International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 3 (6): 505-509, 2007
ISSN 1811-7775

Evaluation of Hypoglycemic Activity of Glycosides and Alkaloids Extracts of *Picralima nitida* Stapf (Apocynaceae) Seed

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Abstract: The blood glucose lowering effect of the seed extract of *Picralima nitida* has been suggested to be due to its rich indole alkaloids; this study, therefore, is aimed at evaluating the hypoglycemic activity of the alkaloids and glycosides extracts of the *Picralima nitida* seed. The alkaloids extract of *Picralima nitida* seed (Apocynaceae) given i.p. caused increase in mean fasting blood glucose levels while the glycosides extract reduced the blood glucose levels in normoglycemic and hyperglycemic rats. Glycosides extract caused significant ($p<0.05$) percentage maximal reduction of 38.6% (250 mg kg^{-1}) and 22.9% (500 mg kg^{-1}) of the mean fasting blood glucose levels in normoglycemic and 64.4% (250 mg kg^{-1}) and 39.0% (500 mg kg^{-1}) in the hyperglycemic rats. The glycosides extract maintained low mean fasting blood glucose levels throughout the 24 h duration of study in hyperglycemic rats. On subchronic treatment of hyperglycemic rats, glycosides extract (250 mg kg^{-1}) caused 82.99% while glyburide (5 mg kg^{-1}) caused 60.81% reduction of mean blood glucose levels. Thus the hypoglycemic activity of seed extract of *Picralima nitida* may be resident in the glycosides of the seed extract.

Key words: *Picralima nitida*, alkaloids, glycosides, hyperglycemia, hypoglycemia

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder (Petal and Rybczynski, 2003; Ozturk, *et al*., 1996) that involves chronic alterations in the carbohydrate, fat and protein metabolism, basically resulting from the secretion of dysfunctional and/or insufficient endogenous insulin by the β-cells of the pancreas. It is characterized by elevated blood glucose concentration that may be accompanied by severe thirst, profuse urination, polyphagia, weight loss, or stupor (Ozturk *et al*., 1996; Bamidele *et al*., 2002).

The number of people that are suffering from this scourge is on the increase and the orthodox antidiabetic agents are relatively expensive and/or unavailable. These reasons have favoured the utilization of alternative medicine to stabilize the blood glucose level in most diabetic patients of some developing countries of the world. Other factors include high cost of hospital management of the disease, non-availability of competent health personnel and the long distance patient has to walk to health facilities (Gyang *et al*., 2004; Akah *et al*., 2002).

In West Africa, plants like *Momordica charantia* (Nadkarni, 1994; Sanjay, 2002), *Bersama engleriana* (Watcho *et al*., 2005), *Vernonia amygdalina* (Gyang *et al*., 2004; Akah and Okafor, 1992), *Musa sapientum* (Jani and Sharma, 1967) and many others are used in the folklore management of diabetes mellitus. The seed extract of *Picralima nitida* (commonly named Akuamma seed in Ghana and Osi-Igwe seed in Igboland, Eastern Nigeria) has been evaluated for blood glucose lowering activity and has been proved effective for such pharmacological action (Aguwa *et al*., 2001; Inya-Agha *et al*., 2006). It has been suggested that the hypoglycemic activity of this plant extract is due to the rich indole alkaloids (Inya-Agha, 1999; Aguwa *et al*., 2001). Since *Picralima nitida* extract is also richly endowed with glycosides, which include biologically active compounds such as saponins, flavonoids, steroids and triterpenes (Vladimir and Ludmila, 2001), the aim of this study, therefore, is to evaluate the hypoglycemic activity of the alkaloids and glycosides extracts of the *Picralima nitida* seed so as to propose the class of the secondary metabolites that has the hypoglycemic activity.

MATERIALS AND METHODS

Plant collection and extractions: The pods of the plant were collected from Ihiala, Anambra state, Nigeria in 2004

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through a herbalist and authenticated by Dr (Mrs.) Inya-Agha of Department of Pharmacognosy, University of Nigeria, Nsukka. The seeds were extracted from the pods, air-dried for six weeks in the tropical sunlight and pulverized. The powdered seed (301.89 g) was defatted with chloroform and macerated with 1 L of 90% methanol for 7 days with intermittent shaking. The filtrates were concentrated to solid residues at room temperature.

Alkaloids extraction: The methanolic extract (9.6 g) was weighed out and dissolved in 70% methanol and made alkaline with ammonia hydroxide (basicity confirmed with litmus paper). The mixture was continuously shaken gently for one hour and extracted with equal ratio mixture of chloroform after acidifying with enough dilute HCl (0.1N). The chloroform phase was basified with enough ammonia hydroxide (pH >7) and dried to constant weight by heating over a water bath at 45°C (Brain and Turner, 1975). The standard phytochemical tests were carried out on the chloroform extract in accordance with Harborne (1984) procedures.

Glycosides extract: The methanolic extract (14.9 g) was dissolved in water (60 mL) and mixed with 200 mL of n-butanol and left to partition in a separating funnel overnight. The butanol phase was percolated through activated carbon bed (2 g). The n-butanol phase was concentrated in a rotary evaporator into solid residue, tested for glycosides (Harborne, 1984) and stored until evaluated.

Animals: Albino mice (20-35 g) and rats (85-200 g) of either sex, bred in the animal unit, Department of Pharmacology, University of Nigeria Nsukka and handled according to the stated guidelines of the Ethical Committee, were used for these studies. They had access to water before and during the experimental stages, fed with standard feed from Pfizer Plc, Lagos. The animals were kept for one week prior to experimentation at room temperature in the Department of Pharmacology, University of Nigeria, Nsukka, Nigeria where the research was conducted in 2006.

Effect of alkaloids and glycosides extracts of *Picralima nitida* on mean fasting blood glucose levels in normal rats: Wistar albino rats of either sex were fasted for 24 h but had access to water *ad libitum*. Five groups of four rats per group were used, two groups for alkaloids extract at doses of 250 and 500 mg kg^{-1}, two groups for glycosides extract at doses of 250 and 500 mg kg^{-1} and one group received glyburide at 5 mg kg^{-1}; all the animals received their drugs ip. At the end of the fast, blood was withdrawn from the tail vein of each animal and the blood glucose levels were determined as the 0 h level. After the 0 h blood withdrawal, the rats in all the groups received their drugs and blood samples were withdrawn at fixed time intervals (1, 2, 4, 8, 12 and 24 h) and the blood glucose levels determined using Glucose meter kit (Life scan, USA).

Effect of alkaloids and glycosides extracts of *Picralima nitida* on mean fasting blood glucose levels in alloxanized rats: Twenty rats of either sex, with blood glucose levels between 78-110 mg dL^{-1} were fasted for 12 h before use. They were given 120 mg kg^{-1} of alloxan monohydrate (Sigma, USA) intra peritoneally. The alloxanized rats were kept for 7 days for hyperglycemia to develop and stabilize but had free access to food and water. The rats were fasted on the day 8 for 12 h and their blood glucose levels were determined using the glucose meter kit (Lifescan, USA). The rats with blood glucose level above 150 mg dL^{-1} were randomly distributed into four groups of 4 rats per group while the normal rats formed the fifth group. Two groups were given 250 and 500 mg kg^{-1} of glycosides extract respectively, one group received glyburide (5 mg kg^{-1}), one group of the rats received 250 mg kg^{-1} of alkaloids and one other group received distilled water (3 mL kg^{-1}). All the drugs were administered ip after the blood samples for 0 h blood glucose levels were collected. At fixed time intervals (1, 2, 4, 8, 12 and 24 h), the blood samples were collected and blood glucose levels determined.

Subchronic treatment effect of glycosides extract of *Picralima nitida* on mean fasting blood glucose levels in alloxanized rat: Sixteen rats of either sex were fasted for 12 h on the first day of the experiment after twelve of the rats were treated ip with alloxan monohydrate, 120 mg kg^{-1} (Sigma, USA) and tested for hyperglycemia 72 h post induction. Animals with blood glucose levels above 150 mg dL^{-1} were considered hyperglycemic. They were grouped into four of four rats per group, one group received glycosides extract of 250 mg kg^{-1}, one group received glyburide (5 mg kg^{-1} positive control) and a group received distilled water (3 mL kg^{-1} negative control) and the last group received distilled water but were normoglycemic rats; all the animals received their drugs i.p for ten days. At the end of the fast in the first day, blood was withdrawn from the tail vein of each the animals and the blood glucose levels were determined as the first day level. After the blood withdrawal, the animals received single dose of the respective drugs for the next ten days. Blood samples were withdrawn from the animals again on the tenth day and the blood glucose levels determined using Glucose meter kit (Life scan, USA).

Acute toxicity test: Of the methanolic extract, LD_{50}, was determined intra peritoneally in mice using Lorke's method (1983).

Statistical Analysis: Results are given as mean blood glucose levels ± SEM (standard error of mean). One-way ANOVA with post hoc Dunnett's multiple comparison tests and student t-test. p values of 0.05 and less were taken to imply statistical significance between the means

RESULTS AND DISCUSSION

The acute toxicity test (LD_{50}) of methanolic extract, the parent extract of the alkaloids and glycosides, in mice through intraperitoneal administration was calculated to be 707.11±32 mg kg^{-1}.

The qualitative phytochemical analysis of extracts revealed the presence of the following secondary metabolites in glycosides extract: Flavonoids, saponins, steroids and triterpenes (Vladimir and Ludmila, 2001) while the alkaloids extract had majorly alkaloids.

The normoglycemic rats treated with 250 mg kg^{-1} of alkaloids showed consistent increment in mean fasting blood glucose levels above the 0 h blood glucose baseline value while some of rats of the 500 mg kg^{-1} treated group died of severe hyperglycemia at the 4th h. This maybe due to the inflammation and necrosis of liver hepatocytes (Fakeye *et al*., 2005) as hepatic damage can lead to insulin resistance (Yeh *et al*., 2003). The normoglycemic rats treated with lower dose of glycosides (250 mg kg^{-1}) experienced consistent reduction ($p<0.05$) in the mean fasting blood glucose levels from 108.33±4.2 at 0 h to 67.17±2.9 mg dL^{-1} at 12 h, while those treated with 500 mg kg^{-1} showed reduction in mean fasting blood glucose levels from 94.67±5.1 at 0 h to 73.00±7.6 mg dL^{-1} at 12 h (Table 1). The 250 and 500 mg kg^{-1} doses of glycosides fraction of *Picralima nitida* seed extract caused percentage maximal reduction of 38.6 and 22.9% respectively in normoglycemic rats.

In hyperglycemic rats, 250 mg kg^{-1} dose of glycosides significantly reduced ($p<0.05$) the mean fasting blood glucose levels from 416.67±17.0 at 0 h to 148.33±21.3 mg dL^{-1} at 12 h while the 500 mg kg^{-1} dose caused mean fasting blood glucose levels reduction from 425.83±4.2 at 0 h to 259.58±12.5 mg dL^{-1} at 12 h. The blood glucose reduction in these diabetic rats treated with glycoside doses commenced from the first hour till the twelfth hour of treatment just like the standard glyburide

Table 1: Effects of alkaloids and glycosides fractions on blood glucose levels of normoglycemic rats (mean±SEM)

	0	1	2	4	8	12	24
Drug	----------(h)----------						
Alkaloids							
250 mg kg^{-1}	70.01±2.1	95.50±1.0	102.17±2.3	79.17±3.5	74.17±2.8	75.83±5.2	76.42±4.5
	(0.00)	(-36.40)	(-46.00)	(-13.10)	(-5.90)	(-8.30)	(-9.50)
Glycosides							
250 mg kg^{-1}	108.33±4.2	110.83±2.9	94.67±4.6	91.33±4.2	79.17±2.7*	67.17±2.9*	86.45±5.7
	(0.00)	(-2.30)	(12.60)	(15.70)	(26.91)	(38.60)	(20.20)
500 mg kg^{-1}	94.67±5.1	88.83±3.7	80.83±6.7	82.17±2.5	75.50±6.2	73.00±7.6	98.65±3.4
	(0.00)	(6.17)	(14.62)	(13.20)	(20.25)	(22.90)	(-4.20)
Glyburide							
5 mg kg^{-1}	84.75±2.4	79.23±4.1	73.45±2.3	64.51±4.8	57.32±5.2	53.45±3.2	82.31±4.1
	(0.00)	(6.51)	(13.33)	(23.88)	(32.37)	(36.90)	(2.88)

No. of animals per group = 4, No. in parenthesis represent percentage blood glucose reduction, Standard Error of the Mean (±SEM). All values of mean fasting blood glucose levels were in mg dL^{-1}, *: $p<0.05$ level of significance against 0 h of the same dose of drug

Table 2: Effect of glycosides (gly) extract of *Picralima nitida* seed on mean fasting blood glucose levels of alloxanized rats (mean±SEM, n = 4)

	0	1	2	4	8	12	24
Drugs	----------(h)----------						
Glycosides extract							
250 mg kg^{-1}	416.67±17.0	390.42±27	290.42±4.9*	211.67±21.5**	195.00±23.9***	148.33±21.3a***	178.91±3.7
	(0.00)	(6.30)	(30.20)	(49.20)	(53.20)	(64.40)	(57.06)
500 mg kg^{-1}	425.83±4.2	328.75±8.9	295.00±12.8	316.25±14.2	327.50±2.5	259.58±12.5*	267.56±3.9
	(0.00)	(22.80)	(30.72)	(25.73)	(23.09)	(39.00)	(37.17)
Glyburide							
(5 mg kg^{-1})	267.75±2.6	153.75±6.3	119.00±4.9	96.75±2.7	94.50±8.4	91.60±12.5	104.56±2.4
	(0.00)	(42.58)	(55.56)	(63.87)	(64.71)	(65.80)	(60.95)
Dist. water							
(3 mL kg^{-1})	186.25±4.7	183.75±2.1	197.50±3.9	190.00±18.3	187.37±11.3	189.15±7.8	198.9±6.9
	(0.00)	(1.34)	(-6.04)	(-2.01)	(-0.06)	(-1.56)	(-6.79)
Alkaloids							
250 mg kg^{-1}	234.26±8.3	238.01±17.4	241.97±5.7	248.54±4.7	253.26±6.9	241.46±13.8	245.45±15.3
	(0.00)	(-1.60)	(-3.29)	(-6.10)	(-8.11)	(-3.07)	(-4.78)

**: $p<0.01$ and *: $p<0.05$ level of significance against 0 h of the same dose of drug, a: $p<0.05$ significant against Dist.water treated group. No. in parenthesis represent percentage reduction in mean fasting blood glucose levels

Table 3: Effect of glycosides extract of *Picralima nitida* seed extract on mean fasting blood glucose levels of alloxan-induced diabetic albino rats after subchronic treatment

	Blood Glucose level (mg dL^{-1}) (mean±SEM)		
Groups	Day 1	Day 10	(%) Max. red.
Non-diabetic 3 mL kg^{-1} b.wt. Distilled water	89.00±0.58	86.00±3.46	3.37
Diabetic control 3 mL kg^{-1} b.wt. Distilled water	217.25±40.03	201.75±32.63	7.13
Glyburide 5 mg kg^{-1} b.wt.	203.50±18.52	79.75±12.29*[a]	60.81
Glycosides extract 250 mg kg^{-1} b.wt.	393.00±12.54	66.85±6.51*[a]	82.99

No. of animal per group = 4, $p<0.05$ sig. vs. the 1st day glucose level of the same drug [a]: $p<0.05$ significant against diabetic control

treated group (Table 2). The 250 and 500 mg kg^{-1} doses of glycosides caused 64.4% and 39.0% maximal reduction in mean fasting blood glucose levels of diabetic rats respectively while the glyburide caused 65.8% reduction. The alkaloids only increased mean fasting blood glucose levels of the treated rats. This result contradicts the suggestions that the hypoglycemic activity of *Picralima nitida* extract is due to its rich indole alkaloids by Inya-Agha (1999) and Aguwa *et al*. (2001).

On subchronic treatment at one dose per day, the 250 mg kg^{-1} glycosides extract caused significant reduction of mean blood glucose levels from 393.00±12.54 mg dL^{-1} in day one to 66.85±6.51 mg dL^{-1} in day ten that amounted to 82.99% maximal reduction in ten days treatment while the standard drug, glyburide, caused the reduction of mean blood glucose levels from 203.50±18.52 mg dL^{-1} at day one to 79.75±12.29 mg dL^{-1} at day ten that amounted to 60.81 % maximal reduction (Table 3). All the groups treated with drugs had significantly ($p< 0.05$) different mean blood glucose levels from the diabetic control group on the tenth day of treatment.

Alloxan is a known insulin dependent diabetes mellitus diabetogenic agent that selectively and permanently destroys the pancreatic β-cells through production of free radicals and excessive calcium concentrations in cell cytoplasm (Ozturk *et al*., 1996; Aguwa *et al*., 2001). This pancreatic β-cells destruction invariably causes absolute insulin deficiency that leads to hyperglycemia in the diabetic rats (Szkudelski, 2001). Since the glycosides extract of *Picralima nitida* seed reduced the blood glucose in normoglycemic rats, it implies that the extract may have reduced the blood glucose through pancreatic effect by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhan's or its release from bound insulin (Pari and Satheesh, 2004) and in alloxanized rats through extra pancreatic effects (Esimone *et al*., 2001; Inya-Agha, 1999) like the glyburide. These pancreatic and extra-pancreatic effects on the blood glucose levels could be through prevention of hepatic glucose overproduction, increase in glucose uptake by the muscle, inhibition of gastric emptying (Nolte and Karam, 2004) and/or increase in glucose permeability of plasma cell membrane (Mahler and Alder, 1999; Shane-McWhorter, 2001).

Since the subchronic treatment of hyperglycemic rats with the glycosides extract of *Picralima nitida* seed effected higher percentage maximal reduction than the standard antidiabetic drug, glyburide, it may be of use in the management of non insulin dependent diabetes mellitus (NIDDM) patients that majorly have relative insulin deficiency (Smith and Tadayyon, 2003). These findings vividly established the fact that the glycosides extract exhibited more potent hypoglycemic effect than alkaloids extract as it contains secondary metabolites like saponins, steroids, triterpenes and flavonoids from *Picralima nitida* plant; it may be useful in the management of NIDDM patients since it showed significant activity in normoglycemic and hyperglycemic conditions in animal model.

CONCLUSION

This study has shown that glycosides of the seed extract of *Picralima nitida* plant have the antihyperglycemic activity and not the indole alkaloids. However, further research to isolate and characterize the active phytochemical constituent(s) responsible for the hypoglycemic effect of glycosides extract is ongoing.

ACKNOWLEDGMENTS

The authors are grateful to Mr Igboeme Sabastin of Department of Clinical Pharmacy Laboratory for his technical assistance and Dr Anaga of Vet. Pharmacology of this University who supplied us with the alloxan sample used.

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