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Full Length Research Paper

Anti-diabetic effect of ethanol leaf extract of *Cissampelos owariensis* (lungwort) on alloxan induced diabetic rats

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***Cissampelos owariensis* (lungwort) is a medicinal plant used in Ayurveda for treating diseases. One of such disease is diabetes mellitus. In the present study, ethanol leaf extract of this plant was prepared, and phytochemical composition, acute toxicity, blood glucose lowering effect and improvement of body weight gain in alloxan monohydrate (150 mg/kg weight) induced diabetic rats were measured and compared with that of a patent drug glibenclamide. Preliminary phytochemical screening of the ethanol leaf extract of *C. owariensis* revealed the presence of tannins, flavonoids, alkaloids and saponins. The median lethal dose (LD_{50}) in rats was 2154 mg/kg body weight (b.wt). Rats were administered *C. owariensis* extract at dose rate of 100 and 200 mg/kg b.wt orally for 14 days, respectively. Blood glucose concentration and body weight was measured by Accu Chek Glucometre test kit and electronic balance and compared with a patent drug glibenclamide at a dose rate of 100 mg/kg b.wt. The data were compared statistically by using analysis of variance (ANOVA). The herbal preparation of *C. owariensis* significantly ($P<0.05$) increased body weight gain and decreased blood glucose when compared with patent drug. The present study clearly indicated anti-diabetic activity of *C. owariensis* and supports the traditional usage of the herbal preparations for the therapy of diabetics.**

Key words: *Cissampelos owariensis*, glibenclamide, alloxan, toxicology, diabetes, rats.

INTRODUCTION

Diabetes mellitus is a multi factorial disease which is characterised by hyperglycaemia (Scoppola et al., 2001; Ugochukwu et al., 2003), lipoprotein abnormality (Scoppola et al., 2001), raised basal metabolic rate (Avesani et al., 2001; Nawata et al., 2004; Owu et al., 2006), defect in reactive oxygen species scavenging enzymes (Kesavulu et al., 2000) and altered intermediary metabolism of major food substances (Avesani et al., 2001; Unwin et al., 2001; Nawata et al., 2004). Diabetes is a major degenerative disease in the world today (Ogbonnia et al., 2008) affecting at least 171 million people and having complications which include hypertension, atherosclerosis and micro circulatory disorders. Nowadays, various medicinal plants are becoming very popular in the treatment of different diseases all over the world. There are a number of plants that control blood glucose level such as *Azadirachta Indica, Catharanthus roseus, Allium sativum, Memordica judaica, Aloe vera, Trigonella foenum greacum*, etc.

Cissampelos is a medicinal plant belonging to the kingdom plantae, phylum tracheophyta, class mangoliopsida, order ranunculales, genus Cissampelos and species *Cissampelos owariensis* (Baerts and Lehmann, 2006). It is known in Nigeria as jokoje in Yoruba and ebubueka enwe in Ukwuani. Through out the distribution of *C. owariensis*, people take the infusion of the bitter rhizome, leaves or stems to cure gastrointestinal complaints such as diarrhoea, dysentery, colic, intestinal worms, and digestive complaints, and also urogenital

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problems such as menstrual problems, venereal diseases and infertility. It is also used to induce contraction of the uterus to start labour or abortion and to expel the placenta. Women of the Bini in Nigeria use the leaves to promote foetal growth (Burkhill, 1994).

Plants which had been shown to have hypoglycaemic action, act on blood glucose through several mechanisms. Some of them may inhibit endogenous glucose production (Eddouks et al., 2003) or interfere with gastrointestinal glucose absorption (Musabayane et al., 2006); some may have insulin-like substances (Collier et al., 1987; Gray and Flatt, 1999); some may inhibit insulinase activity and some may increase secretion of insulin from β cells of the pancreas (pancreatotrophic action) (Khan et al., 1990; Trivedi et al., 2004; Yadav et al., 2008), while others may proliferate β cells in pancreas by activating regeneration of these cells (Shanmugasundaram et al., 1990). Very few traditional treatments for diabetes mellitus have been scientifically examined. The aim of this study was to evaluate the possible anti-diabetic action of *C. owariensis* and to examine the safety and efficacy of this plant in the management of diabetes mellitus.

MATERIALS AND METHODS

The experiment was performed in the laboratory of the Department of Chemical Sciences, Biochemistry Unit, Novena University, Ogume, Delta State, Nigeria for a period of 2 months to evaluate the efficacy of lungwort and glibenclamide on alloxan monohydrate induced diabetic rats.

Collection and acclimatisation of rats

Mixed albino rats aged 2 months, with average weight of 113 g were collected from the Animal House of the College of Health Science, Delta State University, Abraka, Delta State, Nigeria. Rats were grouped into five groups, each containing seven animals. Each group of rats were housed at serene bottomed wire cages arranged in rows. The animals were housed in standard environmental conditions of temperature (21 ± 2°C), humidity (55 ± 10%) and a 12 h light-dark cycle. The animals were fed with palletised feed and water *ad libitum*. The rats were maintained in this condition for a period of three weeks to acclimatise them prior to experimental uses.

Collection and preparation of plants extract

Fresh leaves of *C. owariensis* were collected from Amai, Delta State, Nigeria in the month of May and June, 2011. The plant was botanically identified and authenticated by Professor J. M. O. Eze of the Laboratory of Ethno Botanical Research, Biological Sciences Department of Novena University, Ogume in Delta State, Nigeria through comparison with a voucher specimen present in the herbarium.

Preparation of ethanol extract

One litre of ethanol (Analar Grade) was added to 37 g of shaded, dried and ground leaves that was packed into a container, cocked and left to stand for 48 h with occasional shaking. The whole mass was filtered out in a cotton cloth and then through Whatman No. 1 filters paper and concentrated in a water bath at 65°C into slurry.

Phytochemical screening of the extract

The phytochemical study for the presence of flavonoids, alkaloids, tannins and saponins in the ethanol extract was done according to the method described by Harbone (1984).

Flavonoids

0.1 g of each of the extract was added to a mixture of 10 ml of lead acetate solution (90% w/v) and 20 ml of 50% aqueous ethanol in a 200 ml conical flask. The mixture was placed on boiling water for 2 min, cooled and filtered. Five milliter (5 ml) of dilute ammonia was added to a portion of the aqueous filtrate followed by the addition of concentrated sulphuric acid (1 ml) to 2 ml of potassium hydroxide solution and allowed to mix. Then, into the acid base mixture, a small quantity of aqueous filtrate of the sample was added and observed for colour change.

Alkaloids

0.2 g of the extract was added to 5 ml of 2% hydrochloric acid and heated on boiling water for 10 min. It was then allowed to cool and then filtered. To 1 ml of the filtrate in a test tube was tested alkaloids reagent, Wagner's and Mayer's reagent and results were compared with blank. Turbidity or precipitation indicated the presence of alkaloids.

Tannins

0.2 g of the extract was boiled with 5 ml of 45% ethanol for 5 min. The mixture was filtered hot using a filter paper and filtrate was collected in a beaker. Two milliter (2 ml) of the filtrate was mixed with 10 ml of distilled water and then a drop of iron chloride solution was added. A blue-black or blue-green precipitate indicates the presence of tannins.

Saponin

0.1 g of each of the extract was measured into a beaker and 20 ml of distilled water was added, the beaker was heated in a water bath for over 5 min. The mixture was filtered using a filter paper into another beaker to obtain a filtrate. Two milliter (2 ml) of the filtrate was poured in another test tube and 10 ml of distilled water was added, it was shaken vigorously for over a minute. Frothing which persist on warming indicated the presence of saponin.

Acute toxicity study

Acute toxicity study on the ethanol leaf extract was carried out according to the method of Lorke (1983) using 21 mice of average weight of 22.8 g that were dosed orally with different gradual doses (0.01 to 5 g/kg body weight). In the first phase, 9 mice were divided into 3 groups of 3 mice each and were treated with the ethanol extract of the plant at doses of 0.01, 0.1 and 1.0 g/kg body weight orally. They were observed for 24 h for signs of toxicity. In the second phase, 12 mice were divided into 4 groups of 3 mice each and were also treated with the aqueous extract at doses of 1, 1.6,

Table 1. The phytochemical composition of the ethanol leaf extracts of *C. owariensis*.

Phytochemical	Ethanol leaf extract
Alkaloids	++
Flavonoids	+++
Tannins	+++
Saponins	+++

+++ = Present in high amount, ++ = present in moderately high amount, + = present in trace amount, - = absent.

2.9 and 5.0 g/kg bodyweight orally. The median lethal dose (LD_{50}) was calculated as the geometric mean of the highest non lethal dose (with no death) and the lowest lethal dose (where death occurred).

LD_{50} = Square root of the product of minimum toxic dose and maximum tolerated dose

Collection and preparation of the anti-diabetic drug

The oral anti-diabetic drug used was glibenclamide Daonil®. The glibenclamide was dissolved in distilled water to make a concentration of 5 mg/ml. It was given orally at 100 mg/kg daily for 14 days.

Doses for biological investigations

The doses used for pharmacological studies were 100 and 200 mg/kg body weight of the ethanol extract, chosen based on our result of the toxicity study of the extract on mice.

Experimental design

From the thirty five rats collected, twenty eight were rendered diabetic after single intraperitoneal injections of alloxan monohydrate in a dose of 150 mg/kg body weight (Edem, 2009). The rest (untreated rats) served as non-diabetic rats (control group). The diabetic and non diabetic rats were divided into five groups of rats and were treated as follows: Group 1, non diabetic rats (control group) were not administered alloxan monohydrate; Group 2, diabetic non-treated rats (diabetic control); Groups 3 and 4, diabetic rats were orally given the ethanol extract of *C. owariensis* at doses of 100 and 200 mg/kg body weight, respectively for 14 days; Group 5, diabetic rats were given single oral dose of anti-diabetic drug glibenclamide as a reference drug at 100 mg/kg body weight for 14 days.

Rats induced with alloxan monohydrate were administered 5% glucose solution during the first 24 h. Blood glucose levels in alloxan treated mice were measured after 72 h. Alloxan injection was done by intra peritoneal injection of 150 mg/kg body weight of alloxan monohydrate (Sigma) dissolved in sterile distilled water and animals that remained alive after the alloxan injection and with blood glucose levels above 200 mg/dl were considered diabetic. The administration (p.o) was started from the same day, except normal control and diabetic control groups for a period of 14 days. During this period, animals in all groups had free access to standard diet and water. The blood glucose level as well as the body weight of all treated animals (Groups 2 to 5), normal group (Group 1) and diabetic group (Group 2) were taken pre and post day 0 and 7^{th}, and 14^{th} days of post treatment with Accu Chek Glucose Monitoring Kit (Roche Diagnostics, GmbH, Mannheim, Germany) and weighing balance, respectively. Blood was collected from overnight fasted rats using the orbital technique (Ekeanyanwu and Njoku, 2010)

Table 2. LD_{50} estimation.

Treatment	Number used/number dead
Phase I	
Group 1 (10 mg/kg)	3/0
Group 2 (100 mg/kg)	3/0
Group 3 (1000 mg/kg)	3/0
Phase II	
Group 1 (1000 mg/kg)	3/0
Group 2 (1600 mg/kg)	3/0
Group 3 (2900 mg/kg)	3/1
Group 4 (5000 mg/kg)	3/1

Statistical analysis

Results were expressed as mean±standard deviation. Differences between normal and treated groups were the criteria for the pharmacological activities. Statistical analysis of results were done using one way ANOVA, calculated using SPSS (statistical tool for social sciences), version 17.0.

RESULTS

Phytochemical composition

The phytochemical analysis of the ethanol leaf extract of *C. owariensis* showed that it contains in high concentration, phytochemicals like saponins, tannins and flavonoids but alkaloids were found in moderately high concentration (Table 1).

Acute toxicity study (LD_{50})

The result of the acute toxicity study showed that mice administered ethanol leaf extract of *C. owariensis* for doses between 10 and 1600 mg/kg did not experience any fatality. But mice administered doses of 2900 and 5000 mg/kg experienced fatality (Table 2). The LD_{50} was calculated as the geometric mean of the highest dose that did not kill mice and the least dose that killed mice.

$LD_{50} = \sqrt{(1600 \times 2900)} = 2154$ mg/kg

Effect of ethanol leaf extract of *C. owariensis* on body weight of control and experimental rat

Normal control rats were found to be stable in their body weight but diabetic rats showed significant reduction in

Table 3. Effect of ethanol leaf extract and glibenclamide tablet on body weight gain in normal and alloxan treated diabetic rats.

Group	Treatment (mg/kg b.wt) (PO)	Body weight (g) (n=7)		
		Day 0	Day 7 (Mean±SD)	Day 14 (Mean±SD)
1	Normal control	100.00±16.33	127.33±15.53*	121.33±16.74*
2	Diabetic control	115.50±17.33	101.33±11.02*	92.50±0.70*
3	Ethanol leaf extract (100mg/kg b.wt)	123.75±17.29	142.00±7.21*	141.33±2.31*
4	Ethanol leaf extract (200mg/kg b.wt)	114.50±12.92	137.33±8.33*	134.67±5.77*
5	Glibenclamide tablet	112.75±17.23	124.00±15.87*	120.00±19.29*

PO = Per Os (orally), n = number of rats; values are mean ± SD for each group of five rats. *Means significantly different at P<0.05.

Table 4. Effect of ethanol leaf extract and glibenclamide tablet on blood glucose in normal and alloxan treated diabetic rats.

Group	Treatment (mg/kg b.wt) (PO)	Blood glucose concentration (g/dl) (n=7)		
		Day 0	Day 7 (Mean±SD)	Day 14 (Mean±SD)
1	Normal control	129.00±6.24	108.00±14.73	102.00±17.54
2	Diabetic control	214.00±1.41	222.00±32.97*	263.67±12.89*
3	Ethanol leaf extract (100mg/kg b.wt)	217.00±2.82	209.33±16.07*	175.50±9.19*
4	Ethanol leaf extract (200mg/kg b.wt)	214.50±6.03	207.00±7.00*	189.67±7.23*
5	Glibenclamide tablet	213.00±7.00	188.00±8.54*	82.50±9.19*

PO = Per Os (orally), n = number of rats; values are mean ± SD for each group of five rats. *Means significantly different at P<0.05.

body weight during the 14 days of experiment. Diabetic treated rats placed on 100 and 200 mg/kg extract and glibenclamide experienced significant increase in the body weight during the 14 days experiment (Table 3).

Effect of blood glucose level on normal and experimental rat

Ethanol leaf extract of *C. owariensis* was subjected to anti-diabetic activity in rats where alloxan monohydrate (150 mg/kg b.wt. i.p) was used as the diabetogenic agent. A marked rise in fasting blood glucose level was observed in diabetic control when compared with the normal control rats. Ethanol extract of *C. owariensis* (at 100 and 200 mg/kg b.wt.) significantly reduced blood glucose at 7^{th} and 14^{th} day of administration. The hypoglycaemic effect of ethanol leaf extract was found to be less effective than the reference standard; glibenclamide produced a significant reduction in blood glucose as compared to the diabetic control (Table 4).

DISCUSSION

Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In Africa, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately, only a few of such African medicinal plants have received scientific examination.

The percentage yield of the ethanol leaf extract of *C. owariensis* was found to be 5.4%. Phytochemical analysis of the ethanol extract of *C. owariensis* leaves extract revealed the presence of flavonoids, saponins, tannins, alkaloids as shown in Table 1. Secondary plant metabolites like polysaccharides, coumarins, flavonoids, terpenoids, arginine and glutamic acid are known to have hypoglycaemic effects in various experimental animal models (Akah and Okafor, 1992; Marles and Farnworth, 1995; Ross, 2001; Ojewole, 2002). Tannin containing drugs have also been shown to demonstrate anti-diabetic activity (Iwu, 1980, 1983). Effect of the flavonoids on pancreatic β cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini et al. (2004) as the mechanism by which they reduced hyperglycaemia in diabetic rats. The presence of flavonoids in the ethanol crude extract of *C. owariensis* may also be acting similarly, thereby decreasing the high blood glucose levels of alloxan-diabetic rats.

The observed reduced activity of the treated mice in the initial period of extract administration showed that the extract possessed depressing effect. The LD_{50} was calculated to be 2154 mg/kg.

After 14 days administration of *C. owariensis* leaf extract, blood glucose concentration decreased signifycantly (P<0.05) in comparison with day zero. Glibenclamide also decreased blood glucose concentration. After 14 days of administration of glibenclamide, the blood glucose concentration reduced significantly (P<0.05) which agreed with the report of Sharma et al.

(2010). Signs of regeneration of β cells, potentiating insulin secretion from surviving β cells of the islets of langerhans and decrease of blood glucose concentration have been reported following consumption of some plant extracts (Shanmugasundaran et al., 1990; Yadav et al., 2008). *C. owariensis* leaf may have some chemical components that exerts regenerative effects on β cells, stimulate these cells to produce more insulin (pancreatotrophic action) or may have some insulin-like substances, and induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreases (Adewole and Ojewole, 2007). It has been reported that flavonoids and tannins present in plants extracts possess antidiabetic activity (Ojewole, 2002; Iwu, 1983). In the present study, the observed anti-diabetic potential of our test extract may be due to the presence of similar phytochemical constituents which was evident by our preliminary phytochemical screening.

Conclusion

From this study, we can state that the ethanol extract of *C. owariensis* has beneficial effects on blood glucose concentration. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

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