Effects of Methanolic seed extract of *Hunteria umbellata* (abere) on blood glucose level, hematological and lipid profile parameters in alloxan-induced diabetes in male rats

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ABSTRACT

Adult male albino rats weighing between 130–160 g were induced intraperitoneally with alloxan. The male albino rats were grouped into five groups containing Group I were not induced with alloxan, Group II served as the negative control and was given distilled water *ad labium*, Group III served as positive control and was treated with glibenclamide, Group IV and V were treated with 100 and 250 mg/kg body weight of methanolic extract of *Hunteria umbellata* seed respectively. The extracts were given to the animals orally for 14 days. At the end of the experimental period, the animals from each experimental group were starved for 16 hours and sacrificed by cervical dislocation. The phytochemical constituents of *Hunteria umbellata* extract indicates the presence of secondary metabolites like tannins, alkaloids, cardiac glycosides, reducing sugar, saponins, flavonoids and anthraquinones, The weight of diabetic untreated rats (Group II) were significantly (P<0.005) reduced when compared to other groups. The group of rats given glibenclamide, 100 and 250mg/Kg B.W of *HU* extract showed significant decrease (P<0.05) of blood sugar level compared to the untreated rats. This suggests that the plant extract possesses anti-diabetic and hypoglycemic effect. The extracts of *HU* significantly increased RBC, HGB and HCT; and the WBC was significantly reduced in the treated group compared to the untreated group. There was a significant decrease in plasma TC, TG, LDC-Cholesterol and an increase in HDL-Cholesterol values of the treated groups compared to the negative control group. This is an indication of the hypolipidemic effect of the extract and its uses in the treatment of diabetes. The extract significantly increased (P<0.05) plasma total protein level in the treated groups.

Keywords: Alloxan-induced diabetes in male rats, *Hunteria umbellata* seeds, hypoglycaemic effect and lipid profiles.

INTRODUCTION

Diabetes mellitus (DM) is one of the major complex and chronic disorders of carbohydrate, lipid, and protein metabolism characterized by persistent elevation of blood glucose, resulting from a partial or complex cessation of insulin secretion or synthesis, or peripheral resistance to insulin action. Medicinal plants are known to contain a variety of substances and are used in the treatment of many kinds of ailments in traditional medicine. Most importantly is that they are taken by the majority of the population because they are inexpensive and available (Sofowora, 1982). *Hunteria umbellata* (K. Schum.) Hallier belongs to the family Apocynaceae, is a medicinal plant with a long standing use in the treatment of various ailments in Nigeria and Ghana (Adegoke and Alo, 1986). Among the Yoruba and Biniis (Southwest Nigeria), it is locally known as "Abeerere". Various parts of the plant have been used in herbal medicine for the treatment of Diabetes (Raman, 1994), peptic ulcers, piles, yaws, dysmenorrhea, fever, infertility (Eliuba, 1995), helminthic infection, and as an oxytotoxic (Oluwemimo, 2001). Water decoction made from the dried seeds of *Hunteria umbellata* (*HU*) Hallier f. is highly valued in the local management of diabetes mellitus, obesity, stomach aches, pains and swellings, hypertension and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009a).

In addition, the anti-obesity and hyperlipidaemic activities of *HU* have also been reported to be mediated via inhibitions of intestinal lipid absorption and *de novo* cholesterol and triglyceride syntheses (Adeneye et al,2010).

AIM OF STUDY

The study explains the hypoglycemic effect of the methanolic seed extract of *Hunteria umbellata* (abere) and its effect on hematological and lipid profile parameters in alloxan-induced diabetes in male rats.

METHODOLOGY

Collection and identification of *Hunteria umbellate* (*H U*) seeds

The seeds of *Hunteria umbellata* were gotten from Ikorodu market in Lagos State, Nigeria and authenticated by Miss Shokefun, a botanist from the Department of Science Laboratory Technology (Environmental Biology Unit), Lagos State Polytechnic Ikorodu, Lagos-Nigeria.

Preparation of methanolic seeds extract of *Hunteria umbellata*
The normal Laboratory Standard for preparing extract was used in this study. The seeds were air dried under shade in the Biochemistry laboratory. The dried seeds were pounded to coarse powder in a mortar and then to fine powder with a blender. Extraction was carried out by dispersing 200g of the grounded plant material in 1L of 70% Methanol and shaking was done with GFL shaker for 72 hours. This was followed with vacuum filtration and evaporation at a temperature not exceeding 40°C. The concentrate was heated over a water bath to obtain a solvent-free extract, which was stored in a refrigerator at 4°C.

**Phytochemical analysis of methanolic seed extract of Hunteria umbellata**

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures (Trease and Evans (1995), Harborne (1993), and Sofowora (1993).)

**Administration of alloxan**

Male albino rats of about ten weeks old with weight range of 130-160g were made diabetic by injecting them with alloxan intraperitoneally. Development of diabetes was confirmed after 72 hours of alloxanisation by using "Accuchek Active Glucometer" (Roche Diagnostics) and blood glucose test strips.

**Grouping of animals**

The animals were grouped as follows:

- Group I - control (non-diabetic rats)
- Group II- Negative control (diabetic without treatment)
- Group III- Positive control (diabetic + glibenclamide)
- Group IV- Diabetic + 100mg/Kg B.WT of Hunteria umbellata
- Group V- Diabetic + 250mg/Kg B.WT of Hunteria umbellata

**Determination of hematological parameters.**

The total red blood cell (RBC), hemoglobin concentration (HGB), white blood cell count (WBC), platelet count and other hematological parameters were determined in the blood using ADVIA 60 Closed Tube (CT) Automated Hematology System in Yaba, Psychiatric Hospital, Lagos, Nigeria.

**Collection of blood samples for plasma preparation**

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. The blood was later centrifuged for 10 min at 3000rpm using a centrifuge. The clear supernatant was used for the estimation of lipid profiles and liver function tests.

**Determination of plasma lipid profiles**

The plasma total protein (TP), Total cholesterol (TG), Triglyceride (TG) and HDL-Cholesterol (HDL-Chol) were determined using Randox diagnostic kit [Trinder,1969 and Tietze, 1990]. Low density Lipoprotein-Cholesterol (LDL-Chol) was calculated using formula from [Friedwald, et al 1972].

**Data Analysis**

Data analysis was done using the GraphPad prism computer software. Students’t’-test and one-way analysis of variance (ANOVA) were used for comparison. A P-value < 0.05 was considered significant.

**RESULTS**

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins (Ferric chloride test)</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides (Salkowski test)</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids (Wagners test)</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids (Lead acetate test)</td>
<td>++</td>
</tr>
<tr>
<td>Reducing power (Fehling A and B test)</td>
<td>++</td>
</tr>
<tr>
<td>Saponins (Frothing test)</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
</tr>
</tbody>
</table>

Table I: Phytochemical screening of methanolic seeds extract of *Hunteria umbellata*

present at low levels = +, present at moderate levels = ++ and present at high levels = +++

**Figure 1** Body weight of animals after treatment with glibenclamide, 100 and 250 mg/kg B.W of *Hunteria umbellata*
There is a progressive decrease in the body weight of diabetic untreated rats compared to other groups.

Table II. Effect of standard drug (glibenclamide) and methanolic seed extract of Hunteria umbellata (100 and 250mg/Kg B.W) on blood glucose level of albino rats induced with alloxan.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial glucose concentration (mg/dl)</th>
<th>Glucose conc. after Alloxan induction (mg/dl)</th>
<th>Glucose conc. after 7 days of treatment (mg/dl)</th>
<th>Glucose conc. after 14 days of treatment (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>101 ±8</td>
<td>102 ±10</td>
<td>102 ±11</td>
<td>107 ±13</td>
</tr>
<tr>
<td>Group II</td>
<td>103 ±10</td>
<td>526±33</td>
<td>553±43</td>
<td>571±42</td>
</tr>
<tr>
<td>Group III</td>
<td>98 ±11</td>
<td>538±42</td>
<td>220 ±150</td>
<td>107 ±6</td>
</tr>
<tr>
<td>Group IV</td>
<td>100 ±11</td>
<td>557±29</td>
<td>260 ±95</td>
<td>98 ±6</td>
</tr>
<tr>
<td>Group V</td>
<td>104 ±9</td>
<td>568±31</td>
<td>216 ±110</td>
<td>89 ±8</td>
</tr>
</tbody>
</table>

Group I animals were not induced with Alloxan while Group II animals were not treated.

Table II above shows that Hunteria umbellata seed extract and the standard drug have hypoglycaemic effects on alloxan-induced diabetic rats.

Table III. Effect of Methanolic seed extract of Hunteria umbellata on hematological parameters.

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
<th>GROUP V</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁹/L)</td>
<td>9.9 ± 1.1*</td>
<td>14.6±5.2</td>
<td>11.3±1.4*</td>
<td>11.1±1.1*</td>
<td>10.2 ±1.5*</td>
</tr>
<tr>
<td>HGB g/dl</td>
<td>13.2 ± 1.2*</td>
<td>8.7 ± 1.4</td>
<td>12.7 ± 3.1*</td>
<td>13.1 ± 1.2*</td>
<td>13.9 ± 2.1*</td>
</tr>
<tr>
<td>RBC (×10¹²/L)</td>
<td>11.2 ± 2.6*</td>
<td>6.4 ± 1.3</td>
<td>9.4 ± 1.3*</td>
<td>10.3± 1.0*</td>
<td>10.9 ± 2.1*</td>
</tr>
<tr>
<td>HCT %</td>
<td>43.5 ± 2.3*</td>
<td>31.1 ± 10.2</td>
<td>39.8 ± 2.2*</td>
<td>40.2 ± 2.5*</td>
<td>44.1 ± 1.1*</td>
</tr>
<tr>
<td>MCH pg</td>
<td>16.7 ± 1.2</td>
<td>16.9 ± 2.1</td>
<td>17.4 ± 1.8</td>
<td>18.1 ± 0.1</td>
<td>18.3 ± 0.7</td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>29.1 ± 2.1</td>
<td>31.2 ± 1.3</td>
<td>31.1 ± 0.4</td>
<td>30.9 ± 0.8</td>
<td>32.2 ± 1.2</td>
</tr>
<tr>
<td>MPV fl</td>
<td>7.1 ± 0.6</td>
<td>7.3 ± 0.7</td>
<td>7.2 ± 0.1</td>
<td>7.5± 0.9</td>
<td>7.1 ± 0.8</td>
</tr>
<tr>
<td>PCT %</td>
<td>0.464 ± 0.0541</td>
<td>0.413 ± 0.065</td>
<td>0.450 ± 0.053</td>
<td>0.449 ± 0.052</td>
<td>0.481 ± 0.027</td>
</tr>
</tbody>
</table>

The values are the Means ± SD for five rats in each group. White blood count (WBC), Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean platelet volume (MPV) and Plateletcrit (PCT).

Figure II. Plasma Cholesterol values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 250mg/kg body weight of extract.

Figure III. Plasma Triglyceride values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 250mg/kg body weight of extract.

Figure IV. Plasma HDL-Cholesterol values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 250mg/kg body weight of extract.

Figure V. Plasma LDL-Cholesterol values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 250mg/kg body weight of extract.
The *Hunteria umbellata* seed extract however produce hypolipidaemic effect after two weeks of treatment with 100 and 250 mg/kg body weight of the extracts (Figure II-V). Figure VI shows that the extract significantly increase plasma protein level.

**DISCUSSION**

Several hypoglycemic herbs have been used as non-prescription treatment for diabetes. Few herbal medicines have been shown to have hypoglycaemic effect, however there test result is subjected to several factors. First each herb contains thousands of components, only a few of which may be therapeutically effective. Secondly extraction of active component is not easy (Karashima, 1988 and Angelova et al, 2008). The phytochemical screening of methanolic seed extract of *Hunteria umbellata* indicated the presence of secondary metabolites like tannins, cardia glycosides, flavonoids, saponins, alkaloids, anthraquinones, polyphenols and terpenoids etc (Table 1). The presence of these phytochemicals in high concentration account for the significant hypoglycemic effect of *H U*. It has been showned that medicinal plants with hypoglycemic and anti-diabetic effect usually contain high concentration of alkaloids and flavonoids (Oladele et al 1995). The presence of flavonoids and alkaloids in *HU* could account for its protective effects on the liver and kidney, since these phytocomponents have been documented to confer protection on the kidneys and liver through prevention of tissue lipid peroxidation which explains their anti-oxidant and free-radical scavenging activities (Fraga et al, 1987; Laughton et al, 1989; Sanz et al., 1994). The untreated diabetic rats have significantly loss of weight (P<0.05) when compared to the normal and treated groups (Figure 1). This may be due to loss in muscle and adipose tissue protein and fatty acids (Granner 1996). Studies have also reported significant weight reduction in untreated diabetic rats (Ahmed et al 2005). Neminibi-audia, 2003 showed that treatment with extract of *Vernonia amygdalina* resulted in appreciation in weight of the animals after 14 days. The present study observed that 5mg/Kg B.W. of glibenclamide, 100 and 250 mg/Kg of *H U* seed extract showed significant (P<0.05) blood glucose lowering effect compared to the untreated group from the period of 1- 14 days respectively (Table II). Adeneye and Adeyemi, 2009 showed that 50-200mg/kg of *H U* significantly reduce the blood glucose level in glucose and nicotine-induced hyperglycaemic rats.

In the present study, methanolic seed extract of *Hunteria umbellata* treatment for 14 days produced significant elevations in RBC, PCV and Hb indicating the haematopoietic effect of *HU*. Thus, the significant elevations in RBC, PCV and Hb strongly suggest that *HU* could be useful in the management of anemia. The graph II-V above showed that the standard drug (glibenclamide) and extract of *H U* significantly reduce (P<0.005) total cholesterol, triglyceride and LDL-Cholesterol in the diabetic treated groups compared to the untreated group (group II). Kwiterovich 1997 showed that treatment or drug therapy to regulate cholesterol can reduce subsequent cardiovascular diseases (CVD) associated mortality and morbidity. Yokozawa et al 2006 and Zhang et al 2007 explained the therapeutics benefits of plant foods in reducing the risk of CVD through the regulation of cholesterol. The HDL-Cholesterol values of Group I, III, IV and V were significantly (P<0.05) higher when compared to Group II. This is an indication that the diabetic untreated albino rats (Group II) are prone to CVD. Assmann and Gotto 2004 observed through epidemiological and clinical studies that low level of HDL-Cholesterol is associated with increased risk of CVD. Figure VI shows that the extract significantly increase (P<0.05) plasma protein level.

**CONCLUSION**

*Hunteria umbellata* seeds extract contain some secondary metabolite like tannins, cardia glycosides, flavonoids, saponins, alkaloids, anthraquinones, polyphenols and terpenoids. The extract reduces the blood glucose levels, reduces cholesterol level, triglycerides and LDL-cholesterol level while the HDL-cholesterol level increases significantly.
H.U significant elevates RBC, PCV and Hb levels, which strongly suggest that HU could be useful in the management of anemia.

Recommendations for further studies

Further studies are needed to determine the exact component in Hunteria umbellata seed extract responsible for the observed effect and such component may be use as a prophylactic agent against hypercholesterolemia.

REFERENCES


