International Journal of Biochemistry Research & Review
4(6): 536-549, 2014

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Comparative Effects of Antidiabetic Drug, Metformin and Deferoxamine, on Serum Lipids, Serum Ferritin and Endocrine Indicators of Diabetes Mellitus Complications in Sreptozotocin Diabetic Rats

P.O. Okonkwo[1,2*] and ZSC Okoye[1]

[1]Department of Biochemistry, Faculty of Medical Sciences, University of Jos, Jos, Plateau state, Nigeria.
[2]Department of Chemical Pathology, Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom, Plataeu state, NigeriaP.M.B. 02 Vom, Nigeria.

Authors' contributions

This whole work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

Received 6th June 2014
Accepted 25th June 2014
Published 10th July 2014

ABSTRACT

Aims: Increasing evidence has indicated that iron overload not only increases risks of insulin resistance and diabetes, but also causes cardiovascular diseases in non-diabetic and diabetic subjects. The present study compared the effects of metformin (met) and deferoxamine (DFX) on the serum lipids, serum ferritin and endocrine indicators of diabetes mellitus complications in streptozotocin experimental diabetes in rats.
Study Design: Experimental diabetes was induced in overnight fasted rats by a single dose i.p each of nicotinamide and, 15min after, STZ followed by administration of the anti-diabetic drugs, met (os, 250mg/kg b.wt) and DFX (i.p,150mg/kg b.wt), daily for 14 days. Blood and histological samples were collected and prepared for biochemical and histopathological analysis of indicators of cytotoxic side effects. Results were analysed statistically by Student t-test and analysis of variance (ANOVA).

**Corresponding author: Email: itspatonline@yahoo.com;*

Results: Both drugs caused progressively increased hypoglycaemic effect with repeated doses. However, Metformin showed markedly higher potency in hypoglycaemic activity than DFX. STZ diabetes caused hyperlipidaemic effect with respect to lipid profile parameters except HDL and treatment with antidiabetic drugs metformin and deferoxamine reversed the hyperlipidaemic effect. On the other hand, STZ caused hypolipidaemic effect with respect to HDL but the antidiabetic drugs reversed it. However, DFX is more potent than metformin in reversing the effect of STZ diabetes on HDL. STZ diabetes induced elevation of serum ferritin while inducing reduction in serum insulin level. Metformin treatment reversed the adverse effects of STZ on insulin secretion only, whereas, DFX significantly reversed the adverse side effects of STZ on both serum ferritin and serum insulin. However, metformin-treatment and DFX-treatment exhibited comparable potency in their effects on insulin secretion.
Conclusion: Evidence from histological study of the pancreas suggests that both metformin and DFX were sufficiently biologically significant to effectively reverse the disruptic effects on the pancreatic exocrine tissue of diabetic rats.

Keywords: Streptozotocin; serum ferritin; type 2 diabetes; dyslipidaemia.

1. INTRODUCTION

Diabetes mellitus (DM) is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries [1]. Increasing incidence in the developing countries, especially in the younger age group, affecting mainly the people in the productive years of their lives is also of great concern [2]. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3,4].

Type 2 diabetes (T2DM) is the most common form and comprises of 90% of people with diabetes around the world [5]. Type 2 diabetes is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells [6,7]. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease [8]. Increased triglyceride (TG) and reduced high density lipoprotein cholesterol (HDL-C) levels are the key characteristics of dyslipidaemia in Type 2 diabetes [8].

Epidemiological studies have reported a strong association between elevated serum ferritin concentrations and increased risk for diabetes [9,10]. Excess iron impairs pancreatic β cell function and causes β cell apoptosis [10,11]. Increasing evidence has indicated that iron overload not only increases risks of insulin resistance and diabetes, but also causes cardiovascular diseases in non-diabetic and diabetic subjects [12,13]. Epidemiologic studies in overt iron overload states have shown that the incidence of cardiac disease is increased and that treatment with iron chelation improves cardiovascular outcome [14,15].

The management of diabetes mellitus is considered a global problem and a successful treatment is yet to be discovered [16]. Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with Type 2 diabetes [17]. It is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality which is a major cause of death in patients with Type 2 diabetes [18]. Metformin was approved by the Food and Drug

Administration (FDA) for use in the United States in 1995 [19]. Metformin major effect is to decrease hepatic glucose output [20]. In addition, metformin decreases glucose absorption in the small intestine, increases insulin-mediated glucose utilization in peripheral tissues (such as muscle and liver), and has an antilipolytic effect that lowers serum free fatty acid concentrations, thereby reducing substrate availability for gluconeogenesis [20,21].

Deferoxamine is the most common iron chelator in clinical use [22,23]. The capacity of deferoxamine to chelate iron (Fe) and mediate its excretion in Fe-overloaded patients is well documented [24]. It has been approved for use in the USA since the late 1970's [25,26]. Deferoxamine acts by binding free iron in the bloodstream and enhancing its elimination in the urine [27]. Deferoxamine works in treating iron toxicity by binding trivalent (ferric) iron (for which it has a strong affinity), forming ferrioxamine, a stable complex which is eliminated via the kidneys [28]. By removing excess iron, the agent reduces the damage done to various organs and tissues [27].

The social and financial burden of diabetes is mainly due to the complications. Because of this, a growing number of researches have focused on diabetes and its complications, with the aim to expand our knowledge about pathogenic and pathophysiological mechanisms, preventive strategies and potential novel therapies. This study was carried out to compare the effect of metformin on serum lipids, serum ferritin and endocrine indicators of DM with that of deferoxamine which act by a different mechanism in streptozotocin-induced diabetic rats to determine their effectiveness in treating/preventing diabetes and its complications and as an effort in the search for more effective therapeutic initiatives for Type 2 DM.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Streptozotocin (STZ), deferoxamine (DFX), nicotinamide (NA), citric acid, trisodium citrate, sodium hydroxide, sodium chloride, chloroforms, and formaldehyde were purchased from Zayo-Sigma Aldrich Chemicals Ltd, St Louis, MO, USA, metformin HCl (Diabetmim, Hovid Pharmaceutical, Pune Ma Harashtra, India) while insulin and ferritin ELISA kits were purchased from Syntron Bioresearch Inc., Carlsbad, California, USA.

2.2 Experimental Animals

Thirty five male Wistar strain albino rats weighing 172-233g were used in this study. The rats were obtained from the Animal House Unit of the Federal College of Veterinary and Medical Laboratory Technology (FCVMLT), Vom. The rats were housed in clean metallic cages, kept in a well ventilated room and allowed to acclimatize to the laboratory condition of the Nigerian Institute for Trypanosomiasis Research (NITR) Vm, animal experiment room at 25±2°C with 12hour light/dark cycle for two weeks before being used. Animals were fed a standard animal pellet obtained from Dagwom farm of National Veterinary Research Institute (NVRI), Vom, and had free access to water *ad libitum*. All animals were carefully monitored and all the experimental protocol with the animals was in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the Institutional Ethical Committee of NITR.

2.3 Experimental Induction of Type 2 Diabetes in Rats

Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p) injection of freshly prepared streptozotocin (35mg/kg b.wt) dissolved in 0.1M citrate buffer (pH 4.5), 15min after the intraperitoneal administration of nicotinamide (110mg/kg), dissolved in normal physiological saline. The control (group A) animals were injected i.p with the equivalent volume of the citrate buffer solution [29,30].

Diabetes was confirmed by the symptoms of polydipsia, polyuria, glycosuria and elevated fasting blood glucose concentration, 48hours and then one week after STZ injection. Diabetes was developed and stabilized in STZ treated rats over a period of 7 days [31]. Following the criteria of previous workers [32,33], rats with stable glycosuria and blood glucose levels of more than 11mmol/L (>11 mmol/L) were considered to be diabetic and, therefore, used for the study.

2.4 Animal Treatment

Following induction of diabetes, animals were randomly and evenly distributed into four groups A, B, C and D of six rats each (n=6) in clean metallic standard rat cages. Animals in group A (normal control) and those in group B (diabetic control) were given only water *ad libitum.* While those in group C (diabetic) were administered orally with antidiabetic drug metformin (Met) at a dose of 250mg/kg body weight once daily for 14 days by forceful gavage. Rats in group D (also diabetic) were administered intraperitoneally with iron-chelating drug deferoxamine (DFX) at a dose of 150mg/kg body weight once daily for 14 days. The dosages of drugs used in this study were pre determined in a pilot study. Baseline fasting blood glucose levels of each rat for all groups were measured before diabetes induction and at the time of grouping of the animals. Blood glucose levels were estimated just before drug administration on day 0, 5, 10 and 14.

2.5 Monitoring of Blood Glucose Level during Treatment

All blood samples for monitoring of blood glucose level *in situ* were taken from the tail vein of the rats using 24 gauge needles at intervals of 0, 5, 10, and 15 days. Blood glucose level was determined by the glucose oxidase method using reactive strips and a single touch glucometer (Accu-Chek Active, Roche Diagnostics, Mannheim, Germany). Results were initially recorded in mg/dl and then converted to mmol/l by dividing values in mg/dl by a factor of 18

2.6 Blood Collection and Preparation

After overnight fast, the animals were sacrificed on the 15^{th} day under mild chloroform anaesthesia and blood was obtained via cardiac puncture. Blood sample was transferred into plain centrifuge tube and allowed to clot at room temperature. It was then centrifuged within 1 hour of collection at 4000x g for 10min on a digital centrifuge (Biofuge 200, Bosch Medical Systems, Corinth, TX, USA) to separate the serum from the clot. The resultant serum sample were stored frozen at -20°C. Prior to assay, frozen sera were completely thawed and well mixed and all reagents were allowed to attain room temperature.

2.7 Histopathological Examination

After sacrificing the animals on the 15th day post treatment, the pancreas of two animals from each group were excised and immediately fixed in 10% neutral buffered formalin solution after washing with normal saline. The resultant fixed tissue samples were used for histopathological examination in the Histopathology Laboratory of FCVMLT and Diagnostic Center of NVRI, Vom, using the routine procedures developed in the respective laboratories. The tissues were washed, dehydrated with alcohol and cleared with xylene. Serial sections of 4 µm thickness were cut using a rotary microtome (ERM-200P, Erma, Tokyo, Japan).The sections were deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water. These were examined and later counterstained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted. The slides were observed using a light microscope.

2.8 Biochemical Analysis of Rat Serum Sample

Serum triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDL-c) levels were measured colorimetrically by enzymatic point methods using Roche-Hitachi kit via Automatic Analyzer (HITACHI 902, Roche Diagnostics, Mannheim, Germany) according to the procedure described in the manufacturers' operation manual. Serum insulin and ferritin levels were estimated by enzyme linked immunosorbent assay (ELISA) technique using ELISA kits (Syntron Bioresearch Inc., Carlsbad, California, USA).

2.9 Statistical Analysis

All statistical analysis was performed with SPSS software version 16.0. Data are expressed as the means standard error of mean (S.E.M). Student's t-test and analysis of variance (ANOVA) were employed in comparing means of continuous variables as appropriate. Differences were considered statistically significant if $p < 0.05$.

3. RESULTS

The results on the effect of metformin and deferoxamine on the fasting blood glucose levels of diabetic rats are summarised on Fig. 1. The diabetic control animals (group B) exhibited gradually increased hyperglycaemia. There was a significant ($p<0.05$) elevation in fasting blood glucose level in the diabetic control (Group B) when the values for Days 0, 5, 10 and 15 were compared to the corresponding values in the normal control rats. Oral administration of metformin (250mg/kg/day) significantly ($p<0.05$) reduced the elevated levels of blood glucose on the 5th, 10th and 15th day of treatment compared to the corresponding values in the untreated diabetic control ($p<0.05$). On the other hand, intraperitoneal administration of deferoxamine (150mg/kg/day) caused significantly ($p<0.05$) reduced blood glucose level on the 10th day and 15th day of treatment only compared to the diabetic control. This would suggest that the onset of induced hypoglycaemic effect occurred 5 days earlier in rats given oral metformin than in those given intraperitoneal DFX. Both drugs caused progressively increased hypoglycaemic effect with repeated doses. As at Day 15, the % reduction in blood glucose level induced by met (35.58%) was significantly ($p<0.05$) higher than that (16.6%) induced by DFX, an indication that metformin was more potent than DFX at the experimental condition. Similarly, from Day 5, the blood glucose level of diabetic rats given DFX (group D) were consistently and significantly ($p<0.05$) higher than

those given met (group C).This is another indication that metformin is more potent than DFX. Normal control rats (Group A) did not exhibit any significant alterations in blood glucose levels during the experiment.

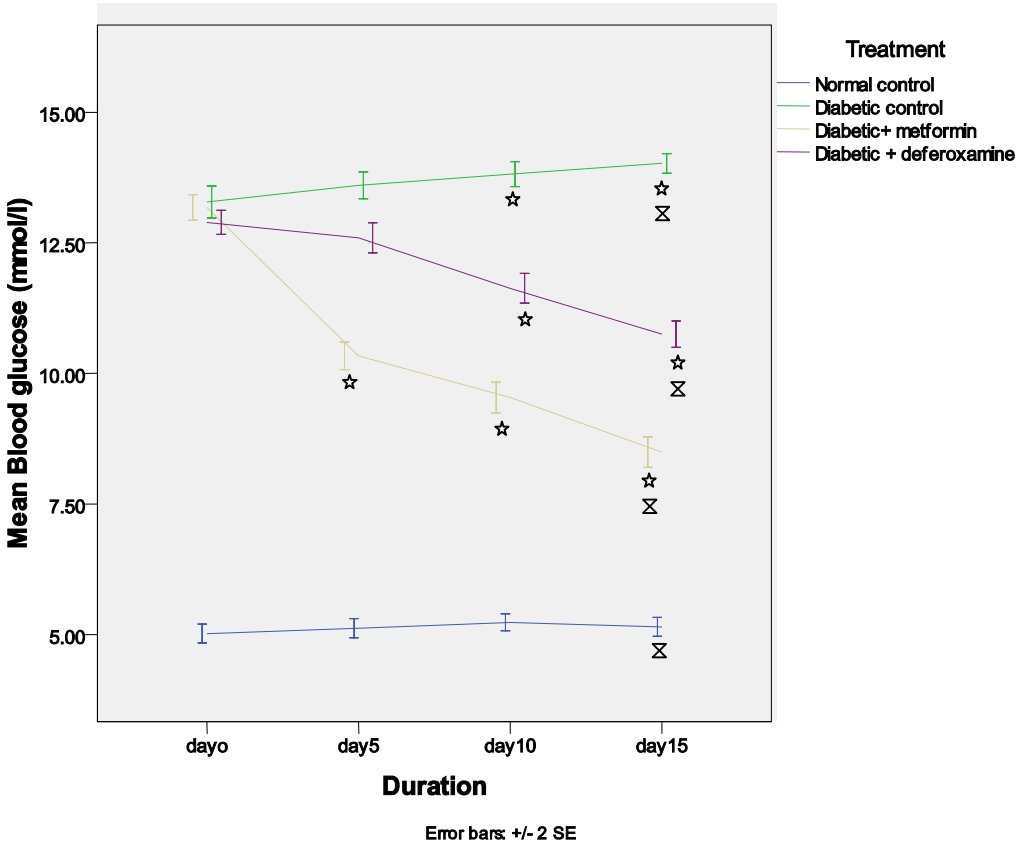

Fig. 1. Effect of metformin and deferoxamine on blood glucose level (Mmol/L) during 14 days treatment in diabetic rats

☆ *Statistically significant at p<0.05 compared with day 0 of same treatment group,*

⧖ *Bars with this superscripts differ from each other statistically at p<0.05 when compared between treatment groups at day 15, Tabulated Values are means±S.E.M for six rats in each group; Statistical significance between means was assessed using one-way analysis of variance followed by Duncan's test as a post-analysis of variance test, [a]Statistically significant difference at p<0.05 compared to the normal control (group A), [b]Statistically significant difference at p<0.05 compared to the diabetic control (group B), [c]Statistically significant difference at p<0.05 compared to the diabetic + met treated (group C), Abreviations: HDL=high density lipoprotein; LDL= low density lipoprotein; Met= metformin; DFX= deferoxamine.*

The results on the comparative effects of metformin and deferoxamine on the various lipid risk factors are summarised on Table 1. There was a significant ($p<0.05$) decrease in the level of serum high density lipoprotein (HDL) and a significant ($p<0.05$) increase in the levels of total cholesterol, triglycerides and low density lipoprotein (LDL) in diabetic control rats (group B) compared to normal control rats (Group A), suggesting that experimental diabetes in rats caused a reduction in serum HDL but elevation of serum total cholesterol, triglyceride and LDL levels. Administration of metformin to STZ-NA induced diabetic rats (group C) led to a significant reduction ($p< 0.05$) in the level of triglycerides, total cholesterol, and LDL and

significant increase ($p<0.05$) in the level of HDL, compared to diabetic control rats (group B). Similarly, deferoxamine treated rats (Group D) showed significant ($p<0.05$) reduction in the levels of triglycerides, total cholesterol and LDL and increased the HDL significantly ($p<0.05$) compared to diabetic control rats after 15 day of treatment. These observations indicate that STZ diabetes caused hyperlipidaemic effect with respect to lipid profile parameters except HDL and that treatment with anti-diabetic drug metformin and iron chelating drug deferoxamine reversed the hyperlipidaemic effect. On the other hand, STZ caused hypolipidaemic effect with respect to HDL but the antidiabetic drug and iron chelating drug reversed it. The mean serum HDL level in DFX-treateed rats was significantly ($p<0.05$) higher than that of metformin-treated rats, suggesting that DFX is more potent than metformin in reversing the effect of STZ diabetes on HDL. Mean serum total cholesterol, triglyceride and LDL levels were higher in metformin treated rats than in DFX- treated animals but the difference were not statistically significant.

Table 1. Mean serum lipid profiles of streptozotocin diabetic rats following 14-Day treatment with metformin and deferoxamine

		Serum lipid concentration (mmol/L)			
Group	**Treatment**	**Total cholesterol**	**Triglyceride**	**HDL**	**LDL**
A	Normal control	1.10±0.06	0.75±0.06	0.60±0.02	0.23±0.07
B	Diabetic control	1.48±0.05[a]	1.50±0.19[a]	0.42±0.02[a]	0.55±0.13[a]
C	Diabetic + Met	1.22±0.05[b]	0.93±0.05[b]	0.49±0.02[ab]	0.30± 0.05[b]
D	Diabetic + DFX	1.18±0.04[b]	0.82±0.05[b]	0.56±0.02[bc]	0.26±0.04[b]

The comparative effects of metformin and deferoxamine on serum insulin and ferritin levels are summarised in Table 2. The mean serum ferritin level was significantly higher ($p<0.05$) while serum insulin level was significantly lower ($p<0.05$) in the diabetic control (Group B) than in the normal control (Group A), suggesting that STZ diabetes induced elevation of serum ferritin while inducing reduction in serum insulin level probably by causing cytotoxic side effects on the pancreatic tissue.

Table 2. Mean serum insulin and ferritin levels in streptozotocin diabetic rats following 14-Day treatment with metformin and deferoxamine.

Group	**Treatment**	**Serum insulin (µu/ml)**	**Serum ferritin (ng/ml)**
A	Normal control	1.42 ± 0.18	1.21±0.01
B	Diabetic control	0.85 ± 0.04[a]	1.25±0.01[a]
C	Diabetic + Met	1.33 ± 0.08[b]	1.24±0.01[a]
D	Diabetic + DFX	1.38 ± 0.19[b]	1.20±0.01[bc]

Tabulated values are means±S.E.M for six rats in each group; Statistical significance between means was assessed using one-way analysis of variance followed by Duncans test as a post-analysis of variance test, [a]Statistically significant difference at $p<0.05$ compared to the normal control (group A) [b]Statistically significant difference at $p<0.05$ compared to the diabetic control (group B) Abreviations: Met= metformin; DFX= deferoxamine

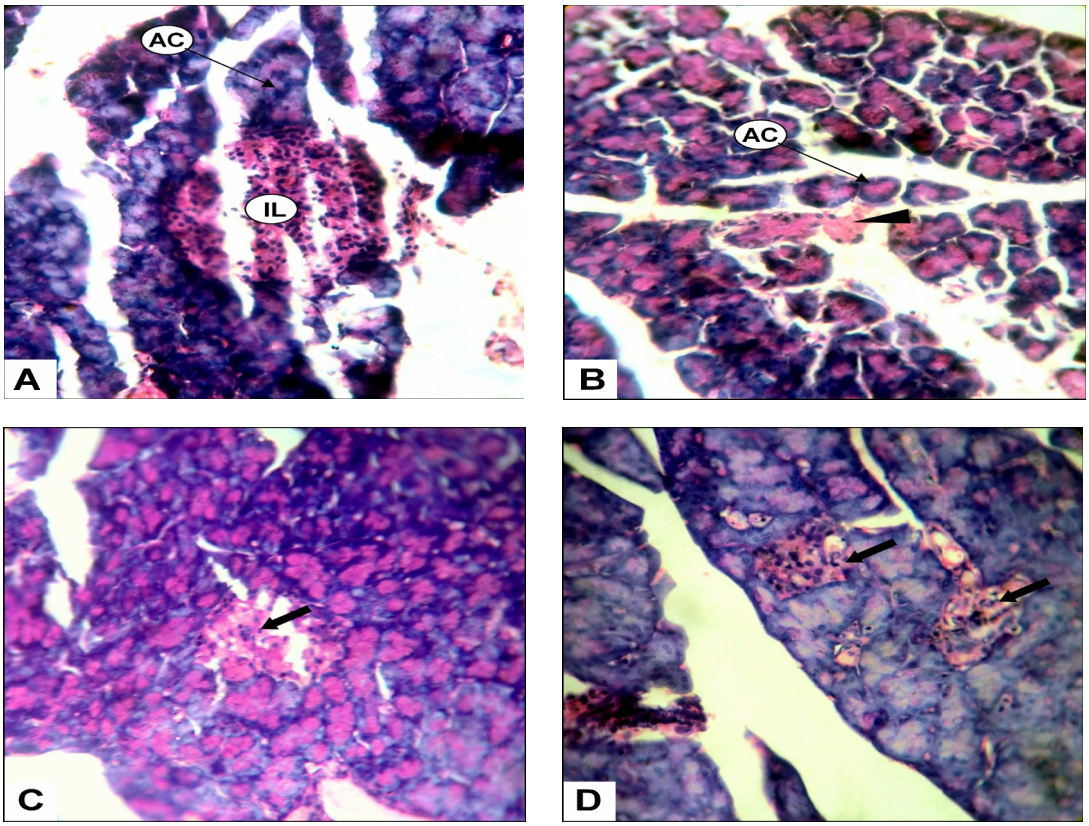

Plate 1. Histopathological changes in pancreatic tissue of control and experimental rats

A. Normal control (Group A) shows normal exocrine acini (AC) and endocrine Islet of Langerhan (IL); B. Diabetic control (Group B) shows a severely reduced number of Islets as well as depleted Islet (arrow head); the Islet cells have been replaced by an eosinophilic acellular substance; C.Metformin treated (Group C) shows normal-appearing pancreatic lobule with preserved Islets (arrow); D. deferoxamine treated (group D) shows a normal pancreatic lobule with increased population of preserved Islets (arrows), H & E Stain; A and C (X10), B and D (X30)

The serum ferritin level was lower while the serum insulin level was higher in STZ diabetic rats treated with metformin (Group C) than in the diabetic control controls (group B) but only in the case of serum insulin was the difference statistically significant ($p<0.05$). This is an indication that metformin treatment reversed the adverse effects of STZ on insulin secretion only. Whereas, DFX significantly reversed the adverse side effects of STZ on both serum ferritin and serum insulin as evidenced by the significantly ($p<0.05$) lower level of ferritin and significantly ($p<0.05$) higher level of insulin in DFX treated rats (group D) compared to diabetic controls (group B). The mean serum ferritin level of DFX-treated diabetic rats was significantly lower than those of metformin-treated diabetic rats, an indication that deferoxamine is more potent than metformin in reducing serum ferritin. On the other hand, there is no significant difference ($p>0.05$) between the mean serum insulin levels of metformin treated diabetic rats and those of DFX-treated diabetic rats, suggesting comparable potency. This observation was corroborated by histopathological findings on the pancreas tissue (see Plate 1). Evidence suggests that they were sufficiently biologically significant to effectively reverse the disruptic effects on the pancreatic exocrine tissue.

Compared to the diabetic control which showed degeneration of the pancreatic beta cells of the Islet of Langerhans (Plate 1B), pancreatic tissues of the rats given metformin (Plate 1 C) and deferoxamine (Plate 1 D) showed normal appearing pancreatic lobular structure and preserved endocrine Islet of Langerhans.

4. DISCUSSION

Streptozotocin (STZ)-nicotinamide (NA) type 2 model shares a number of features with human type 2 diabetes both histologically and metabolically and is characterized by moderate stable hyperglycaemia. Hence, STZ-NA induced diabetes model was used in the present study [34,35]. In the present study, streptozotocin injection caused β cells degeneration in rats, therefore, release of insulin by the pancreas was decreased resulting in hyperglycaemia. This confirms induction of experimental diabetes in rats. This is similar to the findings of several earlier researchers [36,37].

The results indicate that metformin has antidiabetic potentials as has been established by several studies [4]. This is evident in the rapid reduction in blood glucose levels that were noticed once administration of the drug commenced as well as the alleviation of all symptoms of diabetes that were initially noticed before commencement of treatment. Metformin (given o.s) showed markedly higher potency than deferoxamine (given i.p) in hypoglycaemic activity (earlier time onset, higher % reduction); despite the fact that i.p is faster route than o.s, yet metformin acted earlier. The blood glucose lowering effect of metformin is dependent on presence of insulin [21], therefore, according to the results of the present work, it seems that administering STZ-NA in rats was not able to degenerate all pancreatic β-cells and so did not lead to a complete lack of β-cell population as was obvious from the histological findings.

The fundamental mechanism underlying hyperglyceamia in diabetes mellitus involves the over production of glucose and or decreased utilization of glucose by the tissues [21,38]. The mechanisms of metformin-mediated improvement of insulin sensitivity have remained obscure, however, multiple pathways of action have been proposed, including a decrease of hepatic glucose production, an increase of peripheral glucose utilization and a reduction of intestinal glucose absorption [4,39]. Metformin also increases low-affinity and high-affinity receptors of insulin, and improves insulin resistance [40].

Administration of deferoxamine to diabetic rats caused significant reduction of blood glucose and serum ferritin concentration in the present study. The observed effect of deferoxamine on blood glucose and serum ferritin level may be due to improved utilization of glucose by peripheral tissue through increase in insulin receptor activity and signaling in hepatocytes. This is in line with the findings of Dongiovanni et al. [41], who investigated the effect of iron depletion by deferoxamine on insulin signaling and glucose uptake in HepG2 hepatocytes and in rat liver and reported that deferoxamine induced the constitutive glucose transporter Glut1 and the insulin receptor. Use of phlebotomy or iron-chelation therapy to reduce ferritin levels was associated with improved glucose tolerance in patients with hereditary hemochromatosis (HH), healthy blood donors, patients with metabolic syndrome, and patients with T2DM [42,43]. Epidemiological study showed that even at 'normal' levels, iron exerted detrimental effects on pancreatic beta-cell function, and that these effects were reversible with dietary restriction or iron-chelation therapy [11].
In this study, lipid profile alterations produced by STZ-NA administration to experimental rats is in agreement with the findings of other researchers who did report significant changes in lipid abnormalities in type 2 diabetes [4,30]. The beneficial improvement in lipid profile

following metformin administration obtained in the present study seem to corroborate previous findings that this drug does ameliorate dyslipidaemia [39,44]. Lipid abnormalities are due to resistance to insulin and hyperglycaemia [45,46]. Improvement in insulin secretion in the islets of Langerhans by administration of metformin and deferoxamine to diabetic rats which was evident in this study may have led to improvement in the serum lipids levels [37], [47]. It has been documented that metformin exerts direct effects on hepatic glucose and lipid metabolism and suppresses lipogenic enzymes, particularly acetyl-CoA carboxylase (ACC) activity via an 5'AMP activated protein kinase (AMPK)-dependent pathway, thus leading to decreased lipogenesis but increased fatty acid oxidation [20,39]. Epidemiologic studies in overt iron overload states such as transfusional iron overload and hemochromatosis have shown that the incidence of cardiac disease is increased and that treatment with iron chelation improves cardiovascular outcome [14,15].

5. CONCLUSION

The intraperitoneal injection of STZ-NAD induced type 2 diabetes hyperlipidaemic effect with respect to serum TC and TG profile, but hypolipidaemic effect with respect to HDL-C in experimental rats. Treatment with either antidiabetic drug metformin or iron chelating drug deferoxamine, reversed the hyperlipidaemic effect on serum TC and TG and the hypolipidaemic effect on HDL-C.

Deferoxamine was more potent than metformin in reducing serum ferritin and in mitigation of serum HDL in diabetic rats while metformin showed markedly higher potency in hypoglycaemic activity (earlier time onset, higher % reduction). If epidemiological studies confirm an aetiologic role of iron overload in human subjects, chelating iron using deferoxamine may be useful in treating diabetes in future. In addition, iron chelating drug may be a potential drug for ameliorating diabetic complications.

CONSENT

Informed consent was used in the recruitment of the participants and confidentiality was maintained in accordance with standard medical practice

ETHICAL APPROVAL

Ethical approval was given by the Ethics Committees of Jos University Teaching Hospital, Plateau Specialist Hospital and ECWA Evangel Hospital Jos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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