

Antidiabetic Activities of *Picralima Nitida* Powder, an Indigenous Ivorian Edible Plant in Type II Diabetes Mellitus Rat Model

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Received June 03, 2023; Revised August 28, 2023; Accepted November 03, 2023

Abstract Faced with the high prevalence of type 2 diabetes in Africa, sustainable and healthier alternatives (fewer side effects), less expensive are also to be considered compared to expensive conventional medical treatments. In Côte d'Ivoire, neglected edible wild fruits and vegetables including *Picralima nitida*, rich in essential bioactive compounds (vitamins, minerals, fibres, etc.) are used both for food and in the traditional treatment of pathologies linked to oxidative stress including type 2 diabetes. However, their strong perishability limits their uses for a long period towards ready-to-eat, marketable products. Thus, the present study was carried out to evaluate the antidiabetic potentialities of freeze-dried powder of *Picralima nitida* seeds in alloxane-induced type II diabetes mellitus rat model. Experiments were carried out for 28 days with four groups of animals: the negative control (TN), the positive untreated control (TPOS), the orally daily treated with the powder solution of *P. nitida* (SaPn) at 200 mg/kg bw and the rats treated with glibenclamide (REF). Results showed that the SaPn significantly improved ($p < 0.05$) glucose metabolism after one week, ensured the maintenance of body weight, allowed restoration of the globules destroyed by alloxane, healed the lesions recorded at the level of the kidneys, liver, and heart. Thus, dried powder of *Picralima nitida* seeds had pronounced effects in improving health in type II diabetics with no adverse effects and, could be a sustainable alternative, necessary for improved marketable formulations.

Keywords: *Picralima nitida* powder, type 2 diabetes, antidiabetic, indigenous plant

Cite This Article: Konan Jean Noel Yao, Assamoi, Allah Antoine, Hadja Djeneba Ouattara, and Kouadio Eric Donald N'dri, "Antidiabetic Activities of *Picralima Nitida* Powder, an Indigenous Ivorian Edible Plant in Type II Diabetes Mellitus Rat Model." *Biomedicine and Biotechnology*, vol. 8, no. 1 (2023): 14-23. doi: 10.12691/bb-8-1-2.

1. Introduction

Diabetes mellitus is a metabolic disorder recognized by an unusual rise in blood glucose levels (chronic hyperglycemia) [1-3]. This occurs when the body cannot use insulin properly or lacks insulin due to destruction of the β -cells of the islets of Langerhans [4,5]. This state of hyperglycemia would affect other functions and organs of the body, such as the kidneys, heart, eyes, etc [6,7]. This disease causes oxidative stress, intestinal dysbiosis and chronic inflammation [8-11]. Diabetes is recognized in the world as one of the biggest killers and one of the diseases that is growing exponentially, so much so that it is compared to a pandemic [12]. Indeed, between 2000 and 2019, diabetes mortality rates by age increased to 6.7 million (32.6%) in 2021 worldwide [13]. The available statistics on diabetes in Africa show the magnitude of the challenge. Currently, 24 million adults are living with diabetes and this number is expected to increase to 55 million (129%) to adults by 2045 [6]. Diabetes mellitus

caused 416,000 deaths on the continent last year (2021) [7] and is expected to become one of the leading causes of death in Africa by 2030. Among the various types of diabetes mellitus, type II is the most common, both at international level and in sub-Saharan Africa [14]. Côte d'Ivoire is a sub-Saharan African country where non-communicable diseases are currently on the rise (6.9% for diabetes) [15,16]. To solve this public health problem, several anti-diabetic drugs such as glibenclamide, metformin, insulin, are sold at high prices and present several safety problems including unwanted side effects and toxicity (nausea, vomiting, hematological and dermatological reactions, obstructive jaundice, hyponatremia, and intolerance of alcohol and weight gain, ...) and are therefore less acceptable by the patients [17]. Despite the remarkable progress with the drugs, the search for new alternatives continues. One of the avenues considered and encouraged by the WHO is the consumption of plants such as fruits and vegetables [18]. Li and colleagues in 2021, showed that the consumption of fruits and vegetables was correlated with a reduced risk of developing diabetes and also concluded that a 0.2

portion consumed daily corresponded to 13 percent reduction in the risk of diabetes [18]. Côte d'Ivoire is a country, rich in vegetables and fruits and should seize this initiative to enhance them. One of the fruits commonly used for food and in the traditional treatment of diabetes is the fruit of *Picralima nitida*, locally called "diabetes pod"[19]. This fruit of ovoid shape and yellow color at maturity is in rich phytochemical compounds (glycosides, alkaloids, flavonoids, triterpenes, phenolic acids, saponins, tannins, amino acids, vitamin A and E ...) [20]. The seeds of the plant are used to sprinkle dishes or taken as decoction. Although several studies have been conducted to attest to its medical value [1], their post-harvest perishability following microbial attacks limits their uses over a long period. Moreover, there are still no studies in favor of this plant seeds in the form of marketable functional food ready for consumption. The dried powder obtained from the seeds of this plant has therefore been considered for this purpose as an alternative to the direct consumption of the harvest from the surrounding ecosystems where they grow naturally. Hence, this work deals for the time with the antidiabetic effects of *Picralima nitida* seeds from Côte d'Ivoire on rat models.

2. Material and Methods

Preparation of *Picralima nitida* seed powder

The collected fruits from surrounding ecosystems where they grow naturally. Fruits were bought in marketplace in Adjamé (Abidjan, Côte d'Ivoire). A sample of the fruit was transferred to the National Floristic Center of the University Félix Houphouët-Boigny of Abidjan where it was identified using the analytical Flora. The fruits were destemmed, washed with distilled water, and the seeds oven dried (Memmert, Germany) at 50°C for 72 h [21]. The dried materials obtained were ground with a laboratory grinder (Culatti, France) equipped with a 10 µm mesh sieve. The resulting dried powder samples were stored in polyethylene bags at 4°C until further analysis.

Identification of some metabolites and activities of *picralima nitida*

Phenolic compounds were quantified by [22] method after extraction with methanol. Thus, a quantity (1 g) of dried powdered sample was soaked in 10 ml of 70% (w/v) methanol and centrifuged at 1000 rpm for 10 min. An aliquot (1 ml) of supernatant was oxidized with 1 ml of Folin-Ciocalteu reagent and neutralized with 1 ml of 20% (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at room temperature (Préciser la température de la sale) and the absorbance was measured at 745 nm using a spectrophotometer (PG Instruments, England). A standard range was established from a gallic acid stock solution (1mg/mL), under the same conditions as the test to determine the amount of polyphenol in the sample.

Flavonoids were quantified by [23] method after extraction with methanol. So, 0.5 mL of methanolic extract, 0.5 mL of methanol; 0.5 mL of AlCl₃ (10%, w/v); 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water were mixed. The mixture was incubated at room temperature (Préciser la température de la sale) for 30 min. The absorbance was measured with a spectrophotometer

at 415 nm against a blank. A calibration range was performed from a 0.1 mg/mL quercetin stock solution. Subsequently, antioxidant and anti-inflammatory activities were quantified. Anti-inflammatory activity of water-soluble picralima extracts was evaluated by the protein denaturation inhibition method [24]. The reaction mixture (0.5 ml) consisted of 0.45 ml of bovine serum albumin BSA (5% aqueous solution) and 0.05 ml of broths of different concentrations of the reference drug Diclofenac Sodium. Six concentrations were tested from 31.25 to 1000 µg/ml. The pH was adjusted to 6.3 using 1N hydrochloric acid. Samples were incubated at 37°C for 20 minutes, then heated to 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer (pH = 6.3) was added to each test tube. The absorbance was measured spectrophotometrically at 614 nm (Jasco V-630, Deutschland). For the control test tubes, distilled water was used instead of broth, the product control tube did not contain BSA. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = 100 - [(D.O \text{ of sample} - D.O \text{ of control}) / D.O \text{ of control}] \times 10$$

For the antioxidant activity [25], 1 g of sample in 20 ml of 70% methanol was prepared. To a volume of 2 ml of methanolic extract at different concentrations was added 1 ml of freshly prepared DPPH (0.3mM). The negative control was prepared under the same conditions. After incubation in the dark for 30 min the absorbances were read at 517 nm using a spectrophotometer, against a blank for each concentration which contained 50 µl of each concentration of the extract and 1.95 ml of methanol. The results will be expressed as anti-free radical activity or free radical inhibition in percentages (I %) by the following formula:

$$PI = \frac{A_0 - A_1}{A_0} * 100$$

PI: Percentage of anti-free radical activity (AAR%).

A1: Absorbance of the sample.

A0: Absorbance of the negative control

Effects of *Picralima nitida* (SaPn) aqueous solution on rat body weight

Influence of aqueous solution of *P. nitida* on the body mass of treated and control rats was followed every three days throughout the experiment. The body mass was measured with an electronic scale (BIOBASE) [26]. All animal experiments have been carried out in accordance with EU guidelines (2007/526/CE). They were reproduced in Plexiglas cages, at the pet store of the Normal Superior School of Cocody. The resulting litters were fed and watered ad libitum to reach a weight between 110 and 120 g under standard environmental conditions temperature 25°C, with a light-dark cycle of 12 hours.

Method of blood glucose monitoring in rats

Blood glucose was measured with a blood glucose meter (One call plus blood glucose meter) with test strips. The principle of this meter is that it contains a hole into which a test strip is inserted to read the blood glucose. This strip has an absorbent layer on which a drop of blood is placed. The absorbent layer is finely porous or covered

with a membrane on its outer surface which retains the red blood cells and only allows the plasma to diffuse towards the lower layers where the reagent is located: glucose oxidase associated with a chromogen. The resulting color is measured by reflectometry in the meter and the blood glucose value is displayed on the meter screen. This value is given in mg/dL. The blood glucose levels were determined by the following formula: $\% G = ((G_x - G_0)/G_0) \times 100$, G_x = Value of blood glucose value on day x , x = day of collection, G_0 = Blood glucose value on day before test. The evaluation of the effects of the aqueous extract of *Picralima nitida* (Apocynaceae) seeds on blood glucose levels in normoglycemic rats is followed in the short term after gavage of animals with a single dose of 200 mg/kg body weight. This experiment is performed on sixteen male Wistar rats weighing between 150 and 200 g. The animals are divided into 4 batches of 4 rats and fasted for 24 hours. The average blood glucose of each batch of rats is determined before and each week during the experiment. The allocation was done as follows:

- Rats of batch 1 (negative control) received distilled water,
- Rats in batch 2 made sick, received the reference drug (glibenclamide),
- Rats of batch 3 received the aqueous solution of picralima (200 mg/kg PC) after induction of diabetes
- Rats of batch 4 were kept sick after induction of diabetes

Impact of SaPn on hematological and biochemical parameters of treated rats

The blood count performed from whole blood contained in EDTA anticoagulant tubes using an automatic hematology analyzer (Biobase) directly gives values for the following parameters: the concentration of white blood cells WBC ($10^9/L$), lymphocytes LYM* ($10^9/L$), neutrophils MID* ($10^9/L$), granulocytes GR* ($10^9/L$) and their proportions in (%), red blood cell concentration RBC ($10^{12}/L$), hemoglobin HGB (g/L), HCT hematocrit level (%), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCHC), mean cellular hemoglobin concentration (MCHC), red blood cell distribution width (RDW-CV (%)) and RDW-SD (fL)), blood platelet concentration (PLT ($10^9/L$)), mean platelet volume (MPV (fL)), total platelet volume (PCT (%)), thrombocyte size distribution range ((PDW (%)), platelet-to-large cell ratio (P-LCR (%)), and calculation of the platelet-to-large cell ratio (P-LCC ($10^9/L$)). The determination of biochemical parameters such as glucose, transaminases (AST and ALT), creatinine, urea, total cholesterol, bilirubin, triglycerides, C-reactive protein, and total protein was performed automatically in the Cobas C311 automated system. Aliquots of serum (500 μ L) were transferred into test tubes adapted to the automaton and identified. The automaton uses a sample volume of less than 5 μ L for the different assays. The results displayed on the screen are saved as a computer file.

Histopathological study

The histology technique allowed us to obtain thin sections of the following organs: liver, heart, and kidneys. This technique includes several successive main steps.

The organs are fixed in 10% formalin for 48 hours at room temperature. The purpose of organ fixation is to maintain the cells in a state close to the living state. Fixation causes the organ to harden, which keeps the various tissue formations in place and protects the cells from bacterial attack, distortion, and shrinkage. The pieces of each organ were placed individually in labeled cassettes and then dehydrated by successive passage in alcohol baths of increasing degree (80° in 1 hour, 90° in 2 hours and two baths of 100°C in two hours each). After dehydration, the cassettes containing the organ parts were kept in three toluene baths of 2 hours each. This operation allowed us to eliminate the traces of ethanol and to prepare the organs for impregnation. As for the impregnation, it was done in two successive baths of kerosene in the oven (Mettler, Germany) between 58 and 60°C. The cassettes stayed for 2 hours in the first bath and 3 hours in the second bath. The organs were then embedded in kerosene at room temperature in molds and cut with a LEICA microtome model (RM2125 TRS). The blocks were placed on ice and clamped onto the back of the cassettes of the microtome for the effective realization of the cuts. The thickness of the sections was 5 μ m. This allowed us to obtain kerosene ribbons containing organ sections. The ribbons thus formed were placed in a water bath at a temperature of 40°C and then mounted on the slides. The slides with the kerosene strips were placed in an oven (Mettler, Germany) at 58-60°C for 30 min and dewaxed in three successive toluene baths for 15 min each. Rehydration took place in three successive baths of alcohol of decreasing degree (100°, 90° and 80°) for 5 min each. Then, the sections were rinsed with distilled water. After rinsing with distilled water, the sections were stained in a modified Harris hematoxylin solution (2-3 min), rinsed with tap water, and then immersed in a 3% yellowish eosin alcohol solution (3-5 min). After staining, the sections were dehydrated in three successive baths of increasing alcohol concentration (75°, 95°, 100°) for 5 min each. The stained slides were protected to allow microscopic examination and preservation without risk of deterioration. For this purpose, the slides were covered with cover slips using EUKITT glue. The analysis of the sections was done using a tri-ocular electron microscope (Olympus CX31, Philippines) topped by a camera (AmScope, MD130) connected to a computer (HP EliteBook Folio 1040, China) [27].

3. Results and Discussion

3.1. Results

Identification of some biological components of picralima nitida seeds powder

Table 1. Some biochemical characteristics of *Picralima nitida* seeds powder

Dry matter (%)	50.85 $\pm$ 1.50 ^b
Polyphenols (mg/mL)	114.92 $\pm$ 2.18 ^d
Flavonoids (mg/mL)	25.37 $\pm$ 0.92 ^c
Antioxidant activity (%)	62.46 $\pm$ 1.65 ^a
Anti-inflammatory activity (%)	61.91 $\pm$ 1.31 ^a

Data are expressed as mean $\pm$ SEM, n = 4. Statistical significance is: not significant (ns) if $p > 0.05$, significant (*) if $p < 0.05$.

With a dry matter content of $50.85 \pm 1.50\%$, the picralima nitida seeds powder presented a polyphenol and a flavonoid content of 114.92 ± 2.18 and 25.37 ± 0.92 mg/ml with antioxidant and anti-inflammatory activities of $62.46 \pm 1.65\%$ and $61.91 \pm 1.31\%$, respectively (Table 1).

Effect of aqueous solution of Picralima nitida seeds powder (SaPn) on overall measurements

SaPn had no significant influence on the body weight management of the animals ($p > 0.05$). The dose of 200 mg/kg bw of SaPn decreased the blood glucose levels after seven (07) days of gavage from 407 ± 15.09 to 182.75 ± 53.46 mg/dL, i.e. a significant decrease of 62.76% was noticed compared to the initial blood glucose level (Table 3). After fourteen (14) days, this value decreased to 142.5 ± 37.59 mg/dL, (70. 23%). The blood glucose level reached 96.75 ± 16.88 mg/dL (i.e. a maximum reduction of 81.03%) on the 28th. Moreover, the commercial molecule Glibenclamide, gavaged at 5 mg/kg bw significantly decreased the level of hyperglycemia by 48.16% and 65.26% after 21 and 28 days of treatment respectively. In normal control (TN) rats, the blood glucose level hardly changes during the whole experiment. In contrast, blood glucose levels increased sharply in diabetic control rats compared to baseline from 337.5 ± 22.17 to 600 ± 121 mg/dl at the end of the treatment (28 days).

Impact of SaPn on hematological and biochemical parameters of treated rats

Table 4 presented the effects of aqueous solution of Picralima nitida seeds powder on the hematological parameters of rats. The ANOVA of the hematological parameters showed a significant increase in erythrocyte parameters in HCT (%), MCV (fL), MCH (pg) and MCHC (g/L) in the untreated diabetic rat lot ($p > 0.05$). A significant decrease in red blood cell (RBC) count was observed in the same batch compared to the normal control group (TN) ($p < 0.05$). In the lot treated with

P.nitida aqueous solution (SaPn) and in the rats treated with glibenclamide, these parameters were statistically identical to the normal control except for the hematocrit level (HCT). There was no change in hemoglobin levels in any of the treated batches compared to the control. In terms of leukocyte parameters, the number of white blood cells (WBC) and lymphocytes strongly increased in the untreated diabetic batch, slightly changed in the SaPn batch compared to the healthy rats while the number of granulocytes and neutrophils remained the same after four weeks of treatment. The number of platelets and the range of distribution thrombocyte size variability (PDW) were low in the untreated sick rats but high in the reference lot. The number of platelets observed in the SaPn lot was statistically equal to that of the healthy control lot. The mean platelet volume (MPV) did not change significantly in all batches. Analysis of the biochemical parameters showed a significant increase in the levels of each parameter in the untreated sick rats after four weeks ($p < 0.001$). The concentrations of creatinine, urea, and bilirubin were statistically identical to the values of the healthy rats. Enzyme concentrations (ALT and AST) were the same in the reference and SaPn batches but differed from the healthy controls. On the other hand, protein levels (total protein and C-reactive protein) were all statistically different. Regarding lipids, i.e. triglycerides and cholesterol, the concentrations were high in the untreated diabetic lot. In the reference and SaPn lot, the cholesterol concentration was equal to that of the healthy control, which is not the case for triglycerides.

Effects of SaPn on the kidney, heart and liver of rats by a histopathological study

Histological sections of the kidneys, hearts, and livers of the rats after the 28 days of treatment revealed that there was no change in these organs which had their integrity intact and preserved (Figure 2, Figure 3, Figure 4).

Table 2. Monitoring of the weight of the rats during the treatment

Batches	LTN	LTPOS	LREF	SaPn
Day 0	223.86 ± 34.85^a	222.77 ± 31.74^a	195.56 ± 44.43^a	160.85 ± 14.64^a
Day 3	219.56 ± 31.52^a	220.64 ± 32.24^a	195.8 ± 43.15^a	159.36 ± 26.65^a
Day 6	209.97 ± 26.98^a	225.18 ± 36.96^a	192.77 ± 36.85^a	162.85 ± 32.93^a
Day 9	219.73 ± 27.38^a	226.66 ± 37.58^a	194.11 ± 37.63^a	181.03 ± 31.36^a
Day 12	210.51 ± 25.78^a	225.23 ± 36.59^a	177.60 ± 39.91^a	187.77 ± 34.75^a
Day 15	213.37 ± 14.41^a	216.27 ± 38.25^a	179.30 ± 39.63^a	192.34 ± 35.93^a
Day 18	204.56 ± 3.87^a	217.68 ± 37.21^a	187.23 ± 36.70^a	196.45 ± 37.46^a
Day 21	208.47 ± 6.50^a	200.96 ± 31.20^a	181.99 ± 40.47^a	196.39 ± 38.90^a
Day 24	210.61 ± 6.00^a	189.05 ± 29.81^a	182.99 ± 39.10^a	208.17 ± 40.98^a
Day 27	205.9 ± 11.08^a	165.19 ± 4.47^a	182.07 ± 40.18^a	205.97 ± 48.26^a

Data are expressed as mean $\pm$ SEM, n = 4. Statistical significance is not significant (ns) if $p > 0.05$, significant (*) if $p < 0.05$

Table 3. Monitoring of blood sugar levels in rats

Batches	LTN	LTPOS	LREF	SaPn
Day 0	109.25 ± 3.78^b	107.75 ± 2.5^b	113.75 ± 6.08^b	77.75 ± 8.84^a
Day 3	103.5 ± 3.42^c	337.5 ± 22.17^a	565.5 ± 44.04^b	407 ± 15.09^{ab}
Day 7	103.75 ± 2.5^a (-4.50%)	390 ± 0.05^b (41.30%)	321 ± 74.58^b (-6.05%)	182.75 ± 53.46^a (-62.76%)
Day 14	99 ± 4.32^a (-2.57%)	414 ± 0.05^c (50%)	285.5 ± 14.5^{bc} (-13.16%)	142.5 ± 37.59^{ab} (-70.23 %)
Day 21	98.5 ± 5.06^a (0%)	501 ± 0.05^c (81.52%)	163.75 ± 35.99^b (-48.16%)	124.25 ± 28.93^{ab} (-74.18%)
Day 28	98.25 ± 1.71^a (3.46%)	600 ± 0.05^c (117.39%)	133.25 ± 12.97^b (-65.26%)	96.75 ± 16.88^a (-81.03%)

Data are expressed as mean $\pm$ SEM, n = 4. Statistical significance is not significant (ns) if $p > 0.05$, significant (*) if $p < 0.05$

Table 4. Effects of SaPn on hematological parameters after four weeks

Batches	LTN	LTPOS	LREF	SaPn
WBC ($10^9/L$)	12.88 ± 0.61^c	18.25 ± 0.5^d	14.40 ± 1.08^b	16.54 ± 0.57^a
Lym* ($10^9/L$)	9.32 ± 0.63^c	13.75 ± 0.5^b	10.61 ± 1.34^{ac}	12.55 ± 2.47^{ab}
Mid* ($10^9/L$)	2.01 ± 0.47^a	2 ± 0.00^a	2.29 ± 0.08^a	2.48 ± 1.07^a
GR* ($10^9/L$)	1.54 ± 0.69^a	2 ± 0.00^a	1.50 ± 0.26^a	1.51 ± 0.97^a
RBC ($10^{12}/L$)	6.97 ± 1.49^a	4.75 ± 0.5^b	6.86 ± 0.18^a	6.96 ± 0.54^a
HGB (g/L)	126.25 ± 17.67^a	107.75 ± 0.5^a	114.5 ± 2.64^a	117.58 ± 9.22^a
HCT (%)	42.64 ± 9.59^a	30.75 ± 0.5^b	40.89 ± 1.26^{ab}	41.4 ± 3.21^{ab}
MCV (fL)	61.09 ± 1.52^a	65.25 ± 0.96^b	59.56 ± 0.80^a	59.43 ± 0.75^a
MCH (pg)	18.4 ± 1.80^a	22.75 ± 0.50^b	16.69 ± 0.34^a	16.95 ± 0.44^a
MCHC (g/L)	301.88 ± 33.17^a	357.75 ± 0.5^b	280 ± 4^a	284.16 ± 4.80^a
RDW-CV (%)	12.64 ± 0.65^a	14.75 ± 0.5^b	11.89 ± 0.38^a	12.23 ± 0.41^a
RDW-SD (fL)	36.38 ± 2.29^a	34.75 ± 0.5^{ab}	31.88 ± 0.55^b	35.73 ± 2.01^a
PLT ($10^9/L$)	335.13 ± 103.92^{ab}	311.75 ± 0.5^b	478.88 ± 71.76^a	409.66 ± 82.70^{ab}
MPV (fL)	8.48 ± 0.17^a	8.75 ± 0.5^a	8.4 ± 0.08^a	8.4 ± 0.08^a
PCT (%)	0.28 ± 0.09^a	0 ± 0.00^a	0.40 ± 0.06^a	0.34 ± 0.07^a
PDW (%)	17.34 ± 0.61^a	15.75 ± 0.5^b	17.76 ± 0.43^a	17.08 ± 0.65^a
P-LCR (%)	18.36 ± 1.40^b	22.75 ± 0.5^a	17.83 ± 0.98^b	20.05 ± 2.37^{ab}
P-LCC ($10^9/L$)	60.5 ± 13.10^b	68.75 ± 0.5^{ab}	77.63 ± 5.61^{ab}	80.92 ± 10.89^a

Data are expressed as mean $\pm$ SEM, n = 4. Statistical significance is not significant (ns) if $p > 0.05$, significant (*) if $p < 0.05$

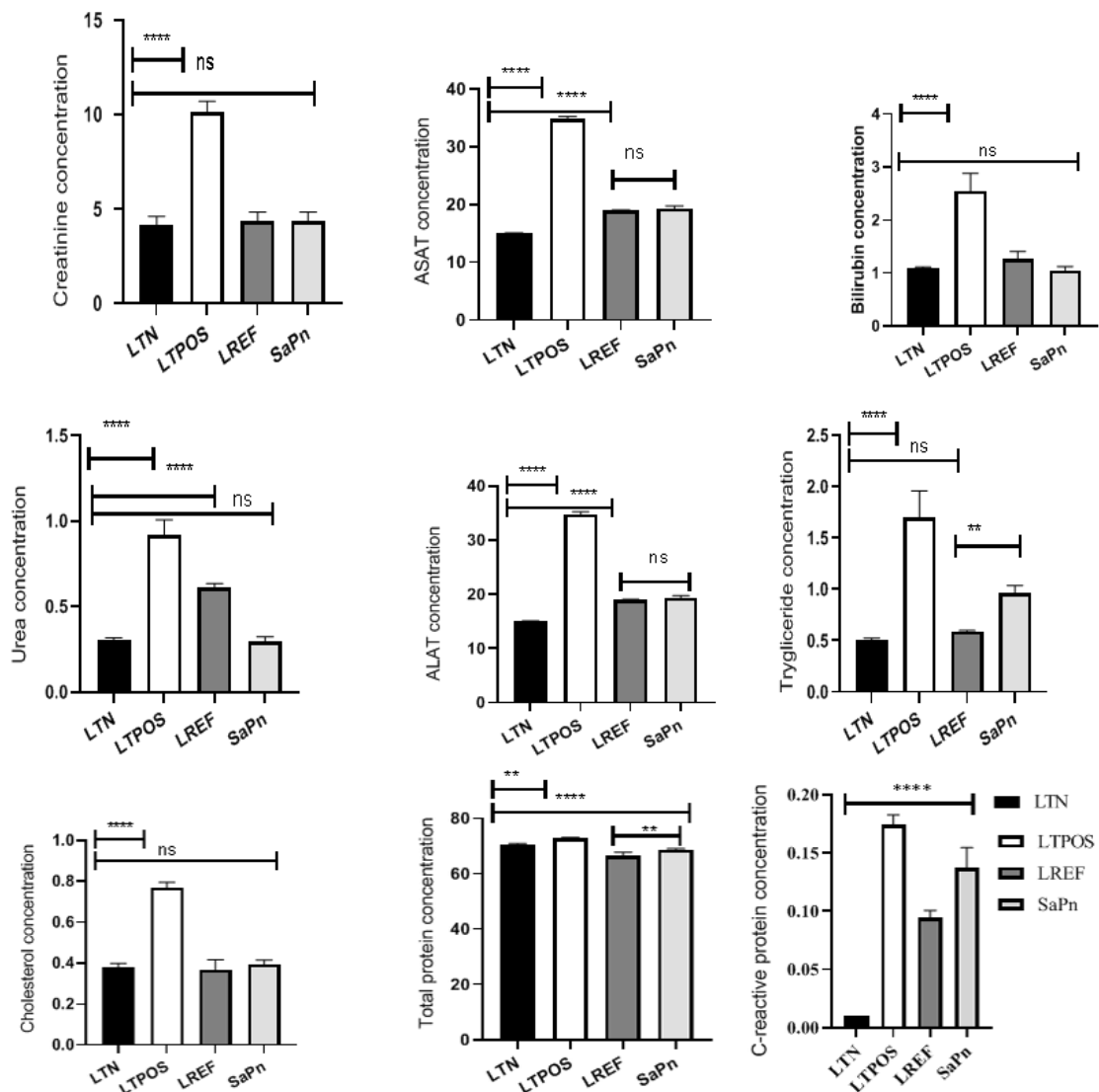


Figure 1. Effects of SaPn (LCD) on biochemical parameters of diabetic rats Data are expressed as mean $\pm$ SD, n = 4. Statistical significance is: not significant (ns) if $p > 0.001$, significant (****) if $p < 0.001$

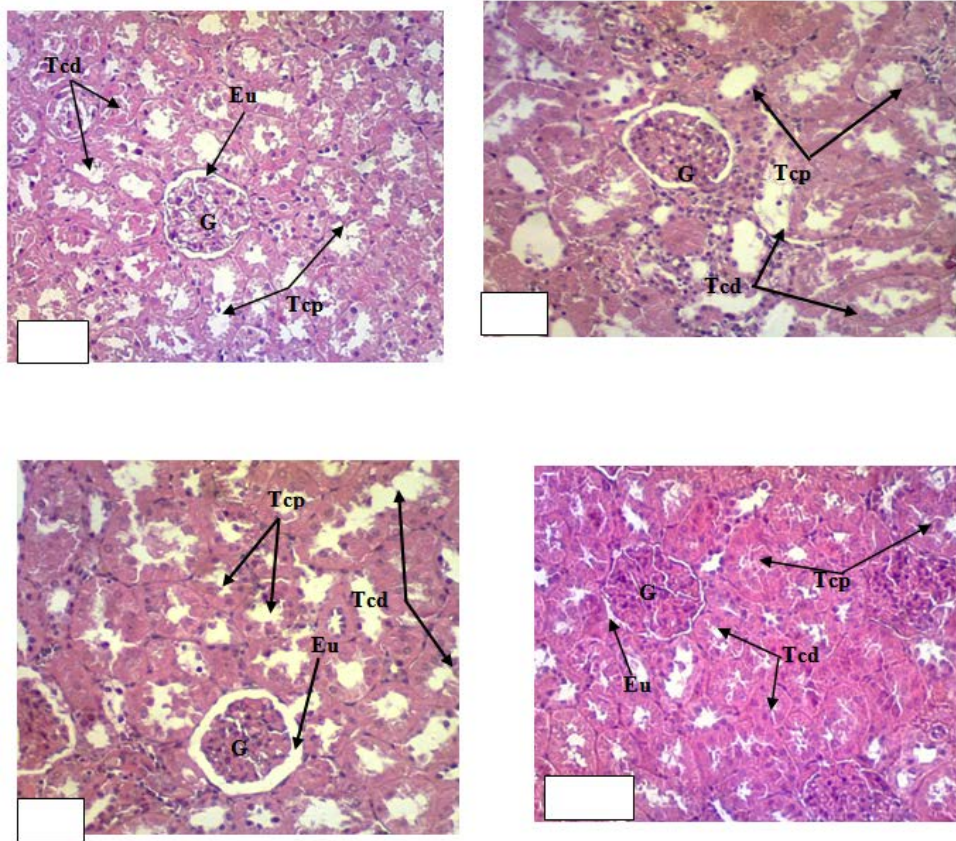


Figure 2. Histological section of rat kidneys

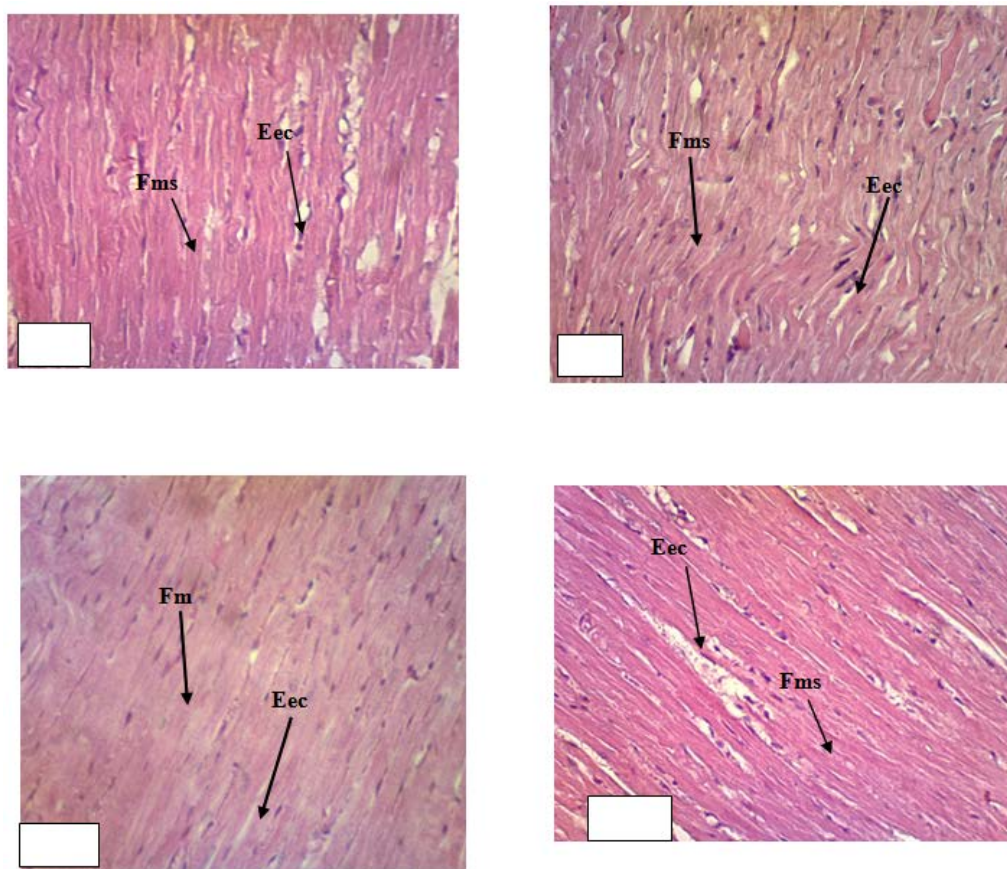


Figure 3. Histological section of rat heart

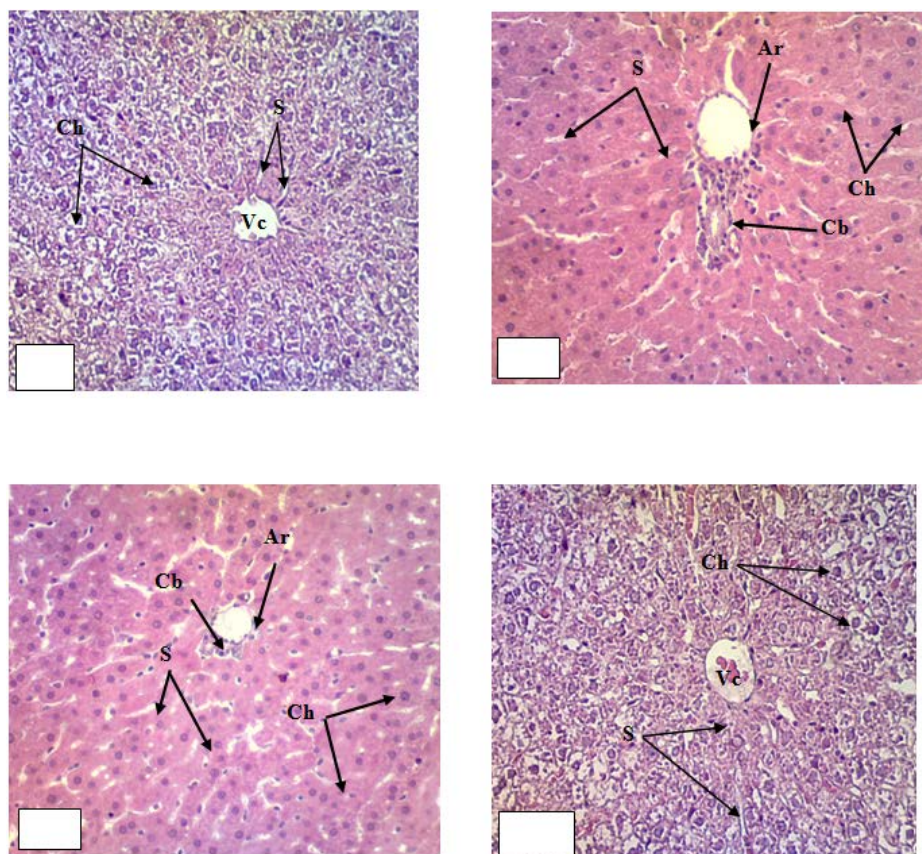


Figure 4. Histological section of rat liver

3.2. Discussion

The use of edible medicinal plants for the prevention and treatment of type 2 diabetes mellitus (T2D) has increased worldwide due to their availability, cultural acceptance and reduced side effects [28]. These plants among which fruits and vegetables contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, vitamins, fibers; ... that seemed to have antidiabetic effects. Several studies have shown the positive impact of the consumption of fruits and vegetables. Their bioactive compounds would exert various activities in glycemic regulation [20]. They could inhibit the action of α -amylase and α -glucosidase, SGLT1, gluconeogenic enzymes that would all promote chronic hyperglycemia which can lead to diabetes [29]. These natural functional or nutraceutical foods would also play a cellular protective role and would increase insulin secretion and GLUT4 expression, improve glucose absorption by GLUT4 and could block the activity of the aldose reductase enzyme as highlighted in a brief mechanism proposed by [30] [21]. The anti-diabetic mechanism of each functional foods (herbs, nutraceuticals) is organ-specific according to WHO [30]. These compounds play antioxidant and anti-inflammatory roles to positively modulate oxidative stress and inflammatory syndrome related to diabetes [21,22]. Due to the high costs of fruits and vegetables in low-income countries [23], special attention should be paid to neglected edible wild fruits and vegetables that are rich in nutrients, vitamins, dietary fiber and also in bioactive pharmaceutical compounds (terpenoids, carotenoids, phenolic compounds, phytosterols, glucosinolates,...) providing several health benefits (antioxidant, anti-cancer,

antihypertensive, antidiabetic properties,...) [24]. In West Africa, one of the fruits (grains) traditionally used is the *Picralima nitida* which is part of the Apocynaceae family. The seed of *P. nitida* is known as diabetes pod in Côte d'Ivoire, Akuamma in Ghana, Osi-igwe seed in Igboland (eastern Nigeria), Eso Abere in Yorubaland (western Nigeria) [31]. The seeds are rich in alkaloids such as akuammine and picraline which are assumed to be the majority [32]. We also find akuammicin, akuammidine, pseudo-akuammigin, akuammiline, burnamine, picranitine, picratidine (N-methyl-picraline), burnamine (deacetyl-picraline), and deacetyl-akuammiline (rhazimol) [31] [33]. All these alkaloids and polyphenols would be the basis of its antimalarial, antifungal, analgesic and antidiabetic activities [31]. The seeds are traditionally ground in powder form and taken orally, against malaria, diarrhea, diabetes... Thus, the powder obtained from the seeds of *P. nitida* had a very high antioxidant and anti-inflammatory activity (more than 60%). These activities would be the result of the presence of alkaloids but also of polyphenols, the presence of which is very significant after dosages [5]. This powder was subsequently diluted in distilled water to constitute our aqueous solution of *P. nitida* (SaPn) and then given to the diabetic Wistar rats. The SaPn had no effect on the weight of the rats after 28 days of treatment contrary to the results obtained by [34] and [35]. It would show that the diabetes induced by alloxane in this case would be that of type 2 because it is very sneaky. Internal anemia is evidenced by a significant decrease in the level of red blood cells, hematocrit, platelets and an increase in the concentration of bilirubin in the untreated diabetic batch [36]. Also, this maintenance of the weight of rats by SaPn would result from its immunoregulatory, cicatrizing

action, and promising in blood coagulation because it increased the level of red blood cells, hematocrit and blood platelets. On the other hand, we observed an increase in blood glucose levels after three days in all the batches of rats that received an intraperitoneal dose of alloxane, unlike the batch that did not receive alloxane. This increase in blood glucose in these batches would be the consequence of the destructive action of alloxane on the β cells of the islets of the pancreas. This can be seen by a significant increase in most leukocyte parameters [37] and biochemical parameters in rats rendered untreated diabetic [38]. Indeed, these cells would be the site of production of insulin, the only hypoglycemic agent in the body. Their destruction would cause chronic hyperglycemia [39]. The treatment of diabetes with the *Picralima nitida* powder solution led to a rapid decrease in blood sugar levels already after one week of treatment and a return to normal after 28 days of force-feeding. This could imply that SaPn has a restorative action on beta cells and would promote an improvement in insulin secretion or its action. These results could be compared to those of [34] [40,41], the antihyperglycemic activity of SaPn was much faster than that of glibenclamide. This would mean that SaPn and glibenclamide would not have the same mechanism of action as that demonstrated by the work of [42]. The evolution of diabetes would cause complications that could affect certain organs such as the liver, kidneys and heart. The safety of the liver would be verified by alanine aminotransferase (ALAT) and aspartate aminotransferase (AST), two enzymes produced by it. An increase in the concentration of ALT and AST, is synonymous to hepatic damage which would be attributable to an excess of synthesis of free fatty acids originating from insulin resistance and oxidative stress as observed by the elevated cholesterol and triglyceride concentrations in the batch of untreated diabetic rat (Figure 1) [43]. On the contrary, the ALT and ASAT concentrations of the SaPn batch seemed identical to that of the negative sample. This action can be due to the antioxidant activity that the seeds of *P. nitida* possess in contrast to hepatoprotective action of glibenclamide. According to Bedou [26], creatinine and urea are excellent markers of kidney function. Their increase reflects renal dysfunction. An ability to improve the elimination of waste products from the blood by the kidneys and a protective effect could be dedicated by SaPn as indicated by the reduction of serum urea and creatinine levels in diabetic rats. This improvement was also confirmed by histopathological examination of the liver and kidneys [44]. Regarding the heart, the histological sections did not reveal any abnormalities, which could justify the cardioprotective effect of SaPn at 200mg/kg of body weight [45]. In regard to these results, it is therefore important to emphasize once again that the powder of the seeds of *P. nitida* owe their actions to their richness in bioactive compounds (alkaloids, polyphenols, flavonoids ...) and could be used as a functional food in the treatment of diabetes.

4. Conclusion

P. nitida powder presented antidiabetic actions on diabetic rats induced by alloxane and could be

recommended in the treatment of diabetes. This dried powder form has reduced water activity and therefore is less subject to physiological and more stable to microbial alterations. It is a sustainable way of preserving foodstuffs. The seeds of *Picralima nitida* which kept the biological activities intact could be exploited in this improved form in functional food or in galenic pharmaceutical formulation to make it more attractive and pleasant but above all regularly available and accessible at any time of the year with no noticeable shortage. Moreover, this powder formulation could also be an interesting way of sustainable development of these neglected edible wild fruits and vegetables whose potentialities are not sufficiently known and valorized by the African populations.

ACKNOWLEDGMENTS

The authors would like to thank the Fonds for Science, Technology and Innovation (FONSTI) for its financial support, the Université Félix Houphouët Boigny for hosting this work, and the agrifood biotechnology laboratory where all the manipulations were carried out.

Authors' Contributions and Consent to Publication

Yao Konan Jean Noel wrote the manuscript. Assamoi Allah Antoine, Ouattara Hadja Djeneba and N'dri Kouadio Eric Donald critically reviewed the manuscript and have given their approval for the publication of the article.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Declaration of Conflicting Interests

The authors had no potential conflicts of interest in the research, writing and/or publication of this article.

Ethical Approval

The Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources of the University Félix Houphouët Boigny of Cocody approved the study protocol. The work had the written or oral consent of all participants.

Funding

The authors have stated that they received the following financial support for the research, writing and/or publication of this article: This study was fully funded by the Fund for Science, Technology and Innovation (FONSTI)

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