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(RESEARCH ARTICLE)

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Blood glucose level, lipid profile and histopathology of alloxan induced diabetic rats fed *Sesamum indicum* compounded diet

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Abstract

The purpose of this study was to examine the blood glucose level, lipid profile, and histology of alloxan-induced diabetic rats fed compounded diet of *Sesamum indicum*. 36 male albino rats were divided into nine groups of four each for a period of 21 days: normal control, diabetic control, treated with the standard medication glibenclamide, diabetic rats fed with 15%, 30%, and 60% compounded diet, and normal rats fed with 15%, 30%, and 60% compounded diet and induced with diabetics after 14 days. single intraperitoneal injection of freshly produced alloxan (120 mg/kg b.wt.) was used to induce diabetes. Acu-check glucometer was used to measure the blood glucose level, and conventional spectrophotometric method was used to measure the lipid profile level. When compared to the diabetic control group, which had glucose level above 200, the groups fed the compounded diet had decreased level. Significant ($p<0.05$) reduction was observed for cholesterol, triglyceride, and low-density lipoprotein when compared with diabetic control while the compounded diets were able to increase HDL that was found to be low in diabetic control. When compared to the diabetic control group, which displayed a damaged pancreas, the results from the histopathology of the pancreas showed that the groups fed with the compounded diet had a repaired pancreas. According to the study's findings, *Sesamum indicum* has the ability to reduce excessive blood glucose and cholesterol levels as well as mend a diabetic rat's deformed pancreas.

Keywords: *Sesamum indicum; A*lloxan; Diabetes; Glibenclamide

1. Introduction

The utilization of plant products is one area of interest that is expanding in diabetes care since numerous plant products have been shown to have therapeutic potential. Numerous medications have been developed to treat diabetes, but the majority of them have unfavorable side effects [1]. And mot times, out of reach. A dietary supplement that makes use of locally accessible food or plant items that have been shown to have a hypoglycemic impact is one solution for this problem [2]. Today, 25% of medicines that are given come from plant sources [3].

A dietary supplement is a product designed to enhance the diet's intake and may include one or more of the following ingredients: vitamins, minerals, herbs, botanicals, amino acids, concentrates, metabolites, extracts, enzymes, or a combination of these [4]. The sesame plant is one of these plants that has been used extensively in food and medicine since ancient times [5].

One of the most difficult health issues of the twenty-first century is diabetes. According to the 2010 diabetes statistics, 285 million individuals worldwide are projected to be carriers of the disease, with type 2 diabetes accounting for 90% of cases. It was the eighth biggest cause of death in 2011 with 1.4 million fatalities worldwide, and by 2030, it is

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predicted to quadruple [6]. The fact that roughly one-third of diabetes cases are still undiagnosed is extremely concerning [7].

Diabetes mellitus is a chronic disease that develops from the pancreas' inability to produce enough insulin (a hormone that regulates blood sugar) or from the condition in which the insulin produced by the body cannot be used effectively. Defective insulin secretion is the main cause of chronic hyperglycemia, which results in impaired function or serious damage to many of the body's systems, such as the eyes, kidneys, nerves, heart, and blood vessels. Early signs of diabetes can be quite subtle, sometimes even going unnoticed [8].

It is essential to provide an alternative with little to no side effects for the treatment of diabetes due to the rising prevalence of diabetes in the world and the noticeable adverse effects of some medications used to treat it. Dietary supplements are now a possibility, and research into diabetes treatment is becoming more and more interested in them. Sesame seed can be used as a dietary supplement because it has been shown in past research studies to have medicinal potential.

A blooming plant belonging to the genus *Sesamum* by the common name of beniseed is *Sesamum indicum*. It is frequently farmed for its edible seeds, which develop in pods, and is found growing in nature in tropical areas all over the world. One of the earliest oil seed crops ever domesticated, it dates back more than 3000 years [9]. It has numerous species, some of which are indigenous to sub-Saharan Africa and are wild. India gave rise to the cultivated variety of *Sesamum indicum* [10]. It is known to thrive in environments where other crops may fail and has a high tolerance for drought-like conditions [9].

Sesamum indicum is reputed to produce some of the seeds with the highest oil content. It is a typical element in cuisines all over the world [11] and has a rich nutty flavor. It is a key source of vegetable oil for cooking in Nigeria and is used as a soup ingredient [12]. *Sesamum indicum* is useful in animal nutrition since it is not only necessary for the production of oil and paste but also for the creation of foods like halaweh, java beans, and bennimix in Sierra Leone [13, 14, 15]. Although it is usually used as a component of soup and is a significant source of edible/cooking oil, there are other alternate uses for it that are advantageous to both people and animals. The seeds are sprinkled on top of hamburger buns and added to breads like bagels. While whole seeds are included in many salads and baked snacks, they can also be baked into crackers, frequently in the form of sticks [16]. They are used in cakes in Greece and in Charleston, South Carolina, as well as in sweet and savory cookies and wafers [12].

The usage of *Sesamum indicum* in the treatment of diabetes has received scant attention. Consequently, this research study examines the hypoglycemic and hypolipidemic effects of a compounded *Sesamum indicum* diet.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

The study's source of alloxan was Qualikem Fine Chemistry Pvt Ltd in Vadodara, India. BDH Chemicals Limited, based in Poole, England, produced the chemicals used. And the USA's Cayman Chemical Company produced the glibenclamide used.

2.1.2. Reagents

Agape Diagnostics Limited, based in Switzerland, produced the reagent used for the lipid profile test.

2.1.3. Experimental animals

At the University of Nigeria Nsukka's Department of Animal Science, 36 male albino rats weighing 120–150g were purchased. Before the studies, the animals had a two-week acclimatization period. The animals were housed in small, well-constructed cages to prevent feed and rat waste from mixing. The institution's Animal Ethics Committee's ethical principles [17] were followed in the handling and care of the animals.

2.2. Methods

2.2.1. Sample collection and preparation

Off-white *Sesamum indicum* growing in Nigeria was acquired from the Ogbete market in Enugu State in the south-east of the country and was identified at the Federal University of Technology Owerri in Imo State Nigeria. According to Esonu et al 2006 [18] for both feed formulation and storage, the seeds were washed in water, allowed to air dry for a week, milled, and stored in a clearly labeled container.

2.2.2. 15%, 30% and 60% Sesamum indicum compounded diet formulation

The compounded diet was formulated using the method of Ezeokeke 2015 [19]. The following constituent were used; Maize, *Sesamum indicum*, wheat offal, Groundnut cake, Fish meal, Bone meal, Premix and Elephant grass. The concentrations are gotten by varying the amount of maize and *Sesamum indicum* used in the formulation.

2.2.3. Experimental induction of diabetes

Diabetes was induced on the rat by intraperitoneal injection of freshly prepared 120 mg/kg b.wt. of alloxan. Diabetes was confirmed after 72hrs of administration. Values above 200mg/dl were considered diabetic [20].

2.2.4. Experimental design

Thirty-Six albino rats were divided into nine groups of four rats each

- Group 1: Normal Control
- Group 2: Diabetic Control
- Group 3: Diabetic Ratss treated with Glibenclamide
- Group 4: Diabetic Rats fed 15% *Sesamum indicum* compounded diet
- Group 5: Diabetic Rats fed 30% *Sesamum indicum* compounded diet
- Group 6: Diabetic Rats fed 60% *Sesamum indicum* compounded diet
- Group 7: Normal Rats fed 15% *Sesamum indicum* compounded diet and induced after 14 days
- Group 8: Normal Rats fed 30% *Sesamum indicum* compounded diet and induced after 14 days
- Group 9: Normal Rats fed 60% *Sesamum indicum* compounded diet and induced after 14 days

2.2.5. Standard Drug (Glibenclamide) Preparation

Group 3 were given 0.5 mg/kg b. wt of glibenclamide orally as a standard drug.

2.3. Biochemical Analysis

2.3.1. Lipid Profile

The level of Trig, HDL, LDL and Cholesterol were estimated using the commercial kit (AGAPE, Switzerland) [21].

2.3.2. Histological Studies of the Organ

Histological screening was carried out on the pancreas of rats representing each group. This is to prove the results obtained from the biochemical analysis.

2.3.3. Statistical Analysis

All data collected in this study were subjected to statistical analysis using SPSS, version 16.0. Means were compared using T-test and the results presented as mean value with the Standard Error of mean (SEM). The statistically significant difference was defined as $p<0.05$.

3. Results

3.1. Blood Glucose Level

Figure 1 below displays the blood glucose levels of the experimental and control groups. According to the findings, the control group's blood glucose level remained below 200 from the third day of the trial, when it was 97, to the fourteenth day, when it was 84. However, after the induction of diabetes on day 3, increase above 200 was seen in the diabetic

group. The high blood glucose level brought on by the introduction of diabetes was gradually reduced by the compounded diet. Rats fed a 15% compounded diet had blood glucose levels that decreased by 53.6% (from 261 on the third day to 121 on the fourteenth day). In contrast, the group given a 30% compounded diet shrank by 58.2% (from 261 on the third day to 109 on the fourteenth). Additionally, the group receiving the 60% compounded diet saw a 58.4% decrease in blood sugar levels (from 243 on the third day to 101 on the fourteenth). The reduction was remarkable when compared to the diabetic group, which experienced a 10% modest decline (due to alloxan regeneration) from 263 on the third day to 212 on the fourteenth.

Since their blood glucose levels were below 200, there was no discernible increase in the group fed the 15%, 30%, and 60% compounded diet after the diabetes was induced.

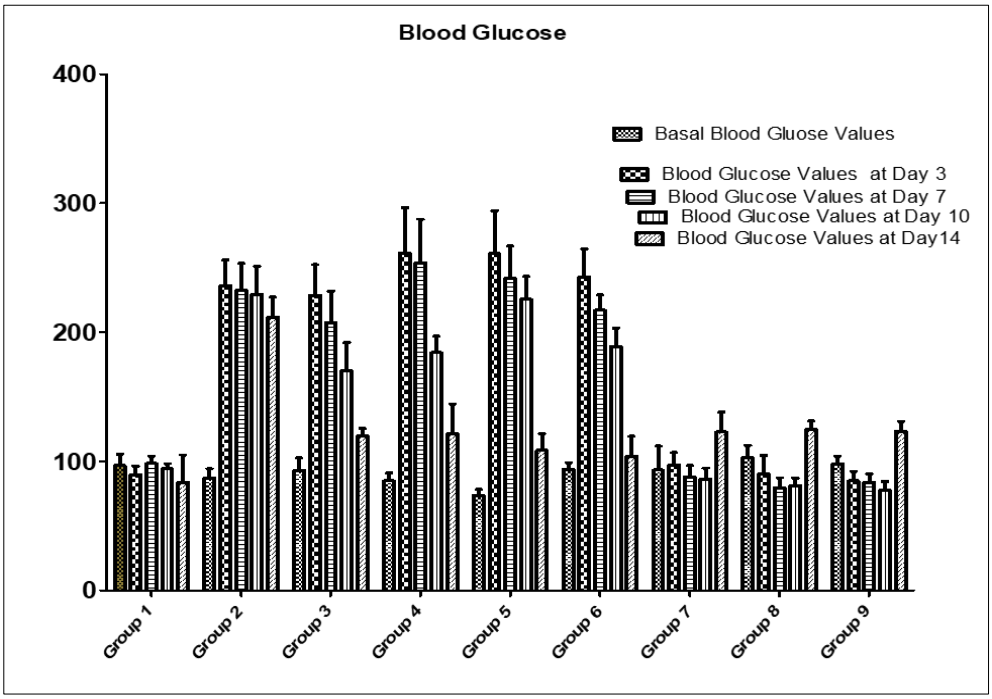

Figure 1 Blood Glucose Level of Albino Rats at 3rd 7th 10th and 14th day. X axis = level of glucose, Y= groups

3.2. Lipid Profile

Table 1 showed the effect of the compounded feed on Lipid Profile parameters of albino rats studied. The results are presented in mean ± standard error of mean deviation and values bearing different superscript letters are significantly different ($P<0.05$). The Lipid Profile parameters presented are Cholesterol, Triglyceride, HDL and LDL.

The result showed that the Cholesterol for the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (105 ± 26^a, 156 ± 26^a, 128 ± 25^a, 140 ± 12^a, 133 ± 8.6^a, and 93 ± 8.4^a respectively) when compared with control group 1 (144 ± 5.9^a) and standard treated group 3(105 ± 12^a). But were significantly different when compared with diabetic untreated group 2 (245 ± 12^b).

For Triglyceride, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (97 ± 9.0^a, 106 ± 8.5^a, 78 ± 1.4^a, 106 ± 1.3^a, 124 ± 1.8^a, and 70 ± 4.0^a respectively) when compared with control group 1 (137 ± 3.4^a) and standard treated group 3 (101 ± 1.7^a). But were significantly different when compared with diabetic untreated group 2 (165 ± 3.0^b).

The result of HDL showed that the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (46 ± 4.0^a, 52 ± 3.7^a, 54 ± 1.5^a, 52 ± 3.3^a, 54 ± 2.1^a, and 55 ± 8.0^a respectively) when compared with control group 1 (54 ± 1.5^a) and standard treated group 3 (46 ± 3.0^a). but were significantly different when compared with diabetic untreated group 2 (34 ± 3.0^b).

The same trend was observed for LDL were the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (115 ± 1.5^a, 121 ± 2.6^a, 101 ± 1.5^a, 112 ± 3.4^a, 81 ± 2.2^a, and 96 ± 1.8^a respectively) when compared

with control group 1 (104 ± 1.7^{a}) and standard treated group 3 (113 ± 1.0^{a}). But were significantly different when compared with diabetic untreated group 2 (156 ± 1.9^{b}).

Table 1 Effect of Compounded Diet on Lipid Profile Function Parameters of Albino Rat

Groups	Cholesterol	Triglyceride	HDL	LDL
1	144 ± 5.9^{a}	137 ± 3.4^{a}	54 ± 1.5^{a}	104 ± 1.7^{a}
2	245 ± 12^{b}	165 ± 3.0^{b}	34 ± 3.0^{b}	156 ± 1.9^{b}
3	105 ± 12^{a}	101 ± 17^{a}	46 ± 3.0^{a}	113 ± 1.0^{a}
4	105 ± 26^{a}	97 ± 9.0^{a}	46 ± 4.0^{a}	115 ± 1.5^{a}
5	156 ± 26^{a}	106 ± 8.5^{a}	52 ± 3.7^{a}	121 ± 2.6^{a}
6	128 ± 25^{a}	78 ± 14^{a}	54 ± 1.5^{a}	101 ± 1.5^{a}
7	140 ± 12^{a}	106 ± 13^{a}	52 ± 3.3^{a}	112 ± 3.4^{a}
8	133 ± 8.6^{a}	124 ± 1.8^{a}	54 ± 2.1^{a}	81 ± 2.2^{a}
9	93 ± 8.4^{a}	70 ± 4.0^{a}	55 ± 8.0^{a}	96 ± 1.8^{a}

Data are presented as means ± standard error mean; n= 4 for each group. For each parameter, values bearing different superscript letters are different ($P<0.05$). Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

3.3. Histopathology of Pancreas

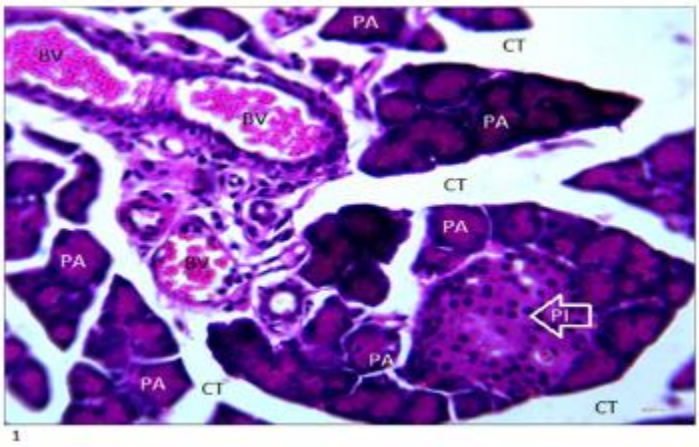

Figure 2 Histopathology of Pancreas for Control Grp 1 showing normal pancreas X 400 H & E

Fig 2 shows the Photomicrograph of the Pancreas of Group 1 which served as Control group. From the fig, it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells (α β δ). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

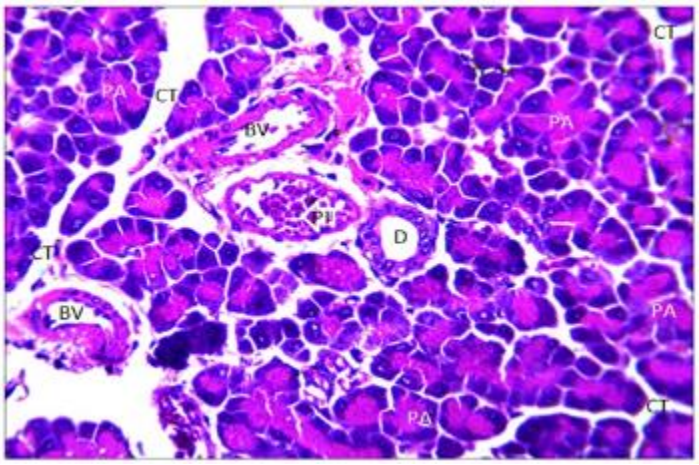

Figure 3 Histopathology of Pancreas for diabetic untreated Grp 2 showing distorted pancreas X 400 H & E

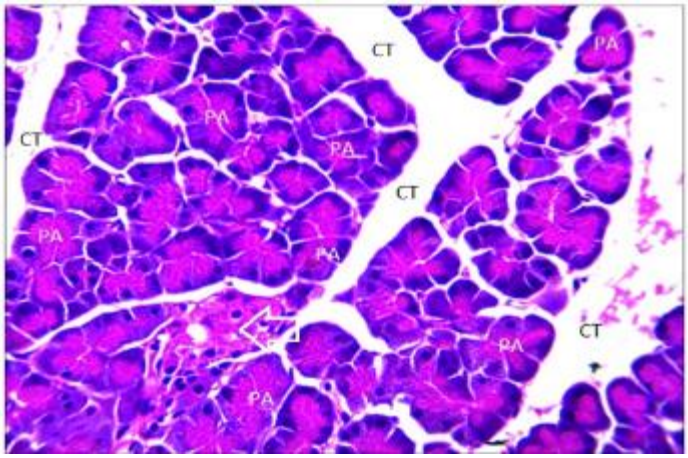

Figure 4 Histopathology of Pancreas for Glibenclamide treated Grp 3 showing normal pancreas X 400 H&E

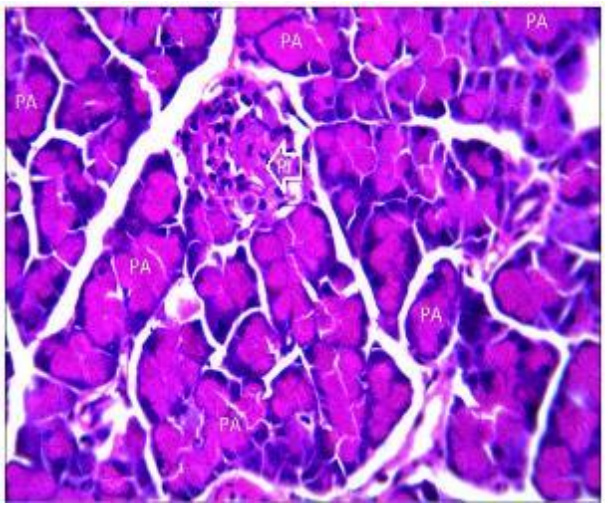

Figure 5 Histopathology of Pancreas for Grp 4 diabetic rat fed with 15% feed showing normal pancreas X 400 H&E

Figure 3 shows the Photomicrograph of the Pancreas of Group 2 which served as diabetic untreated group. From the figure, it shows that the pancreas is histologically distorted with damaged Pancreatic Islet (PI), Intact Pancreatic Acini (PA), Patent Blood Vessels (BV), and Patent Pancreatic Duct (D).

Figure 4 shows the Photomicrograph of the Pancreas of Group 3 which served as Standard drug treated group. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

Figure 5 shows the Photomicrograph of the Pancreas of Group 4 which served as 15% compounded diet fed group. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

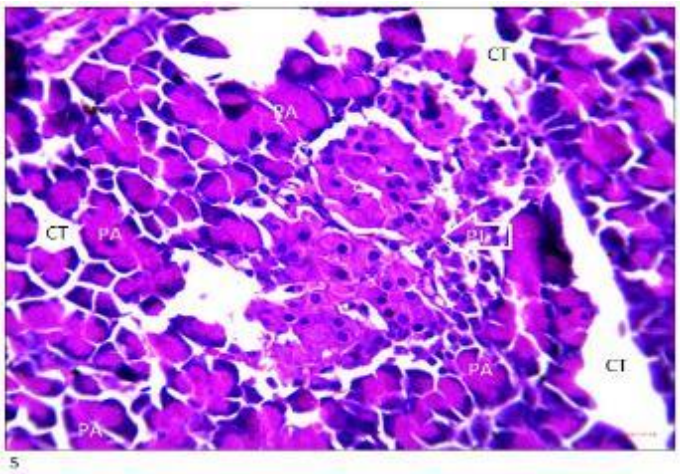

Figure 6 Histopathology of Pancreas for Grp 5 diabetic rat fed with 30% feed showing normal pancreas X 400 H&E

Figure 6 shows the Photomicrograph of the Pancreas of Group 5 which served as 30% compounded diet fed group. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

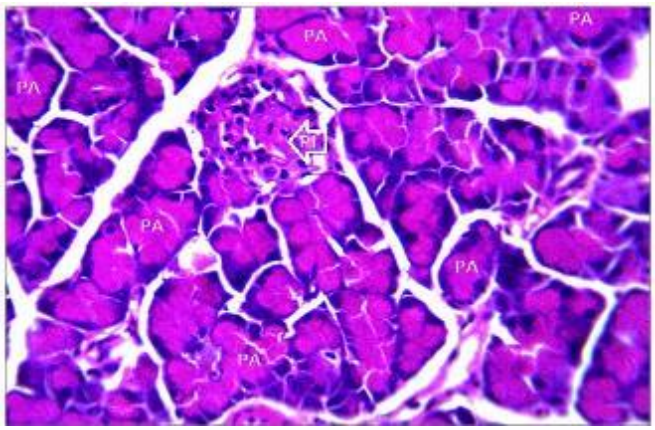

Figure 7 Histopathology of Pancreas for Grp 6 diabetic rat fed with 60% feed showing normal pancreas X 400 H&E

Figure 7 shows the Photomicrograph of the Pancreas of Group 6 which served as 60% compounded diet fed group. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

Figure 8 shows the Photomicrograph of the Pancreas of Group 7 which served as the group that was fed 15% compounded diet for 14 days and afterwards induced with diabetes. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessel (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

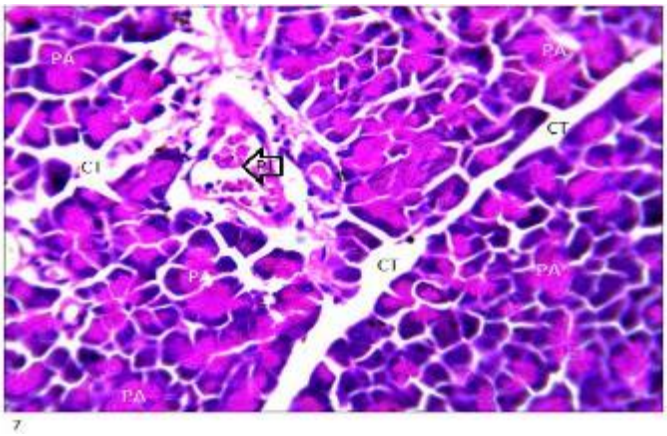

Figure 8 Histopathology of Pancreas for Grp 7 normal rat fed with 15% feed and induced after 14 days showing normal pancreas X 400 H&E

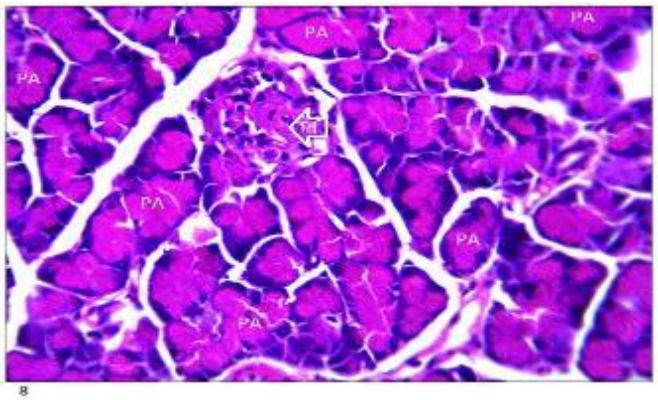

Figure 9 Histopathology of Pancreas for Grp 8 normal rat fed with 30% feed and induced after 14 days showing normal pancreas X 400 H&E

Figure 9 shows the Photomicrograph of the Pancreas of Group 8 which served as the group that was fed 30% compounded diet for 14 days and afterwards induced with diabetes. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessel (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

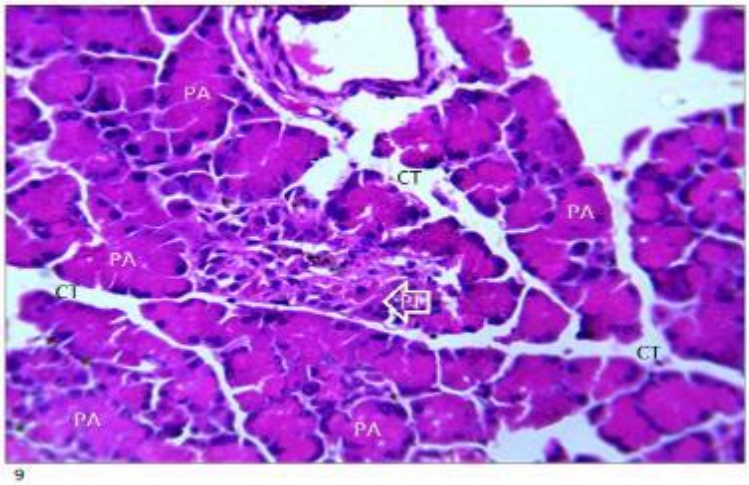

Figure 10 Histopathology of Pancreas for Grp 9 normal rat fed with 60% feed and induced after 14 days showing normal pancreas X 400 H&E

Figure 10 shows the Photomicrograph of the Pancreas of Group 9 which served as the group that was fed 60% compounded diet for 14 days and afterwards induced with diabetes. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells (α β δ). Intact Pancreatic Acini (PA), Blood Vessel (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

4. Discussion

The increased blood glucose levels seen after the onset of diabetes are in line with the literature. It is well-known from the literature that using alloxan to induce diabetes partially destroys the pancreatic insulin and causes diabetes [22]. The presence of several phytochemicals in the seed may explain why blood glucose levels were much lower in groups fed the compounded feed. The seed is reported to contain sesamin, alkaloids, tannins, flavonoids, and saponins according to literature [23]. Flavonoids and sesamin have hypoglycemic effects [24]. The groups fed the compounded diet had a decrease in blood glucose levels as a result of this hypoglycemic feature. When there is some pancreatic beta cell activity, the conventional medication (Glibenclamide) activates the pancreas to release more insulin and is successful [24].

Lipids play a critical function in the onset of sickness or disorder in the body. When a person has diabetes, an increase in blood sugar is typically accompanied with increases in low-density lipoprotein (LDL), triglycerides, and cholesterol, with a decrease in high-density lipoprotein (HDL) levels. This trend was observed in the result of this research; where the diabetic untreated group has elevated Cholesterol, LDL and Triglyceride with a decrease in HDL level while the opposite was observed for the groups fed with the compounded diet or treated with the standard drug. This observation is in line with the report of Ashraduzzaman *et al* [25] on the effect of *Vigna inguiculata* seed oil in diabetic rats. It is also in conformity with the report of Khosla *et al* [26] on administration of Fenugreek seed extract on diabetic rats and report of Kizito *et al* [27] on hypolipidemic effect of irvingia gabonensis fruit on sodium fluoride induced dyslipidemia in rats.

It has been proven by the report of Shih *et al* [28] on Acipinox attenuates hypertriglyceridemia in diabetic patients, that the elevation in triglyceride of diabetic animals may be as a result of insulin deficiency which results in hyperglycemia; when fatty acids from the adipose tissues are mobilized for purpose of energy, excess of it are accumulated in the liver, which are then converted to Triglyceride [28].

The decrease in the Cholesterol level observed in the groups fed with the compounded diet may be as a result of the presence of saponin in the seed, which have been reported to form complexes with Cholesterol and bile acids preventing them from being absorbed through the small intestine thereby decreasing the Cholesterol level in the blood and liver [28].

Report has also shown that insulin elevates the number of Low-Density Lipoprotein receptors. Therefore, insulin deficiency might be linked to a decrease level of LDL receptors that yields more LDL particles and as a result increases the level of LDL-Cholesterol in diabetes. The oil of *Sesamum indicum* has been reported to maintain good Cholesterol (HDL) and decrease bad Cholesterol [29]. Hence the increase in HDL observed in this research work is in line with literature.

5. Conclusion

The result of this research work has shown that the compounded diet has the ability to lower blood glucose level, cholesterol, LDL, and Triglyceride level of diabetes while decreasing the HDL level. Such ability can be put to use in the cure or management of diabetes. Due to some of the phytochemicals like Sesamin and flavonoids found in the seed, possibility abound that the seed could be used in the treatment of hyperlipidemia related diseases.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The ethical committee of the university approved the study protocol prior to commencement of the study and the study was carried out according to the guidelines of the Animal welfare act.

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