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(RESEARCH ARTICLE)

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Evaluation of blood glucose, hepatic biomarkers and histopathology of alloxan induced diabetic rats fed *Sesamum indicum* compounded diet

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Abstract

This study was aimed at evaluating the blood glucose level, hepatic biomarkers and histology of alloxan induced diabetic rats fed with *Sesamum indicum* compounded diet. Thirty-Six male albino rats were divided into nine groups (normal control, diabetic control, standard drug-glibenclamide treated, diabetic rats fed with 15%, 30% and 60% *Sesamum indicum* compounded diet and normal rats fed with 15%, 30% and 60% *Sesamum indicum* compounded diet and induced with diabetics after 14 days) of four rats each and the study lasted for 21 days. The induction of diabetes was done by a single dose of freshly prepared alloxan (120 mg/kg b.wt.) given intraperitoneally. The level of blood glucose was determined using Acu-check glucometer while the hepatic biomarkers was determined using standard spectrophotometric method. The blood glucose of the groups fed with the diet were observed to reduce when compared with the diabetic untreated group. Significant ($p<0.05$) reduction was observed for the hepatic biomarkers when compared with diabetic control. From the histopathology of the liver, the results showed improved liver for the groups fed with the compounded diet when compared with the diabetic control group that showed a damaged liver. The results of this study have shown that *Sesamum indicum* has both protective and ameliorative effect on blood glucose, hepatic biomarkers and restore the distorted liver of a diabetic rat.

Keywords: *Sesamum indicum*; Alloxan; Diabetes; Glibenclamide; Protective; Ameliorative

1. Introduction

One of the growing interests in the management of diabetes is the use of plant products; as many plant products have been known to possess therapeutic potentials. A lot of drugs have been discovered for the management of diabetes but most of them however have noticeable side effects [1]. Most times not at reach for most diabetics. A remedy to this situation is dietary supplement which utilizes food or plant products that has been proven to have hypoglycemic effect and locally available as well [2]. Approximately 25% of drugs prescribed today originate from plant products [3].

Dietary supplement is a product intended to complement the diet by increasing the intake, which might consist of, but not limited to: a vitamin, mineral, herb or botanicals, an amino acid, a concentrate, metabolite, extract, enzymes or a combination of these ingredients [4]. One of such plants that have played a major role in food and drugs since ancient times is the sesame plant [5].

Diabetes has become one of the most challenging health problems in the 21st century. World's record of diabetes as of 2010 reports that 285 million people has been estimated to be carriers of diabetes, with type 2 making up about 90% of the cases. In 2011 it resulted in 1.4 million deaths worldwide making it the 8th leading cause of death and it is

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expected to double by 2030 [6]. It is of great concern that almost one-third of cases of diabetes are currently undiagnosed [7].

Diabetes mellitus is a chronic disease that arises 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 effectively utilize. Defective insulin secretion is the major cause of chronic hyperglycemia resulting in impaired function or serious damage to many of the body's systems like eyes, kidneys, nerves, heart and blood vessels. The common signs and symptoms are excessive thirst, frequent urination, weight loss or gain, fatigue, and influenza-like symptoms. Early diabetes symptoms can be very mild and often even unnoticeable [8].

There are two main (types 1 and 2) and several minor types of diabetes mellitus. Of diabetic patients, 5% suffer from Type 1 diabetes with absolute insulin deficiency, while about 90% of all diabetics are affected by Type 2 diabetes, which is associated with insulin resistance. Due to the increasing prevalence of diabetes worldwide and the noticeable side effects of some drugs used in the management of diabetes, it becomes necessary that an alternative with minimal or no side effect should be provided for the management of diabetes. Dietary supplement becomes an option and it has become of a growing interest in the study of diabetes management.

Sesame seed having proven by other research works to have therapeutic potential can serve as a dietary supplement.

Sesamum indicum commonly known as beniseed is a flowering plant in the genus Sesamum. It widely grows in nature at tropical regions around the world and is being cultivated for its edible seeds, which grow in pods. It has been recorded as one of the oldest oil seed crops ever known domesticated well over 3000 years ago [9]. It has many species; some are wild and are native to sub-Saharan Africa. It has characteristic feature of high tolerance to drought-like condition, and known to grow at grounds or places where other crops may fail [9].

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

Alloxan used for this study is a product of Qualikem fine chem. Pvt ltd, Vadodara, India. Chemicals used were product of BDH Chemicals Limited, Poole England and glibenclamide is a product of Cayman Chemical Company, USA.

2.1.2. Reagents

Reagent used for the Hepatic Biomarker test was product of Agape Diagnostics Limited, Switzerland

2.1.3. Experimental animals

Thirty-six male albino rats weighing 120-150g were purchased from the Department of Animal Science, University of Nigeria Nsukka. The animals were acclimatized for one week before the experiments. The animals were kept in a confined cages that were well designed to avoid mixture of feed and the rat faeces. Ethical guidelines by the institutional Animal Ethics Committee [10] were observed in caring and handling of the animals.

2.2. Methods

2.2.1. Sample collection and preparation

Sesamum indicum seed 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 [11] the seeds were washed in water, allowed to air dry for a week, milled, and stored in a clearly labeled container for feed formulation.

2.2.2. Fifteen percent (15%), Thirty percent (30%) and Sixty percent (60%) Sesamum indicum compounded diet formulation

The compounded diet was formulated using the method of Ezeokeke 2015 [12]. The following constituent were used; Maize, *Sesamum indicum*, wheat offal, Groundnut cake, Fish meal, Bone meal, Premix and Elephant grass. The formulations were gotten by varying the amount of maize and *Sesamum indicum* used in the formulation.

2.2.3. Experimental induction of diabetes

Diabetes was induced in the rat by intraperitoneal injection of freshly prepared 120 mg/kg body weight of alloxan. Diabetes was confirmed after 72hrs of administration and values above 200mg/dl were considered diabetic [13].

2.2.4. Experimental design

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

- Normal Control
- Diabetic Control
- Diabetic Rats treated with Glibenclamide
- Diabetic Rats fed 15% *Sesamum indicum* compounded diet
- Diabetic Rats fed 30% *Sesamum indicum* compounded diet
- Diabetic Rats fed 60% *Sesamum indicum* compounded diet
- Normal Rats fed 15% *Sesamum indicum* compounded diet and induced after 14 days
- Normal Rats fed 30% *Sesamum indicum* compounded diet and induced after 14 days
- Normal Rats fed 60% *Sesamum indicum* compounded diet and induced after 14 days

2.2.5. Standard Drug (Glibenclamide) Preparation

Diabetic rat group treated with standard drugs were given 0.5 mg/kg body weight of glibenclamide orally as a standard drug.

2.2.6. Collection of Blood Sample

Blood sample used for the measurement of fasting blood sugar were collected from the tail of the albino rats while the blood sample for estimation of lipid profile level were collected from the occular vein.

2.3. Biochemical Analysis

2.3.1. Hepatic Biomarkers

The level of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) were estimated using the commercial kit (AGAPE, Switzerland) [14].

2.3.2. Histological Studies of the Organ

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

2.4. Statistical Analysis

All data obtained were analysed using Analysis of variance (ANOVA) and values for $P<0.05$ were considered significant.

3. Results

3.1. Blood Glucose Level

The blood glucose level of the control and experimental groups are shown in fig 1 below. From the result, it showed that the level of blood glucose of the control group remained fairly constant throughout the duration of the study (from 97 mg/dl 3rd day to 84 mg/dl 14th day). However, elevation was observed in the diabetic group after the induction of diabetes at day 3. The compounded diet gradually lowered the high blood glucose level that resulted from the induction of diabetes. The blood glucose level of rats fed with 15% compounded diet reduced by 53.6% (from 261 mg/dl on 3rd day to 121 mg/dl on the 14th day). While the group fed with 30% compounded diet reduced by 58.2% (from 261 mg/dl on 3rd day to 109 mg/dl on the 14th day). And the blood glucose level of the group fed 60% compounded diet reduced by 58.4% (from 243 mg/dl on the 3rd day to 101 mg/dl on the 14th day). The reduction was significant when compared with diabetic group that slightly reduced (which was as a result of alloxan regeneration) by 10% (from 263 mg/dl on the 3rd day to 212 mg/dl on the 14th day).

For the group fed with 15%, 30% and 60% compounded diet before induction of diabetes, there was no significant increase after the diabetes induction, as their blood glucose level was seen to be below 200.

The result shows that there is no significant difference between the group fed with the compounded feed after induction when compared with the groups fed with the feed before induction.

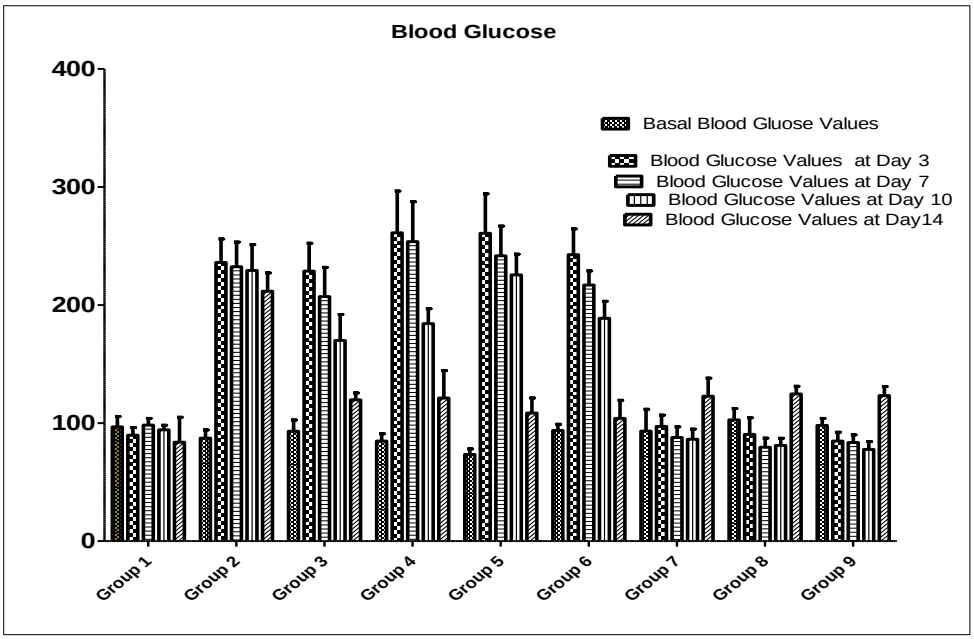

Figure 1 Blood Glucose Level of Albino Rats at 3rd 7th 10th and 14th day X axis = level of glucose, Y= Groups. Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before induction with diabetes.

3.2. Hepatic Biomarkers

Table 1 showed the effect of the compounded feed on Hepatic Biomarkers of albino rats studied. The results are presented in mean ± standard deviation and values bearing different superscript letters are significantly different ($P<0.05$). The hepatic biomarkers presented are AST, ALT and ALP.

The result showed that the AST for the groups 4, 5 and 6, which are the groups fed with the diet after induction of diabetes were not significantly different (36 ± 1.50^a, 30 ± 4.60^a, and 33 ± 0.90^a respectively) when compared with control group 1 (35 ± 1.40^a) and standard treated group 3 (47 ± 1.60^a). But were significantly different when compared with diabetic untreated group 2 (99 ± 1.80^b). Same trend was observed for the group 6, 7 and 8 which was induced after feeding with the compounded diet for 14 days. Their AST value (37 ± 1.50^a, 42 ± 1.50^a, and 37 ± 1.60^a respectively) were not significantly different when compared with control group 1 (35 ± 1.40^a) and standard treated group 3 (47 ± 1.60^a). But were significantly different when compared with diabetic untreated group 2 (99 ± 1.80^b). From the result there was no significant difference between the AST of group 4, 5 and 6 (36 ± 1.50^a, 30 ± 4.60^a, and 33 ± 0.90^a respectively) induced before feeding with the diet and the AST of group 7, 8 and 9 (37 ± 1.50^a, 42 ± 1.50^a, and 37 ± 1.60^a respectively) fed with the diet for 14 days before inducing with diabetes.

For ALT, for the groups 4, 5 and 6, which are the groups fed with the diet after induction of diabetes were not significantly different (30 ± 4.70^a, 19 ± 0.80^a, 22 ± 0.90^a respectively) when compared with control group 1 (23 ± 0.70^a) and standard treated group 3 (44 ± 2.20^a). But were significantly different when compared with diabetic untreated group 2 (57 ± 3.90^b). Same trend was observed for the group 6, 7 and 8 which was induced after feeding with the compounded diet for 14 days. Their ALT value (27 ± 3.00^a, 41 ± 0.80^a, and 36 ± 1.90^a respectively) were not significantly different when compared with control group 1 (23 ± 0.70^a) and standard treated group 3 (44 ± 2.20^a). But were significantly different when compared with diabetic untreated group 2 (57 ± 3.90^b). From the result there was no significant difference between the ALT of group 4, 5 and 6 (30 ± 4.70^a, 19 ± 0.80^a, 22 ± 0.90^a respectively) induced before feeding with the diet and the AST of group 7, 8 and 9 (27 ± 3.00^a, 41 ± 0.80^a, and 36 ± 1.90^a respectively) fed with the diet for 14 days before inducing with diabetes.

The result shows that the same trend observed for ALT and AST was also observed for ALP values of the albino rats

Table 1 Effects of the Compounded Diet on Hepatic Biomarkers of Albino Rat

GROUPS	AST	ALT	ALP
1 (Normal Control)	35±1.40[a]	23±0.70[a]	74±5.20[a]
2 (Diabetic untreated)	99±1.80[b]	57±3.90[b]	164±5.20[b]
3 (Diabetic treated with Glibenclamide)	47±1.60[a]	44±2.20[a]	45±3.90[a]
4 (Diabetic fed with 15% compounded diet)	36±1.50[a]	30±4.70[a]	46±2.30[a]
5 (Diabetic fed with 30% compounded diet)	30±4.60[a]	19±0.80[a]	47±2.30[a]
6 (Diabetic fed with 60% compounded diet)	33±0.90[a]	22±0.90[a]	42±1.40[a]
7 (Rats fed with 15% compounded diet and induced after 14 days)	37±1.50[a]	27±3.00[a]	40±2.40[a]
8 (Rats fed with 30% compounded diet and induced after 14 days)	42±1.50[a]	41±0.80[a]	66±4.40[a]
9 (Rats fed with 60% compounded diet and induced after 14 days)	37±1.60[a]	36±1.90[a]	50±5.70[a]

Values are presented as means ± standard deviation; n= 4 for each group. For each parameter, values on the vertical axis bearing different superscript are significantly different (P<0.05).

3.3. Histopathology of Liver

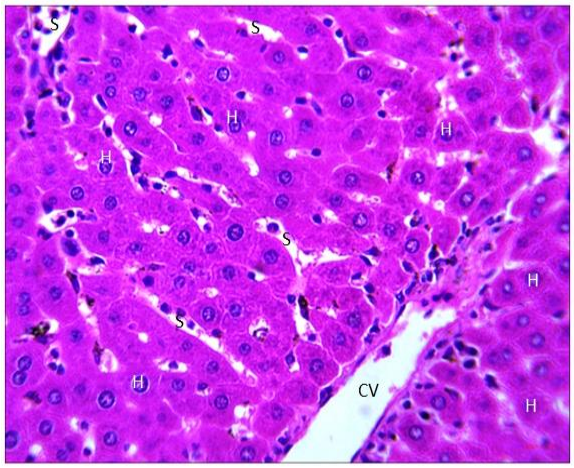

Figure 2 Histopathology of Liver for Control Grp 1 showing Histologically Normal Liver X 400 H&E

Figure 2 shows the Photomicrograph of the Liver of Group 1 which served as Control group. From the result, it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

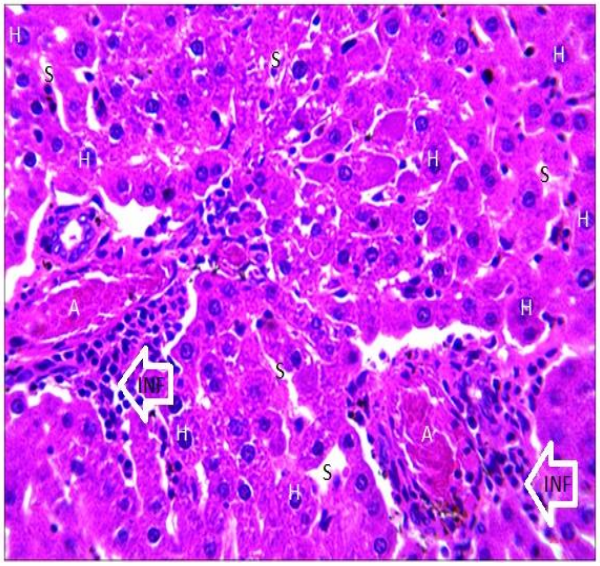

Figure 3 Histopathology of Liver for Diabetic Untreated Grp 2 Showing Distorted Liver X 400 H & E

Figure 3 shows the Photomicrograph of the Liver of Group 2 which served as diabetic untreated group. From the result, it shows that the Liver is histologically distorted with Periportal Inflammation (INF), Congested Hepatic Artery, intact Hepatocytes (H) and Sinusoid (S) containing Kupffer cells.

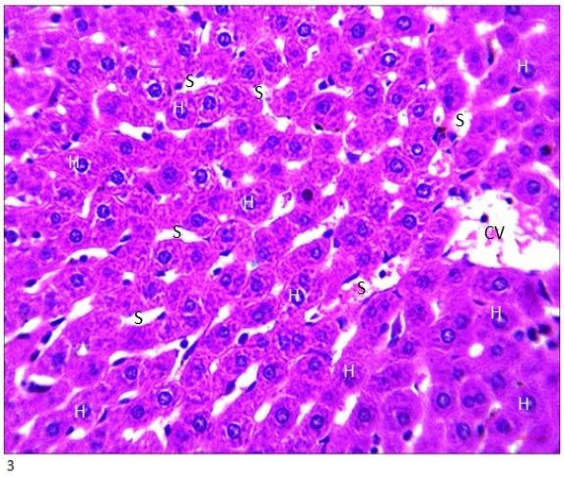

Figure 4 Histopathology of Liver for Glibenclamide Treated Grp 3 Showing Normal Liver X 400 H&E

Figure 4 shows the Photomicrograph of the Liver of Group 3 which served as Standard drug treated group. it shows that the Liver is histologically normal Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

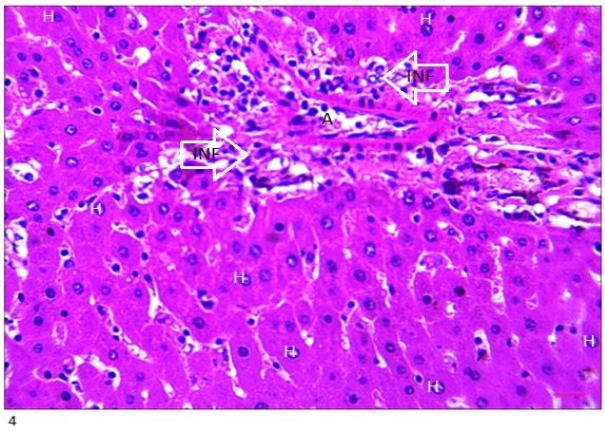

Figure 5 Histopathology of Liver for Grp 4 Diabetic Rat Fed with 15% Feed Showing Normal LiverX 400 H&E

Figure 5, shows the Photomicrograph of the Liver of Group 4 which served as 15% compounded diet fed group. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

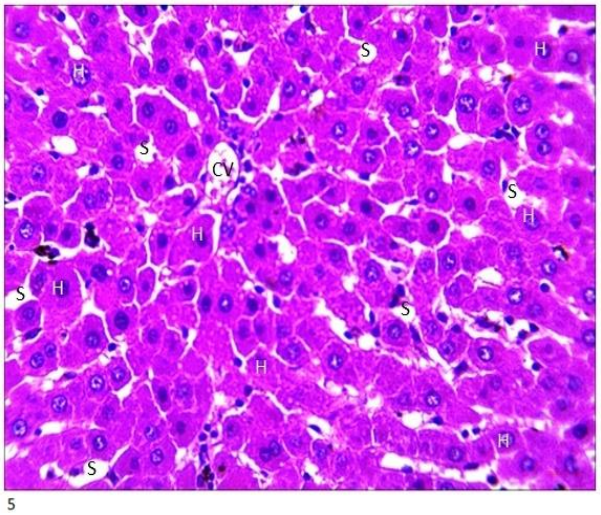

Figure 6 Histopathology of Liver for Grp 5 Diabetic Rat Fed with 30% Feed Showing Normal LiverX 400 H&E

Figure 6, shows the Photomicrograph of the Liver of Group 5 which served as 30% compounded diet fed group. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

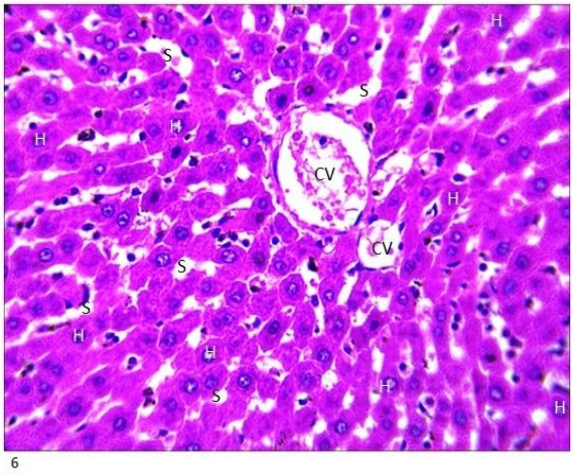

Figure 7 Histopathology of Liver for Grp 6 Diabetic Rat Fed with 60% Feed Showing Normal LiverX 400 H&E

Figure 7, shows the Photomicrograph of the Liver of Group 6 which served as 60% compounded diet fed group. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

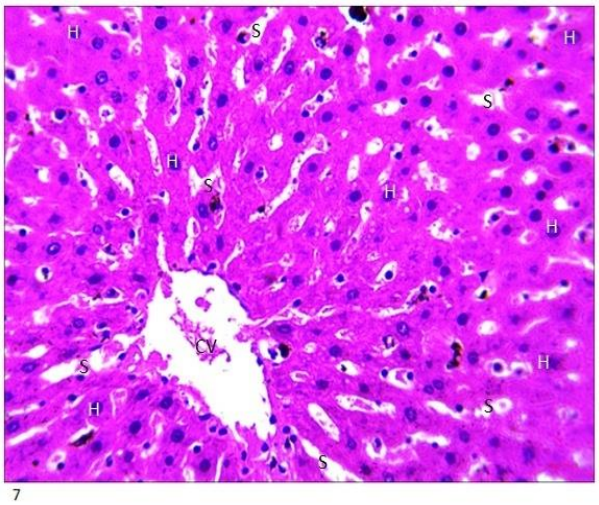

Figure 8 Histopathology of Liver for Grp 7 Normal Rat Fed with 15% Feed and Induced After 14 days Showing Normal Liver X 400 H&E

Figure 8, shows the Photomicrograph of the Liver of Group 7 which served as 15% compounded diet fed group that was induced with diabetes after 14 days of feeding. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

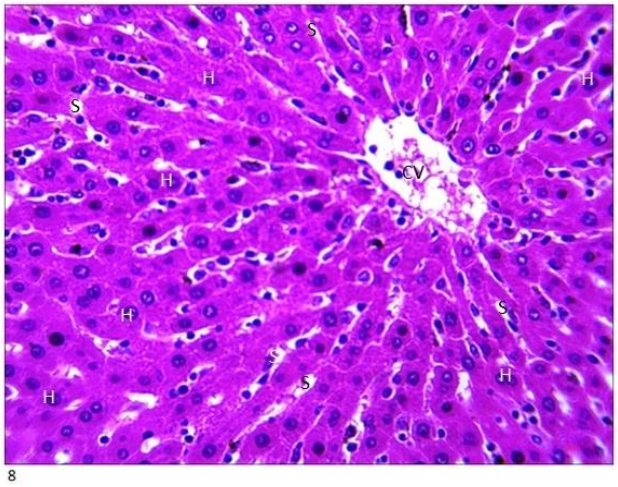

Figure 9 Histopathology of Liver for Grp 8 Normal Rat Fed with 30% Feed and Induced After 14 days Showing Normal Liver X 400 H&E

Figure 9, shows the Photomicrograph of the Liver of Group 8 which served as 30% compounded diet fed group that was induced with diabetes after 14 days of feeding. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

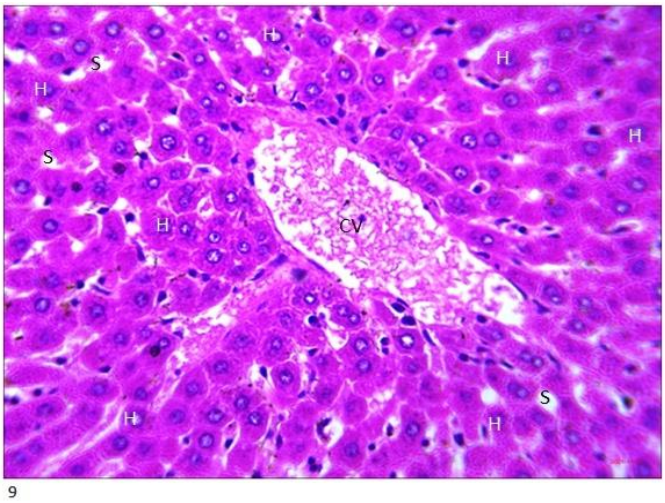

Figure 10 Histopathology of Liver for Grp 9 Normal Rat Fed with 60% Feed and Induced After 14 days Showing Normal Liver X 400 H&E

Figure 10, shows the Photomicrograph of the Liver of Group 8 which served as 30% compounded diet fed group that was induced with diabetes after 14 days of feeding. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

4. Discussion

It has been proven by Kizito et al [15] on effect of combined ethanol leaf extract of moringa oleifera and gongonema on body weight and blood glucose concentration of strep nicotinamide induced rats that increased blood glucose levels are observed after the induction of diabetes hence the increased level of blood glucose in this work is in line with literature. It is well-known from the literature that using alloxan to induce diabetes partially destroys the pancreatic insulin and causes diabetes [16]. 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 [17]. Flavonoids and sesamin have hypoglycemic effects [18]. The groups fed the compounded diet had a decrease in blood glucose levels as a result of this hypoglycemic feature. This is in line with literatures on plant products and its effects on induced toxicity [19].

The increase in the hepatic biomarkers of the albino rats in the diabetic untreated group shows that diabetes has been induced due to liver dysfunction [20]. This might lead to leakage from the cytosol of the hepatic cells into the blood stream. AST, ALT and ALP are used as biomarkers in hepatic injury. The *Sesame indicum* compounded diet were found to significantly reduced the levels of this enzymes. The effect of the compounded diet can be attributed to the presence of sesamin in the seed.

From literature, the seed has been known to contain Sesamin, Alkaloids, Tannins, Flavonoids, Saponins [17]. Sesamin and Flavonoid has hypoglycemic properties [17]. This hypoglycemic property brought about reduction in blood glucose level of the groups fed with the compounded diet.

This hypoglycemic properties of this sesamin found in *Sesamum indicum* which was used in formulating of the diet brought about histologically normal liver which was seen from the histology of the liver of groups fed the compounded diet

5. Conclusion

The result of this research work has shown that for any diabetics that feds on *Sesamum indicum* diet, that the level of blood glucose will be reduced. And also, that feeding on *Sesamum indicum* diet over time, can prevent possible diabetic condition that might arise in future. Hence the study has proven that *Sesamum indicum* has both Ameliorative and protective effect.

Compliance with ethical standards

Acknowledgement

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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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