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Evaluation of the effect of the total aqueous extract of fruits of *Picralima nitida* (Apocynaceae) on glycemiaand the release and storage of hepatic glucose

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Abstract
Picralima nitida (Apocynaceae) is a plant used in traditional Ivorian medicine in the treatment of diabetes. This study aims to evaluate the effects of total aqueous extract of *Picralima nitida* fruits (ETAPN) on glycemia variation, release and storage of hepatic glucose in rats. The diabetes was induced by a single dose of alloxane and animals were treated with ETAPN and metformin for 28 days. Then, the glycemia, glucose levels released into the blood and stored in hepatocytes were determined. The results showed that ETAPN, at doses of 400, 600 and 800 mg/Kg PC, significantly decreased the release of hepatic glucose (glycogenolysis) and increased glucose penetration and storage in hepatocytes. As with metformin, ETAPN, at the above-mentioned doses, lowers and normalizes glycemia in diabetic rats. These results justify the use of *Picralima nitida* in the traditional treatment of diabetes.

Keywords: *Picralimanitida*, glycemia, glucose release, glucose storage; metformin; alloxane.

Introduction
The glucose in excess under the bloodstream is stored in two possible forms: glycogen or lipids (Thibaut Duparc, 2012) [1]. Glycogen is a glucose polymer where molecules are bound in α (1-4) and connected in α (1-6) every 8 to 12 residues. The accumulation of glucose in the blood leads to diabetes. It is a metabolic disease characterized by hyperglycemia resulting from a lack of insulin secretion or action, or both (Hajzadeh *et al.*, 2011) [2]. In a person with diabetes, glucose is not absorbed properly and continues to circulate in the blood, gradually damaging tissues. This damage can lead to life-threatening complications (International Diabetes Federation (IDF), 2013) [3].According to the WHO, diabetes is now a real public health problem (IDF diabetes atlas, 2013) [4]. In the world, there are 366 million people with diabetes with 3.2 million deaths per year (Whiting, 2011) [5]. In Côte d'Ivoire, 1979 data already indicated a national prevalence of 5.7 % (Oga *et al*., 2006) [6]. The works of Oga *et al.*, (2006) indicates a significant increase in diabetic patients between 1979 and 2000. In 2017, there were 217,300 people with diabetes in the country according to the International Diabetes Federation. To treat themselves, populations use synthetic drugs while facing certain difficulties related to access, cost and side effects. In response to the spread of this disease, WHO, in its resolution AFR/RC50/R3 of 31 August 2000, encourages researchers to direct research towards plants with a view to the discovery of new herbal molecules and the development of improved traditional medicines(Comité Régional de l'Afrique, 2000) [7]. In this regard, an ethnobotanical study revealed that *Picralima nitida* (Apocynaceae) is one of the plants used in traditional Ivorian medicine to treat diabetes. This study aims to evaluate firstly the effects of ETAPN on the release and storage of Hepatic glucose and furthermore on the variation ofglycemia in diabetic rats.

Materials and methods
Material
Vegetal material
Fresh and ripe fruits of *Picralima nitida* were purchased at the CHAKA KONE's market in Abobo-Abidjan in September 2016. They were then authenticated at the National Floristic Centre (NFC) of the Félix Houphouët Boigny University (FHBU) in Côte d'Ivoire where they are identified under UCJ 00 2135.

Animal material
White rats, *Rattus norvegicus* (Muriadae) of the Wistar strain, weighing between 150 and 200 g, were used in this study. These rats were raised in the vivarium of the Higher Teacher Training School (HTTS) in Abidjan. The animals had free access to food and water throughout the study. They were stored in a well-ventilated room with an average temperature between 25 and 26°C. They were subjected to a cycle of natural daylight alternating with darkness at night.

Methods
Preparation of the total aqueous extract of *Picralima nitida* (ETAPN)
ETAPN was prepared according to the method described by Guédé-Guina *et al.* (1993) [8]. According to this method,100 g of *Picralima nitida* powder were dissolved in 1 L of distilled water and shaked with an IKA-MAG RCT magnetic shaker for 48 hours at 80°C. The mixture was squeezed into a square of clean cloth, filtered successively twice on hydrophilic cotton, then on büchner with Wattman 3 mm filter paper. The resulting filtrate was evaporated at

reduced pressure at a temperature of 50°C using a BÜCHI rotary evaporator. The fine powder collected is the total aqueous extract of *Picralima nitida*. This powder was used to prepare the different doses used.

Preparation of Mac Ewen's solution

One litre of Mac Ewen solution consists of 130 mM NaCl, 5.63 mM KCl, 12.16 mM $CaCl_2$, 0.91 mM H_2PO_4Na, 11.90 mM HCO3Na and 0.25 mM $MgCl_2$. 2 g of glucose is added to this physiological solution before the experimentations. Mac Ewen glucose is used to study glucose release from rat liver isolated from rat.

Chemicals and pharmacological products

Alloxane (Alfa Aesar, Germany) is a diabetogen whose intraperitoneal administration destroys the β cells of the islets of Langerhans and induces experimental diabetes.

Glycemia measurement of rats with alloxane diabetes

Experimental protocol

Glycemia in rats with diabetes are measured using an Accu-Check Active glucose meter and test strips (Roche diagnostic, Germany). In this study, rats were fasted for 12 hours before the experiences. The test substances were administered orally to them.

Antidiabetic study

Experimental protocol

For this study, 48 Wistar rats were divided into 8 lots of 6 rats each.

Lot 1 as a normoglycemic sample was given distilled water.

Lot 2 was the diabetic sample and received alloxane and distilled water.

Lot 3 consisted of untreated diabetic rats and received alloxane and distilled water.

Lots 4, 5 and 6 were made up of diabetic rats. They received alloxane and were treated with ETAPN's 400, 600 and 800 mg/Kg PC doses respectively.

Lots 7 and 8 were also made up of diabetic rats. They received alloxane and were treated with metformin at doses of 5 mg (Met 5) and 10 mg (Met 10) respectively, a reference antidiabetic agent.

The experimentation lasted 28 days. The glycemia were measured at the beginning of the experience at D0, on day 21 and then on day 28.

Measurement of glucose released from the liver of normoglycemic rats

Experimental protocol

In the presence of glucose oxidase (GOD), glucose is oxidized to gluconic acid. The hydrogen peroxide released during the reaction reacts under the action of peroxidase (POD) with phenol and 4-aminophenazole to form a pink complex. The intensity of staining is proportional to the glucose concentration of the sample. Twenty-four (24) Wistar rats of average weight between 150 and 200 g were used in this study. These rats were divided into four (4) lots of six (6) rats each. Animals in lot 1 or normal sample lot were given distilled water by gavage during the 28 days of the experimentation. Rats in lots 2, 3 and 4 or test lots received by gavage, the total aqueous extract of *Picralima nitida* (ETAPN) at doses of 400, 600 and 800 mg/kg PC respectively. Then, 2 g of liver fragments collected after gentle decapitation from each of the rats in each lot were collected in S1, S2, S3 and S4 solutions of Mac Ewen glucose and incubated at 37°C for 60 min. The quantity of glucose released was measured in the supernatant of each solution collected in the presence of GOD-POD glucose using a 500 nm spectrophotometer at times t0 min, t10, t20, t30, t40, t50 and t60 min.

Measurement of glucose stored in the liver of diabetic rats

Experimental protocol

The glucose reagent GOD-POD was used for the determination of glucose stored in the liver during diabetes. Forty-eight (48) Wistar rats divided into eight (8) lots of six rats each were used in this study.

Lot 1 or normal samplelot received distilled water during the 28 days of the experimentation.Lot 2 or diabetic sample lot received by gavage distilled water and 1 mL streptozotocin at 10 mg/kg.

Lot 3 or untreated diabetic lot received 1mL of STZ at 10 mg/Kg. Lots 4 to 6 or diabetic lots treated with ETAPN received 400, 600 and 800 mg/Kg of PC respectively. Rats in lots 7 and 8 received metformin at 5 and 10 mg doses. After 28 days of treatment, the animals in each lot were sacrificed by gentle beheading and a 5 g fragment of liver was collected from each rat in each lot, cut into small pieces and ground in 30 mL of 4% trichloroacetic acid.

Statistical analysis methods and processing of results

Data analysis was performed using GraphPadInstat software (San Diego CA, USA). The results are given as an average followed by the standard error on the average (M ± ESM). The difference between two values was determined by the Student-Newman-Keuls test and was considered significant for $P < 0.05$. GraphPadPrism software (San Diego CA, USA) is used to plot the graphs.

Results

1. Effects of ETAPN on hepatic glucose released from the liver of normoglycemic rats

Figure 1 shows the results of the effect of ETAPN at different doses on hepatic glucose release. At t0 min, the concentration of glucose released in the solutions (S1 to S4) is 2±0.01 g/L. This concentration increases significantly in the S1 sample solution when it is increased from t0 to t30 min, decreases insignificantly from t30 to t40, and then increases again significantly from t40 to t60 min. The concentration of glucose released in the sample solution S1 ranges from 2±0.01 g/L at t0 to 2.61 ± 0.1 g/L at t30 min, then from 2.61± 0.1 g/L to 2.58 ± 0.4 g/L and finally to 2.67±0.014 g/L at t60 min. Treated with ETAPN at different doses of 400, 600 and 800 mg/Kg PC, the concentration of released glucose decreases significantly ($P < 0.01$) in each solution from S2 to S4 compared to glucose release in the control solution. A very significant reduction was obtained with ETAPN's 800 mg/Kg PC dose. At a dose of 800 mg/Kg PC and at t60 min, the quantity of glucose released in the S4 solution is 2.21 ± 0.014 g/L or 83% reduction compared to the sample. ETAPN reduces the quantity of hepatic glucose released until normalization.

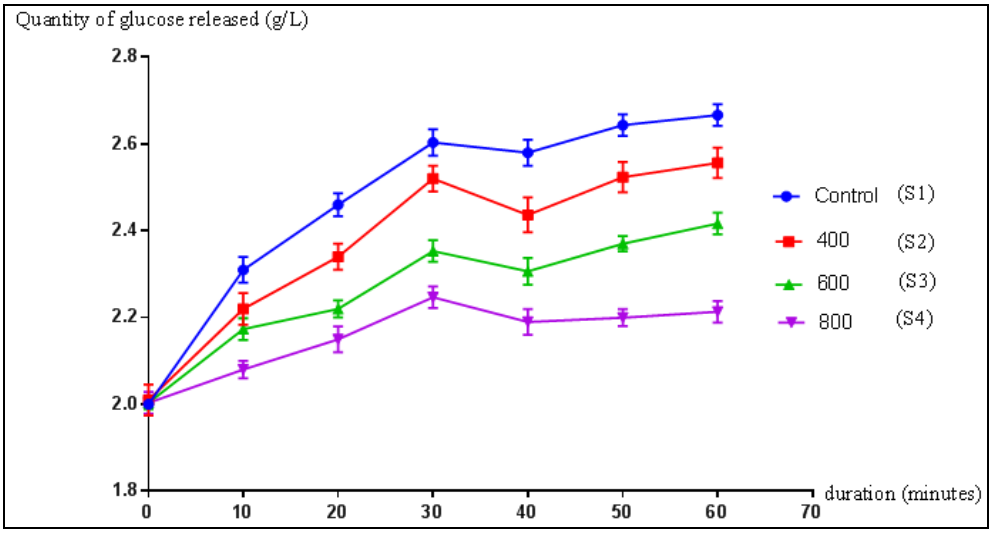

Fig 1: Dose response effect of total aqeous extract of picralima niti da fruits on hepatic glucose release from normolgy rats

2. Effects of ETAPN on glycemia variation in diabetic rats

The results of the effects of ETAPN and metformin on glycemia variation in diabetic rats are presented in Figure 2. The glycemiaof rats in the samplelotat the beginning of the experimentation is 0.68± 0.03 g/L. During the induction of diabetes in rats in the test lots, glycemia will increase significantly (P<0.05). It varies from 0.83 ± 0.165g/L (control lot) at 2.733 ± 0.12 g/L on day 21 and then at 3.56 ± 0.08 g/L in the untreated diabetic rat lot, an increase of 233.29% and 334.14% compared to the sample lot rats. Treatment of diabetic rats with ETAPN at 400, 600 and 800 mg/Kg PC and metformin at 5 and 10 mg doses significantly decreased (P<0.05) glycemia in rats to normalization. When treated with the different doses of ETAPN, the glycemia in rats increased from 2.733±0.12 g/Lto 1.507±0.09, 1.18±0.08and 0.86 ± 0.08 g/L respectively. The same results were obtained with metformin at 5 and 10 mg doses.

3. Influence of total aqueous extract of *Picralima nitida* and metformin on hepatic glucose storage

The histograms in Figure 3 present results on the effects of ETAPN and metformin on hepatic glucose storage. The glucose level stored in the liver of normal sample rats is 0.92±0.038 g/L. This rate decreases significantly during alloxane induction of diabetes. It varies from 0.92±0.038 g/L (sample lot) to 0.49±0.047 g/L in the treated diabetic rat lot and then to 0.32±0.046 g/L in the untreated diabetic rat lot. Treated with ETAPN at 400, 600 and 800 mg/Kg PC and metformin at 5 and 10 mg doses, the stored glucose level increases significantly (P<0,0001) until normalization with 800mg/Kg PC from ETAPN and 10 mg metformin. This rate varies from 0.32±0.046 g/L (DNTlot) to 0.59 ± 0.037 g/L then to 0.69 ± 0.023 g/L and 0.79 ± 0.012 g/L respectively for ETAPN and 0.81 ± 0.029 g/L then 0.87 ± 0.015 g/L for metformin, an increase of 20.41%, 40.81% and 61.22% for ETAPN and 65.30% and 77.55% for metformin.

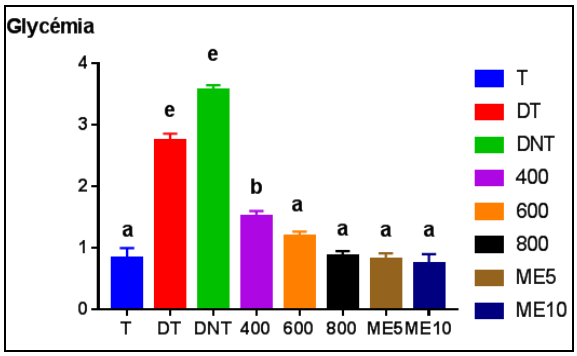

Fig 2: Effects of total aqueous fruits of Picralima nitida (Apocynaceae) on rats glycemia

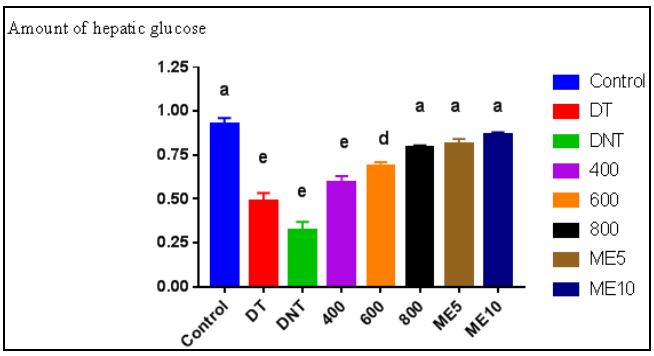

Fig 3: Effects of total aqueous fruits of *Picralima nitida* (Apocynacseae) on hepatic glucose storange in rats

The data are presented as an average ± Error on the average. a : Statistical difference not significant (P>0.05) ; b : significant statistical difference (P<0.01): e : Highly significant statistical difference(P<0.0001) ; DT : Diabetic treated ; DNT : Diabetic untreated ;

The data are presented as an average ± Error on the average. a: Statistical difference not significant (P>0.05); d: Very significant statistical difference(P<0.001); e: Highly significant statistical difference (P<0.0001). DT: Diabetic treated; DNT: Diabetic untreated;

Discussion

Analysis shows that glucose released from the liver of norm glycemic rats, treated or not with ETAPN, increases with time. This suggests that the liver gradually releases glucose. These results are consistent with those of Claude Bernard [9], who showed in 1853 that under physiological conditions, the liver releases glucose that immediately enters the bloodstream of the general circulation. The body's energy needs cause the liver to release glucose stored as glycogen by glycogenolysis. This release provides the body with the energy necessary for the functioning of its cells. This due to the hydrolysis of glycogen by glycogen-phosphorylase. The administration of increasing doses of ETAPN to diabetic rats significantly reduces the release of hepatic glucose. The ETAPN would have an action similar to that of insulin and would act by inhibiting glycogenolysis of hepatic glycogen and/or neoglucogenesis. These results are in agreement with those of Gohi Bi *et al.,* (2016) [10]. Indeed, these authors have shown that the administration of increasing doses of the extract of *Pseudarthria Hookeri Wight & Arn. (Fabaceae)* reduces the release of hepatic glucose in a dose-dependent manner for 60 min. Moreover, this action of reducing the release of hepatic glucose by different doses of ETAPN would be similar to that of insulin. Insulin is a hypoglycemic hormone that acts by inhibiting the release of hepatic glucose by inhibiting glycogenolysis of hepatic glucose and/or neoglucogenesis. ETAPN could inhibit the release of hepatic glucose by using the same mechanism of action as insulin. The administration by gavage of a single dose of alloxane to rats causes a significant increase of blood glucose from 0.68±0.03 g/L in control rats to 2.733±0.0441 g/L. Alloxane is a reference diabetogen. It induces diabetes by destroying the β cells of the islets of Langerhans in the pancreas that can no longer produce insulin (Lenzen *et al.,* 1996) [11]. The treatment of these diabetic rats with different doses of ETAPN and then metformin significantly reduces to normalize blood glucose. These results are consistent with those of N'domou *et al.,* (2014) [12]. These authors showed that methanol extracts from *Gnetum buchholzianum* leaves and ethyl acetate extracts from *Gnetum africanum* leaves significantly reduce and normalize blood glucose in rats exposed to hyperglycemia. The action of ETAPN is believed to be similar to that of metformin and may be explained by its ability to reduce hepatic gluconeogenesis leading to a decrease in hepatic glucose production by inhibiting the functioning of the mitochondrial respiratory chain.In addition, in rats made diabetic by alloxane, the stored hepatic glucose level is reduced by 47.29%, or nearly half, compared to that of non-diabetic control rats. The decrease in hepatic glucose storage observed in diabetic rats may be explained by self-regulation in these rats whose livers release glucose stored by glycogenolysis to maintain glycemic homeostasis and by a change in insulin secretion after alloxane administration. Indeed, Jörns *et al.,* (1997) [13] showed that alloxane causes diabetes by destroying the beta cells that produce insulin in the pancreas and inhibiting the glucokinase activity needed to store liver glucose. These results are similar to those of Gohi Bi *et al.*,(2016) [10]who showed that the administration of alloxane to rats halved liver glucose storage. After a week of treatment of diabetic rats with ETAPN and metformin, the glucose level stored in the liver increases significantly. Among ETAPN's doses, the 800 mg/Kg PC dose appears to be the most effective because of its effect almost identical to that of metformin and promoting glucose storage that is essentially identical to that of normoglycemic control rats. These results show that ETAPN promotes glucose storage in the liver. Similar results have been reported by Cheng *et al.,* (2009) [14] and Ugochukwu and Babady (2003) [15].These authors respectively showed that the aqueous extract of the leaves of *Psidium guajava L.*, and the aqueous extract at 100 mg/kg of the leaves of *Gongronema latifolium* promote the storage of hepatic glucose. The effect of ETAPN on hepatic glucose storage would be similar to that of metformin, which reduces hepatic glucose production by transient mild inhibition of the mitochondrial respiratory chain complex. It may also stimulate the action of glycogen synthetase to promote hepatic glucose storage.

Conclusion

The different doses of ETAPN significantly reduce glycemia in rats with alloxane diabetes and the release of hepatic glucose. This action would justify its use in traditional medicine and would be due to its mode of action. The 800 mg/Kg PC dose offers the best blood glucose reduction by ETAPN. Its action is closest to that of metformin. The use of a dose of ETAPN greater than 800 mg/Kg PC could offer more interesting results and a better knowledge of its active ingredients would be beneficial.

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