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Effects of Metformin in Combination with a Herbal Capsule (Glucoblock) on Insulin Resistance and Oxidative Stress Index in Type 2 Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ONB designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors KNE and JCO wrote the protocol and managed the literature searches. Authors EON and ESB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated the effects of metformin in combination with a herbal capsule (glucoblock) on insulin resistance and oxidative stress index in type 2 diabetic rats.
Methodology: A total of 35 male Wistar albino rats weighing between 120-220 g 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 on the herbal capsule was done using classical methods.

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Results: The results revealed the presence of alkaloids (100.31µg/mg), flavonoids (131.45µg/mg), cardiac glycosides (55.93µg/mg) and saponins (61.47µg/mg) in the herbal drug glucoblock. The results showed significantly lower FPG levels in the treatment groups when compared to the diabetic control. Group 3 administered metformin had significantly higher FPG levels compared to the negative control. Group 4 administered the herbal drug glucoblock and group 5 administered a combination of metformin and glucoblock, showed no significant differences in FPG levels when compared to the negative control. The diabetic control had significantly higher FPI levels compared to the negative control and treatment groups. The treatment groups showed no significant differences in FPI levels when compared to the negative control. HOMA-IR was significantly higher in the diabetic control compared to the negative control and treatment groups. Also, HOMA-IR values in the treatment groups showed no significant difference compared to the negative control except for group 3 (metformin), that was significantly higher than the negative control. SOD was significantly lower in the diabetic control, compared to the negative control and treatment groups. There were no significant differences in SOD levels in the treatment groups compared to the negative control. TOS levels in the negative control group and treatment groups were significantly lower, compared to the diabetic control. TAS was significantly lower in the diabetic control and treatment groups compared to the negative control. OSI in the diabetic control was significantly higher, compared to the negative control and treatment groups. Also, the treatment groups had significantly higher OSI compared to the negative control.

Conclusion: High fat diet and streptozotocin induction produced significant insulin resistance and oxidative stress in the diabetic rats. Glucoblock was more effective in reducing insulin resistance compared to metformin. The combination showed synergistic drug-herb reaction as glucoblock potentiated the actions of metformin. Both showed antioxidant potential but were not effective in lowering oxidative stress to normal levels. There is need to incorporate antioxidant therapy in the treatment protocol for diabetes mellitus.

Keywords: Insulin resistance; oxidative stress; antioxidants; diabetes mellitus; metformin; glucoblock; herbal therapeutics.

1. INTRODUCTION

Insulin resistance is a key factor in the development of type 2 diabetes. It is presented as a suppression or retardation in metabolic responses of the liver, muscle, and adipose tissue to insulin action [1,2]. It leads to impaired regulation of hepatic glucose synthesis, and declining beta-cell function, ultimately leading to beta-cell failure. The characteristic picture is chronic hyperglycaemia, but there is also altered metabolism of lipids, and proteins along with an increased risk of complications from vascular disease [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 [2,4]. Although obesity and physical inactivity are the leading factors for the development of type 2 diabetes, data from research suggests that oxidative stress may increase insulin resistance and impair insulin secretion, thus contributing to its pathogenesis [5].

There is an upsurge in the use of herbal therapy over orthodox drugs for the treatment and management of type 2 diabetes, with manufacturers of these herbals claiming it ameliorates diabetes by affecting metabolic and diabetic pathways, with the promise of better patient outcomes. Also, the cost and perceived lesser side effects of herbal medicines has led to an increase in their usage by diabetic patients [6]. This work looks into the efficacy of a herbal drug in combination with a standard anti-diabetic drug in affecting insulin resistance and oxidative stress parameters in experimental type 2 diabetes, thus evaluated the effects of metformin in combination with a herbal capsule (glucoblock) on insulin resistance and oxidative stress index in type 2 diabetic rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of thirty five (35) male Wistar rats weighing between 120 to 220g were used for the 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 rats were allowed to acclimatize for two (2) weeks prior to the commencement of the study.

2.2 Drugs

The drugs used for the study were glucoblock and metformin. Glucoblock is a polyherbal drug manufactured by Green World Group, Michigan, USA, and commercially sold in Nigeria as an anti-diabetic capsule. Metformin, a biguanide is manufactured by LEK SA, Poland.

2.3 Acute Toxicity Study

Acute Toxicity Study was done by the fixed dose procedure [7], using a group of 3 rats. 2000 mg/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, there were no signs of toxicity, hence the herbal drug glucoblock was deemed safe up to a dose of 2000 mg/kg body weight. Metformin is a standard anti-diabetic drug.

2.4 Dose Calculation

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

2.4.1 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 [8].
= 90mg/kg body weight/day.

2.4.2 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 [8].
= 90mg/kg body weight/day.

2.5 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). On the day of induction, after a 6 hour fast, 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). Diabetes was confirmed after 72 hours in the rats having fasting blood glucose levels above 14mmol/L (250 mg/dl) [9]. 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 herbal drug glucoblock.
Group 5: Diabetic rats treated with a combination of metformin 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 [10,11].

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 [12] 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 [13] as described by Elabscience Biotechnology Company limited, China. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method [14]. Total oxidant status (TOS) and total antioxidant status (TAS) were determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [13,15,16] 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 [17].

2.6 Statistical Analysis

Data generated was analysed using Graph Pad Prism version 8.0.2. 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≤0.05). Values are expressed as Mean ± SD.

3. RESULTS AND DISCUSSION

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. These plant secondary metabolites have various molecular targets and can affect diverse metabolic and diabetic pathways, bringing about biochemical, physiologic and glycaemic responses [18].

Table 2 shows results of FPG, FPI and HOMA-IR (insulin resistance) of the rats after treatment. There were significant improvements in fasting plasma glucose (FPG) levels in the treatment groups after 4 weeks of treatment, as the results showed significantly lower (p< .05) FPG levels when compared to the diabetic control. Group 3 administered metformin had significantly higher (p< .05) FPG levels compared to the negative control. This implies metformin reduced the elevated fasting plasma glucose levels, but not effective enough to bring it to baseline control values. Group 4 administered the herbal drug glucoblock and group 5 administered a combination of metformin and glucoblock, showed no significant differences (P> .05) in fasting plasma glucose levels when compared to the negative control. This implies the herbal capsule glucoblock administered alone, and in combination with metformin was very effective than treatment with metformin alone in reducing FPG level to baseline control values. It also shows the herbal capsule glucoblock potentiated the effect of metformin. Phytochemicals present in herbal drugs act alone or in interaction with the orthodox drugs, affecting metabolic pathways and bringing about glycemic responses as seen in the glucose levels. Lu et al. [19], and Skovso, [20] reported poor glycaemic control in the high fat diet/streptozotocin diabetes model treated with insulin sensitizing therapeutics. Similar research by Briggs et al. [21] revealed metformin was not effective as a stand-alone drug, but had a better control of the glucose level when used in combination with the herbal drug diawell, indicating a synergistic interaction between the herbal drug diawell and metformin. Poonam et al. [22], 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 a similar study, treatment with a polyherbal diarth and its combination treatment with glibenclamide were more effective than treatment with glibenclamide alone in reducing glucose levels to baseline control values [23].

The diabetic control had significantly higher (p< .05) fasting plasma insulin levels compared to the negative control and treatment groups. All the treatment groups showed no significant differences (p> .05) in fasting insulin levels when compared to the negative control. This means the significant hyperinsulinaemia in the diabetic rats due to HFD/STZ was returned to normal levels by the administration of metformin, glucoblock and their combination in the treatment groups. The reduction in insulin levels by these treatments could be as result of insulin sensitizing activity of the drugs, thus increasing insulin sensitivity in the liver and peripheral tissues or by providing a sort of protection to pancreatic beta cells, preventing necrotic cell

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 2. 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 ± 0.86[a b]	3.60 ± 0.12[b]	1.9 ± 0.1[a b]
Group 4 (Gluco) n = 7	4.90 ± 0.78[b]	3.67 ± 0.59[b]	0.8 ± 0.2[b]
Group 5 (Met + Gluco) n = 7	3.42 ± 0.59[b]	4.06 ± 0.30[b]	0.6 ± 0.1[b]
P-value	< 0.0001	< 0.0001	< 0.0001
F-value	183.5	11.0	210.6

n – Number of samples, Met – Metformin, 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. Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Oxidative Stress Index (OSI) and Superoxide Dismutase (SOD) Levels after Treatment

Groups	SOD (pg/ml)	TOS (U/ml)	TAS (U/ml)	OSI
Group 1 (Negative control) n = 7	38.26 ± 2.191[b]	1.61 ± 0.04[b]	1.99 ± 0.06[b]	0.81 ± 0.03[b]
Group 2 (Diabetic control) n = 6[#]	30.33 ± 1.94[a]	2.55 ± 0.05[a]	1.62 ± 0.05[a]	1.58 ± 0.06[a]
Group 3 (Met) n = 7	35.94 ± 1.55[b]	1.74 ± 0.06[a b]	1.40 ± 0.07[a b]	1.25 ± 0.10[a b]
Group 4 (Gluco) n= 7	37.89 ± 3.81[b]	1.54 ± 0.05[b]	1.57 ± 0.06[a]	0.99 ± 0.03[a b]
Group 5 (Met + Gluco) n = 7	35.24 ± 1.59[b]	1.53 ± 0.09[b]	1.43 ± 0.10[a b]	1.08 ± 0.13[a b]
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F-value	10.83	303.6	70.62	79.37

n – Number of samples, Met – Metformin, 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

death and leakage of their contents caused by STZ. The results agree with the works of Reed et al. [24] and Skovso [20], 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 Gupta et al. [25] in which combined treatment with ginseng and metformin significantly improved plasma glucose and insulin levels.

The results revealed significantly higher ($p < .05$) HOMA-IR (insulin resistance) values in the diabetic control compared to the negative control and treatment groups. Also, HOMA-IR values in the treatment groups showed no significant difference ($p > .05$) compared to the negative control except for group 3 (metformin), that was significantly higher ($p < .05$) than the negative control. This implies the significant insulin resistance produced by HFD/STZ in the diabetic rats, was reduced by the administration of metformin, glucoblock, and the combination treatment. Glucoblock administered alone, and in combination with metformin was more effective in reducing HOMA-IR values to normal control values, than metformin administered alone, showing glucoblock potentiates the insulin sensitizing activities of metformin. The results are consonance with the works of Hu et al. [26] in which they found significant improvement in HOMA-IR using a combination of ginseng and metformin, than the individual drugs used alone, suggesting an additive effect. Also, in a randomized control clinical study, the polyherbal drug, green cumin capsule was found to significantly increase insulin sensitivity [27].

Table 3 shows the results of SOD, TOS, TAS and OSI levels of the rats after treatment. It shows levels of the antioxidant enzyme superoxide dismutase (SOD) was significantly lower ($p < .05$) in the diabetic control, compared to the negative control and treatment groups. There were no significant differences ($p > .05$) in SOD levels in the treatment groups compared to the negative control. The results imply type 2 DM may be associated with depressed levels of the antioxidant enzyme SOD, which could be as a result of increased oxidative stress. The treatments with metformin, glucoblock and their combination showed antioxidant potential in effectively improving SOD levels to normal control values.

The findings also showed significantly lower (p< .05) total oxidant status (TOS) levels in the negative control group and treatment groups, when compared to the diabetic control. This shows the significantly elevated TOS levels caused by diabetes (HFD/STZ), was significantly reduced by the treatment with metformin, glucoblock and their combination. Treatment with the polyherbal drug glucoblock, and when used in combination with metformin showed no significant difference (P> .05) in TOS compared to the negative control. However, treatment with metformin alone showed significantly elevated (P< .05) TOS, compared to the negative control. This again shows the better efficacy of glucoblock over metformin and its potentiating effect when used together with metformin.

Total antioxidant status (TAS) was significantly lower (P< .05) in the diabetic control and treatment groups compared to the negative control. This shows a depressed anti-oxidant status in the diabetic rats and even the treatment were not able to restore it to normal values.

The results from this study revealed significantly higher (p< .05) oxidative stress index (OSI) in the diabetic control, compared to the negative control and treatment groups. Also, the treatment groups had significantly higher {P< .05) OSI compared to the negative control. This implies diabetes induced by STZ/HFD is associated with increased oxidative stress and the treatment with metformin, the polyherbal glucoblock and their combination though showed anti-oxidant potential, could not restore the OSI to normal values.

Hyperglycaemia in diabetes is associated with increased production of ROS through a number of mechanisms, leading to increased oxidative stress. Oxidative stress which further worsens the hyperglycaemic state is responsible for the complications of diabetes [28]. 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 defence mechanism in mopping up oxidant molecules [25]. The alteration in oxidative stress and antioxidant parameters in this study, show an increased production of oxidants, which lead to depressed antioxidant defence mechanisms. The treatments have also shown antioxidative potential in addition to their hypoglycaemic effects. The results are in consonance with the work of Gupta et al. [25], 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. [29], 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 CAT were significantly increased, while MDA reduced in the kidneys of the diabetic rats treated with curcumin. In a similar study, Briggs et al. [30] found that glucoblock and glibenclamide, administered individually or as a combination showed antioxidant potentials, by significantly reducing TOS, increasing SOD and improving OSI in treated diabetic rats, compared to the untreated rats.

4. CONCLUSION

Administration of high fat diet and streptozotocin induction produced significant insulin resistance and oxidative stress as seen in the elevated HOMA-IR and OSI of the diabetic rats. The herbal drug glucoblock was more effective in reducing insulin resistance compared to metformin. The combination showed synergistic drug-herb reaction as glucoblock potentiated the actions of metformin. Both showed antioxidant potential but were not effective in lowering oxidative stress to normal levels. This shows the need for the incorporation of antioxidant therapy in the treatment protocol for diabetes mellitus.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It’s not applicable.

ETHICAL APPROVAL

All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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