Original Research Article

Regulatory activity of the aqueous extract of *Picralima nitida seeds* (Apocynaceae) on the glycemia of normoglycemic and hyperglycemic Wistar rats

Abstract

Picralima nitida is a plant commonly used in traditional African medicine for its antimalarial, antidiuretic, antidiarrheal, anti-inflammatory and antioxidant effects. The aim of this work was to evaluate the effect of the aqueous extract of *Picralima seeds nitida on the glycemia of rats. To achieve this objective ,* we performed phytochemical screening using the tube revelation method. The effect of the aqueous extract of *P.* nitida seeds on glycemia was evaluated in normoglycemic and hyperglycemic rats. The screening results indicated the presence of polyphenols, catechic tannins, flavonoids and saponins. The results of the effect of the extract on glycemia showed that the extract did not cause hypoglycemia or hyperglycemia in normoglycemic rats. As for the effect of the extract on the glycemia of hyperglycemic rats, the extract caused a significant reduction in the hyperglycemia induced in rats with glucose. The use of this plant in the treatment of hyperglycemia would therefore be justified.

Keywords : *Picralima nitida, hyperglycemia, hypoglycemia, normoglycemic , hyperglycemic*

Introduction

Diabetes is a chronic and serious metabolic disease, due to insufficient or poor use of insulin by the body **(WHO, 2021).** According to WHO, the current prevalence of diabetes worldwide is around 347 million people **(WHO, 2013)** . Estimates for 2030 are around 4.7% of the world population **(Shaw et al., 2010).** Furthermore, more than 80% of diabetes-related deaths come from low- and middle-income countries. Thus, in 2013, nearly 20 million people had diabetes in sub-Saharan Africa, representing a prevalence of 4.9% (**WHO, 2013).** In Côte d'Ivoire, the International Diabetes Federation (IDF) estimated the prevalence of diabetes at 9.6% in 2014. This morbidity is thought to be caused by hyperglycemia. Today, it is estimated that nearly 3,700,000 deaths are related to hyperglycemia. In sub-Saharan countries, although

treatments in traditional medicine exist, the population continues to turn to plants given the high cost of modern medicine treatments **(Konda et al., 2011).** In Africa, several medicinal plants have been identified in the treatment of metabolic diseases such as diabetes. It is in this context that *picralima nitida,* a plant that has very interesting therapeutic and antioxidant properties, was chosen to evaluate its effects on the regulation of blood sugar. The effects of the seeds of the plant are a remedy against malaria and diabetes **(Teugwa et al., 2013) .**

Material and method

Plant material

The plant material **consists** of the dried grains of *picralima nitida* (Apocynaceae). These fruits were collected in the national floristic center located at the Felix Houphouët Boigny University, after an ethnobotanical survey. The grains were then collected, dried in the shade, at around 25°C, for a month and pulverized using a Vorwerk type grinder. thermomix 3000. The powder obtained was subjected to aqueous extraction.

Animal material

The animals used for this study are male rats, of the *Wistar strain* , aged 8 to 9 weeks and weighing between 145 and 180 g. The rats were raised in the animal house of the National School of Abidjan where the temperature varies between 26° and 27°, with a 12-hour light/dark cycle. The animals were fed with FACI pellets. They received tap water as drinking water ad libitum.

Method

Phytochemical tests of the aqueous extract of *picralima grains nitida*

The phytochemical study was carried out following the methods described by **(Bekro *et al* ., 2007)** in order to detect the presence or absence of certain families of secondary metabolites. For this, characterization tests of the different groups of compounds were carried out on the extract obtained. The flavonoids were characterized by the cyanidin reaction , the tannins were characterized by the STIASNY reagent, the polyphenols were characterized by the 2% ferric chloride test. The LIEBERMANN test made it possible to characterize the polyterpenes and sterols and the alkaloids were characterized by the DRAGENDORF and BOUCHARDART reagents. As for the saponins, they were characterized by the appearance of persistent foam, after stirring the extract solution.

Hypoglycemic activity of the total aqueous extract of *Picralima* grains *nitida*

a. Principles

For this experiment, male Wistar rats **,** aged 10 to 14 weeks, weighing between 150 and 250 g, will be used. The experiments will be carried out in the ENS animal facility. The products were administered by gavage using a probe. The extract was suspended in a solution of distilled water and from the mother solution, the different concentrations were obtained. The normoglycemic animals were divided into six batches containing five rats each, receiving in a single dose the different doses of the extract orally and a negative control group which received distilled water and another batch, glibenclamide (**Begbin *et al* . 2021).**

After administering the different substances to the animals of the different batches, blood glucose levels were measured every 30 minutes up to 150 minutes.

b. Operating mode

Thirty normoglycemic rats were divided into six groups of five rats and were fasted for 12 hours. At time To, i.e. before treatment, blood glucose was first determined (initial blood glucose). Distilled water, glibenclamide (10 mg/kg bw) and the aqueous extract at different doses (100, 200 and 400 mg/kg bw) were then administered to the different groups:

Lot 1 (control): rats received distilled water.
Lot 2 (glibenclamide): rats treated with 10 mg/kg bw of glibenclamide
Lot 3, 4 and 5 (EAPN100, EAPN200, EAPN400): rats from different lots treated respectively with 100, 200 and 400 mg/kg bw of EAP

Blood glucose monitoring is performed at 30, 60, 90 and 120 minutes after administration of the test products. The percentage changes in blood glucose levels are calculated at the different times.

II.4.1.2. Hyperglycemic activity of the total aqueous extract of *Picralima grains nitida*

a. Principles

For this experiment, male Wistar rats **,** aged 10 to 14 weeks, weighing between 150 and 250g, were used. The experiments were carried out in the ENS animal facility. The animals were conditioned in the same way as those used in the acute toxicity study. The products were administered by gavage using a probe.

b. Operating mode

The normoglycemic animals were divided into groups receiving a single dose of 10 g/kg bw of pure anhydrous glucose, orally, and a negative control group which received distilled water (**Begbin *et al* ., 2021)** according to the following protocol.

- Batch 1 (negative controls): batch of normoglycemic rats received distilled water
- Batch 2 (positive control): batch of hyperglycemic rats received distilled water
- Lot 3 (glibenclamide): lot of hyperglycemic rats treated with 10 mg/kg bw of glibenclamide.
- Lot 4, 5 and 6 (EAPN100, EAPN200 and EAPN400): lots of hyperglycemic rats treated respectively with 100, 200 and 400 mg/kg bw of EAPN.

Blood glucose monitoring is done every 30 minutes for 150 minutes after glucose administration. The percentages of changes in blood glucose levels are calculated at the different blood glucose measurement times.

Results

Phytochemical screening

The results of phytochemical characterization revealed that the aqueous extract of *picralima grains nitida* contains saponins, polyphenols, flavonoids and catechol tannins. On the other hand, metabolics such as alkaloids, sterols and terpenes were not revealed in the aqueous extract of *P. nitida* (Table I).

Table I: Chemical composition of the aqueous extract of *Picralima nitida grains* .

Chemical groups	**Aqueous extract of *P. nitida* *grains***
Saponins	+
Polyphenols	+
Flavonoids	+
Sterols and Terpenes	-
Catechin Tannins	+
Alkaloids	-

(+): presence of the chemical group highlighted in the extract; (-): absence of the chemical group highlighted in the extract.

Effect of total aqueous extract of *picralima nitida in* normoglycemic rats

The evaluation of the effect of *P. nitida extract* on the glycemia of normoglycemic rats was followed for six hours after gavage of the animals with different doses of 250, 500 and 1000

mg/kg of MC (Figure 1). The glycemia of the control rats did not experience significant variations (67.2±6.9- 72.75±4.6 mg/dl). The glycemia of the rats treated with the extract also did not experience significant variations. Furthermore, the basal glycemia of the rats was around 61 mg/dl during the experiment. One hour after administration of the *P. nitida extract* , a non-significant increase ($p<0.05$) in blood glucose levels was observed in rats treated with doses of 250 mg/kg MC (83.6±8.2 mg/dl), 500 mg/kg MC (75±.28 g/dl) and 1000 mg/kg MC (79.6±7). At the end of the experiment, blood glucose levels were approximately equal to basal blood glucose levels (65.6±6 mg/dl, 67.8±4.87 mg/dl and 66±6 mg/dl respectively for doses of 250, 500 and 1000 mg/kg MC). Statistical analysis revealed no significant difference ($p<0.05$) between blood glucose changes in rats treated with the *P. nitida extract* and control rats.

As for the rats treated with glibenclamide, a very significant decrease ($p<0.001$) in the blood glucose of the rats (35.6±2.7 mg/dl) was observed . Their blood glucose continued to decrease until the end of the experiment, with an average blood glucose of 22.4±3.29, significantly lower ($p<0.001$) than their basal blood glucose. Statistical analysis revealed a significant difference between the rats that received glibenclamide and the rats from the other groups, namely the control rats and the rats treated with the *P. nitida extract* at the different doses.

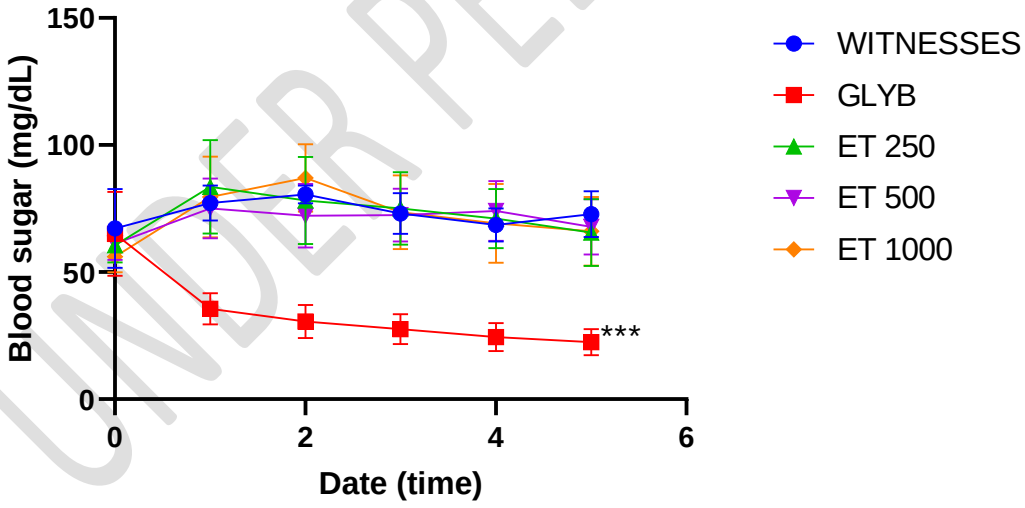


Figure 1: Variation in blood glucose levels of normoglycemic rats after administration of *P. nitida*

Controls: healthy control rats

GLYB: normoglycemic rats receiving glibenclamide

ET: normoglycemic rats receiving the extract

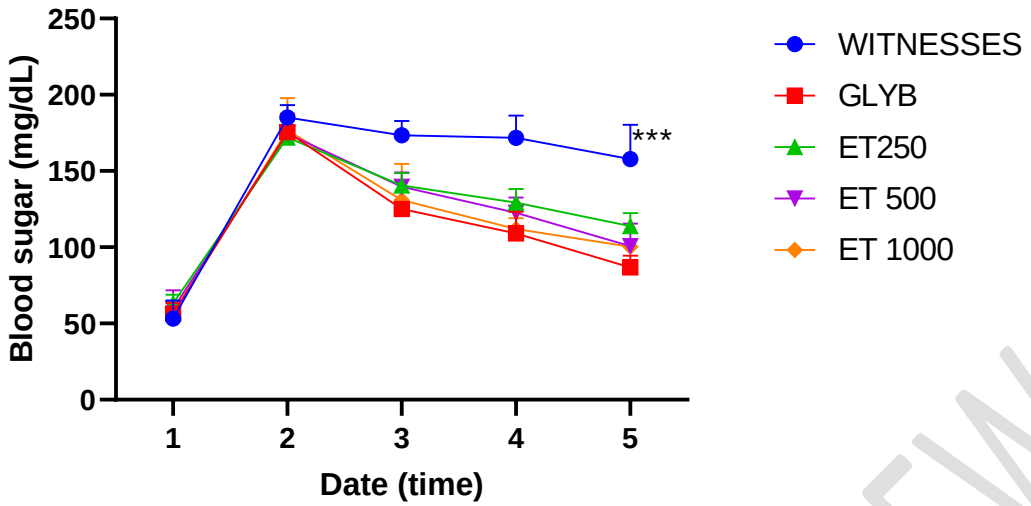
Effect of total aqueous extract of *picralima nitida* in hyperglycemic rats

The evaluation of the effect of *P. nitida extract* on the glycemia of hyperglycemic rats was followed for six hours after gavage of the animals with different doses of 250, 500 and 1000 mg/kg of MC (Figure 2). The basal glycemia of the rats was of the order of 60 mg/dl. One hour after administration of anhydrous glucose, the rats were all hyperglycemic, with a glycemia that went from the order of 60 mg/dl to 174 mg/dl. Statistical analysis revealed a very significant increase ($p<0.001$) in the glycemia of the rats after administration of glucose compared to their basal glycemia. In control animals receiving only glucose, blood glucose levels decreased slightly (non-significant $p>0.05$), from 185±4 mg/dl to 157.75±11 mg/dl at the end of the experiment, a decrease of -14.9±4 mg/dl.

For rats treated with glibenclamide, glibenclamide caused a significant decrease ($p<0.01$) of -28.3±2.4 mg/dl, one hour after its administration to the rats. At the end of the experiment, the blood glucose of rats treated with glibenclamide decreased from 175.5±6 to 86.75±4 mg/dl, a significant reduction ($p<0.001$) of -50.38±3 mg/dl. Statistical analysis revealed a significant difference ($p<0.001$) in the behavior of glibenclamide compared to distilled water received by control rats.

The blood glucose levels of rats treated with the extract showed significant variations. Furthermore, one hour after administration of the *P. nitida extract* , a significant decrease ($p<0.01$) was observed in the blood glucose levels of rats treated with doses of 250 mg/kg MC (-17.87±4.6 mg/dl), 500 mg/kg/MC (-19.46±4.8 g/dl) and 1000 mg/kg /MC (-25.08±7). At the end of the experiment, blood glucose levels decreased from 172±6 to 113.75±4 mg/dl (250 mg/kg MC), from 174.5±6 to 100.75±7.4 mg/dl (500 mg/kg/MC) and from 176.75±10 to 100.25±1 mg/dl (1000 mg/kg/MC); these changes correspond to significant reductions ($p<0.001$) of -33.41±4.42 mg/dl, -42.12±4 mg/dl and -42.58±3.9 mg/dl respectively. Statistical analysis revealed a significant difference between the blood glucose levels of rats treated with P. nitida extract and *control rats* . Moreover, no significant decrease ($p>0.05$) in blood glucose was observed in rats treated with glibenclamide compared to those treated with *P. nitida extract.*

Fig 2- Graph indicating significant variations in the blood glucose levels of rats treated with the extract



hyperglycemic rats after administration of *P. nitida*

Controls: healthy control rats

GLYB: normoglycemic rats receiving glibenclamide

ET: normoglycemic rats receiving the extract

Discussion

The results of the phytochemical screening of the *P. nitida extract* revealed the presence of polyphenols, flavonoids, catechin tannins and saponins. However, the analyses did not reveal alkaloids, sterols and terpenes. These molecules are involved in the treatment of cancers, diabetes, inflammatory pathologies, sickle cell disease, arterial hypertension, oxidative stress **(Guerrero and Ruter , 2002),** antimicrobial, antifungal **(Prior et al., 2005**).

Oral administration of the extract showed a slight increase in blood glucose from the first hour. This slight increase in basal blood glucose in normoglycemic animals would be due to the installation of physiological stress in the animals during their handling **(Rowsey et *al* ., 2002).** Indeed, stress causes an increase in the production of pro-oxidants and a decrease in that of antioxidants **(Ayala et *al* ., 2014).** However, from the second hour, blood glucose levels decrease to reach basal values. The aqueous extract of *P. nitida* therefore did not cause a significant variation (p >05) in basal blood glucose in normoglycemic rats. These results show that the extract of *P. nitida* does not cause either hypoglycemia or hyperglycemia in normoglycemic rats. These results corroborate those of **Kroa et *al* . (2016),** who have shown that the administration of the aqueous extract of the ethanolic extract of *Anthocleista*

djalonensis (2500 mg/kg), did not cause either hypoglycemia or hyperglycemia in normoglycemic animals. Glibenclamide, unlike *P. nitida extract* , induced a considerable decrease in blood glucose levels in rats below basal blood glucose. Glibenclamide therefore induces hypoglycemia in normoglycemic rats, in agreement with the results of **Kroa et *al* . (2016).**

P. nitida aqueous extract and glibenclamide on blood glucose levels in rats showed that they have significant antihyperglycemic activity in treated hyperglycemic rats. Indeed, one hour after the peak of hyperglycemia observed in post-treated rats, administration of glibenclamide or *P. nitida aqueous extract* significantly reduced blood glucose levels. The similar effects of *P. nitida aqueous extract with those of glibenclamide on blood glucose levels suggest that P. nitida* seeds may act by the same mechanism as antihyperglycemic substances. Flavonoids and polyphenols present in this extract may be the origin of these pharmacological effects . Thus , the hypoglycemia and reduction of hyperglycemia observed in rats treated with *P. nitida aqueous extract were significantly reduced . nitida* plant could be explained either by a stimulation of insulin secretion by the pancreas or by an increase in peripheral glucose utilization in the presence **(Yasodha et *al* ., 2008).**

Our results corroborate those of **Nabi et *al* . (2013),** who showed that the extract of *Piper longum* (Piperaceae) roots had an antihyperglycemic effect in rats. Similarly, **Nwakile and Okore . (2011),** showed the oil of *P. nitida seeds* significantly reduced sugar-induced hyperglycemia in rats.

Conclusion

This study is part of the research of drugs in the treatment of diabetes from plants. During this work, the phytochemical screening of the aqueous extract revealed that the seeds of P. nitida represent a source of bioactive molecules including polyphenols, flavonoids, catechin tannins and saponins. These seeds do not have hypoglycemic effects in normoglycemic rats unlike the glibenclamide molecule which had a hypoglycemic effect. However, the seeds of P; nitida led to a significant drop in blood sugar in hyperglycemic rats compared to the glibenclamide molecule. The activity of the aqueous extract of the seeds of P. nitida on the blood sugar of rats shows that these seeds could be a very interesting therapeutic source in the treatment of hyperglycemia in diabetic individuals.

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