THE NOVEL ANTIHYPERGLYCAEMIC ACTION OF HUNTERIA UMBELLATA SEED FRACTIONS MEDIATED VIA INTESTINAL GLUCOSE UPTAKE INHIBITION

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Abstract

The present study evaluated the antihyperglycaemic effect and mechanism of action of fractions of the aqueous seed extract of Hunteria umbellata (K. Schum.) Hallier f. (HU) in normal and alloxan-induced hyperglycaemic rats. HU was partitioned in chloroform, acetyl acetate and butan-1-ol to give chloroform fraction (HUc), ethyl acetate fraction (HUe), butanol fraction (HUb) and the “residue” (HUm), respectively. 200 mg/kg of each of these fraction dissolved in 5% Tween 20 in distilled water was investigated for its acute oral hypoglycaemic effects in normal rats over 6 hours while its repeated dose antihyperglycaemic effect was evaluated in alloxan-induced hyperglycaemic rats over 5 days. In addition, 50 mg/kg of the crude alkaloid fraction (HU3a) extracted from HU was evaluated for its possible antihyperglycaemic activity in alloxan-induced hyperglycaemic rats using oral glucose tolerance test (OGTT) over 6 hours. Using the solvent system, distilled water-butanol-ammonium hydroxide (2:15:1, v/v/v), HUc was chromatographed and stained with Dragendorff’s reagent for confirmatory qualitative analysis for alkaloids. Results showed that oral pre-treatment with 200 mg/kg of HUc, HUb and HUm resulted in a significant (p<0.05, p<0.001) time dependent hypoglycaemic effect, with the butan-1-ol fraction HU causing the most significant (p<0.001) hypoglycaemic effect. In the alloxan-induced hyperglycaemic rats, repeated oral treatment with 200 mg/kg of each HU fractions for 5 days resulted in significant (p<0.05) decreases in the fasting blood glucose concentrations with the most significant (p<0.001) antihyperglycaemic effect also recorded for HUb. Similarly, oral pre-treatment with 50 mg/kg of HU3a significantly (p<0.05, p<0.01 and p<0.001) attenuated an increase in the post-absorptive glucose concentration at 1st -6th h in the alloxan-induced hyperglycaemic OGTT model. In addition, alkaloid was present in most of the separated spots on the TLC plate. In conclusion, results of this study showed that HU contains a relative high amount of alkaloids which could have accounted for the antihyperglycaemic action of HU that was mediated via intestinal glucose uptake inhibition.

Key words: Hunteria umbellata aqueous seed extract, Alkaloid fraction, Intestinal glucose uptake inhibition, Normal and alloxan-induced hyperglycaemic rats

List of Abbreviations: C4H9OH = butan-1-ol; dH2O = distilled water; HCl = hydrochloric acid; HU = aqueous seed extract of Hunteria umbellata (K. Schum.) Hallier f. (HU3a) = alkaloid fraction of HU; HUm = butan-1-ol fraction of HU; HUb = ethyl acetate fraction of HU; HUc = chloroform fraction of HU; Na2CO3 = sodium bicarbonate; NH4OH = ammonium hydroxide; Rf = retention factor ; TLC = thin layer chromatography; UV = ultraviolet light

Introduction

Hunteria umbellata (K. Schum.) Hallier f. (family: Apocynaceae) is a tropical rainforest tree which closely resembles Picralima umbellata K. Schum., Polyphodium umbellata (K. Schum.) Stapf., Hunteria elliotii (Stapf) Pichon, Picralima nitida, Picralima elliptica (Stapf) Stapf., and Picralima gracilis A. Chev. (Hutchinson and Dalziel, 1963). In Nigeria, the plant is ubiquitous to the South-west region where it is locally known as “Abeere” among the Yorubas and the Benis while in French it is known as “Demouain” (Bouquet and Debray, 1974; Boone, 2006). In Ethiopia, the plant is known as “Gebets” and is used as an immune booster (Boone, 2006; Adeneye and Adeyemi, 2009a; 2009b).

Ethnomedical uses of Hunteria umbellata (K. Schum.) Hallier f. include treatment of yaws and sexually transmitted infections, stomach ache and ulcers, diabetes mellitus and dysmenorrhoea (Adeneye et al., 2008). In Nigeria, traditional birth attendants/midwives employ the fresh leaves and pulp of fresh fruits of Hunteria umbellata in the induction and/or augmentation of labour in gravid uterus at term (Falodun et al., 2006). Hot and cold decoctions made from the plant seeds have also been reported to be highly valued in the local treatment of obesity, hypertension, pain and swellings, anaemia and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009a; 2009b). Previous studies have reported the antihyperglycaemic effect of the crude aqueous seed extract of Hunteria umbellata (HU) in rats. In furthering our investigation of the antihyperglycaemic effect of HU, the present study is aimed at separating and investigating the possible antihyperglycaemic effect of the solvent fractions of HU in normal and alloxan-induced

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hyperglycaemic rats. In addition, qualitative analysis of the most active fraction of HU was conducted on thin layer chromatography. The choice of dose used was based on the results of previous studies.

Materials and methods

Plant material

Collection, identification, authentication and the extraction process of *Hunteria umbellata* with voucher no.: FHI 107687 were done as previously described by Adeneye and Adeyemi (2009a). The extraction process was done as previously reported by Adeneye and Adeyemi (2009b).

Solvent partitioning of HU

30 g of the extract was dissolved completely in 100 ml of distilled water. The solution was then transferred into 5 litre burette before it was partitioned using between 1 litre to 1.5 litres of different partitioning solvents (diethyl ether, chloroform, ethyl acetate and butan-1-ol) in the order of their increasing solubility gradients. The fraction obtained with each partitioning solvent was concentrated in vacuo using rotary evaporator (BU’CHI Rotavapor® Model R-215, Switzerland) with Vacuum Module V-801 EasyVac®, Switzerland) set at a revolution of 70 rpm and a temperature of 35°C. The “Marc” and the concentrate of each fraction were then transferred to an aerated oven preset at 35 °C for complete dryness. The residues left after oven drying were then weighed. This procedure was repeated thrice and each residue was pooled together and stored in clean and dry, water and air-proof containers and preserved in the refrigerator until required for experimentation.

Qualitative analytical TLC method for HUb

Thin layer chromatography (TLC) analysis of HUb was performed using TLC plates which were cut along lines into three parts (10 x 3 cm) and dried in a drying oven at 100-105 °C for 1 h. Plates were activated before-hand in a drying oven for at least 1 h at 100-105 °C for sharp separation of the components. The chromatographic chamber was saturated with vapour of the solvent system for 24 h for the same purpose. 0.02 ml of the extract was deposited at the origin of the TLC paper using a micropipette. The plate with the deposited samples was air-dried for 5 min, placed in the chromatography chamber saturated with water: butanol: ammonium hydroxide solution (dH2O: C4H9OH: NH4OH) solvent system at the ratio of 2:15:1 (v/v/v), and chromatographed in an ascending mode. When the solvent front reached about 9 cm, the plate was removed from the chamber and air-dried for 5 min. The plate was examined in UV light at wavelength 366 nm before and after treatment with Dragendorff’s solution for the identification of alkaloids in the extract. A colour change on the TLC plate was then recorded. The ratio of the distance moved each of separated spot (solute) relative to the distance moved by the solvent (solvent front) is expressed as the retention factor (Rf).

Extraction of the crude alkaloid fraction (HUaf) from HU

4.14 g of HU in 10 ml of distilled water (pH = 4.2) was repeatedly titrated with 50 ml of 5% aqueous HCl solution to acidify the solution to a pH of 2. The acidify solution was extracted with chloroform (150 ml x 3) to remove the neutral compounds in the acidify HU solution. The acidify HU solution was carefully basified with 5% sodium bicarbonate (Na2CO3) solution to pH 10. Using a separating funnel, the mixture extracted repeatedly with small portions of chloroform (150 ml x 3) until the last extract was almost colourless and the basic solution gave negative tests with alkaloid detecting reagents. The chloroform extracts were combined followed by evaporation at the room temperature (32 °C) to give total alkaloid fraction (a very deep brown solid residue) weighing 0.30 g (yield: 7.25%) (Yang et al., 1999; Adeoye and Oyedapo, 2004). This procedure was repeated thrice and the total alkaloid fraction pooled together into a tight-capped container which was stored in the refrigerator until required for experimentation.

Experimental animals

Healthy young adult male albino Wistar rats (100-130 g) used in this study were obtained from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria after an ethical approval for the study has been obtained. The rats were housed in polypropylene cages and handled in accordance with international principles guiding the Use and Handling of experimental animals (United States National Institutes for Health, 1985). Rat feed (Ladokun Feeds, Ibadan, Nigeria) and tap water were provided ad libitum. The rats were maintained at an ambient temperature between 28-30 °C, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 h of light (06:30 h - 18:30 h) alternating with approximately 12 hours of darkness (18:30 h - 06:30 h).

Determination of the oral hypoglycaemic effect of the fractions of HU in normal rats

To identify the biologically active fraction(s) of HU, 1 g of the marc, chloroform fraction, acetyl acetate fraction and butan-1-ol fraction each were dissolved in 10 ml of 5% Tween-20 in distilled water to make a concentration of 100 mg/ml solution. 5% Tween-20 in distilled water was used as the solvent in this case because of the insolubility of the fractions in distilled water. Thirty, young in-bred adult male white albino Wistar rats (100-130 g) were randomly allotted to 6 groups. The rats were then fasted overnight for 12-14 hours but had free access to drinking water. The basal fasting blood glucose of each rat was first determined using One Touch Basic Blood Glucose Monitoring System® (LifeScan Inc., Milpitas, California, U.S.A.). This was then followed by the following treatments: Group I rats were orally administered 10 ml/kg of

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and alloxan-induced hyperglycaemic rats were randomly allocated into treatment groups as follows:

- **Group I**: normoglycaemic rats which were orally treated with 10 ml/kg of 5% Tween 20 dissolved in distilled water;
- **Group II**: alloxan-induced hyperglycaemic rats orally pre-treated with 10 ml/kg of 5% Tween 20 dissolved in distilled water;
- **Group III**: alloxan-induced hyperglycaemic rats orally pre-treated with 1 mg/kg of glibenclamide (Daonil®, Hoechst Marion Roussel Limited, Mumbai, India) dissolved in 5% Tween 20 in distilled water;
- **Group IV**: alloxan-induced hyperglycaemic rats orally pre-treated with 10 ml/kg 5% Tween 20 dissolved in distilled water 1 hour before oral treatment with 3 g/kg of D-Glucose (BDH Chemicals, Poole, England);
- **Group V**: alloxan-induced hyperglycaemic rats orally pre-treated with 1 mg/kg of glibenclamide (Daonil®, Hoechst Marion Roussel Limited, Mumbai, India) dissolved in 5% Tween 20 in distilled water 1 hour before oral treatment with 3 g/kg of D-Glucose (BDH Chemicals, Poole, England);
- **Group VI**: alloxan-induced hyperglycaemic rats orally pre-treated with 200 mg/kg of chloroform fraction (%yield = 73.3%), 4 g of butan-1-ol fraction (%yield = 13.3%) and 22 g of the “residue” (%yield = 0%), all of which were insoluble in water.

**Statistical Analysis**

Results were expressed as mean ± S.E.M. of six observations. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-Newman-Keuls test on SYSTAT 10.6. Statistical significance were considered at p<0.05, p<0.01, and p<0.001.

**Results**

**Solvent Partitioning of HU**

Partitioning of HU with diethyl ether, chloroform, ethyl acetate, and butan-1-ol and the eventual oven-drying of the fractions yielded 0 g of diethyl ether fraction (%yield = 0%), 1 g of chloroform fraction (%yield = 3.3%), 2 g of ethyl acetate fraction (6.6%), 4 g of butan-1-ol fraction (%yield = 13.3%) and 22 g of the “residue” (%yield = 73.3%), all of which were insoluble in water.

**Qualitative analyses of the major phytochemicals in HU**

Using the developing solvent water-butanol-ammonium hydroxide (2:15:1, v/v/v) HU was chromatographed and stained with Dragendorff’s reagent to confirm the presence of alkaloids on TLC plate. Chromatographic separation of HU on TLC plate showed spots at different gradients but two distinctive heavy spots that fluorescent white- one at the middle of the spectrum (y1) (with Rf value of 0.40) and the other at the upper end of the spectrum (y2) (with Rf value of 0.93) when view

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under UV light (wavelength: 366 nm) when compared to the chromatogram viewed under the visible light (Figure 1). After spraying with Dragendorff’s reagent in the fume cupboard, spots x₁, x₂, x₄, x₅, x₆, x₇ (with Rₜ values of 0.07, 0.16, 0.27, 0.32, 0.41, 0.59 respectively) turned deep orange-brown confirming the presence of alkaloids at these spots corresponding to spots y₁, y₂, y₃, y₄, y₅, y₆ (with Rₜ values of 0.08, 0.15, 0.29, 0.33, 0.40, 0.59, respectively) on TLC before spraying with Dragendorff’s reagent (Figure 2).

Evaluation of the antihyperglycaemic effect of HU fractions in normal rats

Figure 3 and Table 1 show the acute effect of oral treatment with chloroform, ethyl acetate, butan-1-ol, and “residue”) fractions of HU on the blood glucose concentrations of normal and alloxan-induced hyperglycaemic rats, respectively. Oral treatment with 1 mg/kg glibenclamide, 200 mg/kg glucose concentrations in alloxan-induced hyperglycaemic rats for 5 days resulted in significant (p<0.05) decreases in the fasting blood glucose concentrations with the most significant (p<0.01) hypoglycaemic effect recorded for HUb. This significant (p<0.01) hypoglycaemia was comparable to that recorded for glibenclamide (Table 1).

Table 1: Effect of 5 days of repeated oral administration of 200 mg/kg of HUae, HUam, HUb and HUan on the fasting blood glucose concentrations in alloxan-induced hyperglycaemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>fasting blood glucose (FBG) (mg/dl) on day 1</th>
<th>% FBG inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>76.00 ± 3.21</td>
<td>2.66 ± 3.96</td>
</tr>
<tr>
<td>II</td>
<td>230.20 ± 5.38e</td>
<td>10.69 ± 1.42e</td>
</tr>
<tr>
<td>III</td>
<td>229.40 ± 5.57e</td>
<td>-50.12 ± 0.56f</td>
</tr>
<tr>
<td>IV</td>
<td>231.40 ± 8.47e</td>
<td>-5.79 ± 1.34d</td>
</tr>
<tr>
<td>V</td>
<td>224.00 ± 5.50e</td>
<td>-9.09 ± 1.03d</td>
</tr>
<tr>
<td>VI</td>
<td>221.80 ± 6.71e</td>
<td>-48.53 ± 1.77f</td>
</tr>
<tr>
<td>VII</td>
<td>231.40 ± 7.13e</td>
<td>-15.37 ± 1.96d</td>
</tr>
</tbody>
</table>

* represents a significant increase at p<0.001 when compared to basal value at day 0 while †, ‡ and § represent significant decreases at p<0.05, p<0.01 and p<0.001, respectively, when compared to Group II values on day 6 and ‡ represents a significant increase at p<0.05 when compared to Group I and Group II values on day 0.

Discussion

The current study investigates the antihyperglycaemic potential of the solvent fractions of HU in normal and alloxan-induced hyperglycaemic rats. The profound hypoglycaemic effect induced by the 200 mg/kg of butan-1-ol HU fraction suggests that this fraction contains a high concentration of antihyperglycaemic phytoconstituent(s) that may account for the antihyperglycaemic effect of the crude aqueous seed extract of Hunteria umbellata (K. Schum.) Hallier f. Evaluation

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Figure 1: Comparative chromatograms of HU butanol fraction (HU$_b$) viewed under visible light and UV light at wavelength of 366 nm showing the different spots of separation of HU$_b$.

Figure 2: Comparative chromatograms of HU butanol fraction (HU$_b$) after and before staining with Dragendorff’s reagent (b) to confirm the alkaloid portions of HU$_b$ (indicated as deep orange-brown spots) on the TLC plate under visible light.

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**Figure 3:** Effect of 200 mg/kg of HU fractions on time-dependent blood glucose concentration (mg/dl) of normal rats

- d and f represent significant decreases at $p<0.05$ and $p<0.001$ respectively, when compared to the basal value

Group I = 10 ml/kg 5% Tween 20/distilled water; Group II = 1 mg/kg glibenclamide in 5% Tween 20/distilled water
Group III = 200 mg/kg HUc in 5% Tween 20/distilled water; Group IV = 200 mg/kg HUe in 5% Tween 20/distilled water
Group V = 200 mg/kg HUb in 5% Tween 20/distilled water; Group VI = 200 mg/kg HUm in 5% Tween 20/distilled water

**Figure 4:** Effect of 50 mg/kg of the crude alkaloid fraction (HU_Af) on oral glucose tolerance test (OGTT) in alloxan-induced hyperglycaemic rats

- a, b and c represent significant increases at $p<0.05$, $p<0.01$ and $p<0.001$ when compared to the basal values at 0 hr while d, e and f represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$ when compared to values at 1 hr

I = normoglycaemic rats + 10 ml/kg of 5% Tween 20 in distilled water; II = normoglycaemic rats + 10 ml/kg of 5% Tween 20 in distilled water + 3 g/kg D-Glucose; III = alloxan hyperglycaemic rats + 10 ml/kg of 5% Tween 20 in distilled water IV = alloxan hyperglycaemic rats + 3 g/kg D-Glucose in 5% Tween 20 in distilled water; V = alloxan hyperglycaemic rats + 1 mg/kg of glibenclamide + 3 g/kg D-Glucose; VI = alloxan hyperglycaemic rats + 50 mg/kg of (HU_Af) + 3 g/kg D-Glucose.

[http://dx.doi.org/10.4314/ajtcam.v9i1.3](http://dx.doi.org/10.4314/ajtcam.v9i1.3)
of the alkaloid fraction in HU showed that single oral treatment with this fraction caused profound antihyperglycaemia in the treated rats suggesting that HU could be responsible for the antihyperglycaemic effect of the extract. This antihyperglycaemic effect was mediated via inhibition of intestinal glucose uptake mechanism since the HU significantly attenuated an increase in the post-absorptive glucose concentrations in the alloxan-induced hyperglycaemic rats. Literature has shown that certain alkaloids induce hypoglycaemia via inhibition of intestinal glucose uptake by inhibiting α-glucosidase activities (Yoshikawa et al., 2002; Fattorusso and Taglialetela-Scafati, 2007). In addition, it has been reported that medicinal plants rich in alkaloids usually exhibit hypoglycaemic activities (Svoboda et al., 1964; Yoshikawa et al., 2007; Okonta and Agwu, 2007; Fuji et al., 2009). Thus, result of this study suggests that HU could be mediating its oral hypoglycaemic effect through this mechanism, although other major phytocomponents in HU could be mediating their hypoglycaemic effects via other mechanisms.

Again, based on the higher number of spots positive for alkaloids, it could be deduced that HU contains a higher amount alkaloids than other phytocomponents. Therefore, result of this study is in accord with the earlier report of Adeogoke and Alo (1986). Although, quantification of these phytocomponents was not done in this study but this could constitute an area of future study. However, previous studies have reported intestinal glucose uptake inhibition as one of the mechanisms by which HU lowers and control blood glucose (Adeneye and Adeyemi, 2009a; 2009b). From the results of the qualitative analyses of HU on TLC coupled with that of in vivo antihyperglycaemic study, it could be deduced that alkaloids are responsible for intestinal glucose uptake inhibition mechanism of HU.

In conclusion, results suggest that alkaloids contained in HU accounted for the antihyperglycaemic effect of the extract which was mediated via intestinal glucose uptake inhibition mechanism.

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