World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* compounded feed on liver function markers of alloxan-induced diabetic albino rats

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World Journal of Advanced Research and Reviews, 2023, 19(02), 583–596

Publication history: Received on 28 May 2023; revised on 05 August 2023; accepted on 08 August 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.19.2.1577

Abstract

This study investigated the effect of combined seeds of *Sesemum indicum* (SI) and *Moringa oleifera* (MO) on liver function markers in alloxan-induced diabetic albino rats. 54 albino rats were divided into 9 groups of 6 animals each. Group 1 served as the normal control and received standard feed diet, Group 2 (Diabetic control) received intraperitoneal injection of 140mg/kg of Alloxan, Group 3 (Positive control) received 5mg/kg of glibenclamide, Group 4; diabetic rats fed diet containing 15% of SI. Group 5; diabetic rat fed with 15% of MO diet, Group 6; diabetic rat fed with combination of 15% SI and 15% MO compounded diet. Group 7; diabetic rat fed with 30% of SI diet. Group 8; diabetic rat fed with 30% of MO diet. Group 9; diabetic rat fed with combination of 30% MO and 30% SI diet. The Feed was compounded and was given daily for 14 days. Serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, and bilirubin were estimated to assess hepatic function using spectrophotometric method. Results showed a significant decrease in ALT, ALP, AST, and bilirubin levels when fed with compounded feeds at ($p<0.05$). Also feeding of diet with combined seeds significantly ($P<0.05$) increased the total protein and albumin in induced rats. Overall, compounded feed with combined seeds of SI and MO exhibited a promising anti-diabetic activity and normalized the liver enzyme activities which appeared comparable to the control glibenclamide in alloxan-induced diabetes hence, may be recommended for treatment/management of diabetes mellitus.

Keywords: Anti-diabetic; Diabetes mellitus; Feed formulation; *Moringa oleifera*; *Sesemum indicum*

1. Introduction

Diabetes mellitus (DM) is generally defined as hyperglycemia and is associated with failure of insulin secretion mechanism and/or inactivity of insulin in peripheral tissue. Epidemiological research revealed that diabetes mellitus (DM) is a public health concern in developing nations because its prevalence is constantly rising and proper treatment is expensive or unavailable [17]. Urbanization trends and alterations in lifestyle, such as the adoption of a "Western-style" diet, have been related to the rising prevalence of DM in emerging nations [18]. Centuries before the development of conventional medicine, medicinal herbs were utilized [27]. Plants have long been recognized as authentic sources of a wide range of bioactive compounds with significant therapeutic and medical potential [4]. The control of diabetes mellitus is greatly influenced by diet. Prior to the development of the therapeutic use of insulin, the primary method of disease management was diet, which included the use of conventional medications primarily derived from plants [24]. A modern pharmacological approach, poly-herbal therapy involves combining diverse substances from various plant sources for therapeutic objectives. It has the advantage of achieving the greatest therapeutic efficacy with the fewest adverse effects [8]. The medicinal potential of many plants with antidiabetic properties is tremendous. It is crucial to

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look for a hypoglycemic plant that is both more potent and safer for use in treating or preventing diabetes and its associated issues [29].

Sesame (*Sesamum indicum*) has long been utilized as a traditional plant-based remedy, mostly in Asian countries. The lignan compounds sesamin, sesamolin, sesamol, and episesamin can be found in abundance in sesame seed and its derivatives (oil, flour, and dietary supplements). Most of sesame's medical effects, such as its antioxidant and anti-inflammatory properties as well as its hypoglycemic effects, are a result of these lignans [30].

Moringa oleifera (*M. oleifera*) is a plant that is indigenous to northern India and has been utilized as a food supplement and herbal remedy by people all over the world*. M. oleifera*, often known as the drumstick tree, has a wide range of applications and is thought to have a number of health advantages, including nutritional and therapeutic effects [20]. The phenolics and flavonoids found in moringa seed, which have a scavenging impact on free radicals, are what give the powder its antioxidant properties [1]. Ghiridhari, Malhati, and Geetha [9] also noted that using *M. oleifera* as a pharmaceutical lengthens the course of treatment for diabetic patients, improving their glucose tolerance. The anti-diabetic and antioxidant properties of *Moringa oleifera* seem promising. The current phytochemical analysis of *M. oleifera* found bioflavonoids, which may be in charge of promoting glucose uptake in peripheral tissues and controlling the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism [10]. This study was designed to evaluate the effect of combined seeds of *Sesemum indicum* (SI) and *Moringa oleifera* (MO) on liver function markers in alloxan-induced diabetic albino rats.

2. Material and methods

2.1. Preparation of plant materials.

Seeds of *Sesamum indicum* were bought at Opi Market in Nsukka, Enugu State, Nigeria. and were washed and allowed to air dry. The *Moringa oleifera* seeds were harvested from different trees in Thinker's Corner, Enugu East Local Government Area of Enugu State, Nigeria. The seeds were removed from the pod, washed, hulled, and air dried. Seeds of *Sesamum indicum* and *Moringa oleifera* were grounded into fine powder.

2.1.1 Animals

In this study, male albino rats aged 3weeks old were used. The animals were divided into three (3) control and six (6) fed groups making a total of nine (9) groups. The weight of the animals varied between 95g and 190g. They were housed in clean cages made of stainless steel wire mesh, kept at a consistent temperature, and exposed to the typical day and night cycles. They also received a standard commercial pellet diet and clean water to drink. The rats were allowed to acclimatize for 7 days.

The present study received approval from the Department of Biochemistry's ethical committee for the use of animals in research at the Federal University of Technology in Owerri, Nigeria (Ethics Approval Number: ODVC/REN/998/15). The treatment of the rats and all experimental protocols complied with the National Institutes of Health of the United States' basic guidelines for the care of laboratory animals [15].

2.2. Proximate Analysis

The proximate compositions of the seeds were assessed using standard techniques [3]. Crude protein, was determined by Micro-Kjeldahl distillation method [2]. The amount of carbohydrates was calculated by subtracting the total of the percentages of crude protein, fat, ash, moisture, and fiber from 100%.

2.3. Preparation of Diet and Feed Formulation

Feeds were compounded using the Pearson square method (Ration Formulation using the Pearson Square, 2016). The % constituent of the standard feed contains Variable samples which includes: maize (65.50%), groundnut cake (19.99%), and wheat offal (10.01%) while the Fixed samples (4.50%) includes: fish meal (2.50%), common salt (0.25%), bone meal (0.50%), elephant grass (1.00%), and premix. (0.25%). This sums up 100% which is equal to 1g. Test samples were Sesame seed and Moringa seed. The percentage (%) inclusion of test samples into the standard feed requires maize which is a variable sample to be reduced by that percentage (%).

2.4. Chemicals and Reagents

Analytical-grade chemicals and reagents were used in this investigation. All of the assay's reagents were from commercial kits and goods produced by TECO Diagnostics in the US, Anaheim., Biosystems S.A. in Barcelona, Spain, and Randox Laboratories Ltd. in Antrim, United Kingdom.

2.5. Induction of diabetes mellitus

Prior to the induction of diabetes mellitus (DM), the rats were fasted without access to food or water for 16 hours. The initial fasting plasma glucose concentrations (FPGC) were then determined using a glucometer. A single intraperitoneal (i.p.) injection of 140 mg/kg of alloxan monohydrate (Sigma, St. Louis, USA) in normal saline at a pH of 7.4 was used to induce experimental diabetes mellitus.

2.6. Experimental Design

The rats were divided into nine groups of six animals each after seven days of acclimatization. The formulated feed was given for 14 days.

- **Group 1**: Rat fed with normal standard feed diet (control)
- **Group 2**: Rat induced with 140mg/kg alloxan (negative control)
- **Group 3**: Induced rats administered with 5mg/kg glibenclamide (positive control)
- **Group 4**: Induced rat fed with compounded diet containing 15% Sesame seed.
- **Group 5**: Induced rat fed with compounded diet containing 15% Moringa seed.
- **Group 6**: Induced rat fed with compounded diet combination of 15% Sesame seed and 15% Moringa seed.
- **Group 7**: Induced rat fed with compounded diet containing 30% Sesame seed.
- **Group 8**: Induced rat fed with compounded diet containing 30% Moringa seed.
- **Group 9**: Induced rat fed with compounded diet combination of 30% Moringa seed and 30% Sesame seed.

2.7. Blood collection and serum preparation.

Rats from all groups were sacrificed after administration and feeding. Blood was drawn from the ocular vein, allowed to stand for 10 minutes, and then centrifuged for 15 minutes at 3000 rpm to separate the serum. The determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total Bilirubin (T.B), total Protein (T.P), and albumin, were done using serum.

2.8. Histological study

Following Okoro's [19] methods with a few minor modifications, liver samples were fixed with normal saline, dehydrated, cleared (de-alcoholized), infiltrated, and embedded in paraffin. Serial sections of the samples were made at the appropriate thickness, and they were then stained with hematoxylin and eosin (H&E) [5]. Additionally, tissue sections were examined under a magnification of 100x and 400x with a light microscope.

2.9. Estimation of Liver function markers

The in-vitro determination of albumin (ALB), total protein (TP), and bilirubin were all done using the methods described by Doumas, Watson, & Biggs [7], [26] and Jendrassik & Grof [13] respectively. The activities of alanine amino-transaminase (ALT) and aspartate amino-transaminase (AST) were estimated using Reitman and Frankel's [22] method, as cited in [16]. Serum alkaline phosphatase (ALP) activity was also determined using the methods of Shaheen *et al.*, [23] as cited in [17].

2.10. Statistical analysis

All collected data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for the statistical analysis, and Tukey's multiple comparison tests were used for the posthoc test. The probability level was established at $p < 0.05$. All of the statistical analysis were carried out using GraphPad Prism version 9.0 software.

3. Results

Table 1 shows the proximate composition of *Sesemum indicum* and *Moringa oleifera*. The results showed that the seeds of *Sesemum indicum* are rich in fats with a value of 46.33%, crude Protein content was 32.22% and carbohydrate content was 9.51%, ash content was 5.15%, moisture content was 5.07%, and fiber content was 4.72%. Seeds of *Moringa oleifera*

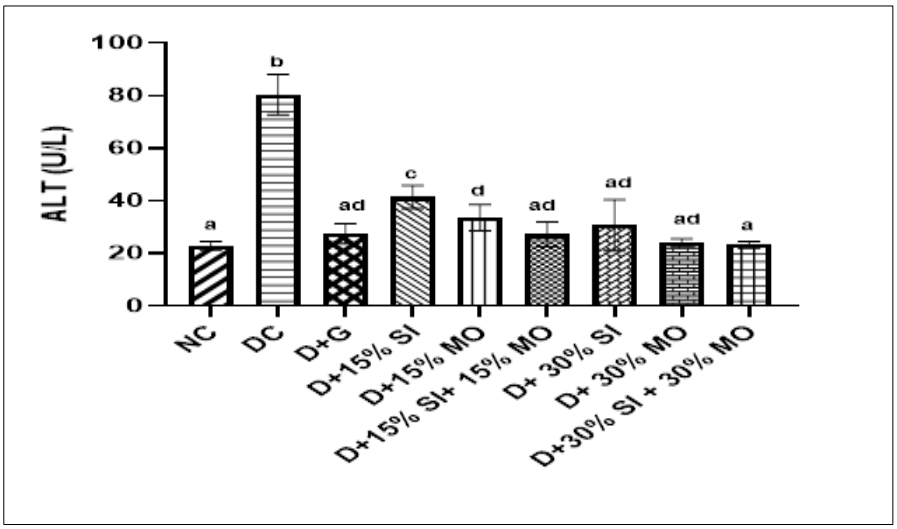
were rich in carbohydrates with a value of 36.56%, fat content was 32.50% and crude protein content was 15.95%. moisture content was 6.38%, fiber content was 5.22%, and ash content was 3.37%.

Table 1. Proximate composition of *Sesemum indicum* and *Moringa oleifera*

Parameters	Moisture %	Fat %	Ash %	Crude protein %	Fiber %	Carbohydrate %
Sesemum indicum	5.07	46.33	5.15	32.22	4.72	9.51
Moringa oleifera	6.38	32.50	3.37	15.95	5.22	36.56

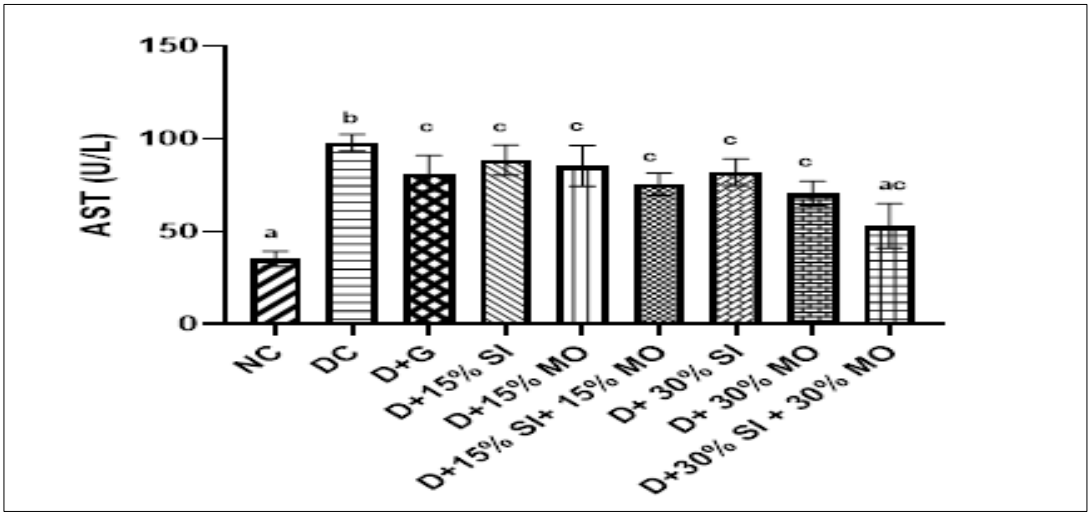
3.1. Liver function enzyme activities

Serum ALT, AST, and ALP activity were determined as indicators of liver injury to assess hepatic toxicity. Figure 1 showed that there was a significant reduction ($p < 0.05$) in serum ALT activities of D+G, D+15%SI, D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO compared with those of DC (Figure 1). There was no significant difference between NC, D+G, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO. The serum ALT activity of D+G was not significantly different ($p > 0.05$) from that D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO. Among the fed groups, there was a significant difference between D+15%MO compared to other fed groups. While D+30%SI+30%MO was significantly different from groups D+15%SI and D+15%MO. Serum AST activity of DC was significantly different ($p > 0.05$) from that of D+G, D+15%SI, D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO. There was also a significant difference between NC and the fed groups except D+30%SI+30%MO. There was no significant difference between D+G and the feed groups. Among the fed groups, there was no significant difference. However, there was a significant reduction in AST activities in D+30%SI+30%MO (Figure 2). For Serum ALP activities, there was a significant reduction in D+G, D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO compared to DC (Figure 3). There was no significant difference between D+15%SI and D+30%SI compared to other fed groups. However, there was a significant difference between NC and the fed groups.



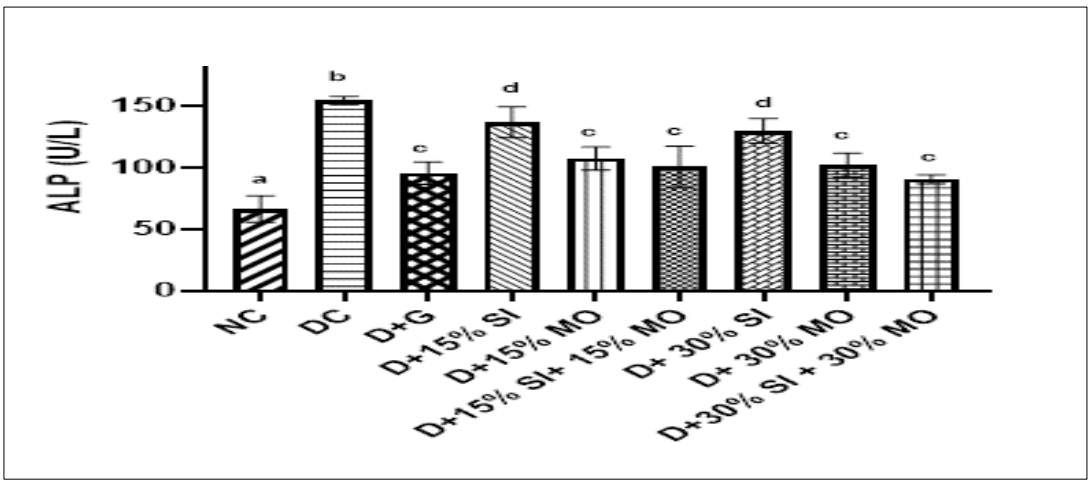
Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 1 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on alanine aminotransferase (ALT) activities (U/L) of alloxan induced albino rats. Groups with superscripts a, c and d showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation ($n = 6$). Values with the same superscript are not significantly different ($p > 0.05$).



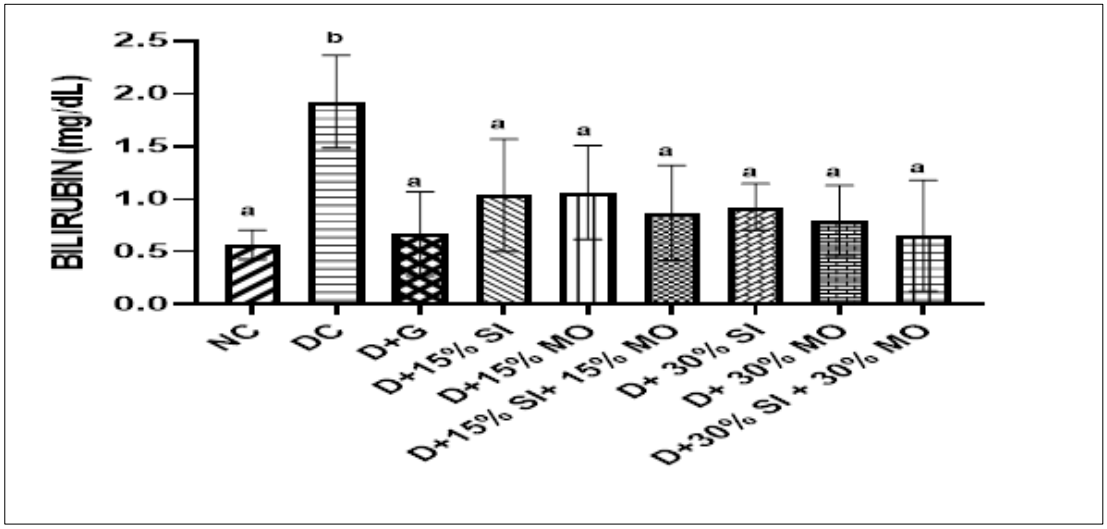
Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 2 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on serum aspartate aminotransferase (AST) activities (U/L) of alloxan induced albino rats. Groups with superscripts a, and c showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =6). Values with the same superscript are not significantly different ($p > 0.05$)



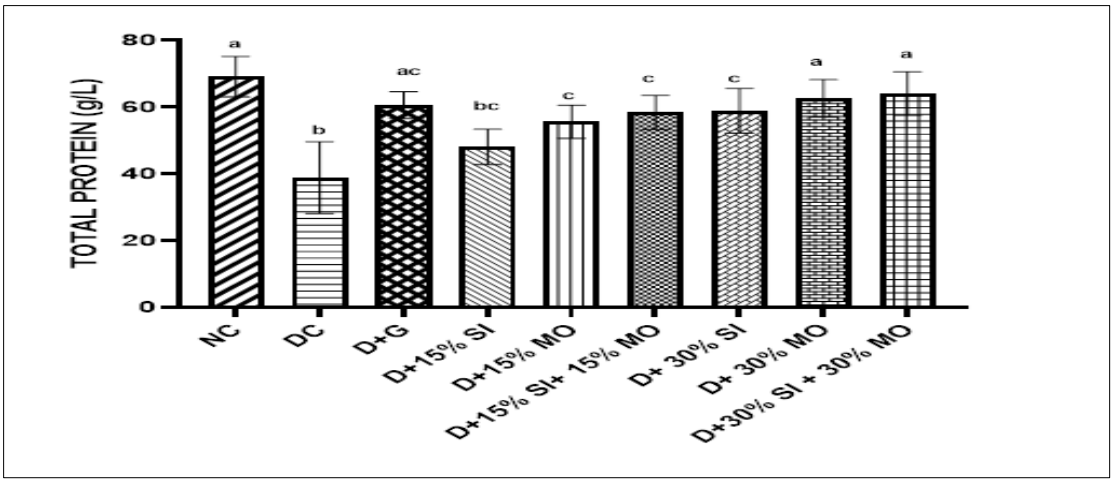
Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 3 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on serum Alkaline Phosphatase (ALP) activities (U/L) of alloxan induced albino rats. Groups with superscripts a, c and d showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =6). Values with the same superscript are not significantly different ($p > 0.05$).



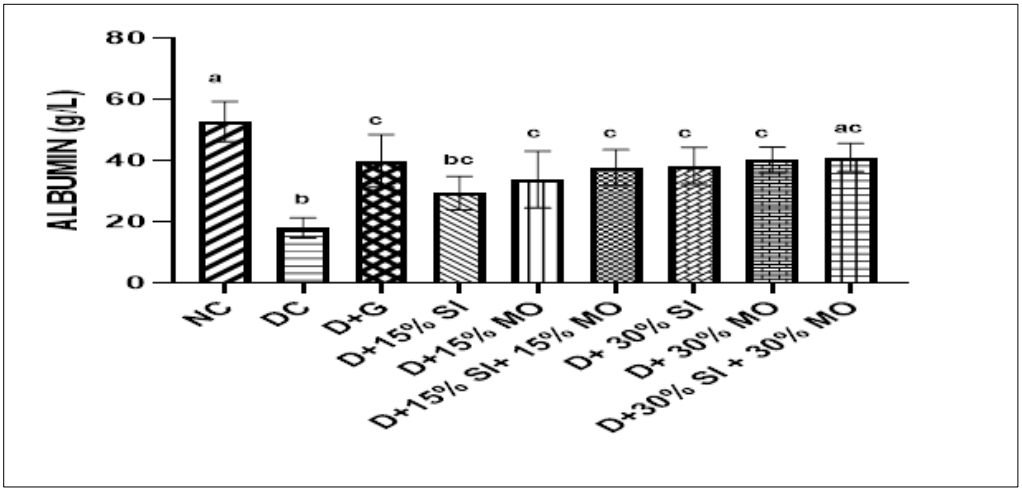
Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 4 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on Serum Total Bilirubin concentration (Mg/dL) of alloxan induced albino rats. Groups with superscript a showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =6). Values with the same superscript are not significantly different ($p > 0.05$).



Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 5 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on Serum Total Protein concentration (g/L) of alloxan induced albino rats. Groups with superscripts a and c showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =6). Values with the same superscript are not significantly different ($p > 0.05$).



Legend: NC: Normal Control; DC: Diabetic Control; D+G: Diabetic rat treated with glibenclimade; D+15%SI: Diabetic rat fed with 15% *Sesamum indicum*; D+15%MO: Diabetic rat fed with 15% *Moringa oleifera*; D+15%SI + 15%MO: Diabetic rat fed with 15% *Sesamum indicum* and 15% *Moringa oleifera*; D+30%SI: Diabetic rat fed with 30% *Sesamum indicum*; D+30% MO: Diabetic rat fed with 30% *Moringa oleifera*; D+30% SI + 30% MO: Diabetic rat fed with 30% *Sesamum indicum* and 30% *Moringa oleifera*.

Figure 6 Effect of combined seeds of *Sesemum indicum* and *Moringa oleifera* on Serum Albumin concentration (g/L) of alloxan induced albino rats. Groups with superscripts a and c showed significant difference ($p < 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =6). Values with the same superscript are not significantly different ($p > 0.05$).

Likewise, serum total bilirubin concentrations of D+G, D+15%SI, D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO were significantly lower ($p < 0.05$) than those of DC (Figures 4). There was no significant difference between NC and the fed groups. Figure 5 showed that there was a significant increase in the serum total protein concentration of D+G and the fed groups. However, D+15%SI was not significantly different ($p > 0.05$) from that of DC. D+30%MO and D+30%SI+30%MO were not significantly different from normal control (NC) compared to other fed groups. Figure 6 showed that after feeding, there was a significant increase in the serum albumin concentration in D+G, D+15%SI, D+15%MO, D+15%SI+15%MO, D+30%SI, D+30%MO and D+30%SI+30%MO ($p < 0.05$). There was no significant difference between DC and D+15%SI (Figure 6).

3.2. Histopathology studies of the Liver

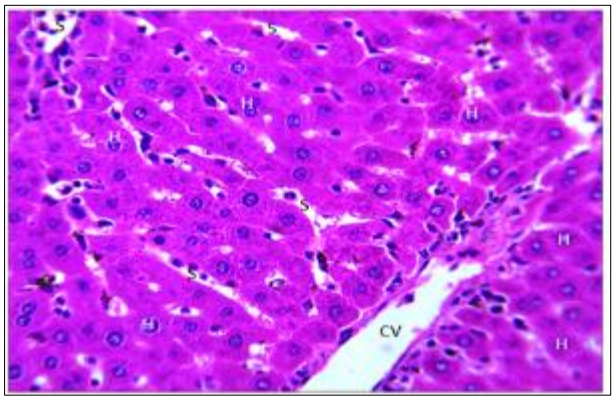


Figure 7 Micrograph of rats in Group 1 (**Normal control**) (x400), Stain: H and E

The photomicrograph of this section of the liver shows a patent central vein (CV) (Centro lobular vein) with arrays of intact hepatocytes (H) which are uniformly distributed throughout the cytoplasmic matrix. Hepatocytes are radially oriented and organized in the liver lobule like bricks in a wall. The Hepatic arteries are in intact. The sinusoids (S) containing Kupffer cells are intact and no pathological lesion seen. The morphological characteristics of the liver match those of a normal liver.

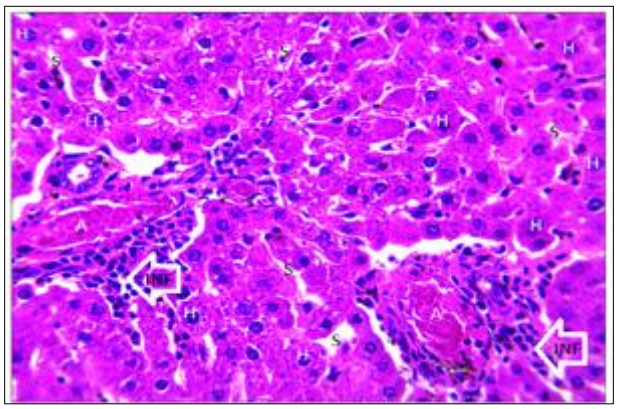


Figure 8 Micrograph of rats in Group 2 (**Diabetic control, with no treatment**) (x400), Stain: H and E

The photomicrograph of this section shows a histologically distorted liver with periportal inflammation (INF) and congested hepatic artery (A). The hepatocytes (H) are intact and the sinusoids (S) still containing kupffer cells.

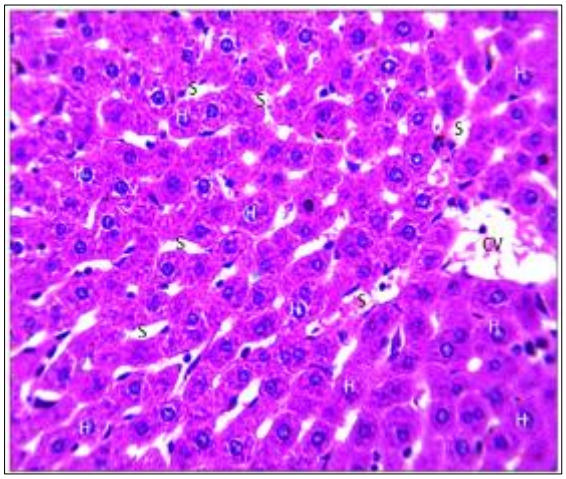


Figure 9 Micrograph of rat in group 3 (**diabetic rat treated with 5mg/kg Glibenclimade**). (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes(H), sinusoid (S) containing kupffer cells and patent central vein(CV).

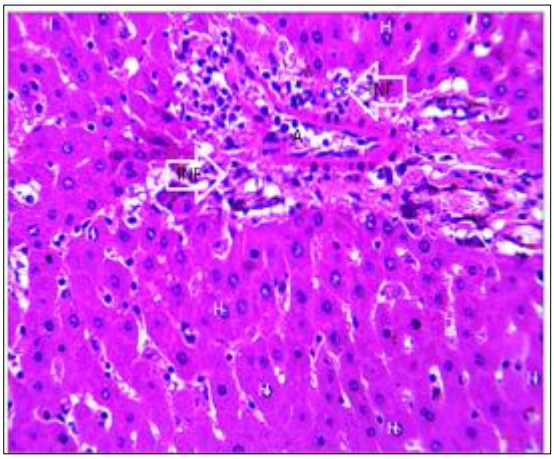


Figure 10 Micrograph of rats in group 4 (diabetic rats fed with compounded diet containing 15% Sesame seeds). (x400), Stain: H and E

The photomicrograph of this section shows histologically distorted liver with periportal inflammation (INF) and congested hepatic artery (A). However, sinusoid (S) containing kupffer cells and hepatocytes (H) are intact.

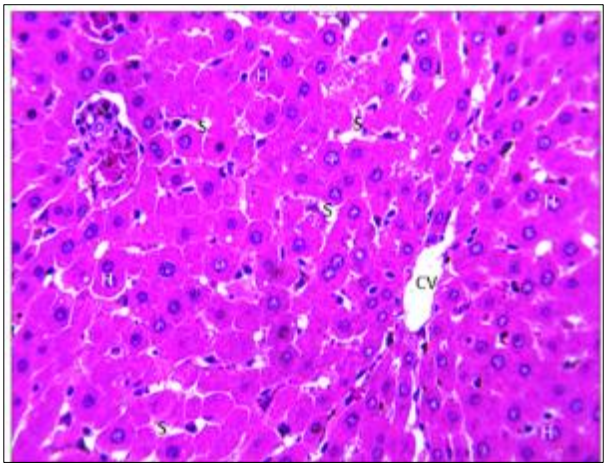


Figure 11 Micrograph of rats in group 5 (**diabetic rats fed with compounded diet containing 15% Moringa seeds**) (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes (H), sinusoid (S) containing kupffer cells, capillaries and congested central vein (CV).

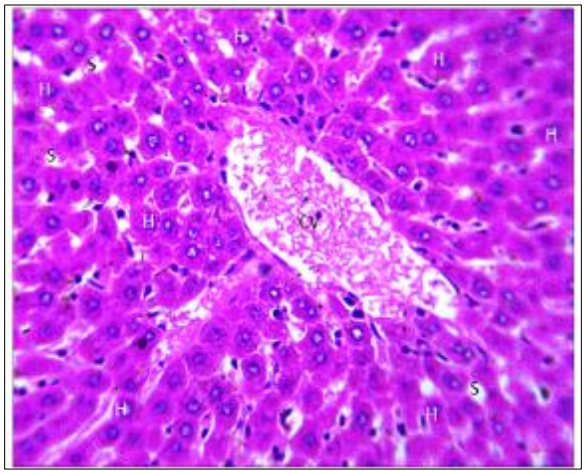


Figure 12 Micrograph of rats in group 6 (**diabetic rats fed with compounded diet containing 15% Sesame seeds + 15% Moringa seeds**) (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes (H), sinusoid (S) containing kupffer cells and patent central vein (CV).

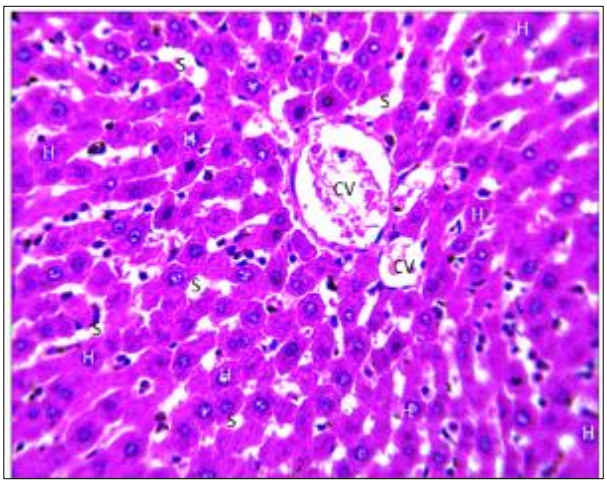


Figure 13 Micrograph of rats in group 7 (**diabetic rats fed with compounded diet containing 30% Sesame seeds**) (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes (H), sinusoid (S) containing kupffer cells and patent central vein (CV).

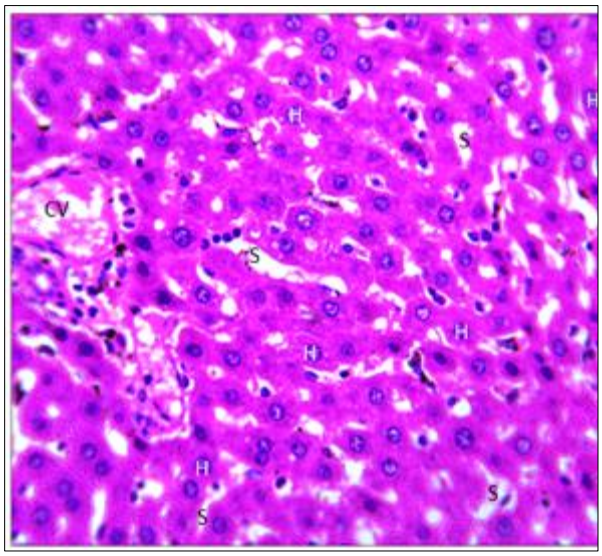


Figure 14 Micrograph of rats in group 8 (**diabetic rats fed with compounded diet containing 30% Moringa seeds**) (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes (H), sinusoid (S) containing kupffer cells and patent central vein (CV).

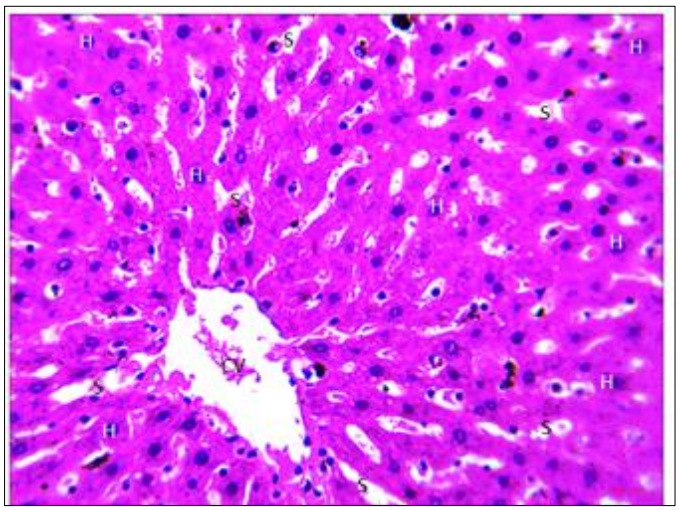


Figure 15 Micrograph of rats in group 9 (**diabetic rats fed with compounded diet containing 30% Sesame seeds + 30% Moringa seeds**) (x400), Stain: H and E

The photomicrograph of this section shows histologically normal liver with intact hepatocytes, sinusoid containing kupffer cells and patent central vein.

4. Discussion

Diabetes mellitus is a metabolic illness that causes hyperglycemia. Hyperglycemia then progresses to micro- and macrovascular problems, where it becomes a significant cause of death [1]. The results of the liver function parameters showed a significant ($p<0.05$) increase of serum alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST) and alkaline phosphatase (ALP) activity in all alloxan induced diabetic rats after induction compared to the normal control. According to studies, the enzymes ALT, ALP and AST are mainly found in the liver, and injury or damage to the liver results in release of these substances into the blood. AST, ALT and ALP are commonly measured clinically as biomarkers for liver health. In this study, these enzyme activities were found to increase in all the diabetic groups after induction due to the toxicity and oxidative stress caused by alloxan in the liver to cellular components, which results in functional and biochemical alterations that appear as liver damage and are seen as an increase in the activity of liver enzymes. This corresponded with the findings of [11] [28] which says that alloxan has been found to be selectively toxic to liver hepatocytes. Feeding with compounded diet of combined seeds of *Sesemum indicum* and *Moringa oleifera* was able to bring the activities of these enzymes, which are crucial in both amino acid degradation and biosynthesis back to normal as seen in the fed rats. The reduction in serum transaminase activity corresponds with the widely held belief that liver parenchyma repair and hepatocyte regeneration lead to a return to normality for serum transaminase activity [25] [14]. This implies that hepatic functions were significantly restored. This observation is comparable to that found in rats given CCl4 treatment [14].

Total protein measures the total amount of two types of protein in the body, Albumin and globulin. Albumin proteins keep fluid from leaking out of the blood vessels and is important for tissue growth and healing. Albumin is made in the liver. Decreased albumin concentrations are seen in hepatic dysfunction. In this study, the total protein and albumin concentrations were significantly ($p<0.05$) reduced after induction of 140mg/kg of alloxan, which signifies liver damage probably due to the effect of alloxan on liver and its parameters, according to Guria *et al.*, [11] Feeding with compounded diet of combined seeds of *Sesemum indicum* and *Moringa oleifera* significantly restored the liver function of amino acid biosynthesis. In a research done by Han *et al.,* [12], a biomarker of liver bilirubin was shown to be connected with levels of diabetes mellitus and has a protective function in both metabolic disorders and cardiovascular diseases (CVD) by serving as an antioxidant and cytoprotective agent. The elevated level of bilirubin in diabetic rats may be an attempt to suppress the generation of ROS to protect cells from damage or may be indicative of haemolytic anemia [6]. After feeding, there was a significant decrease in the bilirubin concentration in diabetic rats fed with compounded diet. This is probably due to the antioxidant capacity of *M. oleifera* and *Sesemum indicum* which has the ability to scavenge oxygen radicals.

Histological results of the liver showed that in normal control group, the photomicrograph of this section of the liver shows a patent central vein (centrolobular vein) with arrays of intact hepatocytes that are uniformly distributed

throughout the cytoplasmic matrix. The hepatocytes in the liver lobule are radially disposed and are arranged like the bricks of a wall. The Hepatic arteries are in intact. The sinusoids containing Kupffer cells are intact and no pathological lesion seen. Morphological features are in line with those of a normal liver. The photomicrograph of group 2 (diabetic control) shows a histologically distorted liver with periportal inflammation (INF) and congested hepatic artery. The hepatocytes are intact and the sinusoids still containing kupffer cells which indicates that alloxan altered the morphological features and caused distortion of the liver cells.

The diabetic rat treated with glibenclamide showed a histologically normal liver with intact hepatocytes, sinusoid containing kupffer cells and patent central vein which indicates that treatment with glibenclamide was able to regenerate most of the constricted hepatocytes and elsewhere. The photomicrograph of group 4 (diabetic + 15% SI) shows a histologically distorted liver with periportal inflammation and congested hepatic artery. However, sinusoids containing kupffer cells and hepatocytes are intact. This shows that this 15% SI inclusion in the compounded diet had no impact on regeneration of distorted liver cells. The photomicrograph of group 5 (diabetic + 15 % MO) shows a histologically normal liver with intact hepatocytes, sinusoid containing kupffer cells, capillaries and congested central vein. This shows that 15% MO compounded diet was able to regenerate distorted liver and reduce the congestion of the central vein. The photomicrograph of group 6 (diabetic + 15% SI +15% MO), group 7 (diabetic + 30 % SI), group 8 (diabetic + 30% MO), and group 9 (diabetic + 30% SI + 30% MO) showed a histologically normal liver with intact hepatocytes, sinusoids containing kupffer cells and patent central vein which shows that the compounded diets was able to regenerate the liver cells back to normal. The improvement effects of the combined seeds on liver histological deterioration in diabetic rats may be attributed to their antioxidant and anti-inflammatory properties.

5. Conclusion

The present study showed that combined seeds of *Sesemum indicum* and *Moringa oleifera* compounded diet possessed potent hepato-protective activity against alloxan-induced liver damage in diabetic rats.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that no competing interests exist.

Statement of ethical approval

The present study received approval from the Department of Biochemistry's ethical committee for the use of animals in research at the Federal University of Technology in Owerri, Nigeria (Ethics Approval Number: ODVC/REN/998/15). The treatment of the rats and all experimental protocols complied with the National Institutes of Health of the United States' basic guidelines for the care of laboratory animals (NIH, 1985).

Authors Contribution

Envisaged and designed the experiments: Iheanacho, K.M.E and Ujowundu, F.N.; Feed formulation, animal study and measurement of biochemical parameters: Ezeokeke C.T., Udebunu, G.O. and Ekeke K.; Data analysis: Udebunu, G.O. and Udebunu, M.U.; Manuscript writing: Udebunu, G.O. All authors reviewed, commented and approved the final manuscript.

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