Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata* in normoglycaemic and glucose- and nicotine-induced hyperglycaemic rats

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Summary: Dry seeds of the plant, *Hunteria umbellata* K. Schum (family: Apocynaceae), are highly valued in African traditional medicine in the treatment of various human diseases, including diabetes mellitus and obesity. In the present study, the hypoglycaemic and weight loss effects of 50 - 200 mg/kg of the aqueous seed extract of *Hunteria umbellata* (HU) were investigated in normal and drug-induced hyperglycaemic rats. In addition, the acute oral toxicity using the preliminary and the main tests of the Up-and-Down Procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity was conducted. Phytochemical analysis of the aqueous seed extract was also carried out. Results showed that HU caused progressive and significant (p<0.05, p<0.01 and p<0.001) dose-related reduction in the blood glucose concentrations in normal and drug-induced hyperglycaemic rats, an effect, which was more than that of glibenclamide and mediated via inhibition of intestinal glucose uptake and adrenergic homeostatic mechanisms. HU also caused significant (p<0.05 and p<0.01) dose-dependent reduction in the average body weight of treated rats when compared to untreated rats. The acute oral toxicity study showed that the plant extract had an LD50 of 1020 mg/kg and as such slightly toxic. Results of the phytochemical analysis of HU revealed the presence of alkaloids, flavonoids, tannins and glycosides. Thus, the data generated in the present study has a strong positive correlation with its folkloric use in the treatment of suspected type 2 diabetic patients, although its use should be with great caution.

Industrial relevance: The data generated in this study suggest that HU could be very useful in the management of type 2 diabetes mellitus although several further studies will still be required at identifying the active hypoglycaemic phytocomponent(s) and their possible mechanisms of action.

Keywords: *Hunteria umbellata* (K. Schum), aqueous seed extract, glucose- and nicotine-induced hyperglycaemic rats, hypoglycaemia, acute oral toxicity, phytochemical analysis

Introduction

Diabetes mellitus (DM) remains one of the major chronic disorders of carbohydrate, lipid, and protein metabolism characterized by persistent elevations of fasting blood glucose equal or greater than 140 mg/dl taken on at least two separate occasions, resulting from a partial or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Murray and Pizzorno, 1997). DM is often associated with increased risk of pansystemic complications which include ischaemic heart disease, nephropathy, retinopathy, neuropathy, ulceration and gangrene of extremities (Murray and Pizzorno, 1997; Rotshney and Zito, 2004). DM could occur alone but more often co-exists with other systemic diseases such as hypertension, dyslipidaemia, ischaemic heart disease, renal diseases etc. As a result, DM and its associated complications have significant impact on health, quality of life and life expectancy of its sufferers. In 1998, World Health Organization estimated that approximately 120 to 140 million people were globally affected by DM (WHO, 1999). In 2000, this figure increased to more than 177 millions (Kalda et al., 2008) and is projected to increase to 221 millions by 2010 (Amos et al., 1997) and to double by the year 2025 (King et al., 1998; Kalda et al., 2008). Also,
epidemiological studies showed that the global epidemic of DM is worse or greater in developing than the
developed countries (Oputa, 2002).

In response to this global health challenge, the WHO Expert Committee on diabetes mellitus recommended
an urgent and further evaluation of the folkloric methods of managing the disease (WHO, 1980; WHO, 2002)
because of high mortality and morbidity arising from its attendant complications and limitations associated with
the use of conventional antidiabetic drugs (Adeneye et al., 2006a). The limitations include inaccessibility to
health facilities where the drugs are available, high cost, and rigid treatment regimen (Adeneye and Agbaje,
2008). In response to this recommendation, several medicinal plants are currently being investigated for their
hypoglycaemic efficacies. One of the several indigenous plants used in the local treatment of DM among Yoruba
(South-West Nigeria) is the dry seeds of Hunteria umbellata.

Hunteria umbellata (K. Schum) Hallier which is synonymous with Picralima umbellata K. Schum, and
Polyadoa umbellata (K. Schum) Stapf., belongs to the family Apocynaceae. The plant is a small glabrous tree
measuring 2 - 5 feet in girth and 25 - 40 feet high which grows well and ubiquitous to the tropical West African
forest grove (Sofowora, 1982). Its leaves are green in colour, broadly elliptic to oblong in shape measuring 11 -
23 cm long, 5 - 9 cm broad with 11 - 18 pairs of lateral veins (Hutchinson and Dalziel, 1937). The axis and
branches of its white, well-scented inflorescence are markedly thickened at their apex (Hutchinson and Dalziel,
1937). The plant is locally known as “Abeere” among the Yoruba (South-West Nigeria).

In African folk medicine, various parts (especially the leaves, roots and barks) of Hunteria umbellata plant
are highly valued for the treatment of various veterinary and human diseases. For example, decoction made from
the plant stem and root is reputed for its anthelmintic activities and in the treatment of swellings (Gill, 1992).
The plant leaves and pulp are equally used by West African traditional midwives to treat pregnancy related
ailments and to induce or augment labour at term (Falodun et al., 2006). Recently, the dose dependent and the
mechanism of action of the contractile effects of the aqueous pulp extract of the plant in isolated non-pregnant
uterus were reported (Falodun et al., 2006). Recent ethnobotanical survey done by us revealed that among the
Yoruba herbalists (South-West Nigeria), pulverized dry seeds of Hunteria umbellata are equally reputed and
highly valued for the treatment of fever, pain, abdominal colic and discomforts, diabetes mellitus and obesity in
its suspected sufferers. In the local treatment of diabetes mellitus, an average of 2 - 3 teaspoonfuls (10 - 15 g)
of the pulverized plant seed soaked in a glass full of hot water for 20 - 30 min is often recommended for an adult to
be taken orally per day. This translation and the result of our preliminary study in rat model informed our
decision to choose the dose range of 50 - 200 mg/kg/day of the extract. However, despite the ancestral use of the
plant decoction in the treatment of suspected diabetic patients, there is a dearth in scientific validation of this
therapeutic use. The present study is, therefore, designed to investigate the effects of acute and repeated graded
oral doses (50 - 200 mg/kg/day) of the aqueous seed extract of Hunteria umbellata in normal rats for 14 days,
and glucose- and nicotine-induced hyperglycaemic rats for 6 hours. In addition, the acute oral toxicity as well as
the phytochemical analysis of the extract was conducted.

Materials and Methods

Collection of Plant Materials

Fresh leaves, inflorescence and mature pods of the Hunteria umbellata plant were collected from the
deciduous forest of Odorasanyin District of Ijebu-Igbo in Ijebu North Local Government Area of Ogun State,
Nigeria, in the month of April, 2007. Botanical identification was done by Mr. T. K. Odewo, Chief
Superintendent Officer, Taxonomy Section, Forest Research Institute of Nigeria, Ibadan, Oyo State, Nigeria.
Voucher specimen was deposited in the institution herbarium with the reference no. FHI 107687, allotted. Plant
authentication was done by Dr. A.B. Kadiri, The Herbarium, Botany and Microbiology Department, the
University of Lagos, Akoka, Lagos State, Nigeria. Hunteria umbellata seeds were harvested from the collected
fresh fruit pods. The seeds were gently washed in tap water and completely dried under room temperature (30 ±
2 °C) for 4 weeks protected from direct heat or sunlight. When dried, the seeds were de-coated of their light-
brown thin coatings.

Preparation of the aqueous seed extract of Hunteria umbellata

100 g of the dry seeds was pulverized to white-to-light brown fine powder using domestic blender. 25 g of
the fine powdered sample was boiled in 500 mL of distilled water in a 1 L Pyrex beaker for 1 hr under
continuous stirring. The homogenate was allowed to cool for about 6 hr before it was rapidly filtered through a
piece of clean white cloth. The filtrate was then transferred to an aerated oven preset at 40 °C and completely
dried until a deep brown, aromatic solid residue was obtained. The yield obtained was 45% (w/w). The residue,
thus obtained, was stored in air- and moisture-tight container which was kept in a refrigerator maintained at -4
°C. From this, a fresh stock was reconstituted in distilled water at a concentration of 100 mg/mL, whenever
needed.

Experimental Animal

Twenty female Wistar rats, 10 - 12 weeks and weighing between 110 - 140 g were employed for acute oral
toxicity study using the Up and Down Procedure according to OECD/OCDE Test Guidelines on Acute Oral
Acute oral toxicity studies of Hunteria umbellata aqueous seed extract using limit dose test and the main test of Up and Down Procedure

An initial preliminary orientation-test of acute oral toxicity study was conducted using the limit dose test of Up and Down Procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided statistical programme - AOT425statPgm, version: 1.0., at a limit dose of 2000 mg/kg body weight/oral route and default at an assumed sigma of 0.5 mg/kg/oral, using the method adopted by Adeneye et al. (2006b). Due to lethality of the two sequentially treated rats at 2000 mg/kg/oral route of HU, the main test of the Up and Down Procedure was subsequently conducted, using the dose progression of 175 mg/kg/oral, 550 mg/kg/oral and 2000 mg/kg/oral of HU. The procedure for the main test was conducted in strict compliance with the guidelines of the main test of Up and Down Procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity under a computer-guided statistical programme - AOT425statPgm (AOT, 2001).

Preliminary Phytochemical analysis

The presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars were determined by the simple and standard qualitative and quantitative methods described by Trease and Evans (1989) and Sofowora (1993). The simple quantitative analysis of the extract was based on the intensity of the colour change.

Briefly described, the qualitative phytochemical analysis of the crude seed powder of Hunteria umbellata was determined as follows:

Tannins: 200 mg of the plant material was dissolved in 10 mL of distilled water and then filtered. A 2 mL of filtrate was pipetted into a test tube after which 2 mL of 15% FeCl₃ was added. Colour change was observed.

Alkaloids: 200 mg of the plant material was extracted with 200 mL of methanol for 20 minutes on a water bath and then filtered. To 2 mL of the cold water extract in different tubes, was added 6 drops of different alkaloids reagents, namely: Dragendorff’s or Mayer’s or Wagner’s reagent. Presence and colours of any precipitate were noted. Creamish precipitate or brownish-red precipitate or orange precipitate indicated the presence of respective alkaloids.

Cyanogenic glycosides: 200 mg of powdered plant material was placed in each of 3 different test tubes labeled A, B and C, respectively. The powder in test tubes A and B were moistened with 5 mL of water, while that in test tube C was left dry. 3 pieces of freshly prepared sodium picrate paper were inserted into the mouth of each tube and stoppered. Test tube B was placed in a water bath while test tube A and C were kept at room temperature. After 30 minutes the colour of the picrate papers in each of the test tube were observed and recorded.

Cardiac glycosides:
- Kedde’s test for lactone ring in cardiac glycosides: 5 g of plant material was boiled with 50 mL of water to obtain a water extract of the plant. The extract was concentrated to dryness and re-dissolved in 10 mL of methanol. To 2 mL of this, 1 mL of a solution of 2% of 3, 5-dinitrobenzoic acid in methanol and 1 mL of 5.7% sodium hydroxide were added. The result was recorded after 5 minutes.
- Liebermann-Burchard reaction for steroidal/triterpenoidal nucleus: 2 g of powdered sample was extracted with 500 mL of methanol. The extract was filtered and the filtrate was gently concentrated to dryness on a water bath. 500 mg of the dried extract was dissolved in 2 mL of acetic anhydride and allowed to cool. With the test tube inside ice pack and slanted at an angle of about 45 degree, 2 mL of concentrated tetraoxosulphate (VI) acid was carefully poured by the side of the test tube. Colour obtained was noted. Blue-green ring indicated the presence of terpenoids.
- Keller-Kiliani test for de-oxy sugars in cardiac glycosides: A methanol extract was obtained and the extract reduced to dryness. 50 mg of this was dissolved in 2 mL chloroform. Tetraoxosulphate (VI) acid was added to form a layer and the colour at interphase recorded.
- Legal test: The extract was dissolved in pyridine and 5 drops of 2% sodium nitroprusside together with 4 - 5 drops of 20% sodium hydroxide were added. Deep colour indicated the presence of cardenolides.
- Salkowski’s test: 200 mg of the extract was dissolved in 2 mL of chloroform. Concentrated tetraoxosulphate (VI) acid was carefully added to form a lower layer. A reddish-brown at the interface indicated the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside).
Reducing sugars:
- Fehling’s test: Water extract of the powdered material was obtained by boiling on water bath. To 2 mL of the extract, in the test tube was added, 1 mL each of Fehling’s solutions A and B. The mixture was shaken and heated in a water bath for 10 minutes. The colour obtained was recorded.

Saponins:
- Frothing test: Water extract was obtained by boiling on water both. The extract was transferred into a test tube and shaken vigorously then was left to stand 10 minutes and the result noted. Frothing persistence meant saponins were present.

Flavonoids:
200 mg of the powdered sample was boiled in 10 mL of absolute ethanol for 10 minutes. The solution was allowed to cool and then filtered. To 2 mL of the filtrate was added concentrated hydrochloric acid and magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids.

Anthraquinone derivatives:
Bontrager’s test: Chloroform extract of the powdered sample was obtained by boiling on the water bath. To 2 mL of this extract, 1 mL of dilute (10%) ammonia was added and the mixture was shaken. Any colour change was recorded.

Acute extract treatment in oral glucose-induced hyperglycaemic rats
In the high oral glucose hyperglycemic model, 12 - 14 hr fasted rats were randomly allotted to 6 groups of 6 rats per group such that the difference within and between groups does not exceed ±20% of the average weight of sample population of rats. Group I rats, which served as the untreated control, were orally pretreated with 10 mL/kg of distilled water 1 hr before treatment with another 10 mL/kg/oral of distilled water. Group II rats served as the model control and were pretreated with 10 mL/kg/oral of distilled water 1 hr before oral treatment with 3 g/kg of D-glucose (Analar). Groups III - VI rats which served as the treatment groups were orally pretreated with 1 mg/kg of glibenclamide (Daonil®, Hoechst Marion Roussel Limited, Mumbai, India), 50 mg/kg, 100 mg/kg, and 200 mg/kg of HU, respectively, for 1 hr before the oral administration of 3 g/kg of D-glucose, as described by Sepici et al. (2004).

Acute extract treatment in nicotine-induced hyperglycaemic rats
Same experimental protocol described for high oral glucose-induced hyperglycaemia except that oral glucose treatment was replaced with 50 µg/kg of nicotine administered as described by Alada (2001) and that nicotine was injected via the intraperitoneal route.

Repeated extract treatment in normoglycaemic rats
In the repeated dose model, 12 - 14 hr fasted rats were randomly allotted to 5 groups of 6 rats per group such that the difference within and between groups does not also exceed ±20% of the average weight of sample population of rats. Group I rats, which served as the untreated control, were orally treated with 10 mL/kg of distilled water while Groups II - V that served as the treatment groups, were treated with single, daily oral 50 mg/kg, 100 mg/kg, 200 mg/kg of HU for 14 days, respectively.

Measurement of fasting blood glucose in acute and repeated extract treated rats
Blood sample from the rat tail vein for fasting whole blood glucose was collected by tail tipping method. The tail was gently squeezed to let out 2 - 3 drops of fresh whole blood which were placed on the test spot of the glucose strip after which the test strip is gently inserted into Test Strip Platform of the Microprocessor digital blood glucometer and the readings were recorded (World Health Organization, 1980). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment, using standard laboratory method of spectrophotometry for the determination of blood glucose concentrations as previously described by Ajala et al. (2003). Blood glucose concentration was estimated using glucose oxidase method of Trinder (1969) on a One Touch Basic Blood Glucose Monitoring System® (LifeScan Inc., Milpitas, California, U.S.A.).

For the acute extract treated hyperglycaemic models, blood glucose concentrations were determined at 0 hr, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, and 6 hr, respectively. For the repeated dose model, the fasting blood glucose concentrations were determined on the 1st and 15th day of the experiment following an overnight fast.

Data Analysis
Results were presented as mean ± S.D. for body weights while data for biochemical indices 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 and Discussion
Several in vivo acute and chronic drug-induced hyperglycaemic models have been developed and used to investigate the hypoglycaemic effects of medicinal plants with antidiabetic potentials. The models include oral glucose loading- and nicotine-induced hyperglycaemia (Grayson and Oyebola, 1985). Other agents that have been used in various hyperglycaemic/diabetic models include diazoxide, alloxan, streptozotocin (Adeneye and
Glucose is the simplest metabolic end-product of carbohydrate metabolism which is most readily absorbed into the portal blood from the gastro-intestinal tract following its oral ingestion (Guyton, 1991a). Its post-absorptive state is marked by postprandial hyperglycaemic state which often is accompanied by increased pancreatic insulin secretion (hyperinsulinaemia), particularly in the first few hours postprandial (Guyton, 1991b). In the present study, single high oral glucose treatment was associated with significant hyperglycaemia in the distilled water-pretreated, high glucose-treated rats, particularly within the first 2 hr post-oral administration (Figure 2). However, the blood glucose levels steadily decreased over the succeeding 5 hr reaching significant ($p<0.05$) hypoglycaemic levels at 5th - 6th hr. (Figure 2).

Oral pretreatment with glibenclamide, on the other hand, was not associated with any significant ($p>0.05$) alterations in the blood glucose concentration when compared to basal value at 0 hr. Over the succeeding 5 hr, the blood glucose concentrations in the glibenclamide and 50 - 200 mg/kg of $HU$ pretreated rats, significantly ($p<0.05$, $p<0.01$, and $p<0.001$) and progressively decreased, becoming most significantly ($p<0.001$) reduced at the 5th and 6th hr when compared to the values at 1 hr. However, the hypoglycaemic effect of $HU$ at 200 mg/kg at 2 - 6 hr was comparable to that of glibenclamide (Figure 2). As shown in figure 2, the significant ($p<0.05$, $p<0.01$, and $p<0.001$) dose related attenuation of significant rise in the post-absorptive blood glucose concentrations in the glibenclamide- and $HU$-pretreated rats is suggestive that glibenclamide and $HU$ could be mediating their hypoglycaemic action via inhibition of intestinal glucose uptake, a mechanism which is similar to those of $\alpha$-glucosidase inhibitors (e.g. acarbose). The significant ($p<0.05$) 5th-6th hr postprandial hypoglycaemia recorded in the distilled water-pretreated, glucose loaded rats could be due to the secondary effect of hyperinsulinaemia following the glucose load. This is a well established physiological response to hyperglycaemia following a high glucose load or intake (Hedeskov, 1980). Literature has shown that extracts with high triterpenoid saponins content mediate their hypoglycaemic effect via inhibition of intestinal glucose uptake, increased hepatic glucose deposition and enhanced hyperinsulinaemia (Shane-McWhorter, 2001).
Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata*

Therefore, it is possible for the saponins contained in *HU* be responsible for the observed attenuation in the post-absorptive glucose concentration. However, further studies will be required to validate this proposed hypothesis.

Nicotine, an alkaloid and the active principle contained in tobacco is usually implicated in causing the acute biological effects of tobacco smoking (Assali et al., 1999). It has been reported to induce hyperglycaemia in canines (Milton, 1966; Grayson and Oyebola, 1985) and rodents (Oyebola and Alada, 1993; Alada, 2001). This effect is believed to be due to its modulatory action on adrenergic mechanisms by stimulating the nicotinic acetylcholine receptors (Westfall, 1965). Thus, nicotine-induced hyperglycaemia as a model of acute drug-induced hyperglycaemia is well established (Oyebola and Alada, 1993).

![Figure 3. Effects of single oral treatment with 10 mL/kg of distilled water, 1 mg/kg of glibenclamide and 50 - 200 mg/kg of *Hunteria umbellata* aqueous seed extract on the fasting blood glucose (mg/dl) in nicotine-induced hyperglycaemic rats](image)

In the present study, significant \( p<0.001 \) hyperglycaemia was reliably established within 1 hr following the intraperitoneal injection of 50 µg/kg of nicotine, which lasted over the next hour (Figure 3). However, the hyperglycaemia was significantly attenuated in rats pretreated with glibenclamide and varying doses of *HU* in a dose related fashion, making the blood glucose fall within the range of basal value at the 2\textsuperscript{nd} hr but significantly \( p<0.05, p<0.01 \) and \( p<0.001 \) lower than the basal value at 0 hr at 3\textsuperscript{rd} to the 6\textsuperscript{th} hr of the study (Figure 3). This is an indication that the hypoglycaemic effect of *HU* became more pronounced within the 3\textsuperscript{rd} to the 6\textsuperscript{th} hr post-administration, an effect which was lower than that of the reference drug, glibenclamide. Thus, from the aforementioned, it is clear that *HU* apart from inhibiting intestinal glucose uptake, it could also be mediating its hypoglycaemic effect via inhibition of the adrenergic homeostatic mechanism on acute course.

In the repeated dose study, 50 - 200 mg/kg/day of *HU* produced a sustained and significant \( p<0.05, p<0.01, \) and \( p<0.001 \) hypoglycaemic effect when compared to the untreated control values (Figure 1).
Adeneye and Adeyemi

Figure 1. Effect of repeated oral treatment with 50 - 200 mg/kg/day of *Hunteria umbellata* aqueous seed extract on the fasting blood glucose (mg/dl) in normoglycaemic rats after 14 days of treatment

The observed hypoglycaemic effects of *HU* extract could be due to the presence of any or combination of the active principle(s) contained in the extract.

Table 1: Phytochemical constituents of the aqueous seed extract of *Hunteria umbellata* (*HU*)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tannins</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Ferric chloride test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Bromine water test</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatinnins</td>
<td></td>
</tr>
<tr>
<td>(i) Draggendoff’s test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Mayer’s reagent</td>
<td>++</td>
</tr>
<tr>
<td>(iii) Wagner’s test</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Draggendoff’s test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Mayer’s reagent</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td></td>
</tr>
<tr>
<td>(i) Keller-Kiliani test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Kedde test</td>
<td>++</td>
</tr>
<tr>
<td>(iii) Legal’s test</td>
<td>++</td>
</tr>
<tr>
<td>(iv) Salkowski test</td>
<td>++</td>
</tr>
<tr>
<td>(v) Liebermann-Burchard’s test</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Reducing sugar</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Fehling A and B test</td>
<td>+</td>
</tr>
<tr>
<td>(ii) Resorcinol test</td>
<td>+</td>
</tr>
<tr>
<td>(iii) Chloramine T test</td>
<td>++</td>
</tr>
<tr>
<td>(iv) Barfoed test</td>
<td>+</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Lead acetate test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Ferric chloride test</td>
<td>+++</td>
</tr>
<tr>
<td>(iii) Sodium chloride test</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Saponins</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Benedict’s test</td>
<td>++</td>
</tr>
<tr>
<td>(ii) Emulsion test</td>
<td>++</td>
</tr>
<tr>
<td>(iii) Frothing test</td>
<td>++</td>
</tr>
<tr>
<td><strong>Anthraquinone</strong></td>
<td></td>
</tr>
<tr>
<td>(i) Borntrager’s test</td>
<td>+++</td>
</tr>
</tbody>
</table>

*p, b, and c* represent significant reductions at *p*<0.05, *p*<0.01 and *p*<0.001 when compared to untreated control values (Group I).

Group I = 10 mL/kg/day of oral distilled water
Group II = 50 mg/kg/day of *Hunteria umbellata* aqueous seed extract
Group III = 100 mg/kg/day of *Hunteria umbellata* aqueous seed extract
Group IV = 200 mg/kg/day of *Hunteria umbellata* aqueous seed extract

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- : not detected; +: present in low concentration; ++: present in moderate concentration; +++: present in high concentration.
Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata*

Results of the phytochemical studies showed *HU* to contain a high concentration of tannins, flavonoids, alkaloids, anthraquinone and cardiac glycosides, while phlobatinnins and saponins were present in low and moderate concentrations, respectively. However, anthocyanosides were absent in the plant extract (Table 1). It is well documented in the literature that medicinal plants with hypoglycaemic and antidiabetic effects usually contain high concentrations of alkaloids, flavonoids (Oladele et al., 1994; Rao and Rao, 2001; Sharma et al., 2008), steroid glycosides (Ivorra et al., 1989; Adallu and Radhika, 2000) and terpenoids (Reher et al., 1991, Shane-McWhorter, 2001). The presence of these phytochemicals in high concentrations could account for the significant hypoglycaemic effect of the extract, either singly or in synergy with one another. Again, this hypothesis will require further validation. Apart from the aforementioned hypoglycaemic mechanisms, it is also possible that *HU* could be mediating its effects via hyperinsulinaemia [which itself, could be caused by increasing secretion of insulin from the pancreatic cells of islets of Langerhans or its release from bound insulin (Pari and Amarnath, 2004)], increased peripheral utilization of glucose or combination of any of the stated mechanisms. In the current study, these possible mechanisms were not investigated but could constitute areas of further studies.

**Table 2:** Sequence and result of the main test of Up-and-Down Procedure of acute oral toxicity of *Hunteria umbellata* aqueous seed extract in treated rats

<table>
<thead>
<tr>
<th>Test Sequence</th>
<th>Animal Identity</th>
<th>Dose (mg/kg/oral)</th>
<th>Short-term Outcome (the first 48 hr)</th>
<th>Long-term Outcome (successive 12 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B</td>
<td>175</td>
<td>survival</td>
<td>survival</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>550</td>
<td>survival</td>
<td>survival</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>2000</td>
<td>death</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>550</td>
<td>survival</td>
<td>survival</td>
</tr>
<tr>
<td>5</td>
<td>G</td>
<td>2000</td>
<td>death</td>
<td>survival</td>
</tr>
<tr>
<td>6</td>
<td>O</td>
<td>550</td>
<td>survival</td>
<td>survival</td>
</tr>
<tr>
<td>7</td>
<td>L</td>
<td>2000</td>
<td>death</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the sequence and results of the main test of the Up and Down Procedure of the acute oral toxicity. As shown in Table 2, acute oral treatment at 2000 mg/kg caused death in the rats for less than 1 hr post-treatment. Death, on each occasion, was preceded by restlessness, perioral tremor, polyapnoea and generalized convulsion. On stepping down the dose to 550 mg/kg, there was no associated death but there were short-term (48 hr) behavioural toxicities which included hyperactivity succeeded by generalized muscular rigidity, piloerection, and bilateral narrowing of the eyelids. The delayed behavioural toxicities include decreased feeding pattern, progressive weight loss and alopecia. Based on the computer-generated result, the calculated LD$_{50}$ value was 1020 mg/kg/oral route. According to the American Society for Testing and Materials (1987), any chemical substance with LD$_{50}$ estimate less than 2000 mg/kg/oral route but greater than 1000 mg/kg/oral could be considered to be slightly toxic, although Clarke and Clarke (1977) consider any compound with an estimated LD$_{50}$ greater than 1000 mg/kg/oral to be safe. Thus, the result of the acute oral toxicity of *HU* suggests it could be toxic at a high dose on acute exposure.

The effect of *HU* on the body weight is also significant. Table 3 shows effect of repeated, single daily oral administration of *HU* on the initial, final and the percentage weight change of average body weight of treated rats. As shown in the table, oral administration of 50 - 200 mg/kg of *HU* for 14 days was associated with significant (p<0.05 and p<0.01) dose related weight loss when compared to that of untreated control (Group I) rats.
Table 3: Effect of 14 days of repeated oral treatment with 10 mL/kg/day of distilled water, 50 - 200 mg/kg/day of *Hunteria umbellata* aqueous seed extract on the average body weight in normal rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Initial weight (g) (on day 1)</th>
<th>Final weight (g) (on day 15)</th>
<th>Percentage weight change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>184.3 ± 40.6</td>
<td>211.5 ± 36.7</td>
<td>+ 15.8 ± 10.3</td>
</tr>
<tr>
<td>II</td>
<td>182.8 ± 28.2</td>
<td>186.3 ± 29.5</td>
<td>+1.9 ± 7.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>161.5 ± 32.8</td>
<td>150.0 ± 31.1</td>
<td>-7.0 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>199.2 ± 38.3</td>
<td>175.0 ± 34.2</td>
<td>-12.1 ± 4.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup> represent significant decreases at p<0.05 and p<0.01, respectively, when compared to untreated control values (group I).

Group I = 10 mL/kg/day of oral distilled water
Group II = 50 mg/kg/day of *Hunteria umbellata* aqueous seed extract
Group III = 100 mg/kg/day of *Hunteria umbellata* aqueous seed extract
Group IV = 200 mg/kg/day of *Hunteria umbellata* aqueous seed extract

The observed significant weight loss and associated hypoglycaemia could not have been mediated via appetite inhibiting mechanism because there was no change in the feeding pattern of *HU*-treated rats. However, the exact mechanism by which the extract induced progressive weight is currently being investigated in our laboratory.

In summary, the present study has shown that the aqueous seed extract of *Hunteria umbellata* caused significant reductions in normal rats and the two experimental models of acute drug-induced hyperglycaemia in rats, which were mediated via glucose uptake inhibition and adrenergic inhibition. Further studies on other hypoglycaemic mechanisms apart from the aforementioned and identification of suspected hypoglycaemic phytocomponents in *HU* are currently on-going in our research laboratory. Also, results obtained in this preliminary study will form a template for subsequent studies on the hypoglycaemic and weight loss effects of *HU* as well as the mechanisms involved.

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References


Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata*


