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Research Article

# Evaluation of the Therapeutic Properties of the Aqueous Extract of *Picralima nitida* Seeds in Diabetic Rats

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## Abstract

*Picralima nitida* seeds are used in traditional medicine to treat cough, bronchitis and headache, hernia, vomiting, diarrhea and finally leucorrhoea. The aim of this work was to evaluate the therapeutic effect of the aqueous extract of *Picralima seeds nitida* on hyperglycemia in rats. The antidiabetic activity of the aqueous extract of *P. nitida seeds* was evaluated in diabetic rats. For this purpose, diabetes was induced in rats by intraperitoneal injection of a single dose of 150 mg/kg MC of alloxan solution. After induction, rats were given anhydrous glucose solution (5%) overnight to overcome the hyperglycemic shock induced by the action of alloxan, and then the animals were treated. *P. nitida extract caused a marked recovery of body mass in diabetic rats. P. nitida* extract resulted in a significant reduction in induced hyperglycemia in diabetic rats. Regarding biochemical parameters, *P. nitida seeds* resulted in a marked improvement in the physiological state of rats by reducing biochemical parameters such as urea, ASAT, ALAT, creatinine, and uric acid as well as lipid and protein parameters. Administration of *P. nitida seeds* promoted the production of alpha amylase and lipase in diabetic rats. The use of this plant in the treatment of diabetic rats would therefore justify the therapeutic properties of *P. nitida seeds*.

## Keywords

*Picralima nitida*, Hyperglycemia, Diabetic, Alloxan

## 1. Introduction

Diabetes is an endocrine disease that affects nearly 10% of the world's adult population [1] According to WHO, the current prevalence of diabetes worldwide is around 347 million people [2]. Thus, in 2013, nearly 20 million people had diabetes in sub-Saharan Africa, a prevalence of 4.9% [2]. Furthermore, more than 80% of diabetes-related deaths occur in low- and middle-income countries. In Côte d'Ivoire, the International Diabetes Federation (IDF) estimated the prevalence of diabetes at 9.6% in 2014. In sub-Saharan countries, although modern treatments exist, the population continues to turn to plants given their precarious means [3]. In Africa, several medicinal plants have been identified in the treatment of diabetes. It is in this context that *picralima nitida*, a plant that has very interesting therapeutic and antioxidant properties, was chosen to evaluate its antidiabetic properties. These bitter seeds are consumed crushed with lemon to treat hernia, vomiting, diarrhea and leucorrhea [4]. In Ivory Coast, the paste, made from young leaves and crushed flower buds, is

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diluted in water and ingested against palpitations. The powder of dry leaves is consumed in food, drunk in water or smoked like tobacco against coughs. Fruit decoctions are used in the treatment of coughs, bronchitis and headaches. Fruit strips are applied to wounds as a dressing [5, 6]. The seeds, bark, roots and leaves of the species are reputed to be a febrifuge and a remedy for malaria and diabetes [4, 7].

## 2. Material and Methods

### 2.1. Plant Material

The plant material consists of the dried grains of *Picralima nitida* (Apocynaceae) collected at the National Floristic Center located at the Felix Houphou ët Boigny University. The grains were then collected, dried in the shade, approximately 25 ℃, and then pulverized using a Vorwerk Thermomix 3000 grinder. The powder obtained was subjected to aqueous extraction.

### 2.2. Animal Material

The animal material consists exclusively of albino rats *(Rattus norvegicus)* of the Wistar strain. These rats are 13 to 14 weeks old depending on the biological test to be performed, with weights between 195 and 250 g. These animals were fed with a standard rodent diet (mixture of foods composed of wheat bread, peanuts, corn, soybean powder and dried fish) and have free access to water (tap water).

### 2.3. Chemical Material

The products used to conduct this study are alloxan to induce diabetes, biochemical and hematological assay reagents, anhydrous glucose for the preparation of glucose stock solution with a concentration of 1 mg/ml, phenol 5%, concentrated sulfuric acid, isotonic saline 0.9% and distilled water.

### 2.4. Induction of Diabetes by Alloxan in Wistar Rats

a) Principle

Diabetes will be induced by injection of a solution composed of alloxan monohydrate with saline (0.9) according to the method written by Iweala et *al*. [8]

b) Operating mode

The animals (04) were deprived of food for 16 hours, but had water available ad libitum. They received intraperitoneally a single dose of 150 mg/kg of MC of the freshly prepared alloxan solution. After induction, the rats received an anhydrous glucose solution (5%) throughout the night in order to overcome the hyperglycemic shock induced following the action of alloxan. Seventy-two hours after the injection, a blood sample was taken by caudal amputation in order to measure the blood glucose level. Rats whose blood glucose levels were higher than 1.9 g/l were retained for the rest of the experiment.

For the treatment of animals, 6 batches of 5 rats each will be formed and the treatment will be carried out over a period of 24 days as follows:

1. Negative control: group consisting of non-diabetic control rats having received distilled water throughout the experiment
2. Positive Control: group consisting of diabetic rats that received no treatment.
3. PN250, PN500, PN1000: batches consisting of diabetic rats treated with the aqueous extract at the respective doses of 250; 500 and 1000 mg/kg of MC.

The blood sugar of the rats was taken daily to assess the variation in blood sugar. At the end of the experiment, the animals were weighed and then anesthetized, and a blood sample was taken by caudal amputation. The blood was collected in two different tubes, one of which contained an anticoagulant (EDTA) for the measurement of hematological parameters. The latter was centrifuged using a centrifuge brand B 4i at 3000 rpm. In order to determine the use of the extract on the body mass and growth of diabetic rats during treatment, the evolution of the body mass of treated and control rats was monitored periodically throughout the experiment.

## 3. Results

*Picralima nitida Extract on Body Mass of Diabetic Rats*

The body mass variations of the rats were monitored during the experiment. In healthy control rats, a clear increase in body mass of the rats was observed throughout the experiment (Figure 1). This variation in body mass is significantly higher than that of diabetic rats ($p<0.0001$) and diabetic rats treated with the extract ($p<0$ 01). Regarding the body mass variations of the diabetic rats, a strong decrease in body mass of the rats was observed from 198 ±6 g to 157.5 ±4.9 g, which corresponds to a decrease of 20.7%. In the treated animals, reduction percentages of 8.12%, 6.09% and 3.29% were observed respectively for the 250 mg/kg, 500 mg/kg and 1000 mg/kg batches on the 8th day of treatment. However, a clear increase was observed from the 12th day, corresponding to an increase of 0.8%, 1.06% and 1.08% respectively from the 8th day to the 14th day.

At the end of the experiment, in the rats treated with the extract, body weights increased from 197 ±1.4 g to 182.5 ±2.12 g (dose of 250 mg/kg), from 197 ±4 to 186.5 ±5.2 (dose of 500 mg/kg) and from 197.5 ±1.4 g to 193.5 ±3.5 g (dose of 1000 mg/kg). This corresponds to percentage decreases of 7.36%, 5.3% and 2.02% respectively.

*Picralima nitida Extract on Blood Glucose Levels in Diabetic Rats*

The evaluation of the effect of *P. nitida extract* on the blood glucose of diabetic rats was followed for 14 days (Figure 2). After the induction of hyperglycemia in the animals with

alloxan, the blood glucose of the rats was determined. This blood glucose was around 360 mg/dl. However, the blood glucose of the rats that did not receive alloxan, the blood glucose remained practically the same (73 mg/dl) throughout the experiment.

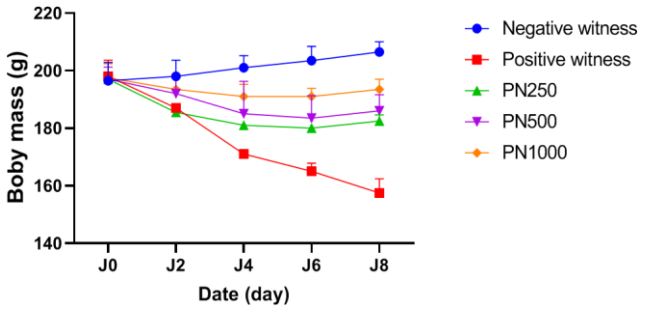


***Figure 1.*** *Effect of Picralima nitida on body mass of diabetic rats.*

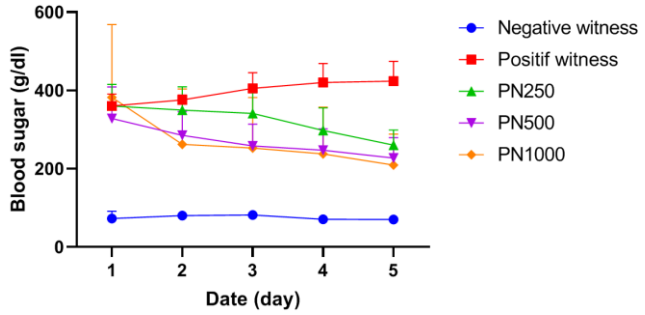


***Figure 2.*** *Effect of Picralima nitida extract on blood glucose levels in diabetic rats.*

*Negative witness: healthy control rats; Positive witness: diabetic control rats*
*PN250, PN500, PN1000: batches consisting of diabetic rats treated with the aqueous extract at the respective doses of 250; 500 and 1000 mg/kg of MC.*

In untreated diabetic rats (negative control), blood sugar remained high, increasing from 360.3 ±17 mg/dl of glucose to 433.66 ±29 mg/dl, corresponding to an increase of 20.86% compared to blood sugar after induction. This increase is significantly different compared to those of diabetic rats treated with the plant.

In diabetic animals treated with the extract, some reduction in blood glucose was observed. Thus, the extract at different doses reduced blood glucose in diabetic rats from 360 mg/dl to 260.33 ±22 mg/dl, to 227.33 ±29 mg/dl and to 209 ±46 mg/dl respectively for doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg of MC. These variations correspond to the percentages of reduction in blood glucose of 27.77%, 36.85% and 41.94% respectively compared to blood glucose after induction of diabetes. Statistical analysis revealed a significant difference ($p<0.05$) in blood glucose levels between diabetic rats treated with the plant extract. However, despite this decrease, the blood sugar level of diabetic rats remained significantly ($p<0.00101$) higher than that of healthy rats (73 mg/dl).

*Picralima nitida extract on biochemical parameters of diabetic rats*

At the end of the experiment, the blood of the animals was collected to measure certain blood parameters in order to assess the serology of the treated or untreated animals. A strong variation in the concentrations of many blood parameters was observed compared to those of the non-diabetic control rats (Table 1). In untreated diabetic rats, an increase in blood concentrations of urea (3.20 ±0.86 g/l), glucose (3.06 ±0.74 g/dl), total cholesterol (1.8 ±0.74 g/l), triglycerides (2.20 ±0.1 g/l), creatinine (9.5 ±0.49 mg/l), uric acid (24.5 ±2 IU/l), LDH (2791.5 ±67 IU/l), GGT (25 ±1 IU/l), ASAT (470.5 ±49 IU/l), ALAT (205.1 ±36 IU/l), CK (9733.5 ±60 IU/l) and ALP (948.5 ±29 IU/l) was observed. However, the concentrations of some parameters were low, namely the concentration of HDL (14.75 ±0.16 mg/l), total protein (21.4 ±0.8 g/l), amylase (683 ±41 IU/l) and alpha lipase (2.02 ±0.4 IU/l).

Statistical analysis revealed that these variations are very significantly different ($p<0.0001$) compared to the concentrations of these same parameters in non-diabetic rats. Similarly, statistical analysis revealed a significant difference ($p<0.001$) compared to diabetic rats treated with the extract at different doses. The behavior of the plant extract on the concentration of blood parameters was dose-dependent. The interesting results were observed in diabetic rats treated with the extract at doses of 1000 mg/kg MC. The mean concentrations measured in diabetic rats treated at this dose are 1.42 ±0.82 g/l (urea), 1.132 ±0.65 g/dl (glucose), 6 ±0 mg/l (creatinine), 20 ±4 IU/l (uric acid), 0.68 ±0.08 g/l (cholesterol), 38.3 ±8.1 mg/l (HDL), 2151 ±172 IU/l (LDL), 74.05 ±2.8 g/l (total protein), 0.88 ±0.19 g/l (triglyceride), 1046.5 ±74 IU/l (amylase), 6 ±3 IU/l (GGT), 235.8 ±42 IU/l (ASAT), 73.1 ±40 IU/l (ALAT), 6245 ±142 IU/l (CK), 7.45 ±0.6 IU/l (lipid), 539.5 ±42 IU/l (ALP).

***Table 1.*** *Effect of P. nitida extract on biochemical parameters of diabetic rats.*

| **Setting** | ***Negative witness*** | ***Negative witness*** | **PN250** | **PN500** | **PN1000** |
|---|---|---|---|---|---|
| Urea (g/l) | 0.237 ±0.04 | 3.20 ±0.86 | 1.80 ±0.11 | 1.24 ±0.14 | 1.42 ±0.82 |
| Gluc (g/dl) | 0.71 ±0.009 | 3.06 ±0.74 | 2.53 ±0.23 | 1.56 ±0.61 | 1.132 ±0.65 |

| Setting | *Negative witness* | *Negative witness* | PN250 | PN500 | PN1000 |
|---|---|---|---|---|---|
| Crea (mg/l) | 5 ±0 | 9.5 ±0.49 | 6 ±0 | 6.5 ±1 | 6 ±0 |
| Ua (UI/l) | 17.6 ±4 | 24.5 ±2 | 21.5 ±0.49 | 20 ±2 | 20 ±4 |
| Chol (g/l) | 0.64 ±0.1 | 1.8 ±.74 | 0.72 ±0.19 | 0.70 ±20 | 0.68 ±0.08 |
| Hdl (mg/l) | 33.95 ±3 | 14.75 ±0.16 | 44.2 ±6.4 | 34.9 ±0 | 38.3 ±8.1 |
| Ldh (UI/l) | 2477 ±60 | 2791.5 ±67 | 2377.5 ±286 | 2041.5 ±149 | 2151 ±172 |
| Tp (mg/l) | 66.4 ±1 | 21.4 ±0.8 | 74.8 ±1.2 | 71.4 ±0.8 | 74.05 ±2.8 |
| Trigl (g/l) | 1.32 ±0.4 | 2.20 ±0.1 | 0.54 ±0.4 | 0.85 ±0.24 | 0.88 ±0.19 |
| Amyl (UI/l) | 1420 ±127 | 683 ±41 | 1002.5 ±123 | 1013.5 ±64 | 1046.5 ±74 |
| GGT (UI/l) | 3.5 ±0.49 | 25 ±1 | 6.5 ±2 | 7 ±1 | 6 ±3 |
| ASAT (UI/l) | 224.5 ±6 | 470.5 ±49 | 214.95 ±31 | 202.8 ±3 | 235.8 ±42 |
| ALT (UI/l) | 65.5 ±27 | 205.1 ±36 | 89.75 ±16 | 74.05 ±4.8 | 73.1 ±40 |
| CK (UI/l) | 6340 ±144 | 9733.5 ±60 | 5722.5 ±146 | 5733.5 ±164 | 6245 ±142 |
| lipase (UI/l) | 9.6 ±0.6 | 2 .02 ±0.1 | 6.75 ±1.14 | 6.5 ±0.4 | 7.45 ±0.6 |
| ALP (UI/l) | 171 ±2 | 948.5 ±29 | 599 ±49 | 515.5 ±96 | 539.5 ±42 |

*Negative witness: healthy control rats; Positive witness: diabetic control rats*
*PN250, PN500, PN1000: batches consisting of diabetic rats treated with the aqueous extract at the respective doses of 250; 500 and 1000 mg/kg of MC.*
*Gluc: glucose; Crea: creatinine; Ua: uric acid; Chol: cholesterol; HDL: HDL cholesterol; Ldh: lactate dehydrogenase; Tp: total protein; Trigl: triglyceride; Amyl: alpha amylase; GGT: gamma-glutamyl transferase; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; CK: creatine kinase; ALP: alkaline phosphatase.*

## 4. Discussion

In order to evaluate the biological effect of *P. nitida* in diabetics, a technical model of experimental diabetes was carried out in animals comparable to diabetes mellitus in humans. The hyperglycemia observed in rats 3 days after the injection of alloxan indicates the onset of diabetes. Indeed, alloxan induces hyperglycemia, a considerable loss of weight followed by physiological disorders [9]. This high hyperglycemia observed would be due to necrosis of the β cells of the pancreas thus leading to an alteration of the carbohydrate, lipid and protein metabolism due to insulin deficiency [10]. In untreated diabetic rats, the weight loss was considerable throughout the experiment. On the other hand, the body mass of diabetic animals treated with the plant extract was characterized by a progressive recovery of their body mass. The ability of the extract to protect rats against body weight loss could be due to its ability to reduce hyperglycemia [11]. These results corroborate those of Abd El Latif et al. [12], who showed that the administration of garlic extract to diabetic rats resulted in a clear recovery of the rats' body mass. In addition to the recovery of body mass in diabetic rats treated with the extract, blood glucose levels decreased considerably by 27.77% at the low dose of the extract (250 mg/kg of MC) and by 41.94% for the high dose of the extract (1000 mg/kg of MC). Our results are similar to those of some authors such as Ladouari [13]. Indeed, this author showed that *Zygphylum album* (Zygophyllaceae) significantly reduced blood glucose levels in diabetic rats. Blood biochemical analysis of diabetic rats showed significant differences in the concentrations of urea, uric acid, creatinine, alpha amylase and lipase, and lipid parameters. The significant variation in lipid parameters, including triglycerides and cholesterol, was linked to the destruction of secretory cells by alloxan, leading to metabolic dysfunction of carbohydrates in rats due to weak insulin action. Also, alloxan may have led to the alteration and lysis of liver cells, an organ responsible for the release of hepatic enzymes (AST and ALT) into the blood. In fact, the increase in the activities of hepatic enzymes in animals indicates the alteration of the liver by alloxan [14, 15]. In addition, administration of the extract reduced the levels of glucose, uric acid, creatinine, urea, amylase and blood parameters. These effects show the antidiabetic effects of the plant *P. nitida* . The antidiabetic activities of the extract (hypolipidemic and hypoglycemic) would be due to its richness in phenolic compounds. Indeed, phenolic compounds participate in the inhibition of enzymes such as amylases, in the reduction of oxidative stress induced by the alloxan molecule in animals thanks to their antioxidant property [16]. Studies have shown that other medicinal plants have antidiabetic effects. Thus, the

antioxidant activity of plants has led to the decrease in the cytotoxicity of free radicals on pancreatic β cells [17]. These plants contain active ingredients capable of acting on insulin metabolism, allowing the storage of glucose in the form of glycogen in the organs [18, 19]. Phenolic compounds through antioxidant properties protect pancreatic cells, improve insulin secretion which promotes the normalization of blood sugar in treated diabetic rats [20]. The administration of polyphenols to diabetics improves diabetes-related complications such as coronary disease and heart failure through its anti-radical effects [21].

The reduction in glucose in diabetic rats treated with the extract would be linked to an increase in insulin levels. This increase in insulin could be due to a stimulation or regeneration of insulin-secreting β cells [17]. These activities of *P. nitida* would offer protection against oxidative stress, cardiovascular disorders such as atherosclerosis by improving lipid profiles and inhibiting hepatic synthesis of cholesterol and fatty acids [22]. The increase in alpha-amylase activity by *P. nitida* in diabetic rats could be explained by protective or regenerative effects on pancreatic cells.

## 5. Conclusion

This study is part of the search for new molecules from plant extracts. The therapeutic properties of P. nitida on diabetic rats showed that the extract promoted an improvement in the body mass of the rats. The biochemical parameters in rats treated with the aqueous extract of P. nitida were significantly improved, unlike untreated diabetic rats. These results reveal that this plant has a powerful therapeutic effect, which supports its traditional use for the treatment of diseases.

## Abbreviations

| | |
|---|---|
| ALAT | Alanine Aminotransferase |
| ALP | Alkaline Phosphatase |
| Amyl | Alpha Amylase |
| ASAT | Aspartate Aminotransferase |
| Chol | Cholesterol |
| CK | Creatine Kinase |
| Crea | Creatinine |
| GGT | Gamma-glutamyl Transferase |
| Gluc | Glucose |
| HDL | HDL Cholesterol |
| IDF | International Diabetes Federation |
| Ldh | Lactate Dehydrogenase |
| Tp | Total Protein |
| Trigl | Triglyceride |
| Ua | Uric Acid |
| UFR | Unit of Formation and Recherch é |
| WHO | World Health Organization |

## Author Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Consent

It is not applicable.

## Ethical Approval

The animals were used under ethical and deontological conditions under the tutelage of our Supervisor.

## Conflicts of Interest

The authors declare no conflicts of interest.

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