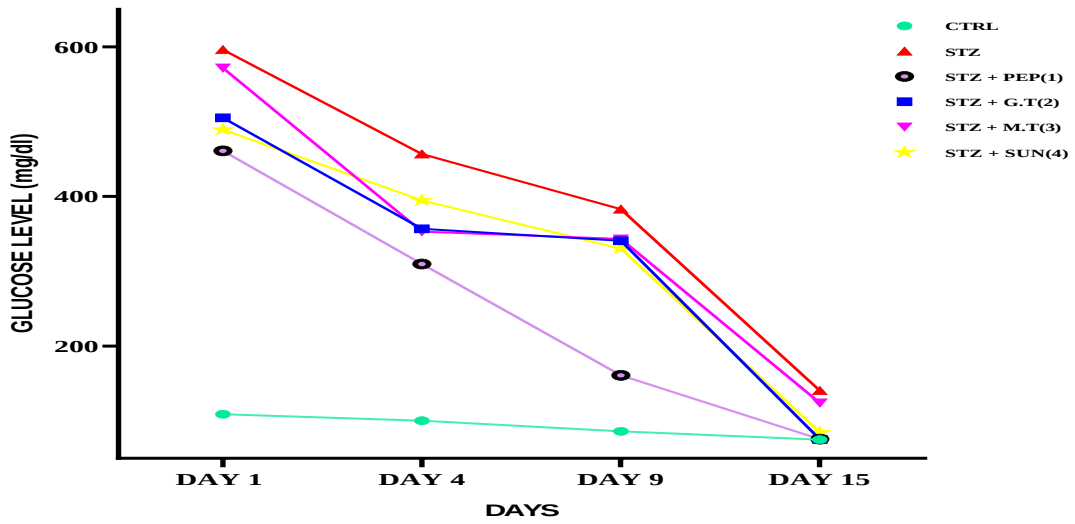


Figure 7: Chart showing changes in blood sugar level of experimental animals over a period of 15 days.

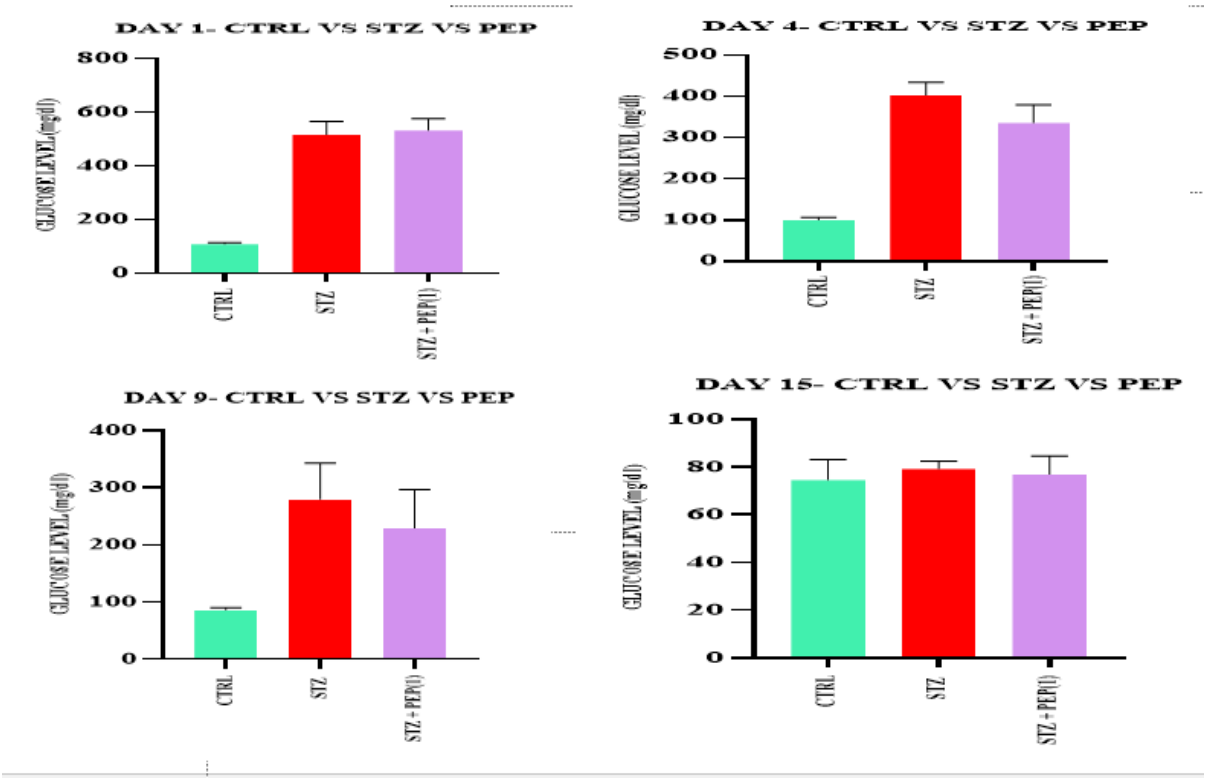


Figure 8: Charts showing varying levels of blood glucose levels of ctrl vs stz vs pep tea at days 1, 4, 5 & 15.

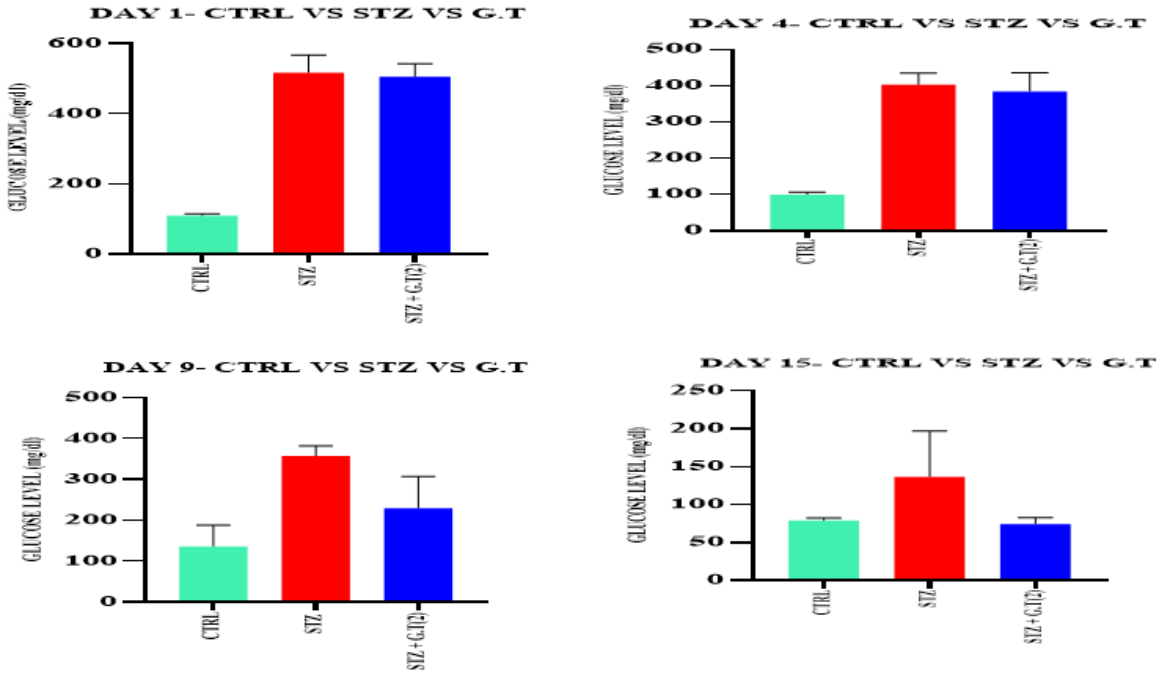


Figure 9: Charts showing varying levels of blood glucose levels of ctrl vs stz vs green tea at days 1, 4, 5 & 15.

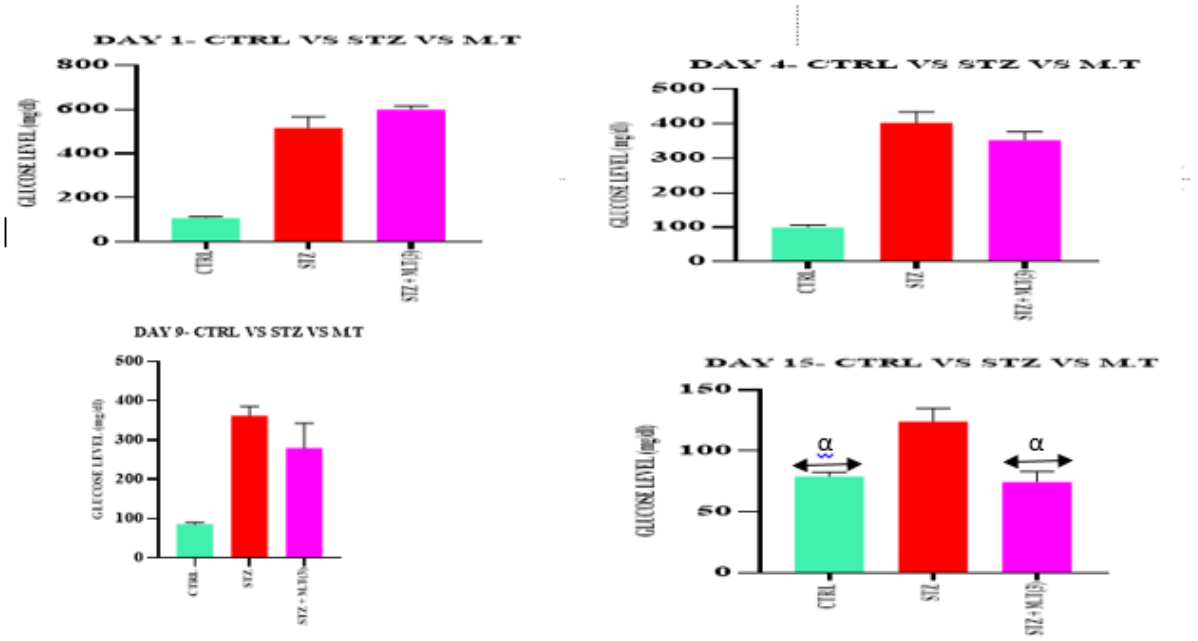


Figure 10: Charts showing varying levels of blood glucose levels of ctrl vs STZ vs mango tea at days 1, 4, 5 & 15.

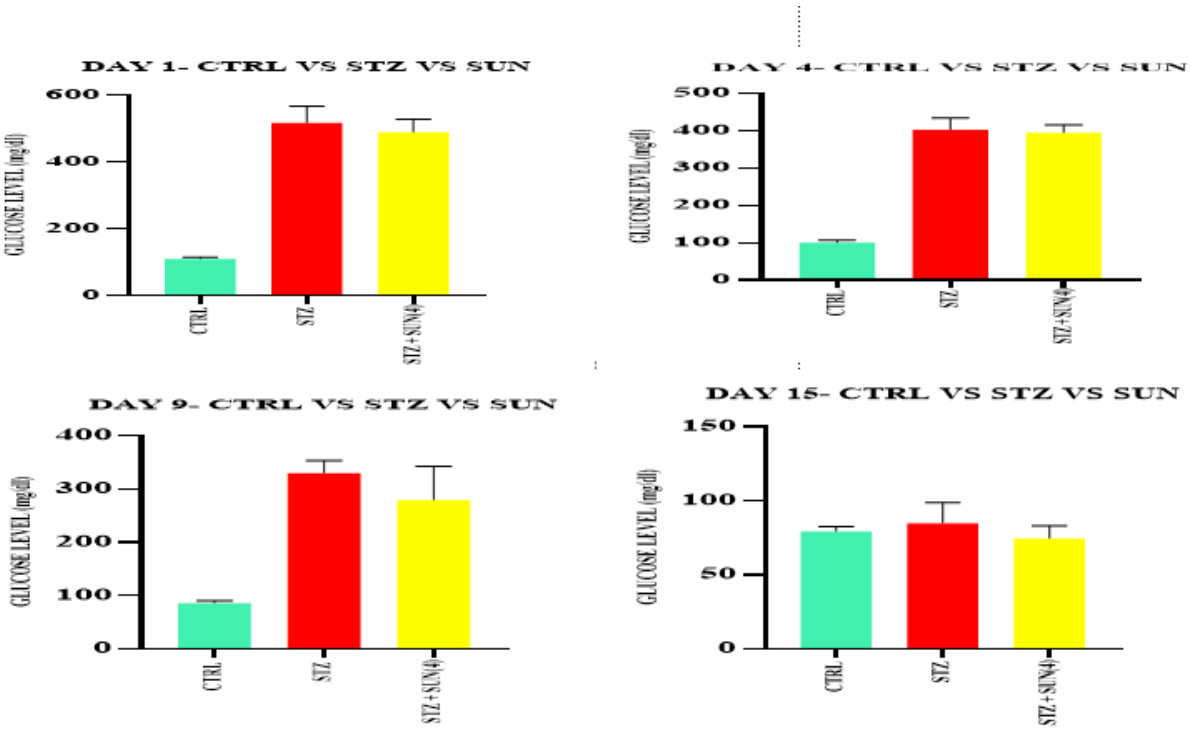


Figure 11: Charts showing varying levels of blood glucose levels of ctrl vs STZ vs sunflower tea at days 1, 4, 5 & 15.

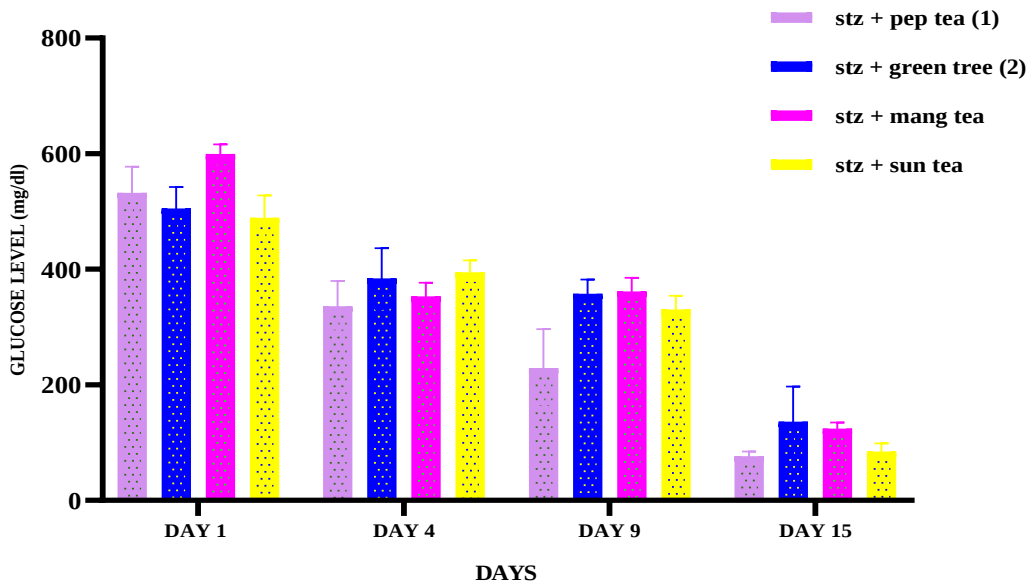


Figure 12: Chart showing varying levels of blood glucose levels of pep tea vs green tea vs mango tea vs sunflower tea at days 1, 4, 5 & 15.

Body Weight

Data on figure 13 showed different significant alterations in body weight of experimental animals across days 1, 4, 9 & 15. At day 1, there were no significant changes between the control and treatment groups likewise the STZ and treatment groups. At day 4, significant changes were seen between control and STZ, control and pep tea, STZ and green tea, mango tea, sunflower tea except the pep tea. At day 9, significant changes were seen between control group and pep tea, mango tea, sunflower tea except the green tea and also between STZ and green tea, mango tea, sunflower tea except the pep tea. Finally at day 15, significant changes were again seen in control group and pep tea, mango tea, sunflower tea except the green tea while no significant changes were seen between STZ group and the treatment groups ($p < 0.05$). Data shown in figure 14 depicts no significant change in all experimental groups. Control group showed higher values, followed by sunflower group, pep tea, mango tea and green tea while the STZ showed the lowest body weight ($p < 0.05$). Data in figure 15 showed significant changes recorded when comparing blood glucose levels against body weight at days 1, 4, 9 & 15. For the control group, there were significant changes between fasting blood sugar and body weight across days 1, 4, 9 & 15. For the STZ group, significant changes between the fasting blood sugar and body weight on days 1 & 4, none on day 9 and again a resurfacing significant change on day 15. Peppermint tea group saw a significant change between fasting blood sugar and body weight on day 1, none on day 4 & 9 and again a change on day 15. Green tea and mango tea recorded significant changes on days 1, 4 & 9 but none on day 15. Sunflower tea recorded significant changes across days 1, 4, 9 & 15 just like the control group.

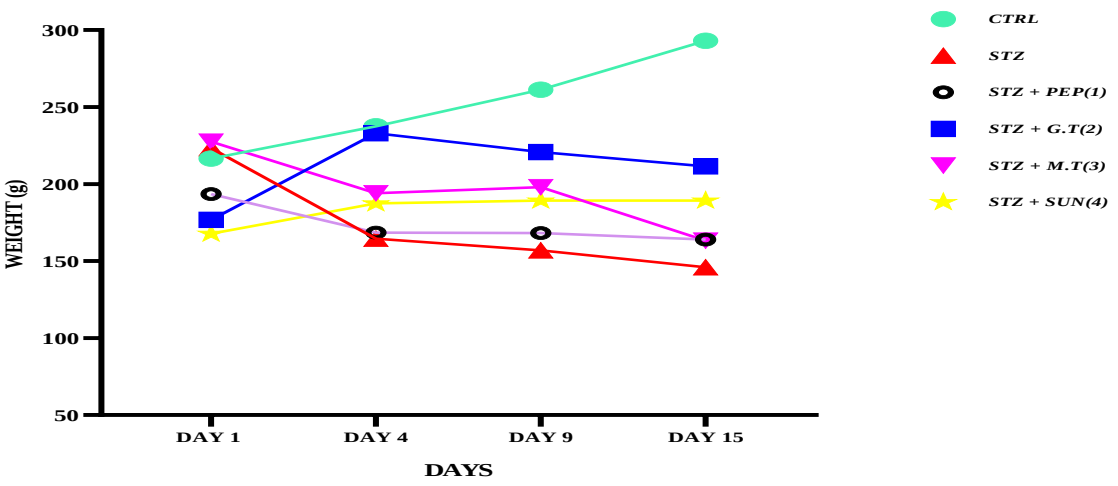


Figure 13: Chart showing varying levels of body weight of experimental rats across days 1, 4, 9 & 15.

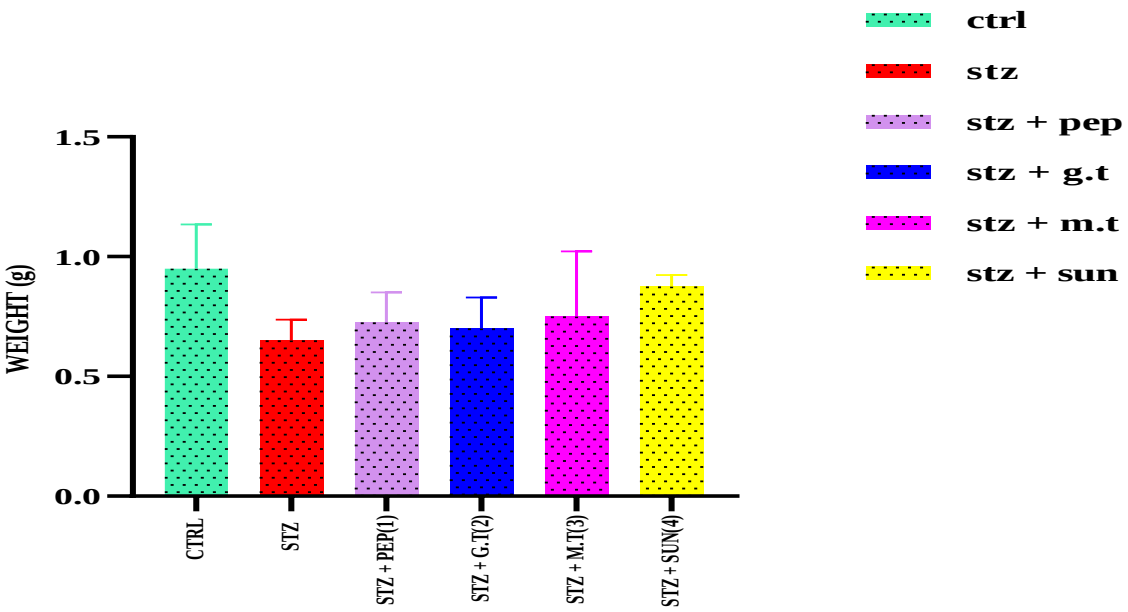


Figure 14: Chart showing organ weight of experimental rats

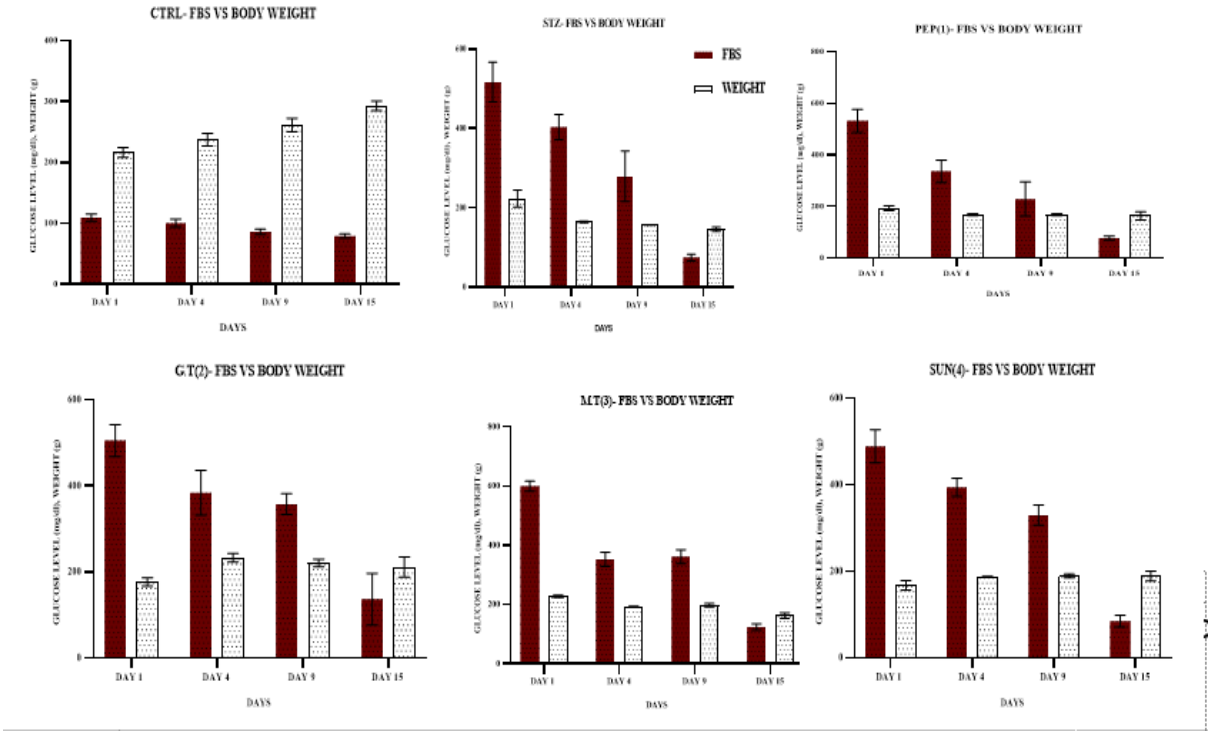


Figure 15: Chart showing blood glucose levels vs body weight across days 1, 4, 9 & 15.

Discussion

Streptozotocin prevents DNA synthesis in mammalian and bacterial cells[9]. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA, this biochemical mechanism results in mammalian cell death[9]. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis. Although Streptozotocin prevents entry of cells into mitosis, no special phase of the cellular cycle is especially sensitive to its mortal effects. Streptozotocin, which is used in an intravenous form by rapid injection or constant short diffusion, stimulates the tissues[9]. Metabolically, a slight deviation of the glucose-bearing pain from the normal limit has been seen in rats treated with a certain dose of Streptozotocin, which is generally reversible, however, the insulin shock, which is one of its other effects, is irreversible. In this study, the clinical manifestations and also the amount of glucose, insulin and C-peptide after using a 100 mg/kg b.w dose of Streptozotocin, ensured induction of diabetes in rats just like[9].

Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of Streptozotocin treatment and, within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover, researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method. Results found in this research were seen to be similar with those of Akbarzadeh *et al* [9] and Michael *et al* [10] with no significant difference between them.

Management of diabetes with the agents devoid of side effects is still a challenge to the medical system. This concern has led to an increased demand for natural products with anti-diabetic activity, having fewer side effects. It was reported that plant extracts causes anti diabetic effect by promoting regeneration of beta cells or by protecting these cells from destruction: plant extracts may activate insulin receptors or affect beta cells to release insulin[11].
Oral administration of the composite teas showed varying degrees of anti-diabetic properties in this research both in the histoarchitecture and also the fasting blood sugar levels. According to a comparative study made by Sailesh[11], it was recorded that peppermint had the lowest anti-diabetic property compared to cinnamon and nutmeg but this study showed that peppermint used in a formulation of different constituents together produced great regeneration of β-cells/islets of langerhans, reduced levels of fasting blood sugar and also no significant difference when compared to the control group. Previous studies showed that peppermint also

possessed weight reduction properties which correlates with the results in this research as well, notable weight loss was noticed alongside improved histoarchitecture, it could be said that this weight loss property also had a role to play in its anti-diabetic property[12].

Sharifzadeh *et al.*, [13] stated that green tea was reported to reduce the elevated blood glucose level in both type 1 and type 2 diabetic animals alongside increased body weight, reduced intake of food and water and better glycemic condition, this corresponds to findings in this research as green tea used alone did not bring about a reduction in weight but an increase with improved glycemic control. Although, when compared with the control, there was significant difference with the control group and less β-cells unlike that of the peppermint group. According to Juśkiewicz *et al.*, [14], green tea extract ingested at high amounts may prove to be a useful therapeutic option in the reversal of diabetic dysfunction.

Although, there has been limited research work on the anti-diabetic properties of mango tea, its anti-obesity effects cannot be overemphasized, Ramírez *et al.*, [15] showed that mango leaves extract has therapeutic potential in treating obesity and related diseases through regulating the expression of transcriptional factors and enzymes associated with adipogenesis. This study found out this anti-obesity property as there was a large drop in final body weight of experimental animals, due to this, it could be said that the anti-diabetic property goes in line with its anti-obesity property as seen in previous research. Histoarchitecture of this group recorded regeneration of the β-cell and also a reduced blood glucose level.

Sebbagh *et al.* [16], showed that sunflower oil supplementation may have a beneficial effect by partly preserving or restoring pancreatic beta-cell mass in the STZ-induced diabetes rat model, its leaves was used in this study and it greatly reversed the necrotic effects of diabetes. In comparison with the control group, there was no significant difference recorded, improved histoarhitecture with regeneration of β-cells and a large drop in blood sugar level.

Researches made on teas so far have largely been based on just a component e.g. green tea only, mango tea only, moringa tea only e.t.c., but this study put together a formulation of different teas (moringa, sunflower, mango, peppermint and turmeric) in order to check for their joint anti-diabetic properties on the pancreas. Based on the results gotten from this study and comparison with other studies, these formulations proved to be better at managing diabetes, in contrast to various studies on just a single type of tea, composite teas produced better glycemic control properties. Peppermint and sunflower tea formulations proved to have the highest anti-diabetic properties amongst the 4 teas from results. From this research as well, it can be deduced that the formulations gave better protective effects when compared to green tea which was administered alone as a single tea with its own components.

Conclusion

This study showed that Streptozotocin, causes type 1 diabetes on adult female wistar rats, which was observed after the administration. The formulations of the composite teas have protective effect on the deleterious changes caused by STZ-induced diabetes on the blood glucose level, body weight and histoarchitecture of the pancreas of female albino wistar rats by regeneration of β-cells in the pancreas of diabetic conditions.

This study, therefore, confirms that the administration of different formulations of teas (composite tea) proved to be a potent anti-diabetic agent in diabetic rats and can be used in place of single teas.

Ethics approval

The experimental procedures were conducted in accordance with the NIH guidelines for the care and use of laboratory animals in line with guidelines of the Department of Anatomy, School of Basic Medical Science, College of Medicine, Federal University of Technology, and the Centre for Research and Development (CERAD), Federal University of Technology Akure, Ondo State.

Acknowledgments

The author is thankful to the Department of Human Anatomy, Federal University of Technology Akure Nigeria for the photomicrograph capturing of slides. Mr Ige of Histology Laboratory, Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ife, Nigeria for preparation of histology slide. Special thanks also to God and my wife and children for their immense contribution all through this research work.

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