Glycaemic, Lipidaemic and Antioxidant Profiles of Alloxan-Induced Diabetic Wistar Rats Treated with Glibenclamide and Aqueous Extract of Gongronema latifolium (Benth)

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Abstract

The current study investigated the ameliorative effects of combined therapy of glibenclamide and G. latifolium (GL) on several biochemical parameters of alloxaized Wistar rats. Thirty adult male Wistar rats assigned into 5 groups of 6 rats each were used for the study. Groups 2-5 were intraperitoneally injected with 160 mg/kg of alloxan monohydrate and upon establishment of diabetes (Fasting Blood Glucose (FBG) ≥ 126 mg/dl) were treated with 10 ml/kg distilled water (DW), 2 mg/kg glibenclamide, 200 mg/kg GL and 2 mg/kg glibenclamide and 200 mg/kg GL respectively. Rats in group 1 were not made diabetic and served as normal control. All the treatments were realized through daily oral route using gastric tube, for 21 days. Results indicated that the treatment of diabetic rats with a combination of glibenclamide and GL significantly reduced the elevated glucose levels, cholesterol, triacylglycerol, low density lipoprotein and malondialdehyde levels, along with increases in the high density lipoprotein, glutathione values and catalase activities, when compared to diabetic untreated group. It was concluded that the combined therapy of glibenclamide and GL showed superior antihyperglycemic, hypolipidaemic and antioxidant effects compared to either of them used alone.

Keywords: antioxidants, glibenclamide, glycemia, Gongronema latifolium, lipid profile

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There is lack of information on the effect of combined therapy of glibenclamide and Gongronema latifolium (GL) on some biochemical parameters of alloxan-induced diabetic rat. This study is therefore aimed to investigate the effects of combining aqueous extract of GL and Glibenclamide on certain biochemical profiles of diabetic Wistar rats.

Materials and Methods

The animals and the plant materials

Adult male albino rats weighing between 150-200 g were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka laboratory animal house. The rats were acclimated for two weeks before the commencement of the experiment. During the acclimatization and in the course of the experiments, the rats were fed with Vital feed grower and clean drinking water was administrated ad libitum. The rats were housed in a stainless wire mesh cage. The experimental protocol used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka and conforms with guide to the care and use of animals in research and teaching of University of Nigeria, Nsukka, Enugu state, Nigeria.

The leaves of Gongronema latifolium were purchased from Ojise Market, Nsukka, Enugu state, Nigeria and identified by a botanist in Botany department, University of Nigeria, Nsukka, Enugu state, Nigeria. The voucher specimen was deposited in the herbarium section of the department.

Chemicals, reagents, drugs

Cholesterol, triacylglycerol and high density lipoprotein assay kits were provided from Randox, UK; Malondialdehyde, Catalase, Glutathione reagents from Sigma Aldrich, UK; Glibenclamide from Hovid, China; glucose strip and glucometer from Accu-Chek Active.

The preparation of the plant extract

Cold maceration method of extraction was employed. The leaves of Gongronema latifolium were air dried at a very low intensity of sunlight to avoid denaturation of the active ingredients. They were pulverized and stored in air tight container pending its usage. About 2 kg of the pulverized plant material were soaked in 10 l of distilled water with intermittent shaking every 2 h for 48 h. The mixtures were filtered using Whatmann No. 1 filter paper and concentrated using oven adjusted to 37 °C.

The induction of experimental diabetes

The method of Venugopal et al. (1998) was used. Induction was done by single intraperitoneal injection of 160 mg/kg of alloxan monohydrate after overnight fasting. The fasting blood glucose values were determined prior to induction. Thereafter, rats with fasting blood glucose levels of 126 mg/dl or above were considered diabetic.

The experimental design

Thirty adult male Wistar rats weighing between 150-200 g were assigned into 5 groups of 6 rats per group. Upon the establishment of diabetes, the rats were treated as summarized in Table 1 below.

Treatments were done daily for 21 days through the oral route using gastric tube. Fasting blood glucose values were assessed 1 h, 3 h, 6 h, 24 h, 7 days, 14 days and 21 days post treatment. Blood sample for lipid profile and in vitro antioxidant determinations were collected on days 7, 14 and 21 post treatment.

The blood sample collection

The method of Parasuraman (2001) was used. Non-heparinized capillary tube was inserted into the capillary plexus in the medial canthus of the eye of the rats and blood was allowed to flow into sample bottles. The blood was kept in slant position and allowed to clot before serum was decanted into a clean bijou bottle for the biochemical analyses.

Determination of serum cholesterol

The serum cholesterol was determined by cholesterol oxidase-peroxidase method (Allain et al., 1974), for the in vitro determination of cholesterol in serum or plasma, using cholesterol test kit. The serum sample (0.01 ml) was reacted with 1.0 ml of cholesterol working reagent containing cholesterol esterase, oxidase and peroxidase to form a coloured quinonic derivative. It was mixed properly and allowed to stand at room temperature for 10 min. The standard was also prepared by adding 1.0 ml of cholesterol working reagent and 0.01 ml of standard. The absorbance of both the sample and standard were read against the working reagent blank at 500 nm within 60 min with a digital spectrophotometer.

The cholesterol concentration in each sample was obtained with the formula:

\[
\text{Cholesterol concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 206
\]

HDL-Cholesterol

Low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant was determined. Five hundred microliter of diluted R1 (phosphotungstic acid and magnesium chloride) were mixed with 200 µL of serum sample or standard and allowed to sit for 10 min at room temperature. Thereafter, the clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOP-PAP method (Allain et al., 1974).

Determination of cholesterol using the supernatant

One hundred microliter (100 µL) of supernatant (or standard or distilled water for blank as the case may be) were mixed with 1,000 µL of reagent, mixed and incubated for 10 min at room temperature.

Table 1. Treatments of experimental rats with drug, plant extract and distilled water

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-diabetic Wistar rats treated with 10 ml/kg distilled water (DW)</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic Wistar rats treated with 10 ml/kg DW</td>
</tr>
<tr>
<td>3</td>
<td>Diabetic Wistar rats treated with 2 mg/kg Glibenclamide</td>
</tr>
<tr>
<td>4</td>
<td>Diabetic Wistar rats treated with 2 mg/kg Glibenclamide and 200 mg/kg Gongronema latifolium</td>
</tr>
<tr>
<td>5</td>
<td>Diabetic Wistar rats treated with 200 mg/kg Gongronema latifolium</td>
</tr>
</tbody>
</table>
Absorbance of the sample (A<sub>sample</sub>) and standard (A<sub>standard</sub>) were measured against the reagent blank within 60 min (Friedwald et al., 1972) and the calculations were as follow:

\[
\text{HDL Cholesterol (mg/dl) = \frac{\text{Change A}_{\text{sample}}}{\text{Change A}_{\text{standard}}} \times \text{Concentration of standard}}
\]

\[
\text{LDL Cholesterol (mg/dl) = Total cholesterol - \left(\frac{\text{Triglyceride}}{5}\right) - \text{HDL Cholesterol}}
\]

\[
\text{VLDL Cholesterol (mg/dl) = \frac{\text{Triglyceride}}{5}}
\]

**Triacylglycerol**

Triacylglycerol measurement was done according to Allain et al. (1974). Triacylglycerol measurement is used in the diagnosis and treatment of diseases involving lipid metabolism and the serum sample or standard (10 µL) was mixed with 1,000 µL of buffer and enzyme reagent R1, and then incubated for 10 min at room temperature.

The absorbance of the sample (A<sub>sample</sub>) and standard (A<sub>standard</sub>) were read against the blank (1,000 µL of R1) at wavelength 500 nm.

\[
\text{Triglyceride conc. (mg/dl) = \frac{A_{\text{sample}} - A_{\text{standard}} \times \text{standard concentration (191 mg/dl)}}{100 \times 0.5271 \times 0.1}}
\]

**Estimation of catalase**

The activity of catalase was assayed by the method of Sinha et al. (1963). This method was based on the development of yellow colour when 5,5'- dithio-bis-2-nitrobenzoic (DTNB) is added to a compound containing sulphydryl groups. The colour developed was expressed as U/ml for plasma (U·umoles of H<sub>2</sub>O<sub>2</sub> utilised / second).

\[
\text{Catalase activity = } \frac{\text{A} \times \text{B}}{0.00691}, \quad \text{where A and B represent the range of the absorbance.}
\]

**Reduced glutathione**

The reduced glutathione level was determined by the method of Beutler et al. (1963). This method was based on the development of yellow colour when 5,5'-dithio-bis-2-nitrobenzoic (DTNB) is added to a compound containing sulphydryl groups. The colour developed was read at 412 nm in a spectrophotometer.

About 0.2 ml of sample was mixed with 1.8 ml of EDTA solution. To this, 3.0 ml of precipitating reagent were added, mixed thoroughly and kept for 5 min before centrifugation. To 2 ml of the filtrate, 4 ml of 0.3 M disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added and the color developed was read at 412 nm in a spectrophotometer. A set of standard solutions containing 20-100 µmoles of reduced glutathione was processed for the test. The activity of catalase was expressed as U/ml for plasma.

\[
\text{Reduced glutathione level (mg/dl) = } \frac{A_{\text{sample}} - A_{\text{standard}} \times \text{standard concentration (191 mg/dl)}}{100 \times 0.5271 \times 0.1}
\]

**Estimation of lipid peroxidation (malondialdehyde)**

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. (1993). A volume of 0.1 ml of the serum was mixed with 0.9 ml of H<sub>2</sub>O in a test tube. A volume of 0.5 ml of 25% TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 min in a water-bath and then cooled in cold water. Then 0.1 ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at 532 nm and 600 nm against a blank.

\[
\%\text{TBA} = \frac{A_{532} - A_{600} \times 100}{0.5271 \times 0.1} \text{ (mg/dl)}, \quad \text{where A is the absorbance.}
\]

**Statistical analyses**

The data were analysed with One Way Analysis of Variance (ANOVA) using SPSS version 20. P values less than or equal to 0.05 were accepted as significant. The results were presented in tables as means (± standard error of mean).

**Results**

**Fasting Blood Glucose (FBG) levels of alloxan-induced diabetic rats treated with combination of glibenclamide and aqueous extract of G. latifolium**

The pre-induction FBG of all the rats in groups 1-5 were statistically the same. Post-induction, the FBG value of rats in group 1 was significantly lower compared to other rat groups. One hour post treatment (PT), the FBG levels of rats in groups 3 and 4 were lower than those of group 2, but higher than those of group 1. Three hours following treatment, the FBG levels of rats in groups 1 and 4 were significantly lower than those of groups 2, 3 and 5. This persisted till the 6th hour. However, 24 h PT, the glucose levels of rats in groups 1, 3 and 4 were comparable until 14 days PT. Twenty one days PT, the FBG value of group 4 rats was lower than those of the normal control rats (group 1). Those treated with glibenclamide (group 3 rats) have their glucose levels comparable with that of the normal control group, while the FBG level of group 2 rats remained very high (Table 2).

**Total mean of cholesterol values**

On days 7 and 14 PT, the total mean of total cholesterol values of rats in groups 1 and 4 were statistically comparable (p > 0.05), but significantly (p < 0.05) lower, than those of groups 2 and 3. On day 21 PT, the mean cholesterol value of rats in groups 1 and 4 was significantly (p < 0.05) lower than that of the rats in group 1. The total mean of cholesterol level of rats in group 3 was good comparing with that of groups 1, 4 and 5 (Table 2).

**The mean of triacylglycerol values**

The mean of triacylglycerol (TAG) level of rats in group 4 was statistically the same (p > 0.05) as those of groups 3 and 5 rats on day 7 PT, but was significantly (p < 0.05) lower than that of the group 2 rats. Rats in group 1 had lower TAG value compared to other rat groups. On day 21 PT, the TAG values of rats in groups 3 and 4 were similar and higher than that of the group 1 rats and lower than that of the group 2 rats. On day 21 PT, the mean TAG levels of rats in groups 1, 3 and 4 were comparable among these groups and higher than those of the other groups under experiment (Table 4).

**The mean of high density lipoprotein-cholesterol values**

The mean of high density lipoprotein-cholesterol (HDL) levels of all the rats in all the groups were statistically similar on
Table 2. Fasting Blood Glucose (FBG) values of alloxan induced diabetic rats treated with combinations of glibenclamide and aqueous extract of *G. latifolium*

<table>
<thead>
<tr>
<th>Group</th>
<th>0 h Post induction (PI)</th>
<th>1 h PI</th>
<th>3 h PI</th>
<th>6 h PI</th>
<th>24 h PI</th>
<th>7 days PI</th>
<th>14 days PI</th>
<th>21 days PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63.33 ± 1.45*</td>
<td>68.33 ± 1.85*</td>
<td>71.33 ± 0.88*</td>
<td>64.00 ± 1.52*</td>
<td>63.00 ± 0.57*</td>
<td>72.66 ± 6.33*</td>
<td>61.33 ± 0.88*</td>
<td>60.33 ± 1.85*</td>
</tr>
<tr>
<td>2</td>
<td>63.00 ± 3.51*</td>
<td>261.66 ± 22.42*</td>
<td>242.00 ± 19.46*</td>
<td>226.33 ± 14.20*</td>
<td>220.67 ± 9.83*</td>
<td>218.33 ± 10.13*</td>
<td>213.33 ± 18.50*</td>
<td>208.33 ± 10.92*</td>
</tr>
<tr>
<td>3</td>
<td>63.66 ± 6.83*</td>
<td>261.33 ± 4.70*</td>
<td>206.33 ± 3.17*</td>
<td>143.33 ± 7.68*</td>
<td>108.66 ± 14.37*</td>
<td>78.66 ± 1.15*</td>
<td>80.00 ± 3.51*</td>
<td>67.00 ± 3.51*</td>
</tr>
<tr>
<td>4</td>
<td>61.33 ± 3.48*</td>
<td>258.66 ± 2.33*</td>
<td>192.00 ± 1.15*</td>
<td>102.20 ± 8.29*</td>
<td>84.33 ± 5.03*</td>
<td>75.00 ± 0.00*</td>
<td>80.00 ± 0.88*</td>
<td>60.33 ± 2.18*</td>
</tr>
<tr>
<td>5</td>
<td>64.00 ± 0.57*</td>
<td>287.00 ± 3.51*</td>
<td>212.33 ± 5.92*</td>
<td>210.66 ± 5.20*</td>
<td>200.33 ± 2.90*</td>
<td>102.66 ± 1.45*</td>
<td>80.00 ± 0.57*</td>
<td>100.00 ± 5.94*</td>
</tr>
</tbody>
</table>

Different superscript along the same column (across groups) indicate significant difference at p < 0.05.

day 7 PT. On day 14 PT, the mean HDL values of rats in groups 3 and 5 were not significantly different from each other, but were significantly higher than that of group 2 rats and lower than that of group 1 rats. On day 21 PT, the mean HDL levels of group 3 rats were similar to those of groups 1, 3, 4 and 5, while that of group 2 rats were lower than all other groups (Table 5).

The mean low density lipoprotein-cholesterol values

The mean low density lipoprotein (LDL) value of rats in group 1 was significantly (p < 0.05) lower than that of the rats in all other rat groups on day 7. On day 14 PT, the mean LDL values of rats in groups 3 and 5 were statistically similar, but lower than that of the group 2 rats and higher than that of group 4 rats. On day 21 PT, the mean LDL values of rats in groups 1, 3 and 4 were statistically the same and significantly lower than those of groups 2 and 5 (Table 6).

The mean of very low density lipoprotein-cholesterol values

The mean of very low density lipoprotein (VLDL) levels of rats in groups 3, 4 and 5 were statistically similar, significantly higher than that of the rats in group 1 and lower than that of the rats in group 2. On day 14 PT, the VLDL values of rats in group 5 were lower than that of the rats in group 2 and higher than those of the rats in groups 1, 3 and 4. On day 21 PT, the mean VLDL levels of rats in groups 1, 3, 4 and 5 were statistically similar and significantly lower than that of the group 2 rats (Table 7).

The mean of malondialdehyde values

The mean of malondialdehyde (MDA) levels of rats in groups 2, 3 and 4 were comparable on day 7 PT, but were higher than that of the group 1 rats and lower than that of group 5 rats. On day 14 PT, the mean MDA values of groups 1 and 3 rats were similar and significantly lower compared to other rat groups. The mean MDA level of group 2 rats was significantly higher than those of the other groups. On day 21 PT, the mean MDA values of groups 1, 3, 4 and 5 rats were comparable between each other, but were lower than that of group 2 rats (Table 8).

The mean of reduced glutathione values

The mean of reduced glutathione (GSH) values of the rats in groups 1, 3, 4 and 5 were statistically similar and higher than that of group 2 rats on day 7 PT. On day 14 PT, the mean GSH values of rats in groups 1 and 3 were statistically similar to those of the rats in groups 4 and 5, while the rats in group 2 remained lower than those of the other groups. On day 21 PT, the mean GSH level of rats in group 3 was similar to those of the rats in groups 1 and 4. The mean GSH of the rats in group 2 was lower than that of the other groups (Table 9).

The mean of catalase activities

On day 7 PT, the mean of catalase activity of rats in group 2 was significantly lower than that of the other groups. On day 14 PT, the mean catalase activities of rats in groups 3 and 4 were statistically similar but higher than those exhibited by rats in groups 1, 2 and 5. The mean catalase activities of rats in groups 1 and 5 were lower than that of the group 4 rats. Rats in group 2 had the lowest mean catalase activity (Table 10).

Discussion

This study investigated possible synergism of glibenclamide and *Gongronema latifolium* combination therapy on amelioration of diabetes and diabetes-associated biochemical parameters of alloxan-induced diabetic rats.
Results of the fasting blood glucose values showed that 48 h post alloxan monohydrate injection there were significant increases in the FBG of groups 2-5 rats (induced groups) compared to that of the group 1 rats (normal control) (Table 2). This was attributed to the effect of alloxan monohydrate on pancreatic beta cells. Alloxan monohydrate is a chemical (analogous to glucose) that has been incriminated in destruction of pancreatic beta cells (Szukuldeki, 2001). This destruction is mediated by free radicals generated by series of redox reactions of alloxan monohydrate and dialuric acid (Munday, 1988). The sequela of this destruction of the pancreatic beta cells is a reduction in the level of circulating plasma insulin with attendant hyperglycaemia (Lachin and Reza, 1991).

Upon treatment of the diabetic rats with glibenclamide or *G. latifolium* extracts alone or in combination, the FBG of groups 2-5 rats (diabetic and treated groups) was reduced significantly (p < 0.05) across the treatment periods (Table 2). These reductions in the FBG values of the treated groups were attributed to the effect of the drug (glibenclamide) or the extract. Glibenclamide, a second generation sulfonylurea, is an anti-diabetic drug used in lowering elevated glucose levels (Bennett et al., 2011). *G. latifolium*, an herbaceous plant used culinarily, has been reported to possess antihyperglycaemic properties (Ugochukwu et al., 2003).

The earlier significant (p < 0.05) reductions in the FBG observed in the group (Group 4) treated with the combination (glibenclamide and *G. latifolium*) compared to the other treatment groups (Table 2) is an indication of synergistic hypoglycaemic actions of the two agents. Synergistic hypoglycaemic actions of *G. latifolium* with other agents have been earlier reported (Aba and Okenwa-ANI, 2015). The observation of severe hypoglycaemia in the group treated with the combination after 21 days is a pointer that subchronic treatment with the two agents might pose hypoglycaemic treat to diabetics at the tested doses. The hypoglycaemic episode is one of the leading side effects of anti-diabetic treatment (Ono 2008; Bennet et al., 2011).

The increases in the serum levels of total cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and decrease in high density lipoprotein levels were observed in diabetic untreated rats (Tables 3-7).

Diabetic dyslipidaemia is a well-known condition in diabetes mellitus (Thomas and Rampersad, 2004; Ozouguw et al., 2013). The decreases in the elevated total cholesterol, triacylglycerol, low density lipoprotein, very low density
lipoprotein and increase in high density lipoprotein following treatments with glibenclamide and or G. latifolium suggest possible hypolipidaemic potentials of these agents. Several studies had also shown hypolipidaemic actions of glibenclamide (Bennett et al., 2011) and G. latifolium (Ugochukwu et al., 2013) when used alone. Much earlier significant ameliorative effects on dyslipidaemia were observed in the group treated with the combination of glibenclamide and G. latifolium, thus indicating synergism of the two agents in mitigating diabetic dyslipidaemia.

The results of oxidative stress markers indicated that following diabetes induction, the level of reduced glutathione and activity of catalase reduced significantly with appreciable increase in the level of malondialdehyde (Tables 8-10). Similar changes in the oxidative stress marker parameters in diabetic conditions had earlier been reported (Aba et al., 2015). During oxidative stress, the plasma levels of malondialdehyde increases with reductions in the levels and activities of antioxidant parameters. All treatment groups showed evidence of amelioration of these diabetes-induced oxidative stresses. Groups treated with combination of glibenclamide and G. latifolium particularly showed remarkable decreases in the MDA levels, increases in glutathione values and catalase activities 21 days PT. This result is in agreement with the reports of Aba and Okenwa-Ani (2015) who noted the ameliorative effects of either the glibenclamide or the G. latifolium on oxidative stress markers.

Conclusions

Treatment of diabetic rats with combination of glibenclamide and G. latifolium reduced the elevated blood cholesterol, triacylglycerol, low density lipoprotein and very low density lipoprotein with an improvement on the level of high density lipoprotein 21 days post treatment. Further studies on dose-response tests should be carried on G. latifolium to determine the appropriate dose to be combined with glibenclamide to achieve the desired effects.

References


