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Evaluation of *Picralima nitida*: Hypoglycemic Activity, Toxicity and Analytical Standards

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Abstract: Hypoglycemic activity in *Picralima nitida* Stapf (Apocynaceae), recorded as an index of blood glucose was confirmed in normal and intraperitonially induced alloxan diabetic albino rats with glibenclamide as reference standard and normal saline as control. Toxicity study was evaluation of acute (15 days) tests. 100, 300 and 900 mg kg^{-1} of the extracts to normal rats resulted in significant ($p<0.01$) lowering of fasting blood sugar after 8 h. Extract maintained hypoglycemic action throughout the 24 h of study indicating a long duration of action. In normal rats, pulp extract (100 mg kg^{-1}) produced a maximum percentage reduction of 38.35%, rind extract (900 mg kg^{-1}) 46.19% and seed extract (100 mg kg^{-1}) 36.81%. Alloxan induced rats were pulp 85.85% (300 mg kg^{-1}), seed 83.26% (300 mg kg^{-1}) and rind 80.25% (900 mg kg^{-1}), respectively. Order of activity recorded as pulp > seed > rind. Acute toxicities (LD_{50}) of pulp, seed and rind were 7071.06, 948.68 and 1364.91 mg kg^{-1}, respectively. Analytical standards were moisture content, ash and extractive values for quality assurance.

Key words: *Picralima nitida*, hypoglycemic activity, toxicity, phytochemical screening, analytical standards

INTRODUCTION

Diabetes Mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now (WHO/Acadia, 1992; ADA, 1997). WHO Expert Committee on diabetes encourages further investigation into traditional methods of treatment and also emphasizes the need to ensure safety and quality control of ingredients used. The presence of abnormally high blood glucose levels is the criterion on which the diagnosis of diabetes is based. The therapeutic goal of treatment within each type of diabetes is to normalize insulin activity and blood glucose levels in an attempt to reduce the development of the vascular and neuropathic complications. Diet and weight control constitute the foundation of diabetes management. While a subsection of NIDDM patients can be managed by diet alone, others require oral hypoglycemic therapy and/or insulin. Although insulin therapy affords a tight and effective hypoglycaemic control, certain drawbacks such as oral ineffectiveness, short shelf life and requirement of constant refrigeration, parenteral therapy and fatal hypoglycaemia in the event of excess dosage limit its usage (Rathi *et al*., 2002). Patient knowledge, willingness, goals, health status and finances may also affect decisions regarding insulin treatment. Pharmacotherapy with sulphonylureas and biguanides is also associated with side effects (Rang and Dale, 1991). The decade 2000-2010 has been declared as decade of traditional medicine in Africa. Hence the popularity of the use of herbal medicine in Nigeria has increased considerably resulting in listing of herbal medicinal products by the Food and Drug regulating agency (NAFDAC).

P. nitida grows as an under storey tree in rain forests and widely distributed in the tropical rain forests of Africa in homesteads or nearby bushes. When fully grown, it is up to 20 m high with white flower in a terminal inflorescence and very large paired fruits (Pods). The fruit (Pod) is several seeded and the seeds are usually embedded in pulpy material known as pulp. The pericarp of the fruit which contains latex is known as the rind (Keay *et al*., 1964)

P. nitida seeds are valued in traditional medicine as good substitute for quinine in treatment of malaria, cough suppressant, chest complaints, including pneumonia, anodyne for topical injuries and also as enema (Burkhil, 1985). In some parts of Africa, decoction of the

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stem bark is used in the treatment of venereal disease .The seeds are used in the treatment of hepatitis, worms, sleeping sickness and malaria in Ihiala town, Anambra state of Nigeria (Iwu, 1982). It was been reported also that the plant has anti trypanosome activity, analgesic, anti-inflammatory as well as anti diabetic activities (Aguwa *et al*., 2001; Tane *et al*., 2002; Duwiejua *et al*., 2002).

Drugs should be uniform in quantity with respect to origin, cleanliness and content of therapeutically active constituent. If these qualities are maintained a particular dose of the drug will invariably have uniform strength for the purpose of authenticity, genuiness and purity. Uniformity in quality is promoted by the use of standards. Standards are numerical quantities by which the quality of drugs may be assessed for accuracy and quality assurance.

The aim of this study is to evaluate hypoglycemic activity in the three major components of *P. nitida* fruit (the pulp, the seed and the rind), mode of action, toxicity, phytochemical constituents and establish standards to enhance optimal use of the plant in traditional medicine practice in Nigeria.

MATERIALS AND METHODS

Collection of plant material and extracts preparation: The pods of *Picralima nitida* were collected in Ihiala, Anambra State in January, 2004 and identified by the Department of Botany, University of Nigeria, Nsukka. A voucher specimen of the pod was deposited in the Department's Herbarium. The pods were broken open and the components were separate into seeds, the pulp and the rind. They were air dried for three weeks during the harmattan season. The dried seed, pulp and rind were differently ground into fine powder with grinding machine and they were stored in waterproof bags.

The pulverized rind (340 g), seed (260 g) and pulp (180 g) were macerated with methanol for 24 h with intermittent shaking. The materials were filtered and concentrated *in vacuo* using rotary evaporator and their percentage yield values were calculated.

Animals: Wistar albino mice (19-35 g) and rats (180-250 g) of both sexes bred in the Department of Pharmacology Animal unit of University of Nigeria, Nsukka were used in the experiment. The animals were kept under room temperature with free access to water and food for two weeks before the commencement of the experiment. After randomization into various groups, the rats were acclimatized for a period of 2-3 days in the new environment before the commencement of the experiment. The animals described as fasting had been deprived of food for at least 12 h but allowed free access to drinking water.

Determination of hypoglycemic effect on normoglycemic rats: Wistar albino rats of either sex were randomly divided into eleven groups (n = 3) and fasted for 12 h before the administration of the drugs. Group A received pulp extract (100 mg kg^{-1}), B (pulp extract- 300 mg kg^{-1}), C (pulp extract (900 mg kg^{-1}), D (Rind extract-100 mg kg^{-1}), E (Rind extract 300 mg kg^{-1}), F (Rind extract 900 mg kg^{-1}), G (Seed extract-100 mg kg^{-1}), H (Seed extract-300 mg kg^{-1}) I (Seed extract-900 mg kg^{-1}), J (Normal Saline-3 mL kg^{-1}) and K (Glibenclamide-10 mg kg^{-1}). The drugs were administrated through intraperitoneal route and blood samples (0.1 mL) was with drawn from the tail vein of the rats at 0, 1, 4, 8 and 24 h after treatment. The blood sugar concentrations were determined using glucometer.

Effects of extracts on hyperglycemic rats: Wistar albino rats were weighed and fasted for 12 h. Hyperglycemia was induced by intravenous administration of 140 mg $kg^{-1}$of alloxan monohydrate (Sigma, USA) prepared in distilled water. The animals were given free access to water and food for 7 days. On the day 8, the survivors with blood glucose level of over 200 mg kg^{-1} were selected and divided into eleven groups (A-K), n = 3. The animals were fasted for 12 h and each group received the extract and dose as indicated in normoglycemic rats. The blood samples were collected from the tail vein at O, 1, 4, 8 and 24 h after treatment and the blood glucose levels were determined using glucometer.

Acute toxicity test: The LD_{50} of the extract was determined in mice using the Lorke method (1983). The animals were dosed with the extracts and kept for (15 days), observed for toxic signs and symptoms, body weight changes and LD_{50} calculated.

Phytochemical studies: The presence of metabolites in the pulp, seed and rind were investigated using standard methods (Trease and Evans, 2002).

Analytical standardization: Total ash, acid insoluble, water soluble, sulphated ash values, alcohol soluble extractive, water soluble extractive values and moisture content were carried out using standard procedures (AP, 1986).

RESULTS AND DISCUSSION

The yield values of the methanolic extracts of *P. nitida* pulp, seed and rind were 35.94, 13.73 and 16.11%, respectively. This high yield of extracts shows that optimal extraction of the constituents requires the use of polar solvents such as water or alcohol which is in consonant with folkloric use.

Oral administration of aqueous extract of the leaves of *Mangifera indica* (1 g kg^{-1}) failed to alter the blood glucose levels in normoglycemic or STZ induced diabetic rats. (Aderibigbe *et al*., 1999). However, the administration of 100, 300 and 900 mg $kg^{-1}$of the extracts to normoglycemic rats resulted in significant ($p<0.01$) lowering of fasting blood sugar after 8 h (Table 1). It was also observed that the extract maintained hypoglycemic action throughout the 24 h of study which shows that it has a long duration of action. The pulp extract (100 mg kg^{-1}) produced a maximum percentage reduction of 38.35% while the rind extract (900 mg kg^{-1}) reduced the blood sugar level by 46.19% after 24 h. When compared with that of glibenclamide (64.90% maximum reduction), the seed extract showed less effect (36.81%). The negative control (normal saline) did not show any significant hypoglycemic activity in normoglycemic rats.

However, the effects of the extracts (Table 2) on alloxanized rats were remarkable since very high hypoglycemic activity was achieved in all the extracts administered. Extracts showed a highly significant ($p<0.01$) antidiabetic activities especially in alloxanized rats. Alloxan and streptozotocin cause hyperglycaemia by selective destruction of the cells of the pancreas. There is a wide variability in the dose of alloxan required to produce long-standing diabetes which is also compatible with life. Different doses of alloxan produce varying intensities of hyperglycemia ranging from 160 to 400 mg dL^{-1} (Dubey *et al*., 1994; Subramoniam *et al*., 1996). In the present study, an injection of alloxan (140 mg kg^{-1}) raised the blood sugar levels. The animals were given free access to water and food for 7 days. On the day 8, the survivors with blood glucose level of over 200 mg kg^{-1} were selected.

Within the 24 h of the study, the pulp, seed and rind extracts showed, maximum reduction of fasting blood sugar of 85.85% (300 mg kg^{-1}), 83.26% (300 mg kg^{-1}) and 80.25% (900 mg kg^{-1}), respectively.

Determination of the LD_{50} was used as a standard measure for assessing the toxicity of the extracts. LD_{50} values were pulp 7071.06 mg kg^{-1}, seed 948.68 mg kg^{-1} and rind 1364.91 mg kg^{-1}. This shows an order of activity and safety as pulp>seed>rind. It is worthy to note that the pulp (300 mg kg^{-1}) and seed (900 mg kg^{-1}) extracts exerted higher hypoglycemic action than glibenclamide (a well known antidiabetic drug,) which proves the potency of the extracts in management of diabetes mellitus. The extracts of these plants differ from insulin in activity upon oral administration and prolonged duration of action thus patient compliance will be high. The extract of *P. nitida* effects hypoglycemia in both normal and alloxanized diabetic rabbits by a

Table 1: Effects *P. nitida* pulp, rind and seed extracts on mean fasting blood sugar of normoglycaemic rats

	Mean fasting blood sugar (mg dL^{-1})					
Group and dose	0 h	1 h	4 h	8 h	24 h	Maximum reduction (%)
Pulp extract 100 mg kg^{-1}	64.33±9.26	63.66±5.36	57.00±2.64	39.66±3.17^{*b}	40.00±2.08^{*b}	38.35
Pulp extract 300 mg kg^{-1}	52.66±2.40	68.33±5.04	64.00±6.11	44.66±2.02^{a}	40.66±0.88^{b}	22.78
Pulp extract 900 mg kg^{-1}	69.00±5.29	74.00±3.50	62.00±1.73	54.33±2.40^{a}	46.33±2.27^{b}	28.02
Rind extract(100 mg kg^{-1})	56.33±5.20	61.66±0.33	60.66±3.75	46.66±2.33	44.00±2.51	21.88
Rind extract (300 mg kg^{-1})	56.66±1.66	63.00±0.57	59.66±5.23	40.66±2.40^{**a}	40.33±2.51^{*b}	28.82
Rind extract (900 mg kg^{-1})	61.33±2.84	63.33±4.19	67.00±5.56	35.66±0.88^{**a}	33.00±0.57^{**b}	46.19
Seed extract (100 mg kg^{-1})	59.33±1.20	65.00±1.73	62.66±11.79	46.66±2.40^{*b}	41.66±0.57^{*b}	32.88
Seed extract (300 mg kg^{-1})	66.66±2.18	64.66±3.52	62.00±5.68	56.33±2.33^{b}	55.33±2.18^{b}	16.58
Seed extract (900 mg kg^{-1})	60.66±1.20	64.66±2.33	66.33±5.78	40.00±1.00^{**b}	38.33±1.20^{**b}	36.81
Normal saline (3 mL kg^{-1})	53.33±3.92	55.00±2.30	56.66±4.09	48.66±1.45	47.66±3.52	10.63
Glibenclamide (10 mg kg^{-1})	88.33±19.40	59.66±2.90^{a}	35.00±2.08^{**c}	32.00±1.20^{**c}	31.00±0.57^{**c}	64.90

Values are expressed as the mean±SEM; n = 3; ***$p<0.001$, **$p<0.01$, *$p<0.05$ significantly different compared with control (normal saline); $^{c}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ significantly different compared with 0 h

Table 2: The effects of the methanolic extract, of *Picralima nitida* pulp, rind and seed on mean fasting blood sugar of hyperglycemic rats

	Mean blood sugar concentration (mg dL^{-1})					
Group and dose	0 h	1 h	4 h	8 h	24 h	Maximum reduction (%)
Pulp extract 100 mg kg^{-1}	318.33±6.93	299.33±8.25^{a}	182.66±5.36^{***c}	69.33±1.45^{***c}	62.66±3.28^{***c}	81.36
Pulp extract 300 mg kg^{-1}	351.00±6.08	241.33±5.48^{c}	137.66±8.37^{***c}	52.66±1.76^{***c}	49.66±1.20^{***c}	85.85
Pulp extract 900 mg kg^{-1}	272.00±32.74	227.66±34.27^{b}	141.33±9.38^{***a}	87.66±3.75^{***a}	50.00±3.46^{***a}	81.61
Rind extract (100 mg kg^{-1})	286.33±24.90	379.00±26.85	335.33±26.73	305.00±30.66	76.66±2.33^{***c}	73.22
Rind extract (300 mg kg^{-1})	356.33±17.26	259.33±18.27^{c}	187.33±18.12^{**c}	150.66±16.16^{**c}	68.33±1.76^{***c}	80.82
Rind extract (900 mg kg^{-1})	217.66±9.38	305.33±14.11	327.33±15.34	244.68±9.53	77.66±4.09^{***c}	64.32
Seed extract (100 mg kg^{-1})	229.00±9.29	263.00±5.50	229.00±5.19	62.33±2.40^{***c}	45.66±1.85^{***c}	80.06
Seed extract (300 mg kg^{-1})	406.33±11.60	325.00±17.21^{b}	159.00±12.12^{**c}	128.00±4.35^{***c}	82.00±3.78^{***c}	79.73
Seed extract (900 mg kg^{-1})	494.00±19.50	413.33±19.71^{b}	521.33±51.84	379.00±29.30^{**b}	82.66±1.85^{***c}	83.26
Normal Saline (3 mL kg^{-1})	306.33±40.06	290.00±35.76	272.00±35.57^{a}	243.33±31.11^{a}	233.33±31.14^{a}	23.83
Glibenclamide (10 mg kg^{-1})	314.00±3.78	291.00±5.68^{a}	184.33±6.00^{**c}	160.33±9.27^{**c}	54.33±0.88^{***c}	82.69

Values are expressed as the mean±SEM; n = 3; ***$p<0.001$, **$p<0.01$, *$p<0.05$ significantly different compared with control (normal saline); $^{c}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ significantly different compared with 0 h

mechanism independent of the availability of insulin from pancreatic β-cell (Inya-Agha, 1999). Lower glucose observed in fasted animals may be due to glucogenesis in the liver. Alloxan destroys Beta cells of Langerhans islets; *P. nitida* lowers blood glucose probably by increasing the permeability of cell plasma membrane to glucose and may be of use in the prevention of T2DM due to lowered blood glucose in normal animals. Phytochemical analysis (Table 3) revealed the presence of steroids, terpenoids, resins, carbohydrates and reducing sugar in all the extracts. A wide array of plant derived active principles with numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM. Among these are alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycans, hypoglycans, guani- dine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions (Bailey and Day, 1989; Bailey *et al*., 1989; Ivorra *et al*., 1988; Marles and Farnsworth, 1995).

The pulp and rind extracts were richer in steroids and terpenoids but the rind extract showed an outstanding positive reaction for flavonoids. Significant positive results for tannins were observed in the seed extract. Alkaloid is abundantly present in the three extracts and has been reported to be of indole origin (Tane *et al*., 2002; Duwiejua *et al*., 2002; Okunji *et al*., 2005).

Results of the analytical standardization (Table 4) showed variable numerical constants for the rind, the pulp and the seed samples. These values can also be used as reference guide for identification and for determining the quality and purity of the drug as any deviation exceeding±0.5% would be an indication of possible adulteration.

Table 3: Phytochemical analysis of *P. nitida* seed, pulp and rind methanolic extracts

Tests	Pulp	Rind	Seed
Flavonoids	++	++++	+++
Resins	+	+++	++++
Alkaloids	++++	++++	++++
Glycosides	+++	+++	+++
Steroids	+++	+++	++
Terpenoids	+++	+++	++
Tannins	+	-	+++
Carbohydrate	+++	+++	++
Acidity	++	+	++
Protein	++	++	++
Reducing sugar	+++	++	+
Saponins	+	+++	+++

(+) = Present (-) = Not present. Multiple pluses indicate degree of abundance

Table 4: Analytical standards

Value analysis (%)	Pulp	Rind	Seed
Total ash	12.50±0.12	25.00±0.06	12.50±0.10
Acid insoluble ash	5.00±0.02	7.50±0.41	2.50±0.27
Water soluble ash	5.00±0.17	17.50±0.70	7.50±0.51
Sulphated ash	19.00±0.39	20.00±0.14	15.00±0.26
Alcohol soluble extractive	22.00±0.48	8.00±0.22	14.00±0.14
Water soluble extractive	22.00±0.35	8.00±0.32	2.00±0.23
Moisture content	3.33±0.61	6.66±0.25	1.66±0.13

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