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ABSTRACT

Plan: The anti-diabetic and anti-hyperlipidemic effects of D-3-O-methylchiroinositol were investigated in male Wistar rats.

Methodology: Diabetes was induced by a single intra-peritoneal injection of 150 mg/kg of alloxan monohydrate. Five groups of rats comprising six animals in each group were used including a positive control group (glibenclamide, 2 mg/kg), treatment groups (8, 4 and 2 mg/kg of D-3-O-methylchiroinositol respectively) and control (untreated) group. Blood samples were obtained from the rat tails every morning before treatment.

Outcome: Alloxan-induced hyperglycaemia was significantly (p< 0.05) reduced by 59%, 48% and 41% after four days at 8, 4 and 2 mg/kg respectively compared with the untreated group (-135%) and Glibenclamide (50%). The in vitro anti-oxidant assays (DPPH and FRAP) showed that D-3-O-methylchiroinositol increased anti-oxidant activities with increasing concentration (10 – 400 μg/ml). D-3-O-methylchiroinositol therefore has anti-diabetic hypolipidemic and anti-oxidant activities.

Keywords: D-3-O-methylchiroinositol; diabetes; hyperglycemia; cholesterol; DPPH; FRAP.

1. INTRODUCTION

Diabetes mellitus remains a global major health problem in the World over with the tropics inclusive. In the past decade, the United States has recorded a 33% rise in diabetes cases1. D-3-O-methylchiroinositol has a structural formula similar to the phosphatidylinositol phosphate, which participates in the insulin signaling pathways that stimulate glucose transport2. It has been observed that D-chiroinositol reduced urinary potency with impaired glucose tolerance, insulin resistance and type 2 diabetes mellitus in rhesus monkeys and human subjects3.

D-chiroinositol also improved glucose tolerance in normal rats and increased gluconeogenesis in the diaphragm4. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas5. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the introduction of cell necrosis6.

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The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration leading to rapid destruction of beta cells. High levels of triglycerides, low density lipoproteins-cholesterol (LDL-C) and very low density lipoproteins-cholesterol (VLDL-C) have been associated with heart disease, insulin resistance and diabetes mellitus.

This study was designed to examine the anti-hyperglycemic and anti-hyperlipidemic effects of D-3-O-methylchiroinositol isolated from the stem bark of *Piliostigma thonningii* on alloxan-induced diabetic rats and to investigate its *in vitro* anti-oxidant effects since it has the same molecular formula as pinitol which is used in the treatment of diabetes mellitus.

2. MATERIALS AND METHODS

D-3-O-methylchiroinositol was isolated from the stem bark of *Piliostigma thonningii* as described by Asuzu *et al*. The stem bark of the plant was exhaustively extracted with 80% methanol in a soxhlet at 40°C for 12 h. The pure compound was isolated using column and TLC, lyophilized and stored in the fridge at 4°C until used for the experiments.

2.1. Animals

Wistar albino rats (120-150 g) of both sexes were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka and used for the experiments. They were kept in clean cages, maintained at normal room temperature and natural daylight/night conditions. They were allowed free access to standard commercial pelleted feed and clean drinking water.

2.2. Experimental

All experiments with animals were approved by the Ethical Committee of the University of Nigeria, Nsukka prior to the commencement of the various tests.

2.3. Determination of blood sugar levels:

The experimental animals were allowed one week to acclimatize to laboratory environment prior to the commencement of the experiment and they were subsequently divided into five groups comprising of 6 rats each. The animals were made diabetic by a single intra peritoneal injection of alloxan monohydrate (Sigma Aldrich, Germany) at 150 mg/kg after an overnight fast. Groups 1, 2 and 3 received 8, 4 and 2 mg/kg of D-3-O-methylchiroinositol (*per os*) respectively. Group 4 was given 2 mg/kg of glibenclamide (positive control) *per os* and Group 5 was used as negative control and treated with distilled water only.

Treatment was once daily for 4 days although fasting blood glucose level was checked at day zero and each day before every treatment. Blood was collected from the tail of the rats. The blood sugar was estimated using the ACCU-CHEK advantage II Glucometer (Roche, Germany). The body weights of the rats were recorded at the beginning and end of the experiment.
2.4. Lipid Profile:

Blood for the determination of lipid profile (cholesterol and triglycerides) was collected from the ocular retrobulbar plexus of the rats and measurements were carried out using an Automated Analyzer (Roche, HITACHI).

2.5. Antioxidant activity

The free radical scavenging activity of the extract was investigated by using the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay described by Iwalewa et al\textsuperscript{10}. Two (2) ml of the test extract, at concentrations of 10 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml and 400 μg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol). The absorbance of the resulting solution was read at 517 nm after 30 min of incubation in the dark at room temperature using a spectrophotometer. One (1) ml of 1000 μM ascorbic acid in 1 ml of DPPH was used as reference standard antioxidant, while a blank of 1ml methanol plus 2 ml of extract was run with each assay. All determinations were carried out in triplicate.

The same procedure was repeated using control sample (DPPH without extract). Mean values were obtained and used for the following calculation:

\[
\% \text{ Antioxidant Activity [AA]} = \left[ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right].
\]

The antioxidant potential of the sample was also determined by using the ferric reducing ability of plasma FRAP assay of Benzie and Strain\textsuperscript{11} as a measure of “antioxidant power”. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe\textsuperscript{1+}− tripyridyltriazine compound from colorless oxidized Fe\textsuperscript{11+} form by the action of electron donating antioxidants.

A standard curve was prepared using different concentrations (100-1000 μmol/L) of FeSO\textsubscript{4} x 7H\textsubscript{2}O. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe\textsuperscript{2+} solution of known concentration, this representing a one-electron exchange reaction. The results were corrected for dilution and expressed in μmol Fe\textsuperscript{1+}/L.

Vitamin C was measured within 1h after preparation. The sample to be analyzed was first adequately diluted to fit within the linearity range. All determinations were performed in triplicate.

2.6. Data analysis

Data obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnett's \(t\)-test. Values of \(p<0.05\) were considered significant in the study.
3. RESULTS

3.1. Effects of D-3-O-methylchiroinositol on body weight

D-3-O-methylchiroinositol increased the body weight of diabetic rats at 2 mg/kg and 4 mg/kg dosage levels (Table 1).

The highest dose (8 mg/kg) did not increase the body weight of rats in that group though the weights (-9.48%) of the rat in the group were significantly higher than those of the diabetic control group (-20.70%). The % weight gain of the rats treated with 4 mg/kg of the extract was not significantly different (P > 0.05) from the untreated control rats (Table 1).

3.2. Effect on blood glucose level

D-3-O-methylchiroinositol produced a dose-dependent reduction of blood glucose level in diabetic rats. The percentage blood glucose reduction was also time-related (Table 2). The highest reduction in blood glucose was achieved on day 3 of treatment at all the treatment doses. The highest % reduction (59%) of blood glucose was achieved by administering 8 mg/kg of D-3-O-methylchiroinositol (Table 2).

3.3. Effect of D-3-O-methylchiroinositol on serum cholesterol and triglyceride

Table 3 shows the effect of D-3-O-methylchiroinositol on serum cholesterol and triglyceride levels in diabetic rats. The compound lowered the serum cholesterol and triglyceride levels compared to those of the treated controls. The effect was not dose-dependent and the values were slightly higher than normal values.

3.4. Anti-oxidant effects of D-3-O-methylchiroinositol

D-3-O-methylchiroinositol showed concentration-related anti-oxidant effect in the DPPH assay. The anti-oxidant activity increased with the concentration of D-3-O-methylchiroinositol up to a maximum at 400 µg/ml (Fig. 1). The anti-oxidant activity was higher than that of ascorbic acid at 200 and 400 µg/ml. The FRAP assay also showed a concentration-dependent anti-oxidant activity of D-3-O-methylchiroinositol. The higher anti-oxidant activity was achieved at 400 µg/ml of the compound (Fig 2).
4. DISCUSSION

D-3-O-methylchiroinositol has a structural formula similar to phosphotidyl inositol phosphate, which takes part in insulin signal pathways and stimulates glucose transport\(^2\). This similarity in structure stimulated the present study to investigate the effects of D-3-O-methylchiroinositol on diabetes mellitus.

It was also necessary to investigate its effect on reactive oxygen species since alloxan induces damage to the pancreas by releasing free oxygen radical species. The results obtained in this study showed that D-3-O-methylchiroinositol isolated from the plant *Pilostigma thonningii* (PT) produced a dose-related reduction of blood glucose level in alloxan-induced diabetic rats. The percentage reduction of blood glucose was better than that of the therapeutic dose (2mg/kg) of glibenclamide when D-3-O-methylchiroinositol was administered at 8 mg/kg.

It was also observed that the blood glucose lowering effect of the plant extract increased as the period of treatment increased. This attribute makes it a good anti-diabetic candidate since diabetes needs prolonged period of treatment, which in some cases is for life. One significant side effect of synthetic anti-diabetic drugs available in the market today is their attendant toxicity. A major challenge today is to discover anti-diabetic drugs that would produce little or no toxic side effects when they are used for treatment over a long period. D-3-O-methylchiroinositol, being a natural product does not have the tendency to cause toxicity when used over a long period particularly as it is showing good anti-diabetic activity at low dose (2-4 mg/kg, *per os*). Previous experiments conducted with the crude extract did not reveal any toxic effects even when used at high doses\(^1\).

D-3-O-methylchiroinositol also showed very high antioxidant activity. Its anti-oxidant activity was concentration-dependent and more effective than ascorbic acid tested at the same concentrations in the two models of antioxidant assay used in the present study. This could be the mechanism by which it protected rats treated with alloxan because the cytotoxic action of alloxan is mediated through the production of reactive oxygen species leading to the destruction of pancreatic β cells. In addition, D-3-O-methylchiroinositol lowered the levels of serum cholesterol and triglycerides in diabetic rats, which is desirable in the treatment of diabetes.

*Diabetes mellitus* is usually accompanied by weight loss. In the present study, it was observed that D-3-O-methylchiroinositol increased the body weight of diabetic rats when it was used at lower doses (2 and 4 mg/kg). Increasing the body weights of diabetic rats is a sign of recovery from the disease.

5. CONCLUSION

D-3-O-methylchiroinositol, a pure compound isolated from the methanol extract of *P. thonningii* has shown significant anti-diabetic effects in alloxan-induced diabetic rats. It probably produced this effect by its anti-oxidant activity. It also increased the body weight of diabetic rats which was a sign of recovery of the rats. The anti-diabetic effects of D-3-O-methylchiroinositol were produced at low doses therefore reducing the chances of causing toxic side effects with prolonged use.
### Table 1: Effect of D-3-O-methylchiroinositol on body weights after 4 days

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Body weight (g)</th>
<th>% increase in body weight</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Alloxan+ D-3-O- methylchiroinositol</td>
<td>8 mg/kg</td>
<td>239.8±8.2*</td>
<td>217±14.8*</td>
</tr>
<tr>
<td></td>
<td>4 mg/kg</td>
<td>237.1±7.1*</td>
<td>275.0±11.7*</td>
</tr>
<tr>
<td></td>
<td>2 mg/kg</td>
<td>236.2±14.2*</td>
<td>267.3±13.4*</td>
</tr>
<tr>
<td>Alloxan+ Glibenclamide</td>
<td>2 mg/kg</td>
<td>241.8±10.7*</td>
<td>270.1±20.1*</td>
</tr>
<tr>
<td>Alloxan+ distilled water</td>
<td>-</td>
<td>244.7±14.9*</td>
<td>205.0±2.1</td>
</tr>
<tr>
<td>Control normal</td>
<td>-</td>
<td>240.1±15.1*</td>
<td>280.2±1.5*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs diabetic control; No of animals per group = 6.

### Table 2: The anti-diabetic effect of D-3-O-methylchiroinositol on alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Drug groups</th>
<th>Mean percent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>Day 1</td>
</tr>
<tr>
<td>8 mg/kg</td>
<td>31.41</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>14.02</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>3.08</td>
</tr>
<tr>
<td>Glibenclamide (2mg/kg)</td>
<td>12.11</td>
</tr>
<tr>
<td>Negative control</td>
<td>-154.15*</td>
</tr>
</tbody>
</table>

*Significantly different from other groups at p < 0.05

### Table 3: Effect of D-3-O-methylchiroinositol on serum cholesterol and triglyceride levels in diabetic rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan+ D-3-O- methylchiroinositol</td>
<td>8 mg/kg</td>
<td>100.7±6.7</td>
<td>115.7±6.0</td>
</tr>
<tr>
<td></td>
<td>4 mg/kg</td>
<td>97.2±7.8</td>
<td>105.0±5.2</td>
</tr>
<tr>
<td></td>
<td>2 mg/kg</td>
<td>129.0±4.5</td>
<td>92.7±4.9</td>
</tr>
<tr>
<td>Alloxan+ Glibenclamide</td>
<td>2 mg/kg</td>
<td>103.4±5.4</td>
<td>118.1±7.7</td>
</tr>
<tr>
<td>Alloxan+ distillwater</td>
<td>-</td>
<td>143.0±5.5</td>
<td>166.4±6.7</td>
</tr>
<tr>
<td>Control normal</td>
<td>-</td>
<td>87.4±7.7</td>
<td>85.1±6.8</td>
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REFERENCES