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Hypoglycemic, antihyperglycemic, and inhibitory effects of intestinal glucose absorption of a medicinal recipe of *Parquetina nigrescens* (Apocynaceae) and *Erythrina senegalensis* (Fabaceae) in the Wistar rat

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Abstract

Diabetes is a disease that is the fourth leading cause of hospitalization and death per year in the world. It is considered by WHO to be a public health problem. This study aims to evaluate the effects of a medicinal recipe, composed of *Parquetina nigrescens* (Apocynaceae) and *Erythrina senegalensis* (Fabaceae), on the glycemia of normoglycemic or carbohydrate overloaded rats and the intestinal absorption of glucose in Wistar rats. It appears that the aqueous extract of the drug recipe (RPNES) administered orally at doses of 600 and 800 mg/kg BW induces dose-dependent hypoglycemia in normoglycemic rats. The effects of this extract at 800 mg/kg BW on blood glucose are similar to that of glibenclamide (10 mg/kg BW), a standard sulfonylurea. Hyperglycemia caused by oral administration of glucose (4 g/kg BW) is significantly reduced in rats pretreated or post-treated with RPNES at 800 mg/kg BW. This extract is therefore a hypoglycemic and antihyperglycemic agent. Besides, RPNES (600 and 800 mg/kg BW) dose-dependently reduced the intestinal absorption of glucose in rats. The effects of RPNES at 800 mg/kg BW are similar to those of acarbose (0.2 mg/mL), a benchmark inhibitor of intestinal absorption. These results suggest that RPNES could contain molecules capable of inhibiting SGLT1 and GLUT2, just like the reference substance (acarbose).The hypoglycemic, antihyperglycemic and intestinal glucose absorption inhibiting effects of the aqueous extract of the medicinal recipe of *Parquetina nigrescens* and *Erythrina senegalensis* justify the use in the traditional medicine of this medicinal recipe against diabetes.

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Introduction

It has been shown that in diabetic rats and type 2 diabetic patients, the capacity of the intestine to absorb glucose is increased. This is due to an increase in the expression of the monosaccharide transporters SGLT1, GLUT2 (Dyer *et al.*, 1997, 2002). Polysaccharides and oligosaccharides are hydrophilic molecules, which cannot diffuse through the plasma membrane. They thus enter enterocytes via the sodium-dependent glucose transporter (SGLT1).

The glucose molecules then exit the basolateral membrane of the enterocyte cell using a transporter (GLUT2) into the blood (Nistor, 2009).

Nowadays, several medicinal plants are used in traditional medicine in the treatment of diabetes. However, for most of them, the effects on intestinal absorption have not been elucidated. This is the case of *Parquetina nigrescens* (Apocynaceae) and *Erythrina senegalensis* (Fabaceae), two plants associated to give a medicinal recipe used in the treatment of diabetes in traditional medicine in Côte d'Ivoire.

This study aims to evaluate the effects of an aqueous extract of this recipe (RPNES) on the blood glucose levels of normoglycemic or carbohydrate overloaded rats, and on the intestinal absorption of glucose in Wistar rats.

Material and methods

Plant material

The plant material consists of the dry leaves of *Parquetina nigrescens* (Apocynaceae) and *Erythrina senegalensis* (Fabaceae). These plants were collected in Dimbokro (Ivory Cost), in October 2018. They were identified and authenticated at the National Floristic Center (CNF) of the Félix Houphouët-Boigny University (Abidjan, Ivory Cost) by ASSI Jean, Technician in this research center, in comparison respectively with the herbaria numbers 15031 and 14625 of the National Floristic Center (CNF) of these plants, discovered on 12/28/1979 and on 01/17/1979 in the forest of Bamoro (Bouaké, Côte d'Ivoire) by the late Ake-Assi Laurent, Emeritus Professor of Botany at the University Félix Houphouët-Boigny (UFHB).

Animal material

White rats, *Rattus norvegicus* (Muridae), are used for blood sugar studies. Their mass varies between 120 g and 150 g. They are raised at the animal facility of the Educational and Research Unit (UPR) of Animal Physiology, of the Biosciences Training and Research Unit (UFR), at the UFHB, at 25 ± 2 ° C, and under light by day and darkness at night. They are fed with food provided by the IVOGRAIN® company in Abidjan, and have free access to water. Breeding and experiments are conducted in accordance with the guidelines for the care and use of laboratory animals published by the National Institute of Health.

Preparation of the drug recipeextract

Seventy grams (70 g) of powder of dried leaves of *Parquetina nigrescens* and 30 g of powder of dried leaves of *Erythrina senegalensis* are put in 1 L of distilled water. The mixture is boiled at 100 ° C for 20 minutes. The decocté obtained is left to cool to room temperature (25-30 ° C) and filtered three times on cotton wool before being filtered with Whatman n ° 2 filter paper, then dried in an oven (Memmert, Germany). at 50 ° C. The powder obtained, very dark brown, constitutes the aqueous extract of the medicinal recipe composed of *Parquetina nigrescens* and *Erythrina senegalensis* (RPNES).

Methods of Measuring Blood Glucose in Rats

The method used is that described by Mansar-Benhamza *et al.* (2013) and Kahou Bi *et al.* (2016). Blood glucose is measured using an Accu-Chek Active® blood glucose meter, complete with test strips. This strip has an absorbent layer on which is deposited a drop of blood from an incision in the tail end of the rat. The blood sugar value is given in g/L.

Study of the effect of the drug recipe (RPNES) on blood sugar in normoglycemic rats

The effects of the drug recipe (RPNES) on the glycemia of normoglycemic rats are monitored in the short term after gavage of these animals with

different doses of RPNES according to the method described by N'doua *et al.* (2015).

This experiment is carried out on a total of 20 Wistar rats weighing between 120 and 150 g. These animals are divided into 4 groups of 5 rats and fasted for 18 hours. The average weight of each lot is determined. Before administration of the substances, blood glucose is measured in all animals (time To).

The rats in batch 1 (control batch) receive 2 ml of distilled water.

The rats of batches 2, 3 and 4 (test batches) receive 2 ml of RPNES at doses of 400, 600 and 800 mg/kg of body weight (mg/kg BW) respectively.

After the treatment of the animals, the blood sugar in these rats is regularly measured at times of 30, 60, 90, 120, 150 and 180 minutes.

Study of the effects of the drug recipe and glibenclamide on the glycemia of rats in carbohydrate overload (hyperglycaemia)

Blood glucose measurement in pretreated rats: Hyperglycemia is caused by oral administration of anhydrous glucose (Cooper, France) to rats at a dose of 4 g/kg BW. For this study, 4 batches of 5 rats are made and the average weight of each batch is determined. These rats are distributed as follows:

Batch 1 (R-T) is the control batch where the rats receive 2 mL of distilled water;

Batch 2 (R-T +) constitutes the hyperglycemic control. The rats in this batch were given distilled water and, 30 min later, 4 g/kg BW glucose;

Batch 3 (R-Glib) consists of rats which receive glibenclamide (DAONIL®, Sanofi-Aventis, France), a sulphonylurea, at a dose of 10 mg/kg BW and, 30 min later, 4 g/kg BW of glucose;

Batch 4 (RPNES) consists of the rats which receive 800 mg/kg BW of RPNES and, 30 min later, 4 g/kg BW of glucose.

The effects of the aqueous extract of the medicinal recipe on the blood sugar of the rats in this series of experiments are followed for 180 minutes during which the blood sugar is measured at 0 min (just before the treatments), then 30, 60, 90, 120, 150 and 180 min after pretreatment. The percentage reduction in induced hyperglycemia is then calculated.

Blood glucose measurement in post-treated rats: Sampling is the same as for pre-treated rats. However, in this series of experiments, the different batches of rats received the test dose of RPNES (800 mg/kg BW) or glibenclamide (10 mg/kg BW), 30 min after the induction of hyperglycemia by administration orally 4 g / kg BW glucose. The glycemia in these rats is measured at time To (just before the treatments), then followed for 180 min, at regular time intervals of 30 minutes (30, 60, 90, 120, 150 and 180 min after the treatments). The percentage reduction in induced hyperglycemia is then calculated.

Study of the effects of the drug recipe and acarbose on the intestinal absorption of glucose in the jejunum of rats

Intestinal glucose absorption is measured according to the method described by Gonzalez-Mujica *et al.* (2003) and Ehoué *et al.* (2018). For this study, 5 batches of 5 Wistar rats weighing between 120 g and 150 g have fasted for 24 h and the average weight of each batch is determined.

After anesthesia of the rats with Lignocaine® (Xylocaine, Aspen Pharma Trading Ltd., Republic of South Africa) at 2.5 mL / kg BW, the abdomen is opened and the jejunum is ligated at one end. At the other end, 0.5 mL of anhydrous glucose (4 g/L) is introduced into the jejunum of the control rats (batch 1), then these animals receive 0.5 mL of distilled water orally.

The rats of lots 2, 3, 4 receive 0.5 mL of RPNES at the respective doses of 400, 600, 800 mg/kg BW and,

after anesthesia, 0.5 mL of anhydrous glucose (4 g/L) is introduced into their jejunum, as for the control rats.

The rats in batch 5 receive 0.5 mL of acarbose (BIOGARAN® 50 mg, BLUEPHARMA, Portugal), a competitive inhibitor of alphas amylase and glucosidase, by gavage, after the introduction of 0.5 mL of anhydrous glucose (4 g/L) in their jejunum, as in the control rats.

The jejunum of the rats is ligated on the second end over a length of 6 centimeters and their abdomen is closed with a few stitches. Sixty (60) minutes after force-feeding the rats with distilled water or their treatment with the substances, the jejunum of the animals is isolated at both ends and the concentration of glucose in this portion of the isolated jejunum is determined by the GOD method. -POD (**Trinder, 1969**) using the BIOLABO Diagnostics (France) type spectrophotometer. The glucose absorbed is that which has disappeared from the intestinal lumen after 60 minutes of experimentation. The level of glucose absorbed is calculated as follows:

$$\text{Glucose absorbed (\%)} = \frac{C_{\text{glucose}}(T0) - C_{\text{glucose}}(T1)}{C_{\text{glucose}}(T0)} \times 100$$

C glucose = glucose concentration

T0 = time just before treatment of rats

T1 = 60 minutes (isolation of the jejunum after 60 min)

Statistical analysis and graphics

Data analysis is done using GraphPad Instat software (San Diego CA, USA). The results are given as the mean followed by the standard error of the mean (M ± ESM). The difference between the two values is determined by the *Turkey-Kramer* comparison test and is considered not significant for P> 0.05, not very significant for P <0.05 (*), significant for P <0.01 (**) and very significant for P <0.001 (***).GraphPad Prism 8 software (San Diego CA, USA) is used to plot the graph.

Results

Dose-response effects of the aqueous extract of the drug recipe (RPNES) on blood sugar in normoglycemic rats

The effects of oral administration of RPNES at doses of 400, 600 and 800 mg/kg BW on the blood glucose levels of normoglycemic rats are shown in Fig.1.

The basal values of the fasting glycemia of the rats, measured before the various treatments, show no significant difference (P > 0.05) between the batches. They are in the order of 0.95 ± 0.05 g/L.

Table 1. Reduction of glucose-induced hyperglycemia in rats post-treated with recipe aqueous extract (RPNES) or glibenclamide.

Rat treatment substance	Time after glucose administration			
	60 min	90 min	120 min	150 min
	Reduction of hyperglycemia			
R-T Glu	35.69 %	46.38 %	61.53 %	100 %
R-RPNES	47.69 %	66.15 %	100 %	–
R-Glib	70.76 %	100 %	–	–

R-T Glu: Rats given glucose (4 g/kg BW), H_2O (hyperglycemic controls)

R-RPNES: Rats given glucose (4 g/kg BW), then RPNES at 800 mg/kg BW

R-Glib: Rats given glucose (4 g/kg BW), then glibenclamide at 10 mg/kg BW.

In control rats receiving only distilled water, blood glucose did not vary significantly (P> 0.05) during the duration of this experiment (180 min). RPNES at a dose of 400 mg/kg BW caused an insignificant (P> 0.05) decrease in blood glucose in rats. This goes from 0.95 ± 0.05 g/L to 0.88 ± 0.02 g/L; or a decrease of 7.36%. In contrast, RPNES at doses of 600 and 800 mg/kg BW produced significant and

dose-dependent decreases in blood glucose in treated rats. These hypoglycemia increase over time. Thus, 180 min after the administration of RPNES at a dose of 600 mg/kg BW, the glycemia of the treated rats increases to 0.74 ± 0.01 g/L; ie a decrease of 22.10% (P <0.01) compared to the initial glycemia. In rats treated with RPNES at 800 mg/kg BW, hypoglycemia was 34.74% (P <0.01), compared to control rats, 180 min after administration of the recipe, with a blood glucose of 0.62 ± 0.019 g/L.

Table 2. Reductions in glucose-induced hyperglycemia in rats pretreated with recipe aqueous extract (RPNES) or glibenclamide.

Rat treatment substance	Time after glucose administration				
	30 min	60 min	90 min	120 min	150 min
	Reduction of hyperglycemia				
R-T Glu	–	7.69 %	46.15 %	61.53 %	100 %
R-RPNES	20 %	55.38 %	93.1 %	100 %	–
R-Glib	37.5 %	87 %	100 %	–	–

R-T Glu: rats receiving H_2O, then 4 g/kg BW of glucose (hyperglycemic controls)
R-RPNES: rats treated with RPNES at 800 mg/kg BW, then given glucose (4 g/kg BW)
R-Glib: rats treated with glibenclamide 10 mg/kg BW, then given glucose (4 g/kg BW).

Blood glucose change in hyperglycemic rats post-treated with RPNES or glibenclamide

In this series of experiments, the rats in the test groups were first given anhydrous glucose, followed 30 minutes later by administration of RPNES at 800 mg/kg BW or glibenclamide at 10 mg/kg BW. Before glucose administration, the blood glucose levels of the rats of the different batches were substantially identical (P> 0.05) with a blood glucose value of 0.90 ± 0.17 g/L.

In rats receiving only distilled water (normoglycemic controls), glycemia did not vary significantly (P> 0.05) until the 180th min of the experiment. In the rats of all the groups receiving glucose (4 g/kg BW), the hyperglycemia peak, obtained after 30 min, is substantially identical (P> 0.05) with a blood glucose level which goes from 0.90 ± 0, 17 g/L at 1.55 ± 0.16 g/L; or hyperglycemia of 0.65 ± 0.16 g/L, representing 72.22% increase in blood sugar, compared to the initial blood sugar. Subsequently, this hyperglycemia gradually decreases, over time, to reach the initial values of blood sugar. However, these drops in glucose-induced hyperglycemia and the time to return to initial basal blood sugar are variables depending on whether or not the rats are treated with RPNES or glibenclamide. Fig. 2 shows the change in blood sugar levels in hyperglycemic rats post-treated or not with RPNES or glibenclamide.

In hyperglycemic rats given 30 min after distilled water (hyperglycemic controls), after peak hyperglycemia, blood glucose is gradually reduced until reaching basal blood sugar after 180 min, after administration of glucose. On the other hand, in the rats post-treated with RPNES (800 mg/kg BW) or glibenclamide (10 mg/kg BW), the return to baseline glycemia takes place respectively 120 min and 90 min after administration of glucose to the rats. In these cases, after the return to basal blood sugar levels, hypoglycemia occurs. Thus, at the 180th stage, the hypoglycemia recorded was 0.71 ± 0.09 g/L and 0.50 ± 0.08 g/L in the rats treated respectively with RPNES or glibenclamide.

Table 1 gives, as a function of time, the percentages of reductions in hyperglycemia-induced by glucose when the rats are post-treated or not with the drug recipe or glibenclamide.

Blood glucose change in hyperglycemic rats pretreated with RPNES or glibenclamide

In this series of experiments, rats are first treated with RPNES at 800 mg/kg BW or with glibenclamide

(standard antihyperglycemic substance) at 10 mg/kg BW. The basal glycemia of these rats (0.90 ± 0.15 g/L) is substantially identical (P> 0.05) in the different batches before the treatments. When the normoglycemic rats received only distilled water (normoglycemic controls), their glycemia did not vary significantly (P> 0.05) during the 180 min of the experiment.

Administration of glucose at a dose of 4 g/kg BW results in significant increases (P <0.01) in blood glucose in all animals, treated or not. The hyperglycemia peak, which appears 30 min after this administration of glucose, is variable depending on whether the rats are treated or not with RPNES or glibenclamide. Fig. 3 shows the results of this series of experiments.

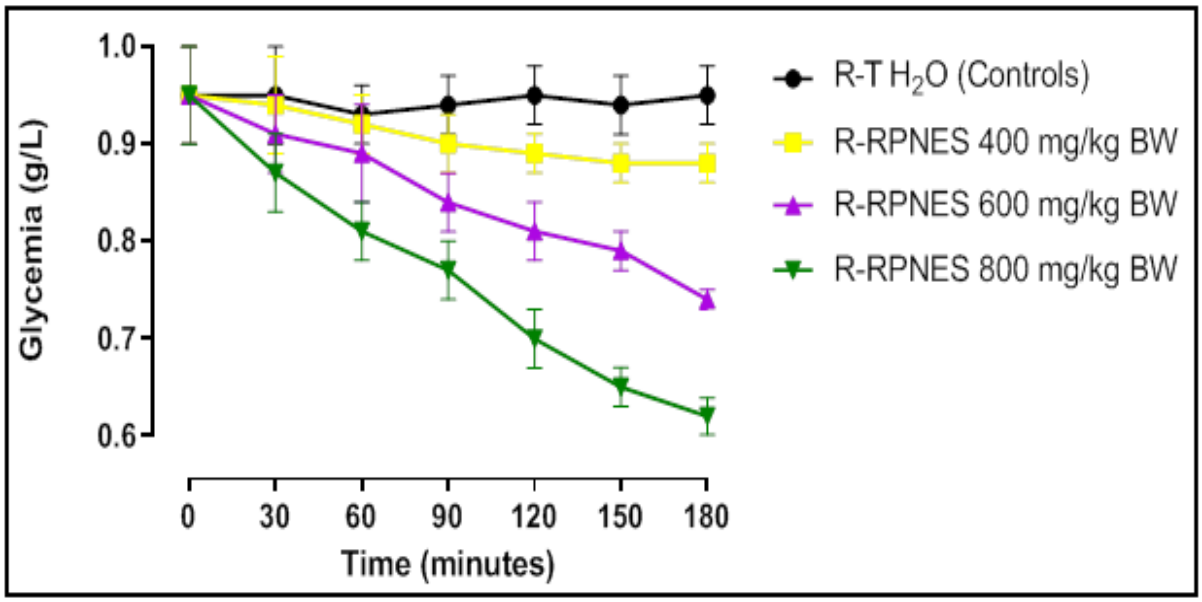

Fig. 1. Dose-response effects of an aqueous extract of a drug recipe of *Parquetina nigrescens* and *Erythrina senegalensis* (RPNES) on the blood sugar levels of normoglycemic rats.
R-T H_2O: Normoglycemic control rats receiving only distilled water
R-RPNES:Rats treated with RPNES at doses of 400, 600 and 800 mg/kg BW.

In the carbohydrate overloaded rats who received distilled water (hyperglycemic control), there was a significant increase (P <0.001) in blood sugar from 0.90 ± 0.15 g/L to 1.50 g/L , after 30 min; i.e. a peak of hyperglycemia of the order of 0.60 ± 0.18 g/L. Subsequently, hyperglycemia is gradually reduced and basal blood sugar is recovered approximately 150 min after administration of glucose. When the rats are pretreated with RPNES at 800 mg/kg BW, the hyperglycemia-induced 30 min after the administration of glucose at 4 g/kg BW is 0.50 ± 0.10 g/L; or a decrease in oral hyperglycemia of 11.11% compared to hyperglycemic control rats. After that, the hyperglycemia is reduced gradually, as a function of time, until it returns to the initial blood sugar after about 100 min, then significant hypoglycemia (P <0.01) of 0.25 ± 0.06 g/L is measured.

In rats pretreated with glibenclamide 10 mg/kg BW, the hyperglycemia-induced 30 min after administration of glucose at 4 g/kg BW was 0.40 ± 0.12 g/L; or a reduction in hyperglycemia-induced by the oral route of 22.22% compared to hyperglycemic controls.

After that, the hyperglycemia is also reduced gradually, as a function of time, until the initial blood sugar level is returned after about 90 min, then hypoglycemia of 0.42 ± 0.07 g/L is measured at the 180th min of experimentation.

Table 2 gives, as a function of time, the percentages of reductions in hyperglycemia-induced by glucose when the rats are pretreated or not with the drug recipe or glibenclamide.

Effects of RPNES and acarbose on intestinal glucose uptake from jejunum in rats

During this experiment, the concentration of glucose introduced into the jejunum (intestinal fragment) of the rats is 4 g/L. After 1 hour, the residual glucose in the control intestine fragments (rats were given distilled water) is 1.77 g/L; or a glucose absorption rate of 55.75%. In the intestines of rats treated with RPNES at doses of 400, 600 and 800 mg/kg BW, the residual glucose concentrations were 1.93 g/L, 2.34 g/L and 2.64 g/L, respectively. at the end of the experiment (after one hour); ie glucose absorption percentages of 51.75% (P> 0.05), 41.5% (P <0.01) and 36.5% (P <0.001) respectively. Likewise, in rats treated with acarbose (0.20 mg/mL), intestinal glucose absorption was 30% (P <0.001), with a residual glucose concentration of 2.8 g/L. The effects of acarbose (0.20 mg/mL) are thus similar (P> 0.05) to those of RPNES at 800 mg/kg BW. Fig. 4 shows, after 1 hour, the percentages of intestinal glucose absorption in rats given distilled water or treated with RPNES or with acarbose.

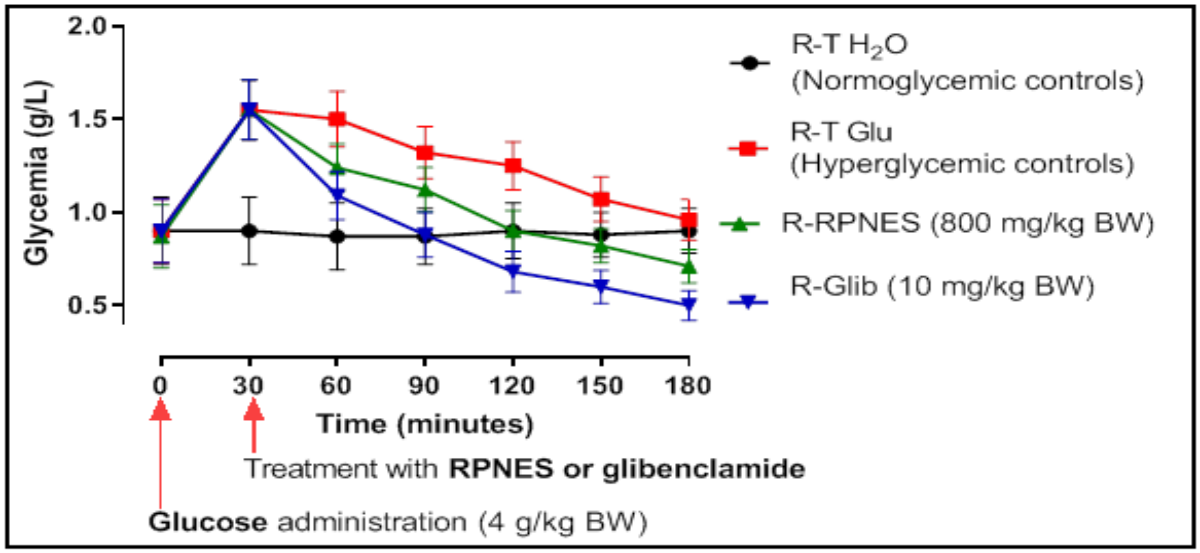

Fig. 2. Evolution over time of glycemia in hyperglycemic rats post-treated with the aqueous extract of a drug recipe (RPNES) or glibenclamide.

R-T H_2O: Rats given distilled water only (normoglycemic controls)

R-T Glu: Rats given glucose (4 g/kg BW), then H_2O (hyperglycemic controls)

R-RPNES: Rats given glucose (4 g/kg BW), then RPNES at 800 mg/kg BW

R-Glib: Rats given glucose (4 g/kg BW), then glibenclamide at 10 mg/kg BW.

Discussion

The study of the pharmacological effects of the aqueous extract of the medicinal recipe, composed of *Parquetina nigrescens* and *Erythrina senegalensis* (7/3, m/m) (RPNES), on glycemia in normoglycemic rats, shows that this extract induces progressive and dose-dependent hypoglycemia. RPNES is therefore a hypoglycaemic substance. These results are similar to those of Mea *et al.* (2017) and Ehoué *et al.* (2018) who also showed, respectively, that the aqueous extract of the leaves of *Justicia secunda* (Acanthaceae) and the aqueous extract of the fruits of *Picralima nitida* (Apocynaceae), administered orally, lead to significant reductions in blood sugar in normoglycemic rats. Administration by gavage of anhydrous glucose 4 g/kg BW to rats causes significant hyperglycemia (increase in basal blood glucose> 70%), the peak of which is recorded after 30 minutes. This induced hyperglycemia is significantly reduced by RPNES in the rats pretreated and in the rats post-treated with this extract. The reduction by RPNES at 800 mg/kg BW of glucose-induced hyperglycemia (4 g/kg BW) is similar to that of glibenclamide (10 mg/kg BW), a sulphonylurea. Thus, like glibenclamide, RPNES has antihyperglycemic effects.

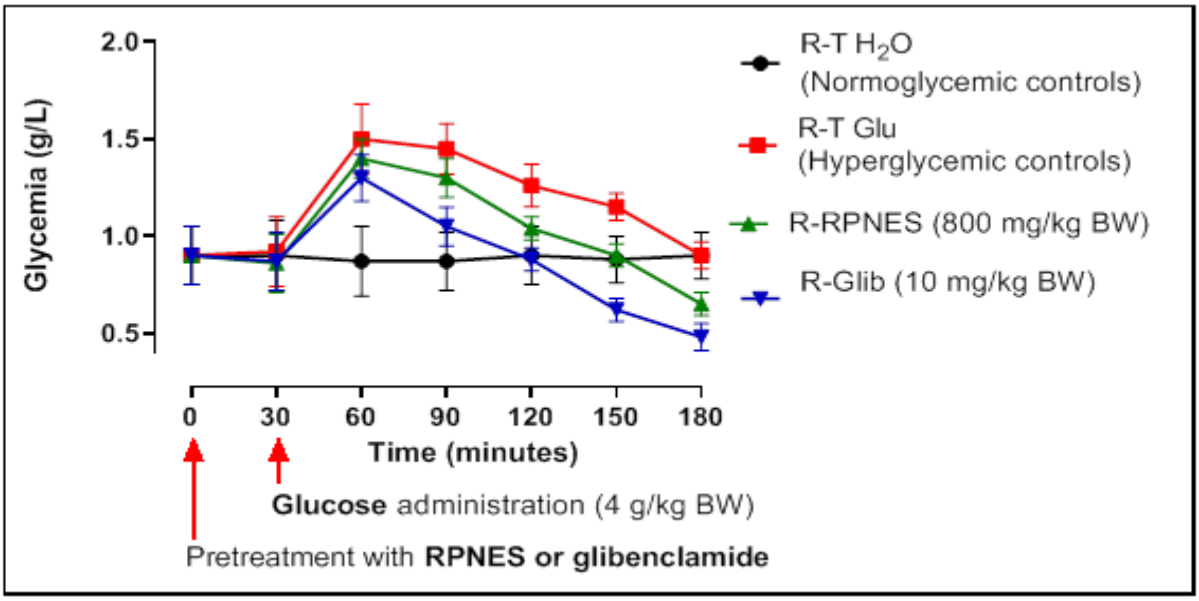

Fig. 3. Evolution over time of glycemia in hyperglycemic rats pretreated with the aqueous extract of a drug recipe (RPNES) or glibenclamide.

R-T H_2O: Normoglycemic rats given distilled water (normoglycemic controls)

R-T Glu: Rats given H_2O, then glucose (4 g/kg BW) (hyperglycemic controls)

R-RPNES: Rats given RPNES at 800 mg/kg BW, then glucose (4 g/kg BW)

R-Glib: Rats given glibenclamide 10 mg/kg BW, then glucose (4 g/kg BW).

The antihyperglycemic and hypoglycemic properties of RPNES are similar to those of many herbal remedies from the traditional African pharmacopeia. This is the case of *Rauvolfia vomitoria* (Apocynaceae) (N'doua *et al.*, 2015), *Pseudarthria hookeri* (Fabaceae) (Kahou Bi *et al.*, 2017) and *Picralima nitida* (Apocynaceae) (Ehoué *et al.*, 2018).

The study of the effects of the aqueous extract of the drug recipe (RPNES) on the reabsorption of intestinal glucose shows that RPNES, at doses of 600 and 800 mg/kg BW, dose-dependently reduces the intestinal absorption of glucose. in rats. Similar results were obtained by Ehoué *et al.* (2018) who showed that the aqueous extract of *Picralima nitida* (Apocynaceae) inhibits glucose uptake in Wistar rats.

Glucose absorption from the intestine is mediated by two transporters: the sodium-dependent D-glucose co-transporter (SGLT1), which is found in the brush border of enterocytes, and the transporter of glucose independent of sodium (GLUT2) found in the basolateral membrane (Freeman *et al.*, 2006). SGLT1 is arguably the main importer of glucose in the gut when luminal concentrations are low. However, in the event of increased glucose in the lumen, SGLT1 facilitates the insertion of GLUT2 on the apical membrane of the enterocyte to facilitate glucose uptake (Kellett *et* Brot-Laroche, 2005).

Acarbose is a pseudo tetrasaccharide that binds to the border of microvilli in the small intestine, causing the absorption of sugars to be slower (Shim *et al.*, 2003).

Acarbose is a competitive inhibitor of D-glucose on SGLT (Sodium-Glucose Transporter). Therefore, it inhibits the absorption of glucose present in the lumen of the gut (Rossetti *et al.*, 1987). Thus, acarbose binds to the receptor transporters SGLT1 and GLUT2 to prevent glucose uptake.

The effects of RPNES (800 mg/kg BW) are similar to those obtained with acarbose (0.2 mg/mL) which also significantly reduces the intestinal absorption of glucose. These results suggest that RPNES may contain molecules that, like acarbose, may act by binding to SGLT1 and GLUT2 receptors to prevent absorption from the intestines.

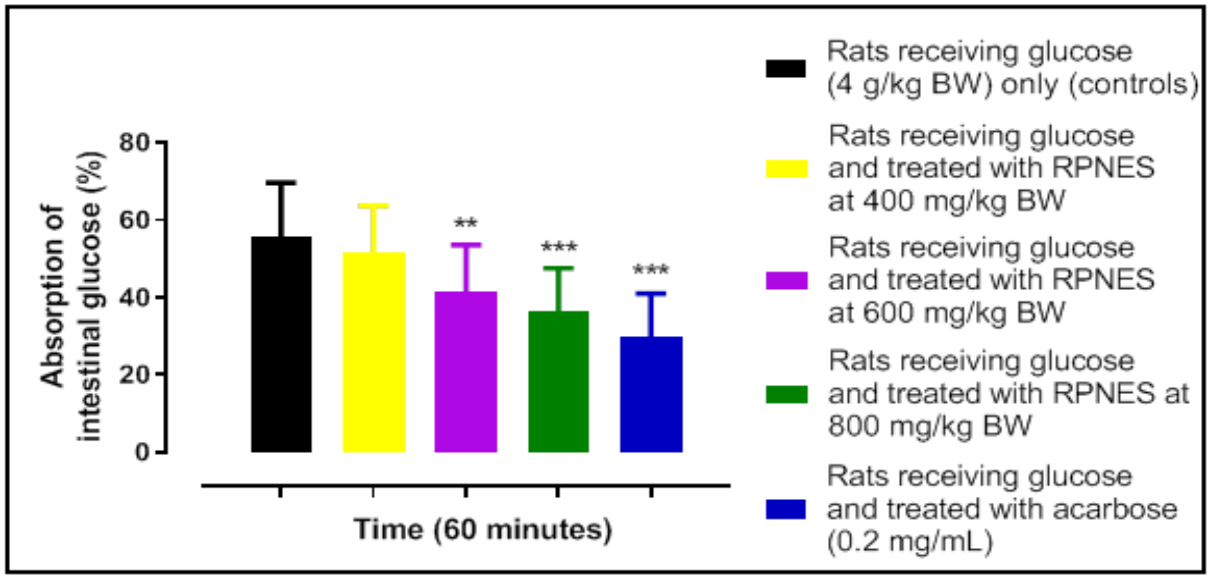

Fig. 4. Effects of RPNES and acarbose on the absorption of intestinal glucose for one hour.
n = 5 ** *P*<0.01; *** *P*<0.001 compared to the control.

Conclusion

This study shows that the aqueous extract of the medicinal recipe, composed of *Parquetina nigrescens* and *Erythrina senegalensis* (RPNES), at doses greater than or equal to 600 mg / kg of EP, has dose-dependent hypoglycemic and antihyperglycemic effects. The antihyperglycemic effects are similar to those of glibenclamide, a standard sulfonylurea. Besides, this extract inhibits the intestinal reabsorption of glucose, as does acarbose, a benchmark inhibitor of intestinal absorption.RPNES has good hypoglycaemic and antihyperglycaemic potential and inhibits the intestinal reabsorption of glucose, which justifies the use in the traditional medicine of the medicinal recipe composed of *Parquetina nigrescens* and *Erythrina senegalensis* in the treatment of diabetes.

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