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Preliminary phytochemical screening and evaluation of hypoglycemic properties of the root extract of *Uvaria chamae*

Preliminary phytochemical screening and evaluation of hypoglycemic properties of the root extract of *Uvaria chamae*

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Abstract

The purpose of this study is to evaluate the hypoglycaemic properties and preliminary phytochemical screening of *Uvaria chamae*. The hypoglycaemic properties of *U. chamae* was assessed on normoglycaemic rat that received single dose of the extract at 250 and 500 mg/kg body weight and blood glucose levels estimated at 2, 4, and 6 hours (single dose study). The hypoglycaemic property of the extract was also evaluated in normoglycemic rats by oral glucose tolerance test. Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods. The extract showed a significant ($p < 0.05$) reduction in blood glucose levels at 2 and 6 hours compared to control. The oral glucose tolerance test result also showed a significant decrease ($p < 0.05$) in blood glucose levels. The study showed that the extract, *U. chamae* has hypoglycaemic properties which may be accounted for by the presence of the phytochemicals.

Introduction

Diabetes mellitus has been described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Walter, 1977; Albert et al., 1998; Kumar and Clark, 2005).

It is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries (Mbaka et al., 2012). It is usually irreversible and its late complications result in reduced life expectancy and major health loss (Kumar and Clark, 2005). Currently diabetes is controlled by diet, exercise, oral hypoglycaemic agents and insulin therapy (Mallick et al., 2007). The high level of treatment failures, unpleasant side effects and enormous cost associated with oral anti-diabetic drugs have generated an urgent need and desire for alternative treatments (Suneetha et al., 2010). The preferred choice of plant

medicine by many might not be unconnected with the historical successes recorded in the use of herbal product in traditional system of medicine in managing diabetes mellitus (Mbaka et al., 2012). Besides, herbal formulations were observed to have fewer side effects and less toxic because of their rich natural source. Based on these and the support provided for its practice by the World Health Organization, several scientific investigations are being conducted with the view of identifying new active ingredient of natural source that would be more effective in the treatment of diabetes mellitus and diabetic complications (WHO, 1980). *Uvaria chamae* is a medicinal plant that belongs to the family, Annonaceae. It is a climbing plant commonly found in West Africa (Irvin, 1961; Okwu, 2004). In this region of the world, it is identified by numerous names such as: Ogholo by Esan people of Edo state, Ayiloko by the Igalas, Kaskaifi by the Hausas, Oko oja by the Yorubas in Nigeria and Akotompo by Fulafainte people of Ghana (James et al., 2013). It has been reported to



have antivenom activity (James et al., 2013). The antibacterial and antifungal activities have also been reported (Okwu et al., 2004). However, no scientific study has been conducted on the anti-diabetic activity of this plant. The aim of this study is to evaluate the hypoglycaemic properties and preliminary phytochemical screening of *U. chamae*.

Materials and Methods

Plant materials

The roots of *U. chamae* were obtained from a farm in Uromi, Edo State, Nigeria. They were authenticated by a taxonomist, Mr T.K Odewo, of Botany Department, University of Lagos. The voucher specimen with number LUH 3572 was deposited in the University herbarium.

Preparation of the plant material for extraction

The roots were washed with clean water to remove foreign materials, chopped into small pieces and dried in an oven at 45 degrees centigrade for four days. They were powdered to coarse particles with electric grinder. The root powder, weighing 500 g, was extracted with 93.3% aqueous ethanol by maceration with frequent stirring for 5 days. The extract was filtered using Whatman filter paper No. 4 and concentrated with a rotary evaporator. The concentrated extract was dried in an oven at 40 degrees centigrade to obtain 22.4 g dry residue (4.5% yields).

Animals

Swiss mice (20-25 g) and Wistar rats (160 ± 20 g) of both sexes were obtained from the Laboratory Animal Center, College of Medicine, University of Lagos, Idi-Araba and were kept under standard environmental condition of 12/12 hours light/dark cycle. They were housed in cages (5 animals per cage), maintained on standard animal pellets (Pfizer Feeds Plc, Nigeria), and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

Acute toxicity study

The toxicity study was carried out using 35 (male and female) Swiss albino mice (each weighing 20–25 g). The animals were randomly distributed into a control group and six treated groups, containing five animals per group. After fasting the animals overnight, the control group was given 0.4 mL of acacia (2%) suspension orally, while each treated group received oral solution of the extract prepared with 2% acacia in the doses of

1.0, 2.5, 5.0, 10.0, and 15.0 and 20.0 g/kg body weight respectively. The animals were observed continuously for the first 4 hours and then for each hour for the next 24 hours and at 6 hourly interval for the next 48 hours after administering the extract to observe any death or changes in general behaviour and other physiological activities (Ayub et al., 1997; Bürger et al., 2005). The dose that results in 50% mortality (LD₅₀) was then determined (Figure 1).

Preliminary phytochemical screening

Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods using the following reagents and chemicals: alkaloids with Mayer's reagent and Dragendorff's reagent (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of 10% lead acetate and 20% sodium hydroxide (Trease and Evans 1983; Sofowora 1993), tannins with 5% ferric chloride solution (Yadav and Agarwala, 2011) and saponins with ability to produce suds (Houghton and Raman, 1998). Terpenes with Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (Shoppee, 1964). Terpenoids with a mixture of extract and chloroform and concentrated H₂SO₄ (Sofowora, 1993).

Assessment of hypoglycemic properties of *U. Chamae* (single dose study)

Fifteen rats were randomly selected into 3 groups, 5 rats per group. The rats were fasted over-night. Fasting blood glucose levels of each group was evaluated. Group I, untreated control was given 0.5 mL of 2% Acacia, while Group II and III were given the extract, orally at doses of 250 mg/kg and 500 mg/kg respectively. Blood samples were collected for estimation of Blood glucose level from the tail vein at 2, 4 and 6 hours after giving the extract (Santosh et al., 2007).

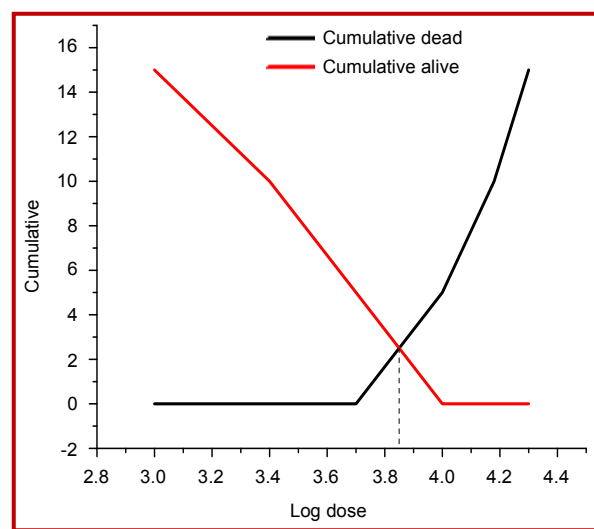


Figure 1: Determination of LD₅₀ using cumulative dead of the mice and cumulative mice that are alive (Colegate et al., 1993)

Table I

Oral acute toxicity of root extract of *Uvaria chamae* in mice

Group	Dose (mg/kg)	Log dose	Mice alive	Dead mice	Cumulative alive	Cumulative dead
I	20,000	4.30	0	5	0	15
II	15,000	4.18	0	5	0	10
III	10,000	4.0	0	5	0	5
IV	5,000	3.7	5	0	5	0
V	2,500	3.4	5	0	10	0
VI	1,000	3.0	5	0	15	0

Control received 0.4 mL of 2% acacia; Group I, II, III, IV, V, VI: 20,000, 15,000, 10,000, 5,000, 2,500, 1,000 mg/kg respectively; n=5

Table II

Assessment of hypoglycaemic properties of *Uvaria chamae*

Group	Blood glucose levels (mg/dL)			
	Pre-treatment	Post-treatment		
		0 (FBG)	2 hours	4 hours
Group I	62.2 ± 2.5	63.8 ± 2.4	53.8 ± 2.5	66.3 ± 7.0
Group II	57.7 ± 1.6	53.3 ± 1.9 ^a	50.4 ± 1.5	30.4 ± 1.1 ^a
Group III	60.8 ± 2.4	52.7 ± 1.5 ^a	52.1 ± 1.8	36.6 ± 3.3 ^a

Mean ± SEM; n=5; ^ap<0.05; ^bp<0.01 vs. control group; Group I: control received 0.5 mL of 2% acacia; Group II, III: 250, 500 mg/kg respectively

Effect of the extract on oral glucose tolerance test

Normal rats male and female were fasted over night and divided into four groups of five rats each. Blood samples were collected from the tail veins of the rats to estimate the fasting blood glucose levels. Group 1, the control, was given 0.5 mL of 2% Acacia and group 2, 3 and 4 were given 100, 250 and 500 mg/kg of extract respectively. Thirty minutes after administering the extract, the three groups were administered 40% (w/v) glucose at a dose of 1 mL/100 g body weight orally (Ogbonnia et al., 2011). Blood glucose levels monitored at 30, 60 and 120 min intervals and reported as the average glucose level of each group.

Statistical analysis

Analysis of data was done using GraphPad Prism 6. One-way analysis of variance and t-test were used to compare means. Means ± SEM are shown in all tables. Level of significance was set at p<0.05 or p<0.01.

Results

In the acute toxicity study (Table I), there was no death among the animals that received 1,000-5,000 mg/kg body weight of the extract. The animals that received 10,000-20,000 mg/kg body weight of the extract died within 24 hours. The LD₅₀ of the drug was calculated to be 7,080 mg/kg body weight.

Table II shows the hypoglycaemic effects of a single oral

administration of two doses 250 and 500 mg/kg body weight of the root extracts of *U. chamae* in normal healthy rats. These doses showed significant reduction (p<0.05) in blood glucose levels at 2 and 6 hours compared to control. Rats treated with 250 mg/kg of the extract showed a maximum reduction of 54.2% in blood glucose level after 6 hours of oral administration. However, the reduction in blood glucose level at the dose of 250 mg/kg body weight at 2 and 4 hours was 16.5 and 6.3% respectively. Also, rats treated with 500 mg/kg of the extract showed a maximum reduction of 44.8% in blood glucose level after 6 hours of oral administration. The reduction in blood glucose level at the dose of 500 mg/kg body weight at 2 and 4 hours was 17.4 and 3.2% respectively.

Figure 2 shows the summary of the oral glucose tolerance test. Following oral glucose load in the control group, the rise in blood glucose level reached a peak at 30 min of glucose load. Decrease in blood glucose level occurred after 30 min but the blood glucose level failed to return to baseline after 120 min. There was a significant decrease (p<0.05) in blood glucose levels at 30, 60 and 120 min with a percentage decrease of 57.5, 28.6 and 41.4% respectively in the group of rats treated with 500 mg/kg dose of extract compared with control.

The preliminary phytochemical screening of the root extract of *U. chamae* revealed the presence of flavonoids, alkaloids, cardiac glycosides, terpenoid and terpenes, saponin, tannin proteins and sugars (Table III).

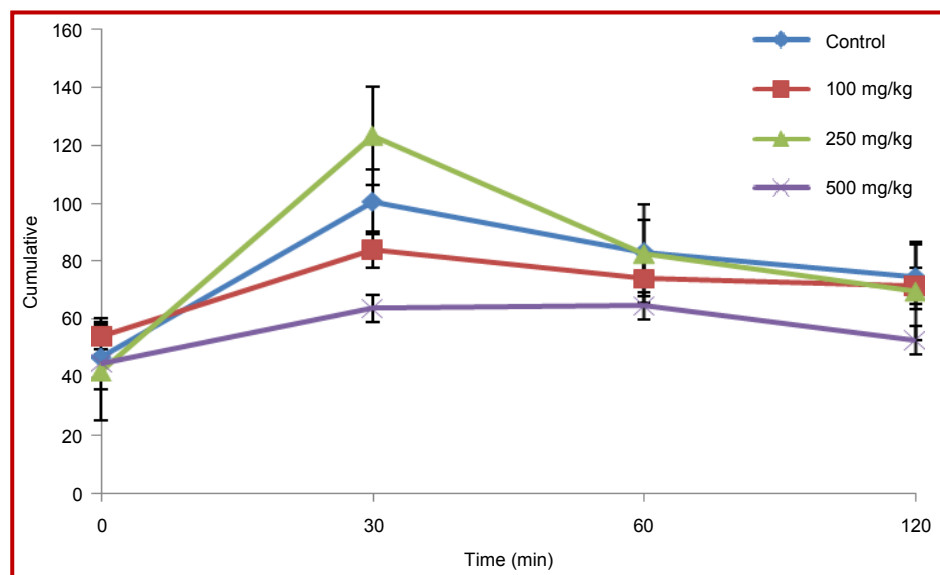


Figure 2: Oral glucose tolerance test

Table III	
Qualitative analysis of phytochemical constituents of root extract of <i>Uvaria chamae</i>	
Compounds	Presence
Alkaloids	+++
Flavanoids	++
Cardiac glycosides	+
Terpenoids and terpenes	+
Saponin	+
Tannin	++
Protein	+
Sugar	+

+++ appreciable amount; ++ moderate amount; + trace amount

Discussion

The major goals in the treatment of diabetes has been to keep both short- and long-term glucose levels within acceptable limits, thereby reducing the risk of long-term complications (Park et al., 2009). This could be achieved by optimizing both fasting blood glucose and postprandial glucose levels which have been found to be very important in achieving near normal glucose levels. Postprandial glucose levels have been reported to serve as a better maker of glycemic control than fasting blood sugar levels (Park et al., 2009).

Drugs that reduce post-prandial hyperglycemia by suppressing hydrolysis of starch have been found useful in the control of diabetes mellitus (Tundis et al., 2010; Kazeem et al., 2013a). Many herbal extracts have been reported for their anti-diabetic activities and are currently being used in traditional medicines for the treatment of diabetes. However, such medicinal plants

have not yet gained much importance as medicines due to lack of sustained evidence (Sudha et al., 2011).

The results of this study showed that the median lethal dose (LD₅₀) of the root extract was determined to be 7.08 g/kg body weight translating to 490 g dose for human adult. According to Loomis and Hayes (1996), the extract can be classified as being practically non-toxic since this value is much higher than Organization for Economic Cooperation and Development (OECD) toxicity index of 2 g/kg (Walum, 1998; OECD, 2001). Therefore, the extract may be considered to be safe for consumption.

This study revealed that the rats treated with 250 and 500 mg/kg of the extract had maximum reduction of 54.15 and 44.80% in blood glucose level after 6 hours of oral administration respectively. The extract exerted hypoglycaemic activity by decreasing the blood glucose level significantly. Low blood glucose level reduces the risk of complications associated with diabetes (Attele et al., 2002; Emordi et al., 2014).

The result of oral glucose tolerance test showed that there was a significant decrease ($p < 0.05$) in blood glucose levels at 30, 60 and 120 min with a percentage decrease of 57.5, 28.6 and 41.4% respectively in the group of rats treated with 500 mg/kg dose of extract compared with control.

Insulin release in response to a glucose load occurs in two phases in humans and in rodents. The early phase peaks within the first 15-30 min and is responsible for limiting the initial rise in glucose upon meal ingestion. The late phase of insulin secretion occurs later than 30 min after a meal, and may persist for several hours. This delayed burst of insulin secretion is responsible for returning glucose to baseline fasting levels. In the face of insulin resistance, the late phase of insulin secretion

persists for an extended period and contributes to excessive insulin levels even after a return to the fasted state, resulting in fasting hyperinsulinemia (Oda, 2012). From this study, the marked reduction in plasma glucose concentration may be as a result of increased release of insulin from beta cells. This may also account for the hypoglycemic activity of the extract.

The health benefits of medicinal plants are attributed in part to their unique phytochemical composition (Okwu, 2005). Phytochemicals are secondary metabolites of plant origin which act as anti-oxidants and stimulate the protective enzymes in the liver or block damage to genetic materials (Okwu, 2004). They prevent the occurrence of oxidative chemical species, stimulate antioxidant repairing mechanism and scavenging capacity for free radicals in the system.

Flavonoids are phenolic compounds that possess anti-oxidant and anti-diabetic potentials due to the presence of hydroxyl groups that confer scavenging ability on them (Mayur et al., 2010). Research has shown that many plants containing flavonoids have been used for the treatment of diabetes (Meiselman et al., 1976; Choi et al., 1991; Hassig et al., 1999).

Tannins induce phosphorylation of the insulin receptors as well as translocation of glucose transporters 4 (GLUT-4), the protein factor involved in the signaling pathway of insulin-mediated glucose transport and the inhibition of the expression of key gene for adipogenesis thereby helping to reduce blood glucose level without increasing adiposity (Liu et al., 2005).

Suba and co-workers reported in 2004 that tannin has anti-diabetic activity.

One of the most important carbohydrate-splitting enzymes is the maltase-glucoamylase which helps to break-down dietary disaccharides into monosaccharide in the small intestine. If its activity is inhibited, the digestion and absorption of monosaccharide can be slowed down, decreasing the post-prandial hyperglycaemia. The potential therapeutic use of polyhydroxylated alkaloids in the treatment of type-2 diabetes due to their ability to inhibit maltase-glucoamylase has been reported (Shang et al., 2013). It is therefore possible that the phytochemicals present in the root extract of *U. chamae* may be responsible for the observed hypoglycemic activity.

The LD₅₀ value 7.1 g/kg obtained was a clear indication that *U. chamae* is safe for use. The study showed that the root extract of *U. chamae* has hypoglycaemic activity which may be as a result of increased release of insulin from beta cells. The hypoglycaemic activity of the extract may also be accounted for by the presence of several bioactive compounds like flavonoids, tannins and alkaloids.

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