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Comparative effects of combinations of metformin, omega-3 and omega-6 oil in the treatment of alloxan-induced diabetic rats

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Abstract

Emerging evidence suggests beneficial effects of omega fatty acids on diabetic complications. This study was designed to investigate the impact of Omega-3 and Omega-6 oil in the management of oxidative stress associated with complications in diabetes in alloxan induced diabetic rats. Experimental rats used in this study were grouped into four as non-diabetics and untreated, alloxan induced diabetic group, alloxan induced and treated with Metformin, lloxan induced and administered Metformin + Omega-3, alloxan induced and administered Metformin + Omega-6 oil. Effect of Metformin + Omega-3 and Metformin + Omega-6 on plasma glucose were also evaluated. Serum lipid profile before and after treatment, lipid peroxidation activity of malondialdehyde, and antioxidant enzyme activity of superoxide dismutase were assessed. Histological examination of pancreas and liver biopsy were also carried out. The results showed that fasting blood glucose (FBS) levels in day 1, day 7, day 14 and day 21 were significantly ($p<0.05$) lower than in the other groups. Superoxide Dismutase (SOD) activity was significantly ($P>0.05$) higher in Metformin + Omega-6 group when compared with diabetic control group at $P= 0.011$. Malondialdehyde (MDA) activity studied among groups was significantly lower ($P< 0.05$) in Metformin + Omega-3 group and Metformin + Omega-6 group when compared with the diabetic control group at $P =0.008$ and 0.016 respectively. There was statistically significant mean weight reduction ($P<0.05$) post intervention for all the alloxan-exposed rats. Metformin + Omega-6 group liver photomicrograph showed marked regenerated hepatocytes with normal histologic architecture. Metformin + Omega-3 group and Metformin + Omega-6 group photomicrograph revealed moderate regeneration of β cells of the Islets of Langerhans. The study demonstrates that treatment with Metformin and Omega-3 / Omega-6 oil intervention in diabetic rats significantly ameliorates hyperglycemia, exhibits pancreatic protective effects and reduces lipid peroxidation activity of MDA.

Keywords: Metformin; Omega-3; Omega-6; Diabetes; Rats

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1. Introduction

Diabetes is the eighth leading cause of death among both sexes and the fifth leading cause of death in women (WHO, 2016). World Health Organization estimates that, globally, 422 million adults aged over 18 years are living with diabetes in 2014 [1]. Several oral hypoglycemic drugs such as biguanides, sulfonylurea, and thiazolidinedione's are commonly used for the treatment of type 2 diabetes mellitus. Metformin is an old and widely used first-line agent, known for its anti-hyperglycemic properties and is also reported to improve lipid profile, fat redistribution [2] and chronic liver diseases [3] and lower microvascular and macrovascular complications associated with diabetes mellitus [4]. One of the major hypothesis proposed to explain the hyperglycemia induced onset of diabetic complications is an increase in oxidative stress [5, 6]. Evidence suggests that reactive oxygen species (ROS) function not only as mediators of destruction, but also as intracellular second messengers that regulate signal transduction cascades and gene expression [7]. After reactive oxygen species are created they deplete cellular antioxidants defenses including vitamin E and C and in many cases reduce antioxidant enzyme activity [7].

Changes in oxidative stress biomarkers such as superoxide dismutase, catalase, glutathione reductase, vitamins, lipid peroxidation, thiobarbituric acid reactants, are consistently observed in diabetes, in addition to changes in nitric-oxide, glycated proteins and hyperglycemia. The effects of antioxidants on these biomarkers for oxidative stress are being explored in an effort to expand treatment options for diabetes [8].

Chemical substances that are currently used for diabetes induction are able to induce all important forms of diabetes. Nevertheless, alloxan and streptozotocin are the most commonly used agents for inducing type 1 diabetes, although streptozotocin in combination with different feeding regimens or other types of manipulations can be used for inducing type 2 diabetes. A better way of using these models is for the purpose of investigation of diabetic complications, and the effects that hyperglycemia induces with regard to physiological functioning of organism [9]. On the other hand, application of alloxan induces a state that is particularly similar to human type 1 diabetes [10]. That is why alloxan application would be preferred and more correct if we consider it as a model of type 1 diabetes, although some authors stated that they have used alloxan for inducing type 2 diabetes [11, 12]. It has been widely accepted that alloxan selectively destroys the insulin producing β cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. After entering beta cells (β), alloxan produces its pathological effect through two independent mechanisms, namely glucokinase inhibition and reactive oxygen species (ROS) cycle generation. Glucokinase is an isozyme of the hexokinases and it is a major glucosephosphorylating enzyme in the liver, as well as in pancreatic β cells [13, 14, 15]. In the liver glucokinase is important for the process of glucose storage in form of glycogen, while in β cells it has a function of glucose sensor and it controls insulin secretion [14, 15].

The most recognizable potent bioactive lipid mediators are Arachidonic Acid (AA,n6) Eicosapentanoic Acid (EPA, n3) and Docosahexaenoic Acid (DHA, n3) synthesized from their dietary essential precursors Linoleic Acid (LA n6) and alpha Linoleic Acid (ALA, n3) [16]. Polyunsaturated fatty acids are of particular interest in the nutritional therapy for diabetes, given their potential role in several pathophysiological processes related to cardiovascular disease. Both Omega-3 and Omega-6 fatty acids are beneficial for improving lipid profiles in healthy individuals and among diabetic patients: Supplementation with omega-3 fatty acids lowers triglycerides and VLDL-cholesterol. However, they might also increase LDL-cholesterol. Emerging evidence suggests beneficial effects of Omega-3 fatty acids on diabetic complications, but their potency to improve treatment with diabetic patients remain poorly characterized [17, 18]. Potential health promoting benefits of omega oil include : Reduction of inflammation in heart diseases and rheumatoid arthritis, lower risk for blocked blood vessels and heart attacks, prevent hardening of arteries, decrease risk of sudden death and abnormal heart rates, decreased triglyceride levels, lower blood pressure [19].

Research suggests potential health promoting benefits of Omega-6 to include: replacing saturated and trans fat with omega-6 fatty acids associated with decreasing risk of heart disease, improve insulin resistance and reduce incidence of diabetes, lower blood pressure and cholesterol levels (Franzen-Castle and Ritter-Gooder, 2010). Furthermore Omega-6 fatty acids have beneficial effects on glucose metabolism, high serum linoleic and arachidonic acid concentrations are linked to lower risk of type 2 diabetes [20].

This study sought to compare the progressive effect of Metformin + Omega-3 and Metformin + Omega-6 on some oxidative biomarkers in alloxan induced diabetic rats. This is because there is a need to continue to explore the relationship between free radicals, diabetes and its complication and to illustrate/ elucidate the mechanisms by which increased oxidative stress accelerates the development of diabetic complications in an effort to expand treatment options.

2. Material and methods

2.1. Experimental animals

Fifty male Wistar rats weighing between 180 -220 g were procured from animal house of Delta State University, Abraka, Delta State, Nigeria. They were housed in clean cages under standard laboratory conditions of temperature, humidity and light (12:12 h dark/light cycle) at 21 ± 2 °C for 2 weeks. The rats were fed with chick growers' mash and distilled water *ad libitum*. All animals were dosed by the same method of intubation (oral) throughout the experiment. At the end of the experimental period, animals were sacrificed. Serum was obtained for further biochemical analysis. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. number 85-23, revised 1985) and in accordance with the University Ethics Committee on the use of laboratory animals.

2.2. Drugs/Reagents

All drugs and reagents used were obtained commercially, and of analytical grade and products of May and Baker, England; BDH, England; Merck, Darmstadt, Germany; Accu-check active glucometer by Roche Diagnostic, Germany; alloxan monohydrate, Sigma-Aldrich Chemical (St. Louis, MO, USA).

2.3. Induction of diabetes

Overnight fasted rats were made diabetic by a single intraperitoneal injection of freshly prepared alloxan of 150 mg/kg in sterile saline. Seventy-two (72) hours after alloxan injection and animals having blood glucose level above or equal to 200 mg/dL were considered were as being diabetic and recruited for this study [21].

2.4. Drug procurement

Metformin and Omega-3 and Omega-6 oils were bought from a Pharmacy out let in Nnewi, Anambra State, Nigeria. Metformin tablets were powdered using a wooden mortar and pestle and reconstituted in distilled water. Omega-3 and Omega-6 oils were separately dissolved in beaker.

2.5. Pharmacological intervention

The experiment was carried out with the method as described by [22]. Thirty (30) male rats were randomized into five groups consisting of six animals in each group. Group A rats (normal control) were administered distilled water (10 mL/kg), group B rats served as diabetic control, and were also administered distilled water (10 mL/kg). Groups C rats were given Metformin (200 mg/kg), group D rats were administered Metformin (200 mg/kg + Omega-3 oil 500 mg/kg, and group rats E were also given Metformin (200 mg/kg + Omega-6 500 mg/kg) respectively. All treatments were carried out through orogastric cannula for a period of 21 days. Blood glucose levels were measured using Accu-Check glucometer test strips (Roche, Germany) at random on days 0, 7, 14, and 21 of the experiment. All animals from each group were sacrificed by halothane 24 h after their respective daily dosages of the agents and distilled water.

2.6. Statistical analysis

The statistical significance was evaluated by one way ANOVA using SPSS (statistical package for social sciences) version 20.0 followed by post –hoc HSD and Turkey tests for individual comparisons (SPSS,2007). The lipid profile, antioxidants and fasting blood sugar results were analyzed using analysis of variance (ANOVA), the comparative analysis between groups was analyzed using students't-test. Results from test were considered significant at $P<0.05$.

3. Results

3.1. Effects on Fasting Blood Sugar

The results of the FBS showed that on the first day of the experiment, all the alloxan-treated animals had significantly increased (P=0.000) FBS mean values from the normal control group. Although the FBS values of the normal control group remained statistically the same throughout the study, there were variations in the trend of FBS values of other groups. The FBS levels in day2, day4, day 6, day10, day12 and day14 studied among the groups were significantly lower at $P<0.05$ [F=21.452, 4.214, 8.752, 6.730, 5.954 and 12.324] respectively, when compared to other groups (Table 4.1). Between group comparison of the FBS results of Metformin + Omega-3 group (group D) and Metformin+ Omega-6 group (group E) showed that metformin + Omega 3 was significantly lower (P=0.002) only on day4 of study. There was generally a significant decrease in the FBS from the first day to the 14th day of the study for all the diabetic treated

groups compared to normal control group P <0.05. Comparison between the diabetic control group (group B) and the Metformin + Omega 3 group (group D) showed FBS was significantly lower in Metformin +omega 3 (group D) on day 2 at P=0.006. Comparison between diabetic control group (group B) and Metformin + Omega 6 (groupE) showed FBS mean values was significantly lower in Metformin +Omega 6 group on day 2, day4, and day 10 at P< 0.05 respectively (Table 1).

3.2. Effects on Lipid Profile

The results presented in Table 4.3 indicated that there was generally no significant mean difference seen in the lipid profile of treatment groups compared to control group (p > 0.05). Comparison between lipid profile of group D and E showed that there is no significant difference (P >0.05) among the Metformin +Omega 3 group and Metformin +Omega 6 group (Table 2).

3.3. Effects on Antioxidants

The results for antioxidant activity interestingly indicated statistically significant low MDA activity among the groups for MDA mean values at P=0.045 [F= 2.847]. Comparison between MDA antioxidant activity of diabetic control group (group B) and the Metformin + Omega 3 group (group D) showed that Metformin + Omega 3 (groupD) was significantly lower,(P=0.008) [t=0.469]. Also between group comparison of diabetic control group (group B) and the Metformin + Omega 6 group (group E) showed that Metformin + Omega 6 group (group E) was significantly lower at P=0.016 [t=0.039]. Between group comparison of antioxidant MDA activity of diabetic Metformin + Omega 3 group (group D) and Metformin + Omega 6 group (group E) showed no significant difference (P>0.05) (Table 3).

The result for antioxidant activity indicated no statistically significant difference among the groups for SOD mean values (P>0.05). Although comparison between antioxidant SOD activity of normal control (group A) and diabetic control (group B) showed significantly lower SOD activity in diabetic control (group B) at P=0.014 [t=0.245]. Also between group comparison of antioxidant SOD activity of diabetic control group (group B) and the Metformin + Omega 6 group (group E) showed significantly higher SOD activity in Metformin + Omega 6 (group E) at P=0.011 [t=0.097]. Again between group comparison of antioxidant SOD activity of normal control group (group A) and Metformin + Omega 3 group (group D) also showed significantly lower SOD activity in Metformin + Omega 3 group (group D) at P=0.045 [t=0.970]. Between group comparison of antioxidant SOD activity of diabetic Metformin + Omega 3 group (group D) and Metformin + Omega 6 group (group E) showed no significant difference (P>0.05).

3.4. Effects of metformin and Omega-3 and Omega-6 on weight on alloxan- induced diabetic rats

The results indicated statistically significant mean weight reduction for all the alloxan-exposed rats while a significant mean weight gain was observed for the normal rats at P <0.05. The weight after mean comparison between normal control group (group A) and diabetic control group (group B) showed a significant weight reduction in diabetic control (group B) (P=0.001). Similarly the weight mean comparison between normal control group (group A) and Metformin group (group C) showed a significant weight reduction in Metformin group (group C) (P=0.039). Also the weight mean comparison between normal control group (group A) and Metformin + Omega 6 group (group E) showed a significant weight reduction in Metformin + Omega 6 group (group E) (P=0.028). Again the weight mean comparison between diabetic control group (group B) and Metformin + Omega 3 group (group D) showed a significant weight gain in Metformin + Omega 3 group (group D) (P=0.015). The weight mean comparison between diabetic Metformin + Omega 3 group (group D) and Metformin + Omega 6 group (group E) showed no significant difference (P>0.05) (Table 4).

3.5. Effects of metformin and Omega-3 and Omega-6 on histology of the liver in alloxan- induced diabetic rats

The figures depict the morphological changes in liver tissues of the control and experimental groups (Figures 1-5)

3.6. Effects of metformin and Omega-3 and Omega-6 on histology of the pancrease in alloxan- induced diabetic rats

Photomicrograph of normal control pancreatic tissue shows lobular arrangement of highly cellular glandular tissue composed of orderly differentiated acinar cells, ductular structures and Islet cells (Islets of Langerhans). Within the lobule are numerous acini (AC) of the exocrine component, an intralobular duct (InD) and islets of Langerhans (IL). In routine section it is not possible to identify the various cell types within the islets. Note, however, the B cells are the most numerous, these produce insulins. The next most numerous are A cells, this produce glucagon. The labels of A and B are not intended to identify specific cells but rather to show those parts of islets where A and B cells are found in greater number. (X400 pixels).

The figures depict the morphological changes in pancreatic tissues of the control and experimental groups where A= α cells, B=β cells, D=δ cells, DD=intrapancreatic ductule, AC=Acinar cells, CC=Capillaries

Table 1 Effect of metformin and Omega-3 and Omega-6 on blood glucose level of alloxan- induced diabetic rats

Blood glucose level (mmol/L)					
Treatment	**Dose (mg/kg)**	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Distilled water (A)	10 mL/kg	4.07±0.76	4.35±0.39	4.56±0.75	4.28±0.83
Diabetic control (B)	10 mL/kg	8.74±4.25	10.70±3.21	11.81±5.57	14.92±3.17
Metformin (C)	200	8.01±2.97	6.25 ±2.26	5.45 ±2.16	4.95±2.21
Metformin (D)	200	8.38±2.18	5.32±1.95	4.65±2.05	4.44±2.20
+Omega-3 (D)	500				
Metformin (E)	200	8.12±349	5.34±2.27	4.67±3.02	4.39±2.24
+Omega-6 (E)	500				

Results are expressed as mean ± SD; (n=6), * p<0.05 compared with control group

Where; Group A = normal control, Group B = Diabetic control, Group C = Metformin, Group D = Metformin + Omega-3, Group E = Metformin + Omega-6.

Table 2 Effect of metformin and Omega-3 and Omega-6 on Lipid profile level of alloxan- induced diabetic rats

Treatment	**Dose (mg/kg)**	**LDL-c (mg/dl)**	**TC (mg/dl)**	**TGL (mg/dl)**	**HDL-c (mg/dl)**
Distilled water (A)	10 mL/kg	39.30±16.27	96.85±16.75	57.24±20.36	46.10±3.10
Diabetic control (B)	10 mL/kg	47.98±17.15	104.99±18.20	48.69±11.80	47.32±2.41
Metformin (C)	200	36.16± 21.24	90.74±20.80	48.54±6.13	44.88±4.64
Metformin (D)	200	36.71±9.30	98.88±7.99	56.02±13.85	50.97±5.43
+Omega-3 (D)	500				
Metformin (E)	200	38.39±10.48	101.33±13.35	61.37±19.67	50.66±7.07
+Omega-6 (E)	500				

Results are expressed as mean ± SD; (n=6), * p<0.05 compared with control group

Table 3 Effect of metformin and Omega-3 and Omega-6 on antioxidant activity of alloxan- induced diabetic rats

Treatment	**Dose (mg/kg)**	**(U/mL) MDA**	**(nmol/mL)**
Distilled water (A)	10 mL/kg	0.90±0.20	1.11±0.16
Diabetic control (B)	10 mL/kg	0.59±0.16	1.27±0.16
Metformin (C)	200	0.63±0.21	1.15±0.32
Metformin (D)	200	0.67±0.30	0.99±0.11
+Omega-3 (D)	500		
Metformin (E)	200	0.83± 0.11	0.98±0.17
+ Omega-6 (E)	500		

Results are expressed as mean ± SD; (n=6), * p<0.05 compared with control group

Table 4 Effect of metformin and Omega-3 and Omega-6 on weight changes of alloxan- induced diabetic rats

Treatment	Dose (mg/kg)	Weight before (g)	Weight before (g)
Distilled water (A)	10 mL/kg	110±8.73	134.83±13.86
Diabetic control (B)	10 mL/kg	111.83±7.65	104.00±8.34
Metformin (C)	200	120.50±17.42	111.17±20.19
Metformin (D)	200	133.00±14.58	129.95±19.64
+Omega-3 (D)	500		
Metformin (E)	200	124.80±16.41	112.17 ±16.57
+ Omega-6 (E)	500		

Results are expressed as mean ± SD; (n=6), * p<0.05 compared with control group

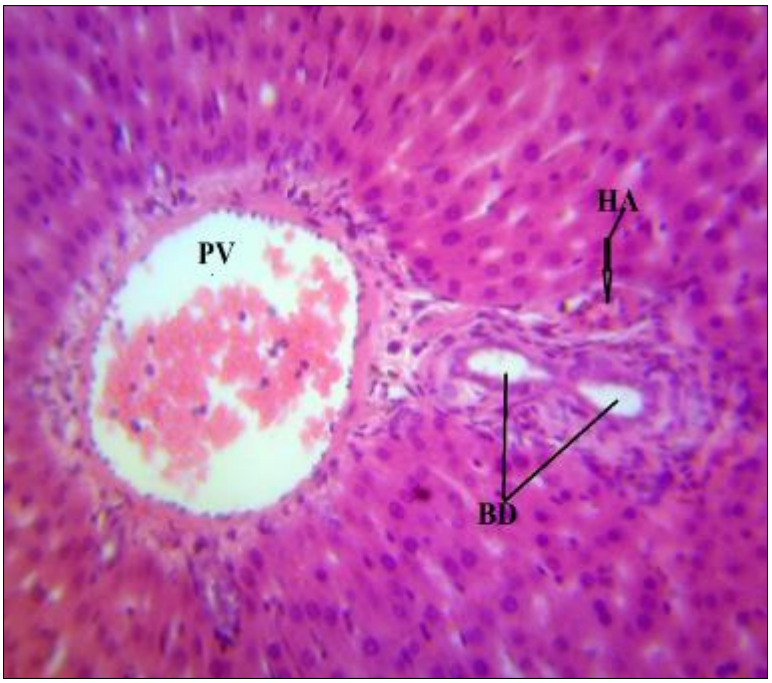

Figure 1 Group A Photomicrograph of liver showing normal histologic architecture in normal control (groupA): central vein (CV) and hepatocytes (H). The portal canal a connective tissue septum that carries the branches of the hepatic artery (HA) and portal vein (PV), bile duct (BD), and lymphatic vessels and nerves shows a normal histologic architecture. The artery and vein, along with the bile duct are collectively referred to as a portal triad. X400

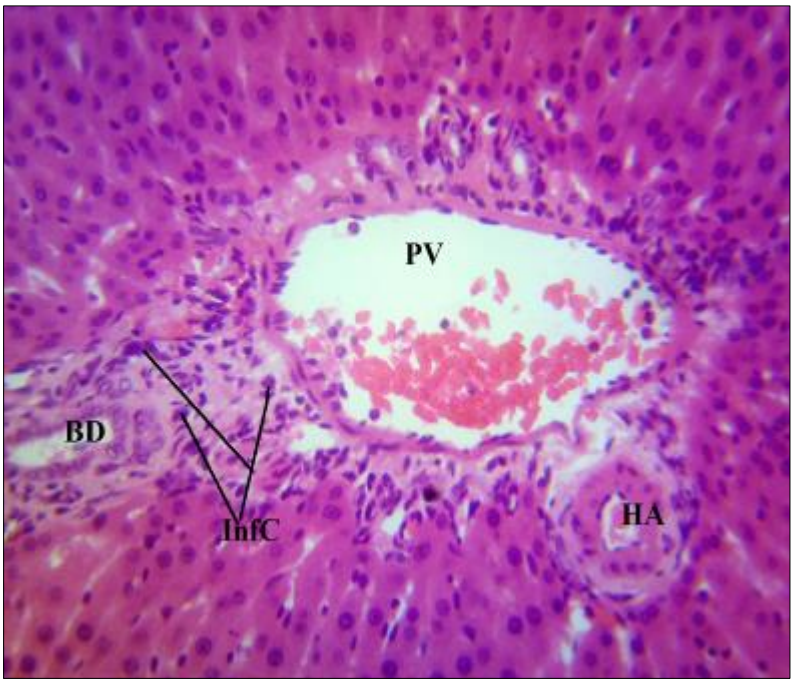

Figure 2 (a) Group B Liver histology

Photomicrograph of a portal triad showing necrosis, and mild inflammatory cells (InfC) in the connective tissue septum in diabetic control group (X400 pixels). InfC = inflammatory cells, BD= bile duct, HA= hepatic artery, PV= portal vein(x400 pixels)

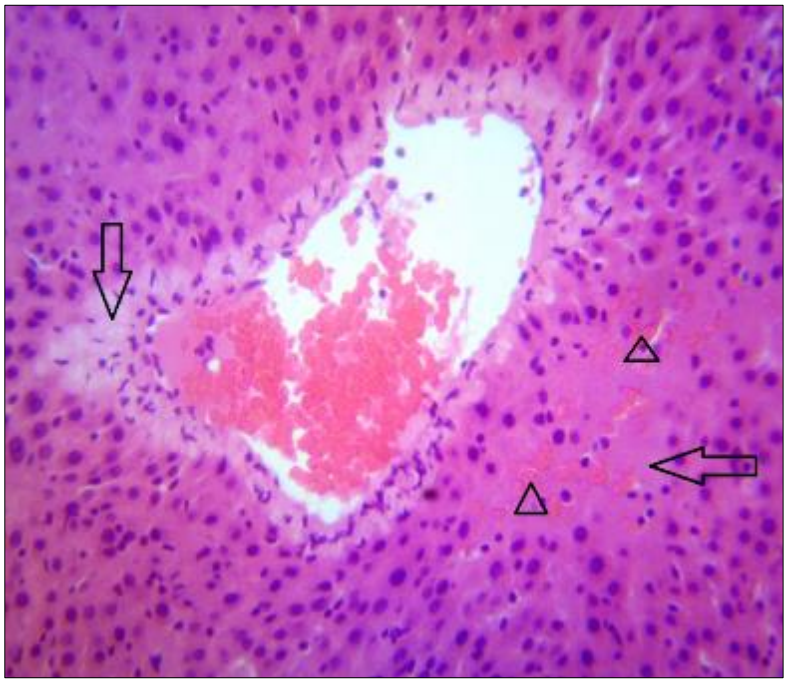

Figure 2 (b) Group B Photomicrograph of liver showing necrosis and depletion of hepatocytes (arrows), and haemorrhages in the parenchyma (triangle) in diabetic control group (group B).

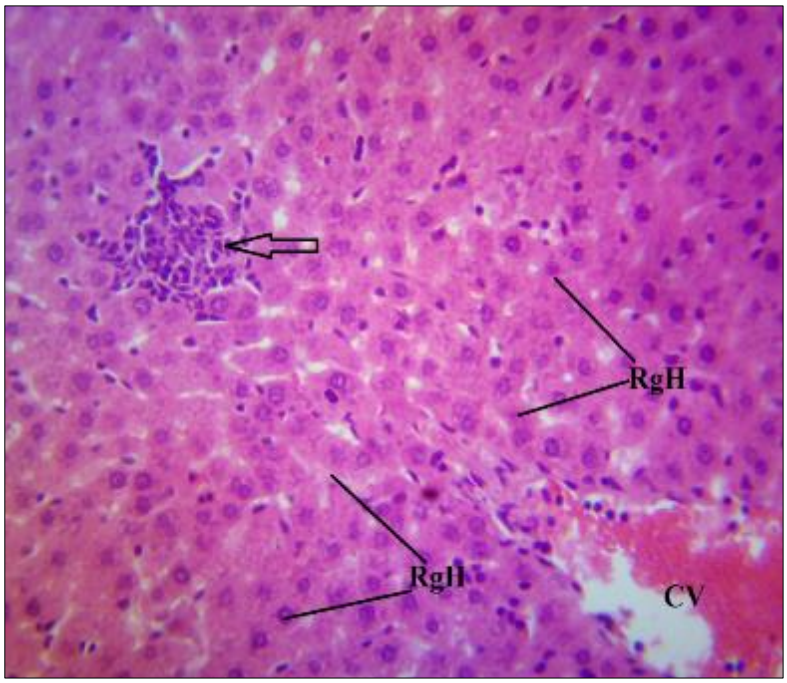

Figure 3 (a) Group C Liver histology

Photomicrograph of liver of Metformin group showing regeneration of hepatocytes (RgH), with multifocal inflammatory cells (arrow) in the parenchyma (arrow). (X400 pixels)

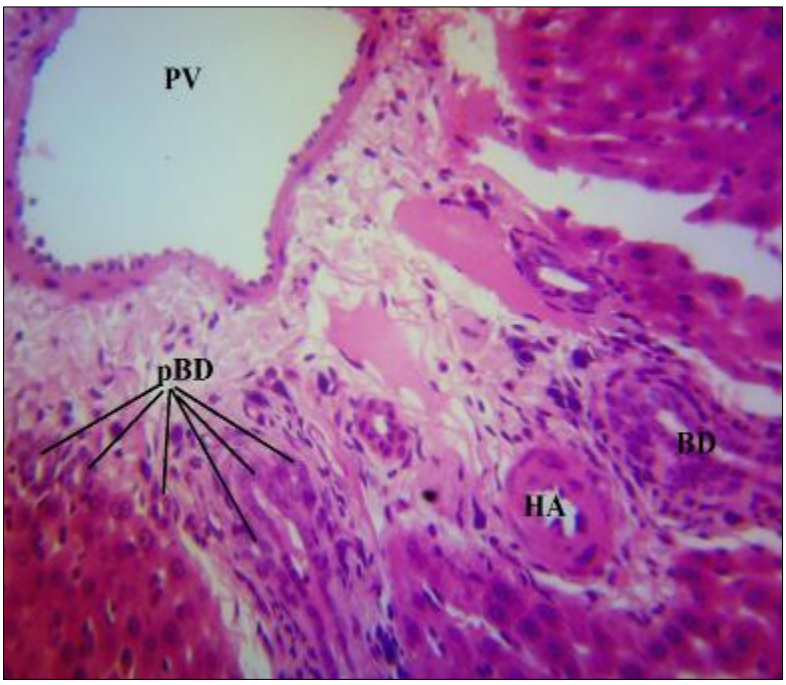

Figure 3 (b) Group C Photomicrograph of a portal triad of Metformin group showing proliferation of bile ducts (pBD). HA= hepatic Artery, BD= bile duct, PV= portal vein.(X400 pixel)

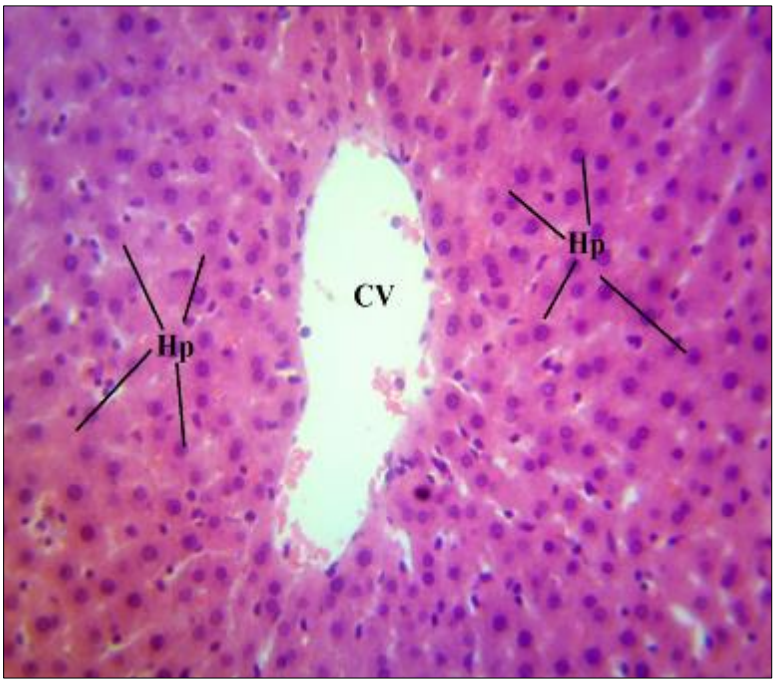

Figure 4 (a) Group D Liver histology

Photomicrogragh of Metformin + Omega 3 group (x400 pixels) showing regeneration of hepatocytes.

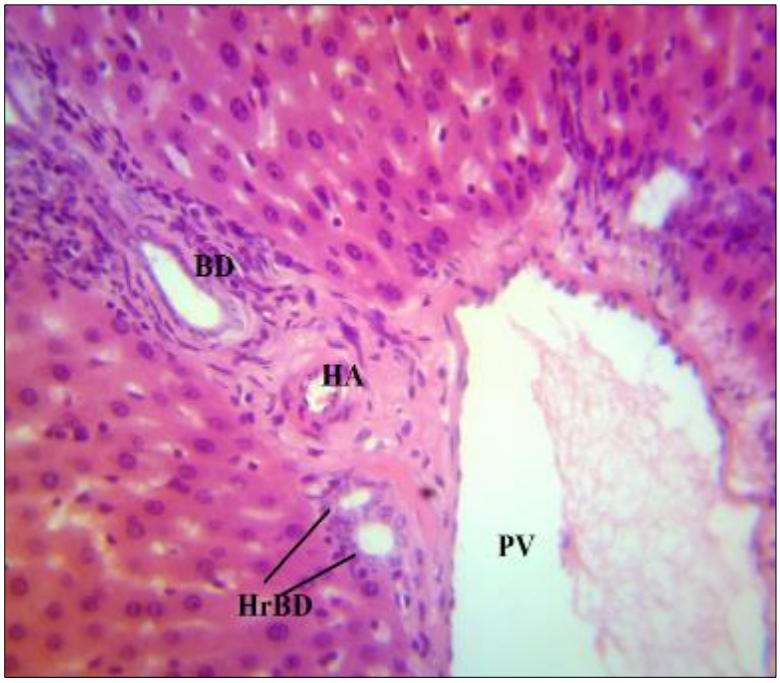

Figure 4 (b) Group D Photomicrogragh of Metformin + Omega 3 group (x400 pixels) portal triad showing hyperplasia of repair of bile duct (HrBD) and mild inflammatory cells in the portal canal

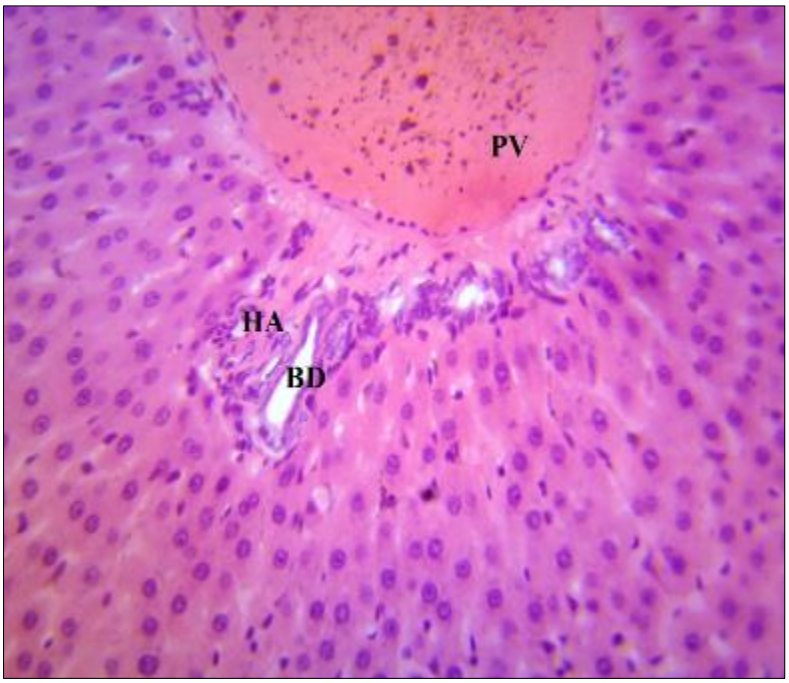

Figure 5 (a) **Group E** Photomicrogragh of Metformin + Omega 6 group (x400 pixels) liver showing marked regenerated hepatocytes (Hp) with normal histologic architecture in group E. (X400pixels)**.**

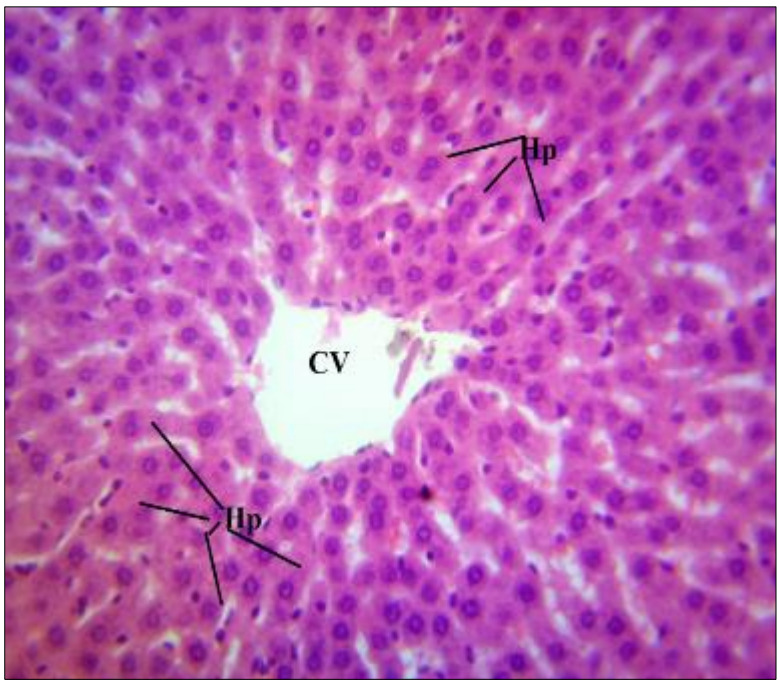

Figure 5 (b) G**roup E** Photomicrogragh of Metformin + Omega 6 group (x400 pixel) portal triad showing normal histologic architecture. Note absence of inflammatory cells in the portal canal in group 5. (X400 pixel

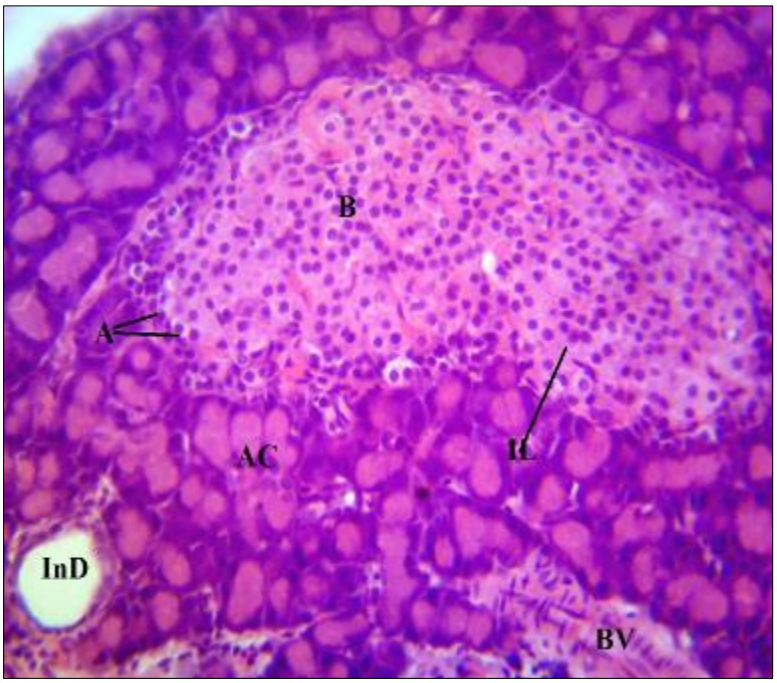

Figure 6 Group A Pancreas histology: Photomicrograph pancreatic tissue shows lobular arrangement of highly cellular glandular tissue composed of orderly differentiated acinar cells, ductular structures and Islet cells (Islets of Langerhans). Within the lobule are numerous acini (AC) of the exocrine component, an intralobular duct (InD) and islets of Langerhans (IL)

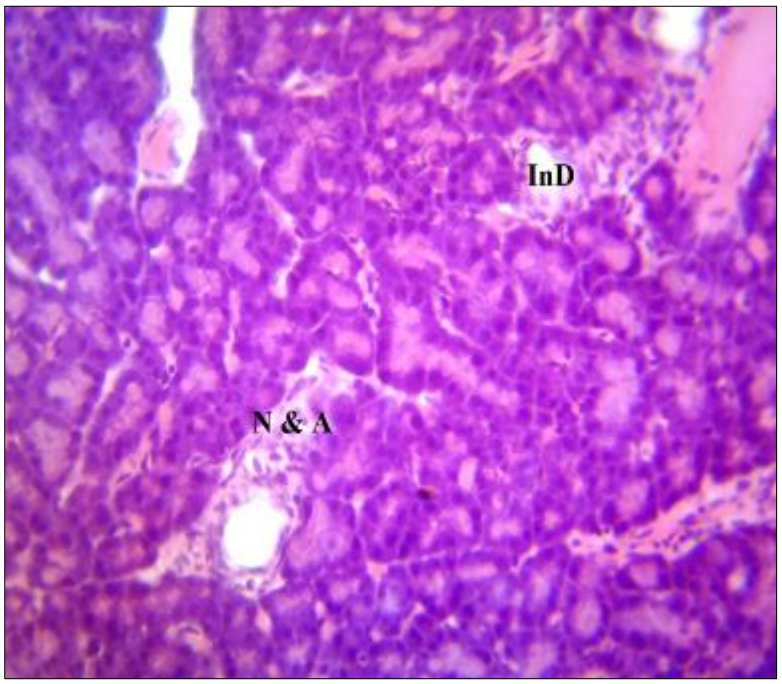

Figure 7 Group B Pancreatic histology: Photomicrograph of diabetic control pancreas showing diffused necrosis and atrophy (N & A) of the islets of Langerhans (B cells) in group B. intralobular duct = (InD) (X400 pixels)

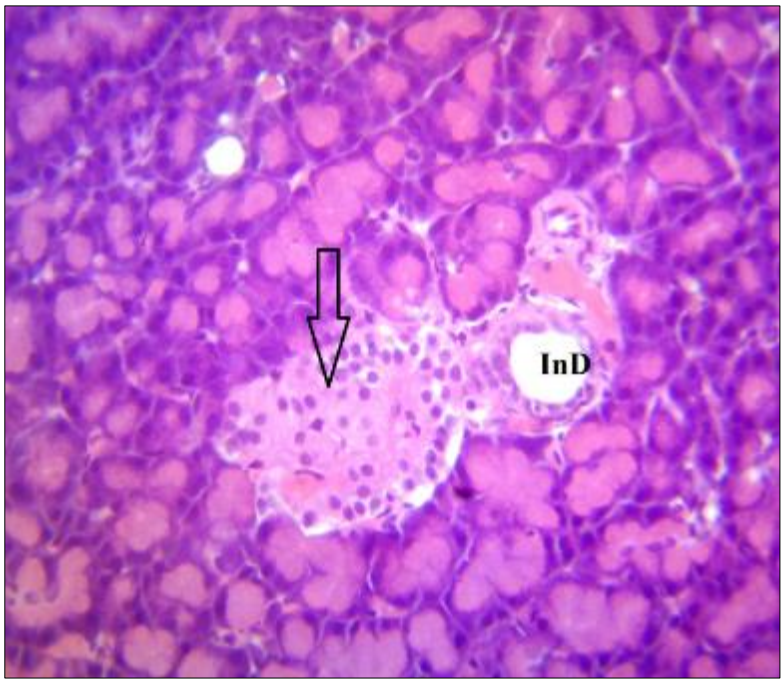

Figure 8 Group C Pancreatic histology:Photomicrograph of Metformin group pancreas showing diffused mild regeneration of islets of Langerhans (arrow) in group C

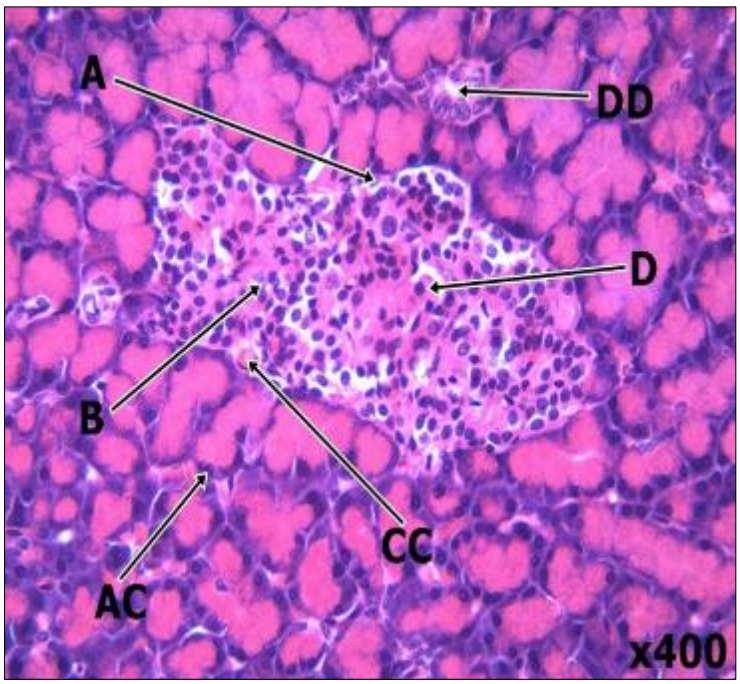

Figure Group D Pancreatic histology: Photomicrograph of Metformin +Omega 3 group pancreas showing diffused moderate regeneration of β cells of the Islets of Langerhans. A= α cells, B=β cells, D=δ cells, DD=intrapancreatic ductule, AC=Acinar cells, CC=Capillaries

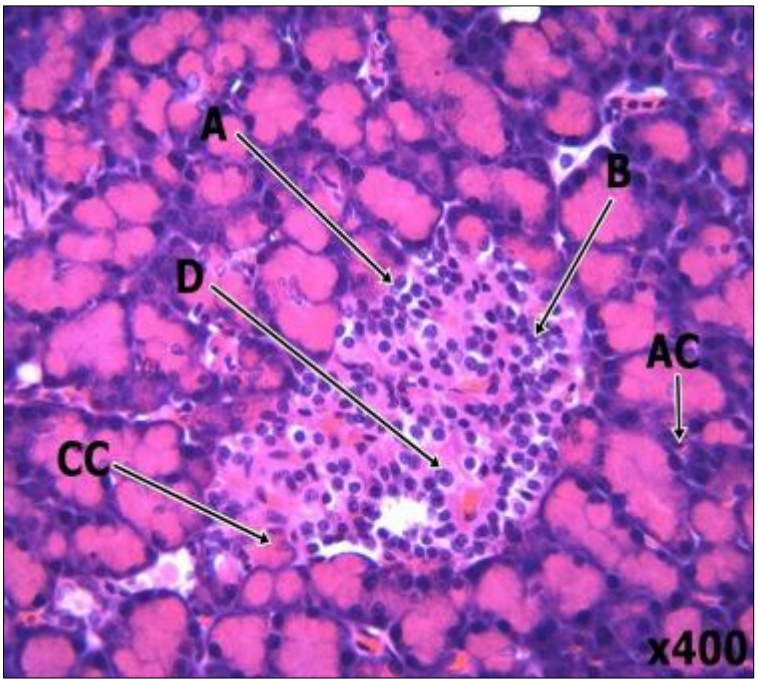

Figure 10 Group E Pancreatic histology: Photomicrograph of pancreas showing diffused moderate regeneration of islets of Langerhans (B cells). A= α cells, B=β cells, D=δ cells, DD=intrapancreatic ductule, AC=Acinar cells, CC=Capillaries

4. Discussion

The results of the present study showed that alloxan at a dose of 150mg/kg body weight apparently caused a significant increase in the fasting blood sugar values ($P < 0.05$) across all the alloxan treated animals resulting in a significant increase in blood glucose indicating establishment of a diabetic state. The use of alloxan to induce diabetes in rats represents a well-established animal model of inducing diabetes mellitus, characteristically similar to type 2 diabetes in humans [23, 24]. There was generally significant decrease in the plasma glucose level for all diabetic treated groups compared to normal control group across the days ($P < 0.05$). The studies of [25, 26] seem to support the proposal that polyunsaturated fatty acids (PUFA) may have a role to play in the treatment of diabetes because of their ability to lower blood sugar levels and reduce the hazards associated with inflammation. Also several studies have reported hypoglycemic and insulin sensitizing effects of Metformin [27, 28]. This is contrary to the findings of [29] which reported that Metformin treatment at a dose of 500mg/kg was not able to lower blood glucose in diabetic rats. Also Ghadge *et al.,* [18] reported that both Metformin and Omega 3 were not able to normalize the glucose levels after 30 days of treatment.

Regarding the lipid profile the result presented indicated that there was generally no significant mean difference seen in the lipid profile of treatment groups compared to control group ($p > 0.05$). Although it was observed that at the end of the experiment that the mean values of total cholesterol levels and low density lipoprotein cholesterol (LDL-C) were higher in the diabetic control group (groupB). The elevation in total cholesterol levels induced by alloxan administered at 150mg/kg bodyweight may be a result of reduced lipoprotein lipase activity secondary to reduced serum insulin levels and represents a risk factor for coronary heart diseases [30]. Increased levels of LDL increase the risk of cardiovascular diseases and stroke [31]. This finding is in line with the report of Kishor *et al*. [32] who reported that alloxan induced diabetic rats administered with 100mg/kg bodyweight did not show significant differences in lipoprotein lipid levels compared with non-diabetic controls. This further confirms that it is not only the onset of diabetes but also its severity that has a direct link with the generation of ROS that affects the lipid and lipoprotein profiles. Alterations in serum cholesterol and triglycerides concentration following the development of diabetes after alloxan or streptozotocin administration to rabbits and rats have been reported [33]. There were elevated total cholesterol levels and triglycerides levels in the Metformin + Omega 6 treatment group (groupE). Elevated total cholesterol and triglycerides could lead to hindrance of blood supply to the heart liver and kidney which could cause coronary heart diseases, stroke or kidney failure. Yuan *et al.* [34] also reported that the levels of triglycerides contributes independently to increased risk of cardiovascular diseases and severe hypertriglyceridemia and is also associated with an increased risk of pancreatitis. The beneficial lipid lowering effects of metformin have been reported in diabetic rats [35]. The intake of Omega 3 polyunsaturated fatty acids (PUFA) have been shown to have an effect on lipoprotein

metabolism with hypocholesterolemic effect [18, 31] which have been attributed to eicosapeentoic acid and Docosahexanoic acid (EPA and DHA). This study did not show any statistical significance in lipid profile effect of Metformin and polyunsaturated fatty acids.

Essentially the results of previous investigations have shown conflicting reports on the antioxidant status in type 2 diabetes mellitus, with reports indicating increased concentrations [36] decreased concentrations [37] or no change at all (Van der-Jagt *et al.,* 2001) depending on the antioxidant investigated. In the present study there was low activity in serum Superoxide Dismutase (SOD) activity of the diabetic control group (group B), Metformin + omega 3 group (group D) when compared with normal control (group A). The low activities of the diabetic control group (group B) supports and confirm previous results that diabetes is associated with impaired antioxidant defenses [38, 33]. Superoxide Dismutase (SOD) is an important defense enzyme which catalyses the dismutation of superoxide radicals [39]. It plays an important protective role against cellular and histological damages that are produced by ROS. However in this study we found that Metformin + Omega-6 (group E) affects the serum SOD activity statistically when compared to the diabetic control group (group B). Over expression of SOD targeted to overcome oxidative stress reduces ROS and increases antioxidant enzymes has been shown to prevent diabetes mellitus [37]. Also alterations in SOD activity in diabetic animals have been reported to be normalized by α-lipoic acid administered prior to or concomitant with the diabetogen [8]. Increase in the activity of SOD is an indication of their ability to scavenge ROS thus contributing to the protective effects against oxidative stress and preventing further damage to membrane lipids. Metformin has been shown to increase SOD activity in previous reports [40]. Urakami *et al* [41] reported that Metformin may present a useful adjuvant to the management of type 1 diabetes mellitus. Malondialdehyde (MDA) is the degradable product of peroxidation by polyunsaturated fatty acids in the cell membrane. Presence of higher MDA levels in the serum is an indication of induced lipid peroxidation and of oxidative stress which has been reported as one of the underlying causes of diabetes mellitus [22]. Increase in Lipid peroxidation is also an indication of decline in defense mechanism of enzymatic and non-enzymatic antioxidants which can exacerbate the occurrence of myocardial infarction [42, 37]. The concentration of MDA across the set groups were statistically significant. Those of Metformin+ Omega treatment groups (group D and groupE) were lower than that of the other set groups and were statistically significant when compared to diabetic control group. This indicates that metformin and polyunsaturated fatty acid supplements may play a role in the reduction of MDA activity in diabetes mellitus in the longterm thereby contributing to the protection against oxidative damage in diabetes. Further studies of longer duration are needed to investigate their effectiveness as antioxidants in the treatment of diabetes.

There was significant reduction in weight for all diabetes treated rats administered 150mg/kg bodyweight of alloxan, while the normal control group was in a generally good condition with normal appetite and progressive weight gain. The weight loss may be due to catabolic processes involved in diabetes mellitus [43].

Liver histology of the diabetic control group (group B) showed necrosis and depletion of hepatocytes with haemorrhages, this depicts that alloxan has hepatotoxic effect. Also liver histology showed marked regeneration of hepatocytes with normal histologic architecture in the metformin + Omega 6 group (groupE). This depicted that Metformin + Omega 6 group has a hepatoprotective effects. The primary site for PUFA metabolism is the liver; a higher n-6 : n-3 PUFA ratio within the liver of non-alcoholic fatty liver disease patients may contribute to the development of fatty liver due to a derangement in the capacity to regulate liver lipid metabolism [44]. This is in agreement with the findings of [45] that reported a hepatoprotective effect of Omega 3 PUFAs in chemically induced hepatotoxicity. Some case report studies also demonstrated that metformin induces hepatotoxicity in diabetic subjects [46, 47]. Pancreas histology showed marked reduction in the density of β cells of the islets of langerhans in all the set groups, thereby depicting the pancreatic cell damaging effects of alloxan. However the metformin + Omega 3 group and the Metformin +Omega 6 group showed marked regeneration of the β cells of the islets of langerhans, this shows that there is a protective effect of Metformin and the polyunsaturated fatty acids. A study published by *The Journal of Clinical Investigation* found that adding omega-3 fatty acids to mice models of type 1 diabetes was effective, among mice fed the omega-3 enriched diet, the investigators noted signs of beta cell regeneration, which suggests that the fatty acids could reverse diabetes [48]. Signs of regeneration of β cells have also been reported following consumption of some plants in STZ-induced diabetic animals. Thus islets cells replacement or regeneration therapy may offer therapeutic benefit to people with diabetes [49].

5. Conclusion

In conclusion, the treatment with Metformin and Omega3/Omega6 oil intervention in diabetic rats has a significant effect of reducing hyperglycemia. Metformin and Omega3/Omega6 oil showed a significant role in MDA activity reduction, and therefore indicate an inclination towards antioxidative defense against damage by lipid peroxidation. Metformin and Omega 6 showed a significant role in SOD activity in diabetic rats which implies that Metformin and

Omega-6 has an antioxidant effect on diabetes through SOD activity. This study did not show any statistical significance in lipid profile effect of Metformin and polyunsaturated fatty acids (Omega 3 and Omega 6). Longer duration investigation is needed in order to have a conclusive evidence of the efficacy of the combination therapy of Metformin and PUFAs (Omega 3 and Omega 6) on diabetes.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest associated with authors of this article.

Statement of ethical approval

The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, as well the National Institute of Health Guide for the care and use of Laboratory Animals.

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