

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, 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

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

45 Type 2 DM leads to the depletion of antioxidant parameters [3], with increased oxidative stress
46 levels resulting in oxidative damage of cellular components [4]. Current oral anti-diabetic agents
47 using orthodox medicine have limited efficacy and undesirable side effects in patients, leading to
48 the development of microvascular and macrovascular complications [5,6]. This has led to an
49 increase in the use of medicinal herbs in the management of type 2 DM [7]. These herbs or
50 herbal products contain phytonutrients which have the potential to affect several metabolic and
51 diabetic pathways, with the promise of better patient outcomes. Also, these agents seem to have
52 become an attractive option because of the lesser-perceived adverse reactions in comparison to
53 prescription medications [8]. This study evaluates the biochemical and oxidative changes in type
54 2 diabetic rats, treated with metformin and the polyherbal drug diawell.

55 **2. MATERIALS AND METHODS**

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

60 **2.1 Drugs**

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

65 **2.2 Acute Toxicity Study**

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

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72 **2.3 Dose Calculation**

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

75 Metformin

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

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

78 = 90mg/kg body wt/day.

79 Diawell

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

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

82 = 324mg/kg body wt/day.

83 **2.4 Study Design and Diabetes Induction**

84 The rats were weighed and grouped into 5 groups of 7 rats each. Group 1 (negative control) was
85 placed on a normal chow diet, while groups 2 to 5 were placed on high fat diet (HFD) with
86 42.1% fat content, 3 weeks prior to induction with streptozotocin (STZ). Diabetes was induced
87 by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body wt) dissolved in 0.1
88 M citrate buffer (pH 4.5), after a 6 hour fast. Diabetes was confirmed after 72 hours in the rats
89 having fasting blood glucose levels above 14mmol/L (250 mg/dl). Treatments (drugs) were
90 administered daily according to the groupings by means of oral gavage for 28 days.

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

92 **Group 2:** Diabetic control

93 **Group 3:** Diabetic rats treated with metformin.

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

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

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

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

117 **2.5 Statistical Analysis**

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

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3. RESULTS

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

Phytochemicals	Diawell	Concentration ($\mu\text{g}/\text{mg}$)
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 $\mu\text{g}/\text{mg}$ and 89.67 $\mu\text{g}/\text{mg}$ respectively. Other phytochemicals such as phenolic acids, saponins, cardiac glycosides, terpenoids, quinones, and tannins were not found.

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140 **Table 2: Fasting Blood Glucose (FBG) Levels of the rats before and after Induction with**
141 **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

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

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

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156 **Table 3: Fasting Plasma Glucose (FPG), Fasting Plasma Insulin (FPI) and HOMA-IR**
 157 **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 ± 0.86 ^{a,b}	3.60 ± 0.12 ^b	1.9 ± 0.1 ^{a,b}
Group 4 (Dia) n = 7	12.10 ± 2.31 ^a	3.75 ± 0.43 ^b	2.0 ± 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

158 n – Number of samples, Met – Metformin, Dia – Diawell, ^a – Significant difference versus
 159 negative control, ^b – Significant difference versus positive control. [#]- A rat died in the diabetic
 160 group in the course of the study
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162 Table 3 shows results of FPG, FPI and HOMA-IR (insulin resistance) of the rats after treatment.
 163 The results show significantly lower (p<0.05) mean FPG levels in all groups, except group 4
 164 (administered diawell) which was not significantly different (p>0.05), compared to the diabetic
 165 control. The results show significantly higher (p<0.05) FPG levels in Groups 3 (metformin), and
 166 4 (diawell) when compared to the negative control. It however shows no significant differences
 167 (p>0.05) in FPG levels in Group 5 (metformin + diawell), compared to the negative control.

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

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

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

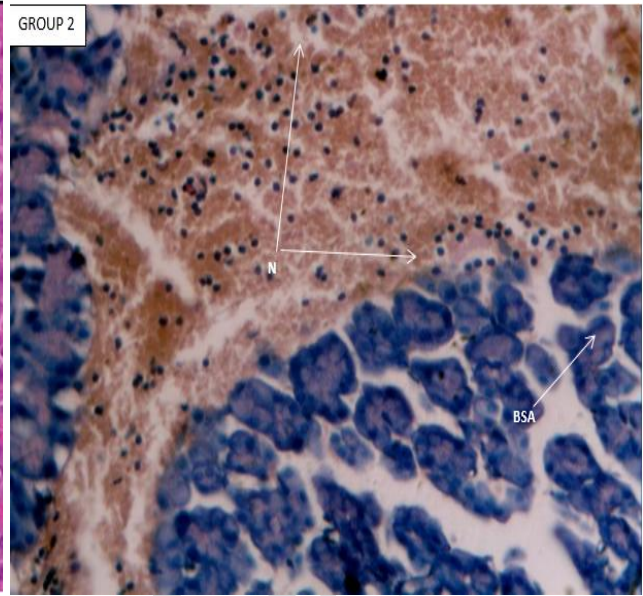
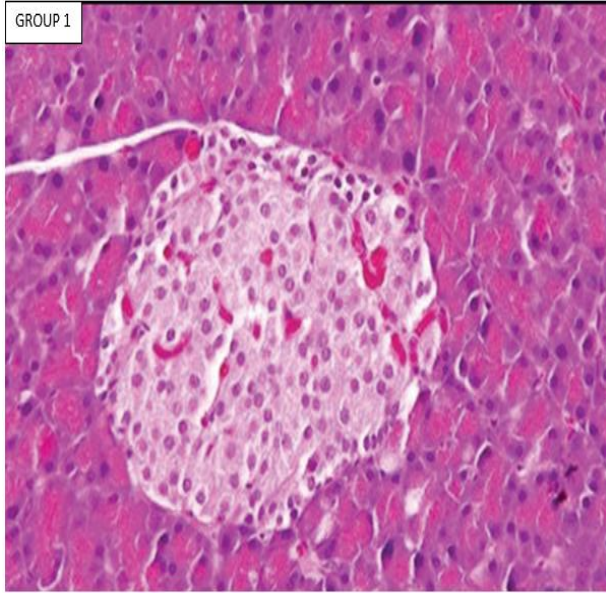
Groups	TOS (U/ml)	TAS (U/ml)	OSI	SOD (pg/ml)
Group 1 (Negative control) n = 7	1.61 ± 0.04 ^b	1.99 ± 0.06 ^b	0.81 ± 0.03 ^b	38.26 ± 2.191 ^b
Group 2 (Diabetic control) n = 6 [#]	2.55 ± 0.05 ^a	1.62 ± 0.05 ^a	1.58 ± 0.06 ^a	30.33 ± 1.94 ^a
Group 3 (Met) n = 7	1.74 ± 0.06 ^{a,b}	1.40 ± 0.07 ^{a,b}	1.25 ± 0.10 ^{a,b}	35.94 ± 1.55 ^b
Group 4 (Dia) n = 7	1.76 ± 0.07 ^{a,b}	1.39 ± 0.06 ^{a,b}	1.27 ± 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 ± 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

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

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

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

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

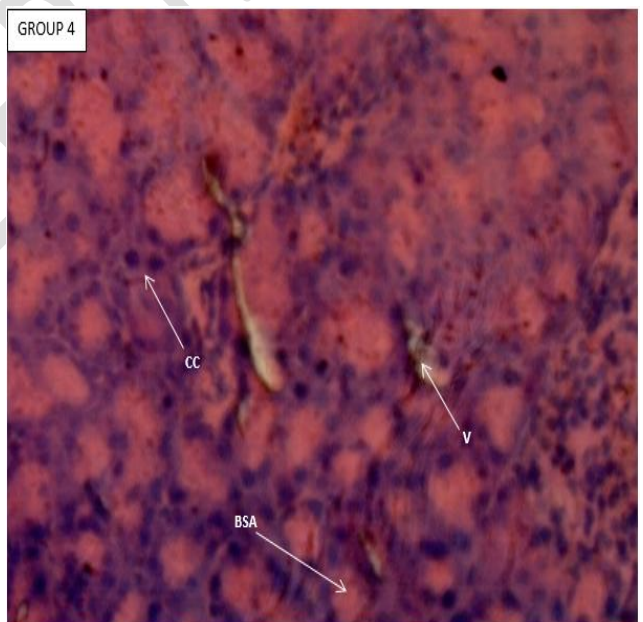
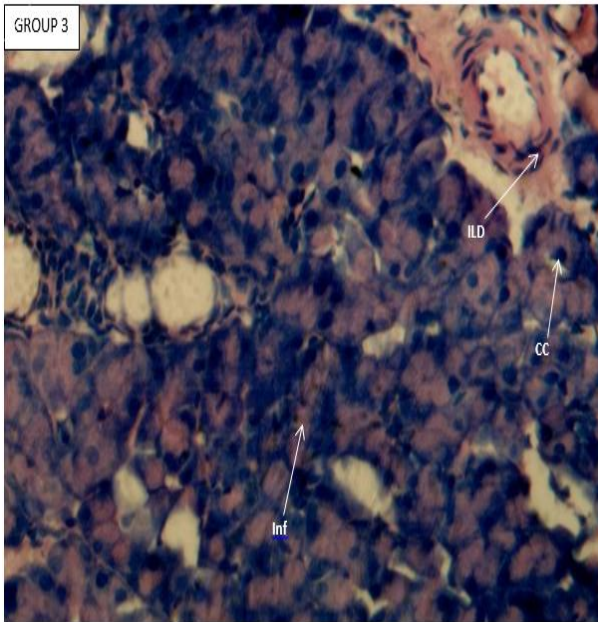


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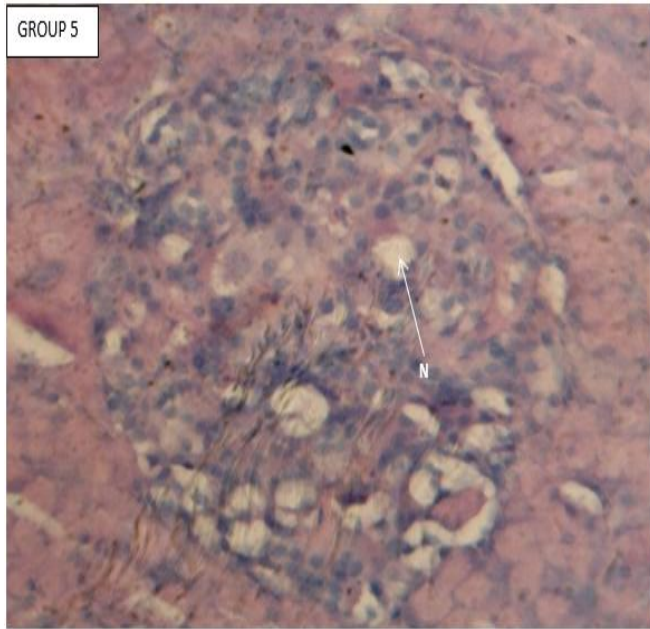


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201 **Figure 1:** (a), (b), (c), (d) and (e): Photomicrograph (X 400) of H&E stained histologic sections
202 of the pancreas of the rats. The negative control shows normal pancreatic islet structure with
203 normal acini. The diabetic group have pancreatic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

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210 4. DISCUSSION

211 Phytochemical analysis of the polyherbal drug diawell revealed the presence of alkaloids and
212 flavonoids in variable amounts. Plant products have been shown to contain different bioactive
213 phytochemicals or secondary metabolites which have nutritive value, but also possess the ability
214 to affect several metabolic pathways and bring about drug-like responses. This forms the basis
215 for their use and application in medicine [13,14].

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

220 bringing about insulin deficiency and hyperglycaemia. It has been used severally to produce
221 different experimental models of animal diabetes [12]. The significant increase in fasting blood
222 glucose levels in the rats could be attributed to the diabetogenic effects of streptozotocin, and this
223 is in consonance with other methods of streptozotocin induction of diabetes [12]. The results
224 agree with the works of Kaur *et al.* [13], in which high fat diet in combination with a sub-
225 diabetic dose of streptozotocin (35mg/kg body wt), produced consistent hyperglycaemia in rats.

226 There was no significant difference ($p>0.05$) in FPG levels in the group administered the
227 polyherbal drug diawell, compared to the diabetic control. The results also showed significantly
228 higher ($p<0.05$) FPG levels in groups 3 (metformin), and 4 (diawell), when compared with the
229 negative control. The results however revealed no significant differences ($p>0.05$) in FPG levels
230 in the combination group (metformin + diawell) compared to the negative control. This shows
231 the combination therapy was very effective in returning fasting plasma glucose levels to baseline
232 control values. Administration of the herbal drug diawell alone had no impact on glucose levels,
233 metformin was not so effective as a stand-alone drug, but had a better control of the glucose level
234 when used in combination, indicating a synergistic interaction between the herbal drug diawell
235 and metformin. Plant products and traditional medicines administered alone or in combination
236 with conventional anti-diabetic drugs have been used in the management of diabetes and have
237 shown different degree of efficacies both experimentally and in clinical trials. These
238 phytochemicals act alone or in interaction with the orthodox drugs bringing about different
239 glycemic responses as seen in the glucose levels. Lu *et al.* [15], and Skovso, [16] reported poor
240 glycaemic control in the high fat diet/streptozotocin diabetes model treated with insulin
241 sensitizing therapeutics. Similar research by Poonam *et al.* [17], reported that the combination
242 therapy of garlic extract and metformin was more effective in reducing blood glucose levels,
243 highlighting that garlic extract potentiates the hypoglycaemic effect of metformin. In another
244 study, by Oluwayemi *et al.* [18], metformin in combination with the extract of *Vernonia*
245 *amygdalina* significantly reduced plasma glucose levels in STZ-induced diabetic rats.

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

250 returned to normal fasting insulin levels by metformin, diawell and their combination in the
251 treatment groups. The reduction in insulin levels by these treatments could be due to increased
252 insulin sensitivity in the liver and peripheral tissues or by providing a sort of protection to
253 pancreatic beta cells, preventing necrotic cell death and leakage of their contents caused by STZ.
254 The results corroborates with the works of Reed *et al.* [19], and Skovso *et al.* [16] in which
255 HFD/STZ induction produced hyperglycaemia, hyperinsulinaemia and established the HFD/STZ
256 treatment as a protocol for inducing animal type 2 diabetes, having the pathological correlation
257 of the human disease. The results are also in agreement with the works of Yoon *et al.* [20], and
258 Gupta *et al.* [21] in which combined treatment with ginseng and metformin significantly
259 improved plasma glucose and insulin levels, compared to their individual treatments.

260 The results revealed significantly lower ($p<0.05$) HOMA-IR values in the negative control and
261 treatment groups as against the diabetic control. This shows the significant insulin resistance
262 produced by HFD/STZ in the diabetic rats, was reduced by the administration of metformin,
263 diawell and their combination. The results also showed significantly higher ($p<0.05$) HOMA-IR
264 values in groups 3 (metformin), and 4 (diawell), when compared to the negative control. This
265 indicates metformin, and diawell reduced insulin resistance, but not so effectively to normal
266 control values. However, there was no significant difference in HOMA-IR values in the
267 combination group (metformin and diawell), when compared to the negative control. Implying
268 the combination treatment effectively reduced insulin resistance to normal control values,
269 highlighting an additive drug-herb interaction in reducing insulin resistance. Zhang *et al.* [22]
270 reported elevated HOMA-IR levels in HFD/STZ-induced diabetic rats. The treatment results are
271 in consonance with the works of Hu *et al.* [23], in which they found significant improvement in
272 HOMA-IR using a combination of ginseng and metformin, than the individual drugs used alone.

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

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

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

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

294 Levels of the antioxidant enzyme SOD were significantly higher ($p<0.05$) in the negative control
295 and treatment groups except group 4 (diawell), which was not significantly different ($p>0.05$),
296 when compared to the diabetic control. There were no significant differences ($p>0.05$) in SOD
297 levels in the treatment groups except group 4 (diawell), which was significantly lower ($p<0.05$),
298 when compared to the negative control. The results imply type 2 DM may be associated with
299 depressed SOD, as a result of increased oxidative stress. Administration of the polyherbal drug
300 diawell did not have any effect on SOD levels. However treatment combinations of the
301 polyherbal drug diawell and metformin were effective in returning SOD levels to normal control
302 levels. This shows a synergistic drug-herb interaction between diawell and metformin showing
303 better antioxidant potential, than when diawell was used alone. Diabetes mellitus and the ensuing
304 hyperglycaemia is associated with increased production of ROS through a number of
305 mechanisms, leading to increased oxidative stress [24]. Various herbs, herbal medicines and their
306 constituent phytochemicals have shown the potential to be able to ameliorate diabetes and
307 oxidative stress, either by directly scavenging ROS generated or by boosting the antioxidative
308 defense mechanism in mopping up oxidant molecules [21]. The alteration in oxidative stress and

309 antioxidant parameters in this study, show an increased production of oxidants or ROS, which
310 lead to depressed antioxidant defence mechanisms even in the treated rats. The results are in line
311 with the works of Chen *et al.* [25], in which HFD/STZ induced diabetic rats had significantly
312 reduced SOD and glutathione peroxidase (GPx) activities and elevated levels of thiobarbituric
313 acid reactive substances (TBARS). The results are in consonance with the works of Gupta *et al.*
314 [21], in which they reported that the combined effect of metformin and ethanol extract of
315 *Scutellaria baicalensis* significantly increased the activity of hepatic antioxidant enzymes while
316 reducing lipid peroxidation, compared to metformin treatment used alone in STZ-induced
317 diabetic rats. The results corroborates with the findings of Asadi *et al.* [26], in which STZ-
318 induced diabetic rats treated with metformin or curcumin had significantly lower TOS, compared
319 to the untreated diabetic rats. In the same study, levels of the antioxidant enzymes SOD, GPx,
320 and catalase (CAT) were significantly increased, while malondialdehyde (MDA) reduced in the
321 kidneys of the diabetic rats treated with curcumin. In other studies, commercially sold polyherbal
322 formulations like 5EPHF, Diabecon® and Glyoherb® significantly improved antioxidant status
323 by increasing levels of antioxidant enzymes and minimizing diabetic complications [27,28].

324 The histological examination of the pancreas of the diabetic control showed disorganized islet of
325 Langerhans, degenerative changes and beta cell necrosis, showing a reduced number of beta cells
326 with inflammatory cells. This could be due to the direct effect of STZ on the pancreas, leading to
327 oxidative damage of beta cell proteins. The histologic analysis of the treatment groups showed
328 minimal beta cell necrosis, slight hypoplasia and inflammation, with a nearly normal population
329 of beta cells. The noticeable reduced injuries in the treated rats could be due to repression of
330 further damage to the pancreas, healing and recovery of injured beta cells and prevention of beta
331 cell death. The results corroborates with the works of Balamash *et al.* [29], in which the pancreas
332 of the diabetic rats had several histopathological changes. Also, treatment with metformin, olive
333 oil and their combination improved the histoarchitecture of the pancreas.

334 **5. CONCLUSION**

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

339 streptozotocin. Administration of metformin and the polyherbal tablet diawell individually, were
340 not effective in correcting the pathological and biochemical changes associated with diabetes.
341 However, the combination treatment produced a better glycemic response and attenuated the
342 oxidant status in the diabetic rats. This study establishes a basis for the need of
343 antioxidant therapy in combination with hypoglycemic agents in the management
344 of diabetes mellitus, and the proper evaluation of anti-diabetic herbal
345 products before they make their way to the markets.

346 **Conflict of Interests**

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

348

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