Original Research Article

Biochemical and Oxidative Changes in High Fat Diet/Streptozotocin-induced Diabetic Rats Treated with Metformin and the Polyherbal Diawell

ABSTRACT

Diabetes mellitus is an epidemic, with a huge disease burden on the patients. This has led to an increase in the use of herbal remedies and combination therapies to reduce this burden.

Aim: This study evaluates the biochemical and oxidative changes in type 2 diabetic rats, treated with metformin and the polyherbal drug diawell.

Methodology: A total of 35 male Wistar albino rats weighing between 120-220g were used for this study. The rats were placed on high fat diet, and diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (45 mg/kg body wt). Fasting plasma glucose (FPG) was determined using the glucose oxidase method. Fasting plasma insulin (FPI), total oxidant status (TOS), total antioxidant status (TAS) and superoxide dismutase (SOD) levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method. Oxidative stress index (OSI) was determined by the ratio of TOS to TAS. Phytochemical analysis was also done on the herbal tablet.

Results: Mean FPG levels were significantly lower ($p<0.05$) in all groups, except the group administered diawell, which was not significantly different ($p>0.05$), compared to the diabetic control. Mean FPG levels were significantly higher ($p<0.05$) in the metformin group, diawell group, but showed no significant difference ($p>0.05$) in the combination group, compared to the negative control. HOMA-IR was significantly higher ($p<0.05$) in the diabetic control compared to the negative control and treatment groups. The metformin and diawell groups had significantly higher ($p<0.05$) HOMA-IR values, whereas the combination (metformin + diawell) showed no significant difference ($p>0.05$) when compared to the negative control. TOS was significantly higher ($p<0.05$) in the diabetic control compared to the negative control and treatment groups. The metformin and diawell groups had significantly higher ($p<0.05$) TOS values, whereas the combination (metformin + diawell) showed no significant difference ($p>0.05$) when compared to the negative control. There was significantly lower ($p<0.05$) TAS levels in the diabetic and treatment groups, compared to the negative control. OSI values were significantly lower ($p<0.05$) in all groups when compared to the diabetic control. Also, OSI values were significantly higher ($p<0.05$) in the treatment groups compared to the negative control.

Conclusion: There was depletion of antioxidant parameters and an increase in oxidative stress in the diabetic rats. Administration of metformin and the polyherbal tablet diawell individually, were not effective in correcting the pathological and biochemical changes associated with diabetes. However, the combination treatment produced a better glycaemic response and attenuated the oxidant status in the rats. Antioxidant therapy should be incorporated in diabetes management, and anti-diabetic herbals properly evaluated.

Keywords: Diabetes mellitus, Oxidative stress, Antioxidants, Herbal therapy, Insulin resistance, Diawell, Metformin, Streptozotocin.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic syndrome characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. There is altered metabolism of carbohydrates, lipids, and proteins along with an increased risk of complications from vascular

disease [1]. It has been predicted that the proportion of adult population with diabetes will increase by 69% for the year 2030 [2].

Type 2 DM leads to the depletion of antioxidant parameters [3], with increased oxidative stress levels resulting in oxidative damage of cellular components [4]. Current oral anti-diabetic agents using orthodox medicine have limited efficacy and undesirable side effects in patients, leading to the development of microvascular and macrovascular complications [5,6]. This has led to an increase in the use of medicinal herbs in the management of type 2 DM [7,8]. These herbs or herbal products contain phytonutrients which have the potential to affect several metabolic and diabetic pathways, with the promise of better patient outcomes. Also, these agents seem to have become an attractive option because of the lesser-perceived adverse reactions in comparison to prescription medications [8]. This study evaluates the biochemical and oxidative changes in type 2 diabetic rats, treated with metformin and the polyherbal drug diawell.

2. MATERIALS AND METHODS

A total of 35 male Wistar albino rats weighing between 120-220g were used for this study. The rats were housed in standard cages at regulated room temperature, with controlled 12 hour light-dark cycles, and allowed access to feed and water *ad libitum*. The animals were allowed to acclimatize for two weeks prior to the commencement of study.

2.1 Drugs

The drugs used for the study were diawell and metformin. The polyherbal drug diawell, is manufactured by Kedi Healthcare Company Ltd, Hong Kong, China and commercially sold in Nigeria as an anti-diabetic tablet. Metformin, a biguanide is manufactured by LEK SA, Poland.

2.2 Acute Toxicity Study

This was done using the fixed dose procedure [9], using 3 rats. 2000mg/kg body weight of diawell was orally administered to each of the rats. The rats were then observed for signs of toxicity for 48 hours. After observation for 48 hours, there were no observed signs of toxicity, hence the herbal drug diawell was deemed safe up to 2000mg/kg body weight dose. Metformin is a standard antidiabetic drug.

2.3 Dose Calculation

The administered dosages were extrapolated from the human dose using the formula by Paget and Barnes.

Metformin

Human daily dose is 1 tablet (500mg) twice daily, that is, 1000mg/day.

Rat dose (mg/kg) = Human daily dose x 0.018 x 5 [10].

= 90mg/kg body wt/day.

Diawell

Human daily dose is 4 tablets (300mg each) three times daily, that is, 3600mg/day.

Rat dose (mg/kg) = Human daily dose x 0.018 x 5 [10].

= 324mg/kg body wt/day.

2.4 Study Design and Diabetes Induction

The rats were weighed and grouped into 5 groups of 7 rats each. Group 1 (negative control) was placed on a normal chow diet, while groups 2 to 5 were placed on high fat diet (HFD) with 42.1% fat content, 3 weeks prior to induction with streptozotocin (STZ). Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body wt) dissolved in 0.1 M citrate buffer (pH 4.5), after a 6 hour fast. Diabetes was confirmed after 72 hours in the rats having fasting blood glucose levels above 14mmol/L (250 mg/dl). Treatments (drugs) were administered daily according to the groupings by means of oral gavage for 28 days.

Group 1: Negative control. The animals were only injected citrate buffer intraperitoneally.

Group 2: Diabetic control

Group 3: Diabetic rats treated with metformin.

Group 4: Diabetic rats treated with the polyherbal drug diawell.

Group 5: Diabetic rats treated with a combination of metformin and diawell.

At the end of the treatments, the rats were fasted for 6 hours, anaesthetized with chloroform and blood samples collected through cardiac puncture. This is in line with the National Institutes of Health (NIH) and the Animal Models of Diabetic Complications Consortium (AMDCC) protocol, on the fasting of laboratory animals [11,12]. The pancreas was also harvested and preserved in 10% formol saline for histological analysis. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly followed. Quality control (QC) samples were run together with the biochemical analysis. STZ was purchased from Sigma-Aldrich, United States of America (USA). Fasting plasma glucose (FPG) was determined using the Glucose oxidase method [13] as described by Randox Laboratories Limited, United Kingdom (UK). Fasting plasma insulin (FPI) and Superoxide dismutase (SOD) levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [14] as described by Elabscience Biotechnology Company limited, China. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method [15]. Total oxidant status (TOS) and total antioxidant status (TAS) were determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [14,16,17] as described by Span Biotech Limited, China. Oxidative stress index (OSI) was determined by the ratio of TOS to TAS. Qualitative phytochemical analysis was done on the herbal drug using classical methods, while the quantitative determination of the phytochemicals was done using spectrophotometric methods [18]. Pancreatic sections were stained using the standard haematoxylin and eosin (H&E) staining technique.

2.5 Statistical Analysis

Data generated was analysed using Graph Pad Prism version 5.03. Groups were compared using one way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test used as Post hoc. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$). Values are expressed as Mean ± SD.

3. RESULTS

Table 1: Qualitative and Quantitative Phytochemical Analysis of the Herbal Drug Diawell

Phytochemicals	Diawell	Concentration (µg/mg)
Alkaloids	**+ve**	119.27
Flavonoids	**+ve**	89.67
Cardiac glycosides	**-ve**	
Phenols	**-ve**	
Phlobatanins	**-ve**	
Saponins	**-ve**	
Tanins	**-ve**	
Terpenoids	**-ve**	
Quinones	**-ve**	

+ve – Present, -ve – Not present

Table 1 above shows alkaloids and flavonoids present in the herbal drug diawell, with concentrations of 119.27 µg/mg and 89.67µg/mg respectively. Other phytochemicals such as phenolic acids, saponins, cardiac glycosides, terpenoids, quinones, and tannins were not found.

Table 2: Fasting Blood Glucose (FBG) Levels of the rats before and after Induction with Streptozotocin (STZ).

Groups	FBG (mmol/l) before Induction	FBG (mmol/l) 72hours after Induction
Group 1 (Negative control) n=7	5.90 ± 0.44	5.75 ± 0.49
Group 2 (Diabetic control) n=7	5.87 ± 0.41	19.88 ± 6.48*
Group 3 n=7	5.85 ± 0.63	16.65 ± 3.50*
Group 4 n=7	5.67 ± 0.57	17.65 ± 3.69*
Group 5 n=7	6.32 ± 0.78	18.78 ± 5.54*
P-value	0.4224	< 0.0001
F-value	1.007	9.922

n – Number of samples, * - Significant difference versus Negative control.

Table 2 shows the FBG of the animals before and after induction with STZ. The results show the mean FBG levels of the animals in all the groups before induction with STZ were not significantly different ($p>0.05$). The results also show significantly higher mean FBG levels ($p<0.05$) in all groups that received HFD/STZ, as compared to the negative control (Group 1) that received only the vehicle (citrate buffer).

Table 3: Fasting Plasma Glucose (FPG), Fasting Plasma Insulin (FPI) and HOMA-IR Values after Treatment.

Groups	FPG (mmol/l)	FPI (mU/l)	HOMA-IR
Group 1 (Negative control) n = 7	4.85 ± 1.12^{b}	3.90 ± 0.24^{b}	0.9 ± 0.2^{b}
Group 2 (Diabetic control) n = $6^{\#}$	14.50 ± 1.02^{a}	4.76 ± 0.28^{a}	3.1 ± 0.3^{a}
Group 3 (Met) n = 7	$11.90 \pm 0.86^{a\,b}$	3.60 ± 0.12^{b}	$1.9 \pm 0.1^{a\,b}$
Group 4 (Dia) n = 7	12.10 ± 2.31^{a}	3.75 ± 0.43^{b}	$2.0 \pm 0.4^{a\,b}$
Group 5 (Met + Dia) n = 7	3.88 ± 1.13^{b}	4.08 ± 0.19^{b}	0.7 ± 0.2^{b}
P-value	< 0.0001	< 0.0001	< 0.0001
F-value	70.60	16.62	93.58

n – Number of samples, Met – Metformin, Dia – Diawell, ^{a} – Significant difference versus negative control, ^{b} – Significant difference versus positive control. $^{\#}$- A rat died in the diabetic group in the course of the study

Table 3 shows results of FPG, FPI and HOMA-IR (insulin resistance) of the rats after treatment. The results show significantly lower ($p<0.05$) mean FPG levels in all groups, except group 4 (administered diawell) which was not significantly different ($p>0.05$), compared to the diabetic control. The results show significantly higher ($p<0.05$) FPG levels in Groups 3 (metformin), and 4 (diawell) when compared to the negative control. It however shows no significant differences ($p>0.05$) in FPG levels in Group 5 (metformin + diawell), compared to the negative control.

The diabetic control had significantly higher ($p<0.05$) FPI levels compared to the negative control and treatment groups. All the treatment groups showed no significant differences ($p>0.05$) in FPI levels when compared to the negative control.

The results reveal significantly higher ($p<0.05$) HOMA-IR values in the diabetic control compared to the negative control and treatment groups. Groups 3 (metformin) and 4 (diawell) had significantly higher ($p<0.05$) HOMA-IR values, whereas the combination in Group 5 (metformin + diawell) showed no significant difference ($p>0.05$) when compared to the negative control.

Table 4: Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Oxidative Stress Index (OSI) and Superoxide Dismutase (SOD) Levels after Treatment.

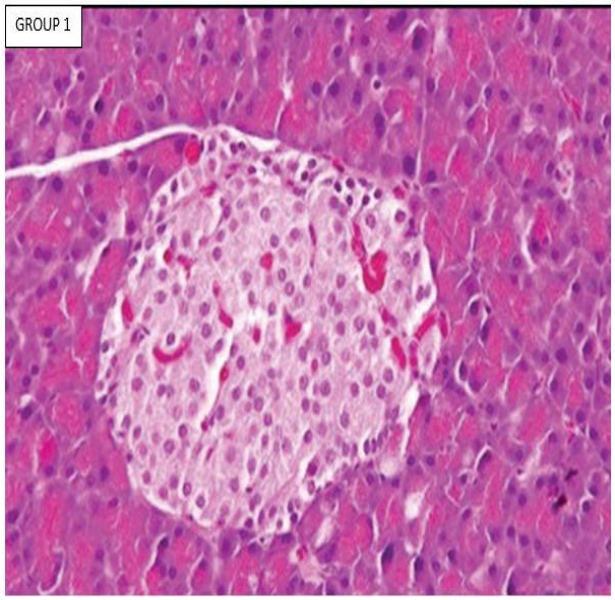
Groups	TOS (U/ml)	TAS (U/ml)	OSI	SOD (pg/ml)
Group 1 (Negative control) n = 7	1.61 ± 0.04^{b}	1.99 ± 0.06^{b}	0.81 ± 0.03^{b}	38.26 ± 2.191^{b}
Group 2 (Diabetic control) n = $6^{\#}$	2.55 ± 0.05^{a}	1.62 ± 0.05^{a}	1.58 ± 0.06^{a}	30.33 ± 1.94^{a}
Group 3 (Met) n = 7	$1.74 \pm 0.06^{a\,b}$	$1.40 \pm 0.07^{a\,b}$	$1.25 \pm 0.10^{a\,b}$	35.94 ± 1.55^{b}
Group 4 (Dia) n = 7	$1.76 \pm 0.07^{a\,b}$	$1.39 \pm 0.06^{a\,b}$	$1.27 \pm 0.07^{a\,b}$	33.15 ± 1.64^{a}
Group 5 (Met + Dia) n = 7	1.54 ± 0.08^{b}	1.62 ± 0.07^{a}	$0.95 \pm 0.08^{a\,b}$	35.33 ± 1.56^{b}
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F-value	259.1	104.0	114.6	16.88

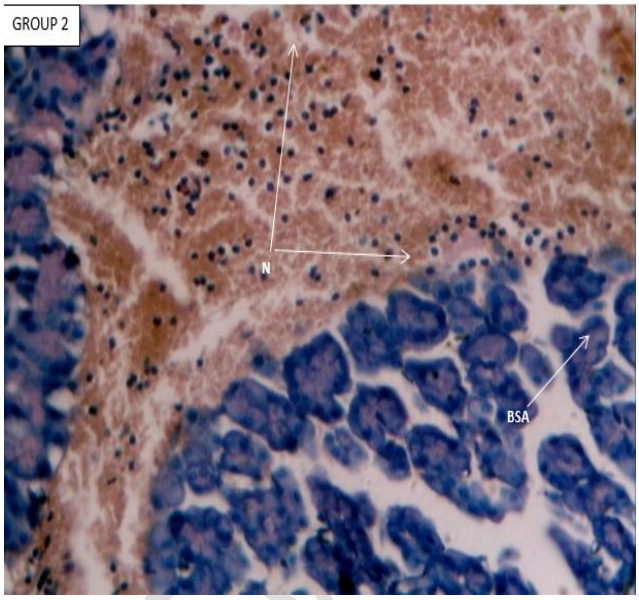
n – Number of samples. Met – Metformin, Dia – Diawell, ^{a} – Significant difference versus negative control, ^{b} – Significant difference versus positive control.

Table 4 shows the results of TOS, TAS, OSI and SOD levels of the rats after treatment. The results show significantly higher ($p<0.05$) TOS levels in the diabetic control compared to all the groups. Groups 3 (metformin) and 4 (diawell) had significantly higher ($p<0.05$) TOS levels compared to the negative control. There was however no significant difference ($p>0.05$) in TOS levels in the combination group (metformin + diawell), compared to the negative control.

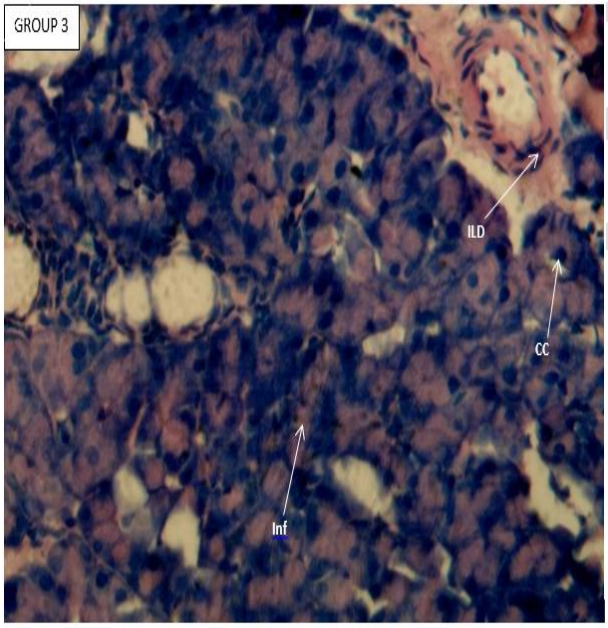
The results show significantly lower ($p<0.05$) TAS levels in the diabetic and treatment groups, compared to the negative control. OSI values were significantly lower ($p<0.05$) in all groups when compared to the diabetic control. Also, OSI values were significantly higher ($p<0.05$) in the treatment groups compared to the negative control.

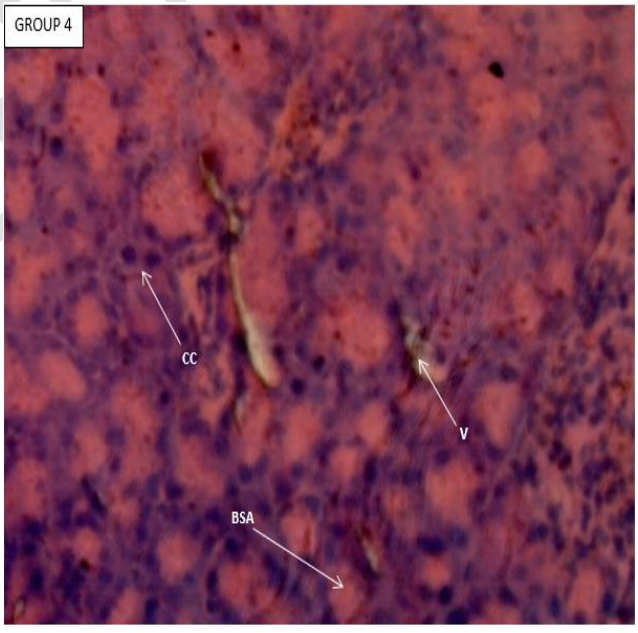
The results reveal significantly higher ($p<0.05$) SOD levels in all groups except Group 4 (diawell) which was not significantly different ($p>0.05$), when compared to the diabetic control. There were no significant differences ($p>0.05$) in SOD levels in the treatment groups, except Group 4 (diawell) which was significantly lower ($p<0.05$), compared to negative control.



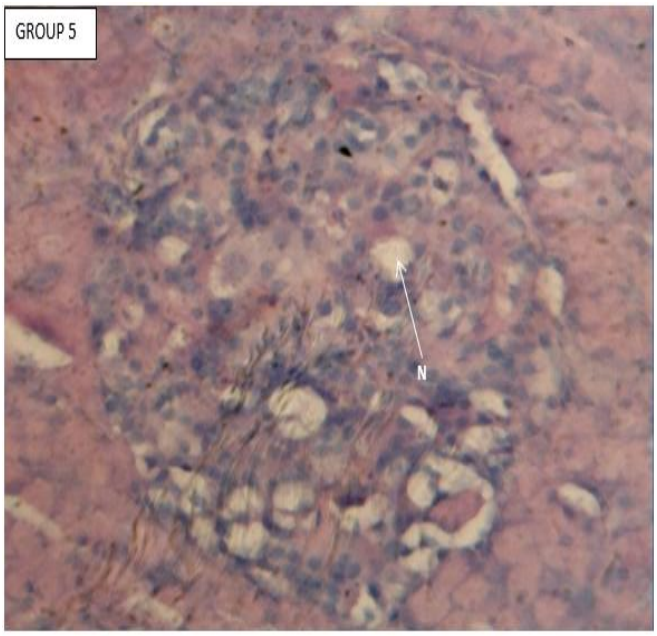


(a) (b)





(c) (d)



(e)

Figure 1: (a), (b), (c), (d) and (e): Photomicrograph (X 400) of H&E stained histologic sections of the pancreas of the rats. The negative control shows normal pancreatic islet structure with normal acini. The diabetic group pancreatic islet cells are disorganised, and show severe beta cell necrosis. There is degeneration of pancreatic islet cell and infiltration with inflammatory cells. The metformin treated group show moderate pancreatic islet hypoplasia and slight pancreatitis. The diawell treated group show severe hypoplasia and reduced number of islet cells. The combination (met + dia) group show moderate pancreatitis, mild beta cell necrosis and normal size islets.

4. DISCUSSION

Phytochemical analysis of the polyherbal drug diawell revealed the presence of alkaloids and flavonoids in variable amounts. Plant products have been shown to contain different bioactive phytochemicals or secondary metabolites which have nutritive value, but also possess the ability to affect several metabolic pathways and bring about drug-like responses. This forms the basis for their use and application in medicine [19,20].

Results from this study showed no significant differences ($p>0.05$) in fasting blood glucose levels in all the groups of rats prior to the administration of STZ. It however, showed significantly higher ($p<0.05$) fasting blood glucose levels in all groups that were induced with HFD/STZ, compared to the negative control. STZ selectively destroys pancreatic beta cells

bringing about insulin deficiency and hyperglycaemia. It has been used to produce different experimental models of animal diabetes [12]. The significant increase in fasting blood glucose levels in the rats could be attributed to the diabetogenic effects of streptozotocin, and this is in consonance with other methods of streptozotocin induction of diabetes [12]. The results agree with the works of Kaur *et al*. [19], in which high fat diet in combination with a sub-diabetic dose of streptozotocin (35mg/kg body wt), produced consistent hyperglycaemia in rats.

There was no significant difference ($p>0.05$) in FPG levels in the group administered the polyherbal drug diawell, compared to the diabetic control. The results also showed significantly higher ($p<0.05$) FPG levels in groups 3 (metformin), and 4 (diawell), when compared with the negative control. The results however revealed no significant differences ($p>0.05$) in FPG levels in the combination group (metformin + diawell) compared to the negative control. This shows the combination therapy was very effective in returning fasting plasma glucose levels to baseline control values. Administration of the herbal drug diawell alone had no impact on glucose levels, metformin was not so effective as a stand-alone drug, but had a better control of the glucose level when used in combination, indicating a synergistic interaction between the herbal drug diawell and metformin. Plant products and traditional medicines administered alone or in combination with conventional anti-diabetic drugs have been used in the management of diabetes and have shown different degree of efficacies both experimentally and in clinical trials. These phytochemicals act alone or in interaction with the orthodox drugs bringing about different glycemic responses as seen in the glucose levels. Lu *et al*. [21], and Skovso, [22] reported poor glycaemic control in the high fat diet/streptozotocin diabetes model treated with insulin sensitizing therapeutics. Similar research by Poonam *et al*. [23], reported that the combination therapy of garlic extract and metformin was more effective in reducing blood glucose levels, highlighting that garlic extract potentiates the hypoglycaemic effect of metformin. In another study, by Oluwayemi *et al*. [24], metformin in combination with the extract of *Vernonia amygdalina* significantly reduced plasma glucose levels in STZ-induced diabetic rats.

The diabetic control had significantly higher ($p<0.05$) fasting plasma insulin levels compared to the negative control and treatment groups. All the treatment groups showed no significant differences ($p>0.05$) in fasting insulin levels when compared to the negative control. This means the significant hyperinsulinaemia caused by the HFD/STZ induction in the diabetic rats, was

returned to normal fasting insulin levels by metformin, diawell and their combination in the treatment groups. The reduction in insulin levels by these treatments could be due to increased insulin sensitivity in the liver and peripheral tissues or by providing a sort of protection to pancreatic beta cells, preventing necrotic cell death and leakage of their contents caused by STZ. The results corroborates with the works of Reed *et al*. [25], and Skovso *et al*. [22] in which HFD/STZ induction produced hyperglycaemia, hyperinsulinaemia and established the HFD/STZ treatment as a protocol for inducing animal type 2 diabetes, having the pathological correlation of the human disease. The results are also in agreement with the works of Yoon *et al*. [26], and Gupta *et al*. [27] in which combined treatment with ginseng and metformin significantly improved plasma glucose and insulin levels, compared to their individual treatments.

The results revealed significantly lower ($p<0.05$) HOMA-IR values in the negative control and treatment groups as against the diabetic control. This shows the significant insulin resistance produced by HFD/STZ in the diabetic rats, was reduced by the administration of metformin, diawell and their combination. The results also showed significantly higher ($p<0.05$) HOMA-IR values in groups 3 (metformin), and 4 (diawell), when compared to the negative control. This indicates metformin, and diawell reduced insulin resistance, but not so effectively to normal control values. However, there was no significant difference in HOMA-IR values in the combination group (metformin and diawell), when compared to the negative control. Implying the combination treatment effectively reduced insulin resistance to normal control values, highlighting an additive drug-herb interaction in reducing insulin resistance. Zhang *et al*. [28] reported elevated HOMA-IR levels in HFD/STZ-induced diabetic rats. The treatment results are in consonance with the works of Hu *et al*. [29], in which they found significant improvement in HOMA-IR using a combination of ginseng and metformin, than the individual drugs used alone.

The findings in this study showed significantly lower ($p<0.05$) TOS levels in the negative control group and treatment groups, compared to the diabetic control. This shows the significantly elevated TOS levels caused by HFD/STZ, was reduced by the treatment with metformin, diawell, and their combination. The results also revealed significantly higher ($p<0.05$) TOS levels in groups 3 (metformin) and 4 (diawell), compared with the negative control. This implies administration of metformin and diawell separately as stand-alone drugs reduced the elevated TOS levels, but not to the normal control values. The results also revealed no significant

differences (P>0.05) in TOS levels in the combination group (metformin and diawell), compared to the negative control. The combination produced a better result than the individual treatments, showing possible additive effect.

The results showed significantly lower ($p<0.05$) TAS levels in the diabetic and treatment groups, compared to the negative control. This indicates none of the treatments could restore the depressed antioxidant status in the diabetic rats to normal control values.

The results revealed significantly lower ($p<0.05$) OSI in the negative control and the treatment groups, when compared to the diabetic control. Also, OSI values were significantly higher ($p<0.05$) in all treatment groups, when compared to the negative control. Meaning the treatments only just reduced oxidative stress, but not to normal control values. OSI which is a ratio of the TOS to the TAS, shows the interplay between reactive oxygen species (ROS) and other oxidants with the antioxidant defense system. The results show the type 2 diabetic rats had increased oxidative stress levels, and although metformin, diawell and the combination showed antioxidant potential, oxidative stress persisted.

Levels of the antioxidant enzyme SOD were significantly higher ($p<0.05$) in the negative control and treatment groups except group 4 (diawell), which was not significantly different ($p>0.05$), when compared to the diabetic control. There were no significant differences ($p>0.05$) in SOD levels in the treatment groups except group 4 (diawell), which was significantly lower ($p<0.05$), when compared to the negative control. The results imply type 2 DM may be associated with depressed SOD, as a result of increased oxidative stress. Administration of the polyherbal drug diawell did not have any effect on SOD levels. However treatment combinations of the polyherbal drug diawell and metformin were effective in returning SOD levels to normal control levels. This shows a synergistic drug-herb interaction between diawell and metformin showing better antioxidant potential, than when diawell was used alone. Diabetes mellitus and the ensuing hyperglycaemia is associated with increased production of ROS through a number of mechanisms, leading to increased oxidative stress [30]. Various herbs, herbal medicines and their constituent phytochemicals have shown the potential to be able to ameliorate diabetes and oxidative stress, either by directly scavenging ROS generated or by boosting the antioxidative defense mechanism in mopping up oxidant molecules [27]. The alteration in oxidative stress and

antioxidant parameters in this study, show an increased production of oxidants or ROS, which lead to depressed antioxidant defense mechanisms even in the treated rats. The results are in line with the works of Chen *et al*. [31], in which HFD/STZ induced diabetic rats had significantly reduced SOD and glutathione peroxidase (GPx) activities and elevated levels of thiobarbituric acid reactive substances (TBARS). The results are in consonance with the works of Gupta *et al*. [27], in which they reported that the combined effect of metformin and ethanol extract of *Scutellaria baicalensis* significantly increased the activity of hepatic antioxidant enzymes while reducing lipid peroxidation, compared to metformin treatment used alone in STZ-induced diabetic rats. The results corroborates with the findings of Asadi *et al*. [32], in which STZ-induced diabetic rats treated with metformin or curcumin had significantly lower TOS, compared to the untreated diabetic rats. In the same study, levels of the antioxidant enzymes SOD, GPx, and catalase (CAT) were significantly increased, while malondialdehyde (MDA) reduced in the kidneys of the diabetic rats treated with curcumin. In other studies, commercially sold polyherbal formulations like 5EPHF, Diabecon® and Glyoherb® significantly improved antioxidant status by increasing levels of antioxidant enzymes and minimizing diabetic complications [33,34].

The histological examination of the pancreas of the diabetic control showed disorganized islet of Langerhans, degenerative changes and beta cell necrosis, showing a reduced number of beta cells with inflammation. This could be due to the direct effect of STZ on the pancreas, leading to oxidative damage of beta cell proteins. The histologic analysis of the treatment groups showed minimal beta cell necrosis, slight hypoplasia and inflammation, with a nearly normal population of beta cells. The noticeable reduced injuries in the treated rats could be due to repression of further damage to the pancreas, healing and recovery of injured beta cells and prevention of beta cell death. The results corroborates with the works of Balamash *et al*. [35], in which the pancreas of the diabetic rats had several histopathological changes. Also, treatment with metformin, olive oil and their combination improved the histoarchitecture of the pancreas.

5. CONCLUSION

High fat diet in combination with 45mg/kg body weight of streptozotocin produced diabetes in the Wistar rats with significant hyperglycaemia, hyperinsulinaemia and insulin resistance. There was depletion of antioxidant parameters and an increase in oxidative stress. The pancreas of the diabetic rats showed histopathological changes which is attributed to the diabetogenic effects of

streptozotocin. Administration of metformin and the polyherbal tablet diawell individually, were not effective in correcting the pathological and biochemical changes associated with diabetes. However, the combination treatment produced a better glycemic response and attenuated the oxidant status in the diabetic rats. This study has established the need for antioxidant therapy in combination with hypoglycemic agents in the management of diabetes mellitus. Also, there should be proper evaluation of anti-diabetic herbal products before they make their way to the markets.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010; 33(1): 62-69.

2. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*. 2010; 87(1): 4-14.

3. Briggs, O. N., Brown, H., Elechi-amadi, K., Ezeiruaku, F., & Nduka, N. Superoxide dismutase and glutathione peroxidase levels in patients with long standing type 2 diabetes in Port Harcourt, Rivers State, Nigeria. *International Journal of Science and Research*. 2016; 5(3): 1282-1288.

4. Bashan, N., Kovsan, J., Kachko, I., Ovadia, H., & Rudich, A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiological Reviews*. 2009; 89: 27-71.

5. Krentz, J. A., & Bailey, C. J. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs*. 2005; 65(3): 385– 411.

6. Yang, W. C., Srinivas Nammi, S., Jeppesen, P. B., & Cho, W. C. S. Complementary and Alternative Medicine for Diabetes. *Evidence-Based Complementary and Alternative Medicine*. 2013; 2013: 831068.

7. Medagama, A. B., & Bandara, R. The use of Complementary and Alternative Medicines (CAMs) in the treatment of diabetes mellitus: is continued use safe and effective? *Nutrition Journal*. 2014; 13: 102.

8. Kumar, D., Bajaj, S., & Mehrotra, R. Knowledge, attitude and practice of complementary and alternative medicines for diabetes. *Public Health*. 2006; 120(8): 705–711.

9. Organisation for Economic Co-operation and Development. Guidance Document on Acute Oral Toxicity Testing: Environmental health and safety monograph series on testing and assessment No.24. 2001; 24. Accessed 14th July, 2018. Available: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd24.pdf.

10. Paget, G. E., & Barnes, J. M. *Evaluation of drug activities*. In Lawrence, D. R & Bacharach, A. L. (Eds.). Pharmacometrics (pp. 161). New York: Academy Press; 1964.

11. Breyer, M. D., Bottinger, E., Brosius, F. C., Coffman, T. M., Harris, R. C., Heilig, C. W., & Sharma, K. Mouse models of diabetic nephropathy. *Journals of the American Society of Nephrology*. 2005; 16: 27-45.

12. Furman, B. L. Streptozotocin-induced diabetic models in mice and rats. *Current Protocols in Pharmacology*. 2015; 70(5): 1-20.

13. Barham, D., & Trinder, P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*. 1972; 97(151): 142-145.

14. Engvall, E., & Perlmann, P. Enzyme-linked immunosorbent assay, elisa. *The Journal of Immunology*. 1972; 109(1): 129-135.

15. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985; 28(7): 412-419.

16. Erel, O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*. 2004; 37: 277-285.

17. Erel, O. A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*. 2005; 38: 1103-1111.

18. Ezeonu, C. S., & Ejikeme, C. M. Qualitative and quantitative determination of phytochemical contents of indigenous nigerian softwoods. *New Journal of Science*. 2016; 2016: 5601327. https://doi.org/10.1155/2016/5601327.

19. Kaur, R., Afzal, M., Kazmi, I., Ahamd, I., Ahmed, Z., Ali, B., Ahmad, S., & Anwar, F. Polypharmacy (herbal and synthetic drug combination): a novel approach in the treatment of type-2 diabetes and its complications in rats. *Journal of Natural Medicines*. 2013; 67(3): 662-671.

20. Van-Wyk, B. E., & Wink, M. *Phytomedicines, Herbal drugs and Poisons*. Briza, Kew Publishing, Cambridge University Press: Cambridge, UK; 2015.

21. Lu, H. E., Jian, C. H., Chen, S. F., Chen, T. M., Lee, S. T., Chang, C. S., & Weng, C. F. Hypoglycaemic effects of fermented mycelium of *Paecilomyces farinosus* (G30801) on high-fat fed rats with streptozotocin-induced diabetes. *Indian Journal of Medical Research*. 2010; 131: 696–701.

22. Skovso, S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *Journal of Diabetes Investigation*. 2014; 5: 349-358.

23. Poonam, T., Prakash, G. P., & Kumar, L. V. Effect of co-administration of *Allium sativum* extract and metformin on blood glucose of streptozotocin induced diabetic rats. *Journal of Intercultural* Ethnopharmacology. 2013; 2: 81–84.

24. Oluwayemi, A. T., Nwachuku, E. O., & Holy, B. Effects of the Interation of Metformin and *Vernonia amygdalina* (Bitter Leaf) On Steptozotocin-Induced Diabetic Rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2018; 1(2): 1-8.

25. Reed, M. J., Meszaros, K., Entes, L. J., Claypool, M. D., Pinkett, J. G., Gadbois, T. M., & Reaven, G. M. A new rat model of type 2 diabetes: The fat-fed, streptozotocin-treated rat. *Metabolism*. 2000; 49: 1390-1394.

26. Yoon, S. H., Han, E. J., Sung, J. H., & Chung, S. H. Anti-diabetic effects of compound K versus metformin versus compound K-metformin combination therapy in diabetic db/db mice. *Biological and Pharmaceutical Bulletin*. 2007; 30(11): 2196–2200.

27. Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K., & Roufogalis, B. D. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetology & Metabolic Syndrome*. 2017; 9(59): 1-12.

28. Zhang, Y., Hu, T., Zhou, H., Zhang, Y., Jin, G., & Yang, Y. Antidiabetic effect of polysaccharides from *Pleurotus ostreatus* in streptozotocin-induced diabetic rats. *International Journal of Biological Macromolecules*. 2016; 83: 126-132.

29. Hu, X., Cheng, D., & Zhang, Z. Antidiabetic activity of *Helicteres angustifolia* root. *Pharmaceutical Biology*. 2016; 54(6): 938-944.

30. Giacco, F., & Brownlee, M. Oxidative stress and diabetic complications. *Circulation Research*. 2010; 107(9): 1058-1070.

31. Chen, L. H., Chien, Y. W., Chang, M. L., Hou, C. C., Chan, C. H., Tang, H. W., & Huang, H. Y. Taiwanese green propolis ethanol extract delays the progression of type 2 diabetes mellitus in rats treated with streptozotocin/high-fat diet. *Nutrients*. 2018; 10: 503.

32. Asadi, S., Goodarzi, M. T., Karimi, J., Hashemnia, M., & Khodadadi, I. Does curcumin or metformin attenuate oxidative stress and diabetic nephropathy in rats? *Journal of Nephropathology*. 2019; 8(1): 8.

33. Lanjhiyana, S., Garabadu, D., Ahirwar, D., Rana, A. C., Ahirwar, B., & Lanjhiyana, S. K. Pharmacognostic standardization and hypoglycemic evaluations of novel polyherbal formulations. *Der Pharmacia Lettre*. 2011; 3(1): 319-333.

34. Maninder-Kaur, V. V. Diabetes and antidiabetic herbal formulations: an alternative to Allopathy. *International Journal of Pharmacognosy*. 2014; 1(10): 614-626.

35. Balamash, K. S., Alkreathy, H. M., Al-Gahdali, E. H., Khoja, S. O., & Ahmad, A. Comparative biochemical and histopathological studies on the efficacy of metformin and virgin olive oil against streptozotocin-induced diabetes in Sprague-Dawley rats. *Journal of Diabetes Research*. 2018; 20: 4692197.