Antidiabetic and Antioxidant Effects of the Polyherbal Drug Glucoblock and Glibenclamide in Type 2 Diabetic Rats.

ABSTRACT

The increased prevalence of diabetes has broadened the search for a more acceptable therapy that reduces the disease burden on patients, as conventional medications are associated with one or more complications. This has led to an increase in the use of herbal remedies and combination therapies to reduce this burden.

Aim: This study evaluates the antidiabetic and antioxidant effects of the polyherbal capsule glucoblock and the combination with glibenclamide in high fat diet/streptozotocin-induced diabetic rats.

Methodolody: 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 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 capsule.

Results: Mean FPG levels were significantly lower ($p<0.05$) in all groups, compared to the diabetic control. Mean FPG levels was significantly higher ($p<0.05$) in the combination group, but showed no significant difference ($p>0.05$) in the glibenclamide group, and glucoblock 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 combination group had significantly higher ($p<0.05$) HOMA-IR values, whereas the individual treatment groups 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 treatment groups showed no significant difference ($p>0.05$) in TOS, 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. SOD was significantly higher ($p<0.05$) in the diabetic control compared to the negative control and treatment groups. The treatment groups showed no significant difference ($p>0.05$) in SOD levels, compared to the negative control.

Conclusion: Increase in total oxidant status and oxidative stress depleted antioxidant parameters. The polyherbal capsule glucoblock was effective when used alone and produced equipotent effect to the treatment with glibenclamide. However, the combination treatment did not fare better. Antioxidant therapy should be used together with antidiabetics in the management of diabetes, and care should be taken in the use herb-drug combinations.

Keywords: Diabetes mellitus, oxidative stress, Antioxidants, Herbal therapy, High fat diet, Glibenclamide, Streptozotocin.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most important diseases worldwide, reaching epidemic levels, with an ever increasing incidence and prevalence [1]. Type 2 DM is a heterogeneous disorder characterized by peripheral insulin resistance, impaired regulation of hepatic glucose

42 synthesis, and declining beta-cell function, ultimately leading to beta-cell failure [2, 3].
43 Hyperglycaemia increases oxidative stress, which contributes to the impairment of the main
44 processes that fail during diabetes, that is, insulin action and insulin secretion. Also, anti-
45 oxidative mechanisms become depleted in diabetes, which could further increase oxidative stress
46 [4, 5]. Oxidative stress induced by hyperglycaemia plays a critical role in the development of
47 diabetic complications. Furthermore, the development and progression of the damage is
48 proportional to hyperglycaemia, thus making the reduction of blood glucose levels the most
49 important goal in preventing complications and treating DM [6].

50 Over the years, herbal therapy has offered an alternative to orthodox medicine with lesser-
51 perceived adverse reactions [7], leading to an increased worldwide trend in the use of
52 complementary and alternative medicine (CAM) [8]. This study evaluates the antidiabetic and
53 antioxidant effects of the polyherbal drug glucoblock and the combination with glibenclamide in
54 high fat diet/streptozotocin-induced diabetic rats.

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 glucoblock, a polyherbal drug manufactured by Green World
62 Group, Michigan, USA, and commercially sold in Nigeria as an anti-diabetic capsule.
63 Glibenclamide, a sulfonylureas was manufactured by Glanil Pharmaceuticals, Nigeria.

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66 **2.2 Acute Toxicity Study**

67 This was done by the fixed dose procedure [9], using a group of 3 rats. 2000mg/kg body weight
68 of glucoblock was orally administered to each of the rats. The rats were then observed for signs
69 of toxicity for 48 hours. After observation for 48 hours, there were no observed signs of toxicity,

70 hence the herbal drug glucoblock was deemed safe up to 2000mg/kg body weight dose.
71 Glibenclamide is a standard antidiabetic drug.

2.3 Dose Calculation

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

Glibenclamide

76 Human daily dose is 1 caplet (5mg) twice daily, that is, 10mg/day.

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

78 $\qquad$ = 0.9mg/kg body weight/day.

Glucoblock

80 Human daily dose is 2 capsules (500mg each) once daily, that is, 1000mg/day.

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

82 $\qquad$ = 90mg/kg body weight/day.

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) having
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
88 0.1 M citrate buffer (pH 4.5), after a 6 hour fast. Diabetes was confirmed after 72 hours in the
89 rats having fasting blood glucose levels above 14mmol/L (250 mg/dl) [11]. Treatments (drugs)
90 were 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 glibenclamide.

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

Group 5: Diabetic rats treated with a combination of glibenclamide and glucoblock.

On the 29th day, the rats were fasted for 6 hours, anaesthetized with chloroform and sacrificed. Blood samples were collected by 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 [12, 13]. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.5 Reagents and Biochemical Determinations

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 gotten from Sigma-Aldrich, USA. Fasting plasma glucose (FPG) was determined using the Glucose oxidase method as described by Randox Laboratories Limited (UK). Fasting plasma insulin (FPI) and Superoxide dismutase (SOD) levels were quantitatively determined by using a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method as described by Elabscience Biotechnology Company Limited (China). Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method. Total oxidant status (TOS) and total antioxidant status (TAS) were determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method 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.

2.6 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

121 as Post hoc. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$).

122 Values are expressed as Mean ± SD.

123 **3. RESULTS**

124 **Table 1: Qualitative and Quantitative Phytochemical Analysis of the Herbal Drug**
125 **Glucoblock**

Phytochemicals	Glucoblock	Concentration (μg/mg)
Alkaloids	**+ve**	100.31
Flavonoids	**+ve**	131.45
Cardiac glycosides	**+ve**	55.93
Phenols	**-ve**	
Phlobatanins	**-ve**	
Saponins	**+ve**	61.47
Tanins	**-ve**	
Terpenoids	**-ve**	
Quinones	**-ve**	

126 +ve – Present, -ve – Not present

127 Table 1 shows alkaloids, flavonoids, cardiac glycosides and saponins present in the herbal drug
128 glucoblock, with concentrations of 100.31μg/mg, and 131.45μg/mg, 55.93μg/mg and
129 61.47μg/mg respectively. Other phytochemicals such as phenolic acids, terpenoids, quinones,
130 and tannins were not found.

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132 **Table 2: Fasting Blood Glucose (FBG) Levels of the rats before and after Induction with**
133 **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	$19.88 \pm 6.48*$
Group 3 n=7	5.82 ± 0.66	$18.38 \pm 6.77*$
Group 4 n=7	6.12 ± 0.63	$19.65 \pm 7.30*$
Group 5 n=7	6.12 ± 0.67	$21.90 \pm 6.86*$
P-value	0.8245	0.0008
F-value	0.3746	6.677

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

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

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146 **Table 3: Fasting Plasma Glucose (FPG), Fasting Plasma Insulin (FPI) and HOMA-IR**
147 **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 (Gli) n = 7	5.13 ± 1.12^b	3.81 ± 0.23^b	0.9 ± 0.2^b
Group 4 (Gluco) n = 7	4.90 ± 0.78^b	3.67 ± 0.59^b	0.8 ± 0.2^b
Group 5 (Gli + Gluco) n = 7	$8.90 \pm 1.09^{a\,b}$	3.87 ± 0.22^b	$1.5 \pm 0.3^{a\,b}$
P-value	< 0.0001	< 0.0001	< 0.0001
F-value	98.74	9.71	121.4

148 n – Number of samples, Gli – Glibenclamide, Gluco - Glucoblock, [a] – Significant difference
149 versus negative control, [b] – Significant difference versus positive control. [#] - A rat died in the
150 diabetic group in the course of the study.

151

152 Table 3 shows results of FPG, FPI and HOMA-IR (insulin resistance) of the rats after treatment.
153 The results show significantly lower ($p<0.05$) mean FPG levels in the negative control and
154 treatment groups, compared to the diabetic control. Mean FPG level was significantly higher
155 ($p<0.05$) in the combination group (glibenclamide + glucoblock), when compared to the negative
156 control. There was however no significant difference ($p>0.05$) in FPG levels in the
157 glibenclamide group and glucoblock group, compared to the negative control.
158 The diabetic control had significantly higher ($p<0.05$) FPI levels compared to the negative
159 control and treatment groups. Also, the treatment groups showed no significant differences
160 ($p>0.05$) in FPI levels when compared to the negative control.
161 The results reveal significantly higher ($p<0.05$) HOMA-IR values in the diabetic control
162 compared to the negative control and treatment groups. HOMA-IR was significantly higher
163 ($p<0.05$) in the combination group (glibenclamide + glucoblock), when compared to the negative
164 control. There was however, no significant difference ($p>0.05$) in HOMA-IR in the
165 glibenclamide group and glucoblock group, compared to the negative control.

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167 **Table 4: Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Oxidative Stress**
168 **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 ± 2.19^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 (Gli) n = 7	1.62 ± 0.07^{b}	1.77 ± 0.07^{ab}	0.92 ± 0.05^{ab}	37.42 ± 1.65^{b}
Group 4 (Gluco) n = 7	1.54 ± 0.05^{b}	1.57 ± 0.06^{a}	0.99 ± 0.03^{ab}	37.89 ± 1.81^{b}
Group 5 (Gli + Gluco) n = 7	1.69 ± 0.04^{b}	1.54 ± 0.06^{a}	1.10 ± 0.04^{ab}	35.39 ± 0.95^{b}
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F-value	432.2	55.77	253.7	12.63

169 n – Number of samples. Gli – Glibenclamide, Gluco – Glucoblock, [a] – Significant difference
170 versus negative control, [b] – Significant difference versus positive control. [#] - A rat died in the
171 diabetic group in the course of the study.
172
173 Table 4 shows the results of TOS, TAS, OSI and SOD levels of the rats after treatment. The
174 results show significantly higher ($p<0.05$) TOS levels in the diabetic control, compared to
175 negative control and treatment groups. The results also revealed no significant differences
176 ($p>0.05$) in TOS levels in the treatment groups, compared to the negative control.

177 The results show significantly lower ($p<0.05$) TAS levels in the diabetic control and treatment
178 groups, compared to the negative control. There were no significant differences ($p>0.05$) in TAS
179 levels in the glucoblock group and the combination group (Gli + Gluco), compared against the
180 diabetic control. However, TAS levels in the glibenclamide treated group was significantly
181 higher ($p<0.05$) than the diabetic control.

182 The results reveal significantly lower ($p<0.05$) OSI levels in the negative control and treatment
183 groups compared to the diabetic control. OSI levels in the treatment groups were also
184 significantly higher ($p<0.05$), compared to the negative control.
185 There were significantly higher ($p<0.05$) SOD levels in the diabetic control, compared to
186 negative control and treatment groups. The results also revealed no significant differences
187 ($p>0.05$) in SOD levels in the treatment groups, compared to the negative control.
188

189 **4. DISCUSSION**
190 Phytochemical analysis of the polyherbal drug glucoblock revealed the presence of bioactive
191 phytochemicals like alkaloids, flavonoids, cardiac glycosides, and saponins in variable amounts,

192 which could have contributed to the changes in the biochemical and oxidative parameters
193 analyzed.

194 The phytochemicals can exert their biological action by modulating molecular targets like
195 enzymes, ion channels etc, to bring about structural and physiological changes, and are thus used
196 in evidence-based medicine [14].

197 The results showed no significant differences (p>0.05) in fasting blood sugar levels in all the
198 groups of rats prior to the administration of STZ. It however, showed significantly higher
199 (p<0.05) fasting blood levels in all groups that were induced with HFD/STZ, compared to the
200 negative control. STZ is selectively accumulated in pancreatic beta cells via the low-affinity
201 GLUT2 glucose transporter in the plasma membrane, is cytotoxic and leads to the degeneration
202 of the islets of Langerhans of the beta cells, giving rise to symptoms of diabetes [15, 16]. It is
203 used severally to produce different experimental models of animal diabetes [13]. The results
204 agree with the works of Kaur *et al*. [17], in which high fat diet in combination with a sub-
205 diabetic dose of streptozotocin (35mg/kg body wt.), produced consistent hyperglycaemia in rats.

206 There were significant improvements in fasting plasma glucose levels in the rats after 28 days of
207 treatment, as the results showed significantly lower (p<0.05) fasting plasma glucose levels in the
208 treatment groups, compared to the diabetic control. There were no significant differences
209 (p>0.05) in fasting plasma glucose levels in the glibenclamide treated group (Group 3) and the
210 glucoblock treated group (Group 4), compared to the negative control, indicating glibenclamide
211 and glucoblock used separately, were equally very effective in returning fasting plasma glucose
212 levels to baseline control values. However, the combination group of glibenclamide and
213 glucoblock had significantly higher (p<0.05) fasting plasma glucose levels, compared to the
214 negative control. This implies that the combination did reduce the elevated glucose levels, but
215 not to baseline control levels, and not as effective as the individual treatments.

216 Orthodox medicines administered alone or in combination with plant products are used in the
217 management of diabetes and have shown different degree of efficacies both experimentally and
218 in clinical trials. These phytochemicals act alone or in interaction with the orthodox drugs
219 bringing about different glycemic responses as seen in the glucose levels. The results are in
220 agreement with the works of Shokoohi *et al*. [18], in which a herbal combination capsule
221 significantly decreased fasting blood glucose levels in diabetics. Al-Omaria *et al*. [19] reported

222   that a concurrent treatment of ginger and glibenclamide significantly reduced blood glucose
223   levels, compared to when glibenclamide was used alone in STZ-induced diabetic rats.

224   The diabetic control had significantly higher ($p < 0.05$) fasting plasma insulin levels compared to
225   the negative control and treatment groups. Also, the treatment groups showed no significant
226   differences ($p > 0.05$) in fasting plasma insulin levels when compared to the negative control. The
227   results indicate the significant hyperinsulinaemia caused by the HFD/STZ induction in the
228   diabetic rats, was returned to normal fasting insulin levels by the treatments with glibenclamide,
229   glucoblock, and their combination in the treatment groups. The reduction in insulin levels by
230   these treatments could be as result of increasing insulin sensitivity in the liver and peripheral
231   tissues or by providing a sort of protection to pancreatic beta cells, preventing necrotic cell death
232   and leakage of their contents caused by STZ. The results are in consonance works of Reed *et al*.
233   [20], and Skovso *et al*. [21] in which HFD/STZ induction produced hyperglycaemia and
234   hyperinsulinaemia. The results are also in agreement with the works of Ali *et al*. [22], in which
235   treatment with glibenclamide and the methanolic extract of *Garcinia pedunculata* (GP) fruit,
236   restored insulin levels in STZ-induced diabetic rats.

237   The results showed significantly lower ($p < 0.05$) HOMA-IR values in the treatment groups
238   compared to the diabetic control. There were no significant differences ($p > 0.05$) in HOMA-IR
239   values in the glibenclamide treated group (Group 3) and the glucoblock treated group (Group 4),
240   compared to the negative control, indicating glibenclamide and glucoblock used separately were
241   equipotent and very effective in returning HOMA-IR values to baseline control values. However,
242   the combination group of glibenclamide and glucoblock had significantly higher ($p < 0.05$)
243   HOMA-IR values compared to the negative control. This indicates the combination did reduce
244   insulin resistance in the rats, but not to baseline control levels, and not as effective as the
245   individual treatments. The results corroborates with the works of Reed *et al*. [20], and Skovso *et*
246   *al*. [21] in which HFD/STZ induction produced hyperglycaemia, hyperinsulinaemia, significant
247   insulin resistance and established the HFD/STZ treatment as a protocol for inducing animal type
248   2 diabetes, having the pathological correlation of the human disease. In a randomized control
249   clinical study, the polyherbal drug, green cumin capsule was found to significantly increase
250   insulin sensitivity [23]. In a similar study, mulberry leaf and glibenclamide significantly reduced

251 HOMA-IR, increased insulin sensitivity (HOMA-IS) and beta-cell function (HOMA-β) in STZ-
252 induced diabetic rats [24].

253 The findings in this study showed significantly lower ($p<0.05$) TOS values in the negative
254 control group and treatment groups, compared to the diabetic control. This shows the
255 significantly elevated TOS levels caused by HFD/STZ induction, was reduced by the treatment
256 with glucoblock, glibenclamide, and their combination. Also, the treatment groups showed no
257 significant differences ($p>0.05$) in TOS when compared to the negative control.

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

261 The results revealed significantly lower ($p<0.05$) OSI in the negative control and the treatment
262 groups, when compared to the diabetic control. Also, OSI values were significantly higher
263 ($p<0.05$) in all treatment groups, when compared to the negative control. This means the
264 treatments only just reduced oxidative stress, but not to normal control values. OSI is a ratio of
265 the TOS to the TAS, and shows the interplay between reactive oxygen species (ROS) and other
266 oxidants with the antioxidant defense system. The results show the diabetic rats had increased
267 oxidative stress levels, and although the treatments glibenclamide, glucoblock and the
268 combination showed antioxidant potential, oxidative stress persisted.

269 SOD levels were significantly higher ($p<0.05$) in the negative control and treatment groups,
270 compared to the diabetic control. There were no significant differences ($p>0.05$) in SOD levels in
271 the treatment groups, compared to the negative control. The results indicate type 2 DM is
272 associated with depressed SOD, which could be due to increased oxidative stress levels.
273 However, treatment with glibenclamide, glucoblock and the combination was effective in
274 returning SOD levels to normal control levels.

275 Hyperglycaemia in diabetes is associated with excessive production of free radicals through a
276 number of mechanisms, leading to increased oxidative stress [6]. Herbal medicines and their
277 constituent phytochemicals have shown the potential to be able to ameliorate diabetes and
278 oxidative stress, either by directly scavenging free radical species or by boosting the antioxidant

279   defense mechanism [25]. The alteration in oxidative stress and antioxidant parameters in this
280   study, show an increased production of free radicals or ROS, which lead to depressed antioxidant
281   defence mechanisms even in the treated rats. The results are in line with the work of Asadi *et al.*
282   [26], in which TOS and malondialdehyde (MDA) were significantly increased in STZ-induced
283   diabetic rats. Activities of the antioxidant enzymes SOD and glutathione peroxidase (GPx), were
284   also decreased in the diabetic rats, pointing to an increase in oxidative stress levels. The activities
285   of the antioxidant enzymes SOD, GPx, catalase (CAT) and levels of reduced glutathione (GSH)
286   were found to be increased in liver and kidney tissues of diabetic rats treated with glibenclamide
287   and/or mangiferin. Levels of thiobarbituric acid reactive substances (TBARS) were also
288   significantly reduced in the kidney and liver of the treated rats, showing antioxidative potential
289   and protection of the organs [27]. Similar studies have also found that commercially sold
290   polyherbal formulations like 5EPHF, Diabecon and Glyoherb significantly improved antioxidant
291   status by increasing levels of antioxidant enzymes and minimizing diabetic complications [28,
292   29].

293 **5. CONCLUSION**

294   High fat diet in combination with a sub-diabetic dose streptozotocin produced type 2 diabetes in
295   the Wistar rats with significant hyperglycaemia, hyperinsulinaemia and insulin resistance.
296   Increase in total oxidant status and oxidative stress index depleted antioxidant parameters. The
297   polyherbal capsule glucoblock was effective when used alone and produced equipotent effect to
298   the treatment with glibenclamide, in the reduction of glycaemic and oxidative stress parameters.
299   However, the combination of the drugs was not as effective as the individual treatments in the
300   reduction of fasting plasma glucose and HOMA-IR. This study establishes a basis for the need of
301   antioxidant therapy in combination with hypoglycaemic agents in the management of diabetes
302   mellitus, as none of the treatments reduced oxidative stress to normal control values. Proper care
303   should be taken in the combination of herbal and conventional medicines, for the risk of adverse
304   drug-herb reactions.

305 **Conflict of Interests**

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

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References

1. International Diabetes Federation. *International Diabetes Federation Diabetes Atlas* (7th ed.). International Diabetes Federation. 2016.

2. Reaven, G. M. The role of insulin resistance in human disease. *Diabetes*. 1998; 37: 1595–1607.

3. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2015; 38(1): 01-93.

4. Rains, J. L., & Jain, S. K. Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine*. 2011; 50(5): 567-575.

5. Briggs, O. N., Brown, H., Elechi-amadi, K., Ezeiruaku, F., & Nsirim, 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.

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

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

8. 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.

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. Deeds, M. C., Anderson, J. M., Armstrong, A. S., Gastineau, D. A., Hiddinga, H. J., Jahangir, A., Eberhardt, N. L., & Kudva, Y. C. Single dose streptozotocin induced diabetes: Considerations for study design in islet transplantation models. *Lab Animal*. 2011; 45(3): 131–140.

12. 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.

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

14. Wink, M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*. 2015; 2(3): 251-286.

15. Ikebukuro, K., Adachi, Y., Yamada, Y., Fujimoto, S., Seino, Y., & Oyaizu, H. Treatment of streptozotocin-induced diabetes mellitus by transplantation of islet cells plus bone marrow cells via portal vein in rats. *Transplantation*. 2002; 73: 512-518.

16. Lenzen, S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008; 51: 216–226.

17. 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.

18. Shokoohi, R., Kianbakht, S., Faramarzi, M., Rahmanian, M., Nabati, F., Mehrzadi, S., & Huseini, H. F. Effects of an herbal combination on glycemic control and lipid profile in diabetic women: a randomized, double-blind, placebo controlled clinical trial. *Journal of Evidence-Based Complementary & Alternative Medicine*. 2017; 22(4): 798-804.

19. Al-Omaria, I. L., Afifib, F. U., & Salhaba, A. S. Therapeutic effect and possible herb drug interactions of ginger (*Zingiberofficinale Roscoe*, *Zingiberaceae*) crude extract with glibenclamide and insulin. *Pharmacognosy Communications*. 2012; 2: 12–20.

20. 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.

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

22. Ali, Y., Sudip, P., Tanvir, E. M., Sakib, H., Nur-E, N. R., Moumoni, S., Nikhi, C. B., Aminu, I., Sabir, H., Nadia, A., Siew, H. G., & Khalil, I. Antihyperglycemic, antidiabetic, and antioxidant effects of *Garcinia pedunculata* in rats. *Evidence-Based Complementary and Alternative Medicine*. 2017; 2017: 2979760. https://doi.org/10.1155/2017/2979760.

23. Jafari, S., Sattari, R., & Ghavamzadeh, S. Evaluation the effect of 50 and 100 mg doses of *Cuminum cyminum* essential oil on glycemic indices, insulin resistance and serum inflammatory factors on patients with diabetes type II: a double-blind randomized placebo-controlled clinical trial. *Journal of Traditional and Complementary Medicine*. 2017; 7(3): 332-338.

399   24. Sheng, Y., Zheng, S., Ma, T., Zhang, C., Ou, X., He, X., Xu, W., & Huang, K. Mulberry
400   leaf alleviates streptozotocin-induced diabetic rats by attenuating NEFA signaling and
401   modulating intestinal microflora. *Scientific reports*. 2017; *7*(1): 12041.
402   doi:10.1038/s41598-017-12245-2
403
404   25. Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K., & Roufogalis, B. D.
405   Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action
406   and clinical implications. *Diabetology & Metabolic Syndrome*. 2017; 9(59): 1-12.
407
408   26. Asadi, S., Goodarzi, M. T., Karimi, J., Hashemnia, M., & Khodadadi, I. Does curcumin
409   or metformin attenuate oxidative stress and diabetic nephropathy in rats? *Journal of*
410   *Nephropathology*. 2019; 8(1): 8.
411
412   27. Sellamuthu, P. S., Arulselvan, P., Kamalraj, S., Fakurazi, S., & Kandasamy, M.
413   Protective nature of mangiferin on oxidative stress and antioxidant status in tissues of
414   streptozotocin-induced diabetic rats. *International Scholarly Research Notices:*
415   *Pharmacology*. 2013; 75: 1-9.
416
417   28. Lanjhiyana, S., Garabadu, D., Ahirwar, D., Rana, A. C., Ahirwar, B., & Lanjhiyana, S. K.
418   Pharmacognostic standardization and hypoglycemic evaluations of novel polyherbal
419   formulations. *Der Pharmacia Lettre*. 2011; 3(1): 319-333.
420
421   29. Maninder-Kaur, V. V. Diabetes and antidiabetic herbal formulations: an alternative to
422   Allopathy. *International Journal of Pharmacognosy*. 2014; 1(10): 614-626.