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

ABSTRACT

The increased prevalence of diabetes, and the huge disease burden on patients has led to an increase in the use of complementary and alternative medicine in diabetes treatment and management.

Aim: This study evaluates the antidiabetic and antioxidant effects of the polyherbal capsule glucoblock and glibenclamide in type 2 diabetic rats.

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 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 of 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, Glucoblock, 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 synthesis, and declining beta-cell function, ultimately leading to beta-cell failure [2, 3].

Hyperglycaemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, that is, insulin action and insulin secretion. Also, anti-oxidative mechanisms become depleted in diabetes, which could further increase oxidative stress [4, 5]. Oxidative stress induced by hyperglycaemia plays a critical role in the development of diabetic complications. Furthermore, the development and progression of the damage is proportional to hyperglycaemia, thus making the reduction of blood glucose levels the most important goal in preventing complications and treating DM [6].

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

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

2.2 Acute Toxicity Study

This was done by the fixed dose procedure [9], using a group of 3 rats. 2000mg/kg body weight of glucoblock 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 glucoblock was deemed safe up to 2000mg/kg body weight dose. Glibenclamide is a standard antidiabetic drug.

2.3 Dose Calculation

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

Glibenclamide

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

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

= 0.9mg/kg body weight/day.

Glucoblock

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

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

= 90mg/kg body weight/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) having 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) [11]. 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 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 of 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

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 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**	

+ve – Present, -ve – Not present

Table 1 shows alkaloids, flavonoids, cardiac glycosides and saponins present in the herbal drug glucoblock, with concentrations of 100.31µg/mg, and 131.45µg/mg, 55.93µg/mg and 61.47µg/mg respectively. Other phytochemicals such as phenolic acids, 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	5.82 ± 0.66	18.38 ± 6.77*
Group 4 n=7	6.12 ± 0.63	19.65 ± 7.30*
Group 5 n=7	6.12 ± 0.67	21.90 ± 6.86*
P-value	0.8245	0.0008
F-value	0.3746	6.677

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, and established the pathological state of diabetes in the rats, as compared to the negative control 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 (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 ± 1.09[a b]	3.87 ± 0.22[b]	1.5 ± 0.3[a b]
P-value	< 0.0001	< 0.0001	< 0.0001
F-value	98.74	9.71	121.4

n – Number of samples, Gli – Glibenclamide, Gluco - Glucoblock, [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 the negative control and treatment groups, compared to the diabetic control. Mean FPG level was significantly higher ($p<0.05$) in the combination group (glibenclamide + glucoblock), when compared to the negative control. There was however no significant difference ($p>0.05$) in FPG levels in the glibenclamide group and glucoblock group, compared to the negative control.

The diabetic control had significantly higher ($p<0.05$) FPI levels compared to the negative control and treatment groups. Also, 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. HOMA-IR was significantly higher ($p<0.05$) in the combination group (glibenclamide + glucoblock), when compared to the negative control. There was however, no significant difference ($p>0.05$) in HOMA-IR in the glibenclamide group and glucoblock group, 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 ± 2.19[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[a b]	0.92 ± 0.05[a b]	37.42 ± 1.65[b]
Group 4 (Gluco) n = 7	1.54 ± 0.05[b]	1.57 ± 0.06[a]	0.99 ± 0.03[a b]	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[a b]	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

n – Number of samples. Gli – Glibenclamide, Gluco – Glucoblock, [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 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 negative control and treatment groups. The results also revealed no significant differences ($p>0.05$) in TOS levels in the treatment groups, compared to the negative control.

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

The results reveal significantly lower ($p<0.05$) OSI levels in the negative control and treatment groups compared to the diabetic control. OSI levels in the treatment groups were also significantly higher ($p<0.05$), compared to the negative control.

There were significantly higher ($p<0.05$) SOD levels in the diabetic control, compared to negative control and treatment groups. The results also revealed no significant differences ($p>0.05$) in SOD levels in the treatment groups, compared to the negative control.

4. DISCUSSION

Phytochemical analysis of the polyherbal drug glucoblock revealed the presence of bioactive phytochemicals like alkaloids, flavonoids, cardiac glycosides, and saponins in variable amounts, which could have contributed to the changes in the biochemical and oxidative parameters

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

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

There were significant improvements in fasting plasma glucose levels in the rats after 28 days of treatment, as the results showed significantly lower ($p<0.05$) fasting plasma glucose levels in the treatment groups, compared to the diabetic control. There were no significant differences ($p>0.05$) in fasting plasma glucose levels in the glibenclamide treated group (Group 3) and the glucoblock treated group (Group 4), compared to the negative control, indicating glibenclamide and glucoblock used separately, were equally very effective in returning fasting plasma glucose levels to baseline control values. However, the combination group of glibenclamide and glucoblock had significantly higher ($p<0.05$) fasting plasma glucose levels, compared to the negative control. This implies that the combination did reduce the elevated glucose levels, but not to baseline control levels, and not as effective as the individual treatments. Orthodox medicines administered alone or in combination with plant products are 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. The results are in agreement with the works of Shokoohi *et al*. [18], in which a herbal combination capsule significantly decreased fasting blood glucose levels in diabetics. Al-Omaria *et al*. [19] reported that a concurrent treatment of ginger and glibenclamide significantly reduced blood glucose levels, compared to when glibenclamide was used alone 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. Also, the treatment groups showed no significant differences ($p>0.05$) in fasting plasma insulin levels when compared to the negative control. The results indicate the significant hyperinsulinaemia caused by the HFD/STZ induction in the diabetic rats, was returned to normal fasting insulin levels by the treatments with glibenclamide, glucoblock, and their combination in the treatment groups. The reduction in insulin levels by these treatments could be as result of increasing 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 are in consonance works of Reed *et al*. [20], and Skovso *et al*. [21] in which HFD/STZ induction produced hyperglycaemia and hyperinsulinaemia. The results are also in agreement with the works of Ali *et al*. [22], in which treatment with glibenclamide and the methanolic extract of *Garcinia pedunculata* (GP) fruit, restored insulin levels in STZ-induced diabetic rats.

The results showed significantly lower ($p<0.05$) HOMA-IR values in the treatment groups compared to the diabetic control. There were no significant differences ($p>0.05$) in HOMA-IR values in the glibenclamide treated group (Group 3) and the glucoblock treated group (Group 4), compared to the negative control, indicating glibenclamide and glucoblock used separately were equipotent and very effective in returning HOMA-IR values to baseline control values. However, the combination group of glibenclamide and glucoblock had significantly higher ($p<0.05$) HOMA-IR values compared to the negative control. This indicates the combination did reduce insulin resistance in the rats, but not to baseline control levels, and not as effective as the individual treatments. The results corroborates with the works of Reed *et al*. [20], and Skovso *et al*. [21] in which HFD/STZ induction produced hyperglycaemia, hyperinsulinaemia, significant insulin resistance and established the HFD/STZ treatment as a protocol for inducing animal type 2 diabetes, having the pathological correlation of the human disease. In a randomized control clinical study, the polyherbal drug, green cumin capsule was found to significantly increase insulin sensitivity [23]. In a similar study, mulberry leaf and glibenclamide significantly reduced HOMA-IR, increased insulin sensitivity (HOMA-IS) and beta-cell function (HOMA-β) in STZ-induced diabetic rats [24].

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

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

SOD levels were significantly higher ($p<0.05$) in the negative control and treatment groups, compared to the diabetic control. There were no significant differences ($p>0.05$) in SOD levels in the treatment groups, compared to the negative control. The results indicate type 2 DM is associated with depressed SOD, which could be due to increased oxidative stress levels. However, treatment with glibenclamide, glucoblock and the combination was effective in returning SOD levels to normal control levels. Hyperglycaemia in diabetes is associated with excessive production of free radicals through a number of mechanisms, leading to increased oxidative stress [6]. Herbal medicines and their constituent phytochemicals have shown the potential to be able to ameliorate diabetes and oxidative stress, either by directly scavenging free radical species or by boosting the antioxidant defense mechanism [25]. The alteration in oxidative stress and antioxidant parameters in this study, show an increased production of free radicals or ROS, which lead to depressed antioxidant defence mechanisms even in the treated rats. The results are in line with the work of Asadi *et al*. [26], in which TOS and

malondialdehyde (MDA) were significantly increased in STZ-induced diabetic rats. Activities of the antioxidant enzymes SOD and glutathione peroxidase (GPx), were also decreased in the diabetic rats, pointing to an increase in oxidative stress levels. The activities of the antioxidant enzymes SOD, GPx, catalase (CAT) and levels of reduced glutathione (GSH) were found to be increased in liver and kidney tissues of diabetic rats treated with glibenclamide and/or mangiferin. Levels of thiobarbituric acid reactive substances (TBARS) were also significantly reduced in the kidney and liver of the treated rats, showing antioxidative potential and protection of the organs [27]. Similar studies have also found that commercially sold polyherbal formulations like 5EPHF, Diabecon and Glyoherb significantly improved antioxidant status by increasing levels of antioxidant enzymes and minimizing diabetic complications [28, 29].

5. CONCLUSION

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

Conflict of Interests

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

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