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EVALUATION OF THE ANTI-DIABETIC ACTIVITY OF THE HYDRO-ETHANOLIC EXTRACT OF THE NUCLEI OF PERSEA AMERICANA (LAURACEAE) IN THE WISTAR RAT.

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ABSTRACT

Faced with the alarming increase in the number of diabetics around the world and the cost of modern treatment, the African pharmacopoeia offers an alternative to synthetic antidiabetics. Thus, the present study was undertaken to evaluate the anti-diabetic activity of the hydro-ethanolic extract of Persea americana Mill nuclei in rats. Thus, the hypoglycemic, hyperglycemic and anti-diabetic activities of this extract were evaluated. The phytochemical study by characterization reactions in tubes demonstrated the existence of tannins, alkaloids and flavonoids in this extract. The extract administered by the oral route made it possible to lower the glycemia of normal rats, hyperglycaemic (by overloading with glucose at a rate of 3g / kg) and diabetics by injection of streptozotocin, compared to rats having received only distilled water. At a dose of 400 mg / Kg the extract decreases the baseline glycemia of normal rats at the 4th hour ($p < 0.05$). In diabetic rats, the extract at a dose of 400 mg / kg significantly ($p < 0.01$) reduced blood sugar from the 1st hour, compared to diabetic rats given distilled water and the extract at the dose of (200mg / kg. The observed antidiabetic activity can be explained by the action of flavonoids or their association with the other chemical families highlighted in this extract. These results can justify the use of these nuclei in traditional medicine for the treatment of diabetes.

KEYWORDS: Persea americana, diabetes mellitus, streptozotocin, blood sugar.

1. INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by permanent hyperglycemia which disrupts the use of carbohydrates and consequently the metabolic disorder of proteins and fats. This general metabolic disorder affects the normal functioning of the body, causing specific organ complications affecting the eyes, kidneys, nerves, liver, heart and blood vessels.[1]

Diabetes is a disease on the rise throughout the world and is nowadays a real public health problem: According to the International Diabetes Federation, the prevalence in 2015 was 415 million, or 8.8% of the adult population of world. And she estimates that this prevalence will increase to one in ten adults by 2040, or 642 million diabetics worldwide.[2]

The clinical course of this pathology requires treatment and good lifelong monitoring. Treatment for diabetes involves keeping blood sugar at normal levels to prevent complications from permanent hyperglycemia from developing. All modern therapeutic approaches end up generating resistance problems and are not accessible to all social strata because of their high costs. Thus, to seek treatment, many patients turn to traditional herbal treatments. According to the World Health Organization, more than 80% of African populations use traditional medicine and pharmacopoeia for treatment.[4]; [5] This is how extracts from the kernels of Persea americana have been tested for their anti-diabetic activity.

2. MATERIALS AND METHODS

2.1. Plant Material

The fresh stones of Persea americana were extracted from ripe fruits harvested in April 2020 in Arrondissement 2 (Bacongo) of Brazzaville. These cores were stripped of their shells and air dried at room temperature for two weeks. They were pulverized with a mortar, then macerated in a hydro-ethanolic solution (50:50).

2.2. Animal material

Our experimental study was carried out on male Wistar rats with a body weight of between 250-350 g. The breeding of these animals was done in the animal house of the Faculty of Sciences and Techniques of the University Marien NGOUABI of Brazzaville. These animals were housed in plastic cages and kept under standard conditions: 12 hours of light and 12 hours of darkness, at room temperature with free access to food and water.

2.3. Preparation of hydro-ethanolic maceration

100g of powder from the Persea americana stones were introduced into a flask containing 500 mL of distilled water and 500 mL of 90 ° ethanol. The solution was stirred regularly and kept in the dark for 72 hours. The mixture was then filtered through cotton wool and the resulting filtrate was evaporated in an oven at 55 ° C.

2.4. Induction of type II diabetes

The diabetes was induced by injection through the dorsal vein of the penis of the rat, of a single dose of streptozotocin, previously dissolved in a 0.9% sodium chloride solution, at a rate of 60 mg / kg of body weight, after a fast of 14 to 16 hours. 72 hours later, blood was taken from the main tail vein to collect blood for blood sugar control, using an accu-check brand glucometer.[6] The rats exhibiting a glycemia of between 1.29 and 2 g / l were selected as type II diabetics.

2.5. Phytochemical profile

Tube reactions using appropriate reagents revealed some chemical groups.[7]

2.6. Evaluation of hypoglycaemic activity

The normal - glycemic male rats fasted for 16 hours before the tests were divided into four (4) groups of five rats each and treated per os as follows.

- Lot 1 (negative control) received distilled water at 10 mL / Kg;
- Lot 2 (positive control) received Glibenclamide at 5 mg / Kg;
- Lots 3 and 4 respectively received the doses of 200 and 400 mg / Kg of the macerated stones of Persea americana.

Blood glucose levels were taken every hour for 5 hours.

2. Evaluation of anti-hyperglycaemic activity

This activity was evaluated by inducing hyperglycemia by oral injection of 10% glucose in normal rats at a rate of 3 g / kg. The animals were divided into four Lots of five rats each and treated as follows.

- Lot 1 (negative control) received distilled water at 10 mL / Kg
- Lot 2 (positive control) received Glibenclamide at 5 mg / Kg;
- Lots 3 and 4 respectively received the doses of 200 and 400 mg / Kg of hydro-ethanolic macerated from the stones of Persea americana.

At the start of the experiment, the animals received distilled water, glibenclamide and macerated at doses of 200 and 400 mg / Kg.

One hour later, the animals received the glucose overload;

The blood glucose levels were taken half an hour, one and two hours after the glucose overload (3g / Kg).

2.8. Evaluation of antidiabetic activity

The diabetic rats subjected to a 16 hour fast were divided into four groups of five rats each and treated as follows:

- Lot 1 (negative control) received distilled water at 10 mL / Kg.
- Lot 2 (positive control) received Glibenclamide at 5 mg / Kg;
- Lots 3 and 4 respectively received doses of 200 and 400 mg / Kg of the hydro-ethanolic extract of the stones of Persea americana.

At the start of the experiment, the baseline blood glucose levels were taken, then glycemic monitoring was done every hour and for 5 hours.

3. RESULTS

3.1. Phytochemical profile of the hydroethanolic extract of Persea americana kernels

The results reported in Table I, from the reactions in tubes, show that the hydroethanolic extract of the nuclei of Persea americana contains: alkaloids, tannins and flavonoids in large quantities. There is an absence of oses, saponosides and mucilages in this extract.

Table I: Phytochemical profile of the hydro-ethanolic extract of the stones of Persea americana.

Chemical compounds	Observations	Results
Alkaloids	Above red or yellowish	+
Flavonoids	Orange color	++
Saponosides	0.9 <1cm	-
Tannins	Blackish blue	+
Dares	/	-
Mucilage	/	-

Symbol meanings: "+": Presence; "+ +": Strong presence; "-": absence

3.2. Effect of hydroethanolic extract of Persea americana kernels on blood sugar levels in normal rats

The hydroethanolic extract of the stones of Persea amaricana at a dose of 400 mg / Kg decreases the basal blood sugar level of normal rats (Table II). Indeed, oral administration of this extract at this dose does not lead to a significant reduction ($p < 0.05$) in blood sugar until the 4th hour, with a percentage reduction in blood sugar of 13.36 %, compared to controls given distilled water. At the 5th hour, this reduction is more significant ($p < 0.01$; PRG = 21.12%), compared to the rats given distilled water. It should be noted that this reduction is significant from the first hour with Glibenclamide at 5mg / Kg.

However the hydroethanolic extract of Persea amaricana at a dose of 200mg / Kg does not reduce the blood sugar of normal rats until the 5th hour (p <0.05) with a percentage reduction in blood sugar equal to 33.65 %, greater than that of the 400 mg / kg dose (Table II).

Table II: Effect of the hydroethanolic extract of Persea americana nuclei on the mean blood glucose levels of normal rats.

Treatments	Average blood sugar values (in g / l) and percentage reduction in blood sugar					
	0 hour	1 hour	2 hours	3 hours	4 hours	5 hours
ED 0.5mL / kg	1.03 ± 0.07	1 ± 0.11	1.07 ± 0.12	1.03 ± 0.12	0.95 ± 0.21	0.89 ± 0.11
	/	(2.91%)	(-4.07%)	(-0.38%)	(7.18%)	(13.39%)
Glib 5mg/kg	1.18±0.10	0.75±0.11	0.61±0.05	0.63±0.10	0.62±0.36	0.56±0.12
	/	(36.54 %) *	(48.05 %) **	(46.53 %) **	(47.54 %) **	(52.28 %) **
Ext HE 200 mg/kg	1.25±0.06	1.18±0.10	1.13±0.10	1.12±0.11	0.94±0.28	0.83±0.14
	/	(5.90 %) ns	(9.56 %) ns	(10.52 %) ns	(25.03%) ns	(33.65 %) *
Ext HE 400 mg/kg	1.21±0.05	1.25±0.06	1.23±0.02	1.18±0.02	1.05±0.04	0.95±0.04
	/	(-3.30 %) ns	(-1.98 %) ns	(2.64 %) ns	(13.36 %) *	(21.12 %) **

Ext HE: Hydroethanolic extract; Glib: Glibenclamide; * p <0.05; ** p <0.01: Significant difference from the control batch treated with distilled water; ns: not significant. (): Percent reduction in blood sugar (PRG)

3.3. Effect of Hydro-Ethanolic Extract of Persea americana Kernels on Blood Glucose in Normal Rats Tested for Orally Induced Hyperglycemia

The hyperglycemia induced by oral glucose overload (3g / Kg) was illustrated by a maximum increase in blood glucose level in all rats, half an hour after administration (Figure1). However, it is noted that the average spike in blood sugar in rats not having received only distilled water is higher than all others. During the first hour before the administration of glucose, a significant decrease in the mean glycemia of the rats treated with glibenclamide and the extract at a dose of 400 mg / kg is observed.

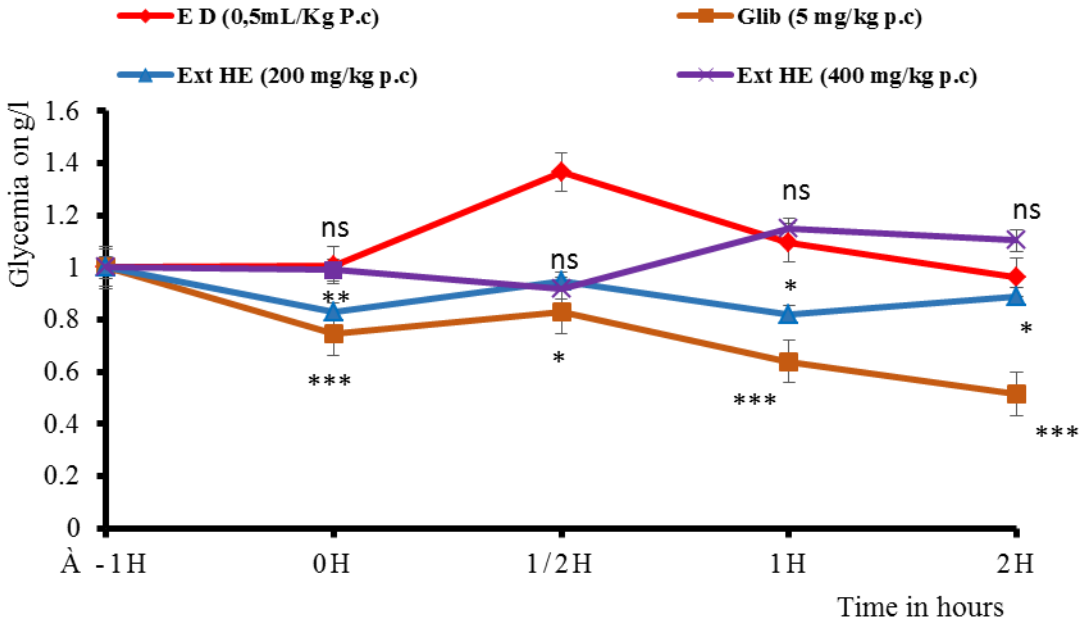

Figure 1: Blood glucose trend in normal rats tested for oral hyperglycemia.

ED:distilled water; Ext HE: hydro-ethanolic extract; Glib: Glibenclamide; Significant difference from the control batch treated with distilled water: * p <0.05; ** p <0.01; *** p <0.001; ns: not significant

3.4. Effect of hydro-ethanolic extract of Persea americana kernels on blood glucose levels in diabetic rats.

Table III shows the effect of the hydro-ethanolic extract of the stones of Persea americana at doses of 200 and 400 mg / Kg on the glycemia of type II diabetic rats. The five-hour glycemic monitoring shows, one hour after administration of the extracts, a decrease in the mean blood sugar in all the diabetic rats compared to the starting blood sugar levels. However, this decrease is only significant in diabetic rats given glibenclamide (p <0.05) and hydro-ethanolic extract at a dose of 400 mg /

kg ($p < 0.01$), compared to rats. Diabetics who received only distilled water.

The 200 mg / Kg dose does not become significantly active until the 2nd hour. Both doses of the extract remain significantly active until the 5th hour, compared to rats given only water; but glibenclamide remains significantly more effective with regard to the percentage reduction in blood sugar (40.94% versus 33.28% and 28.73% respectively for doses of 200 and 400 mg / kg.

Table III: Evolution of the average blood sugar levels of diabetic rats as a function of the doses of the aqueous extract.

Treatments	Average blood sugar values (in g / l) and percentage reduction in blood sugar					
	0 hour	1 hour	2 hours	3 hours	4 hours	5 hours
ED 0.5mL/kg	1.29±0.02	1.38±0.08	1.29±0.02	1.38±0.08	1.29±0.02	1.38±0.08
	/	(-6.81 %)	(-5.57 %)	(-1.85%)	(-2.47%)	(-1.85 %)
Glib 5mg/kg	1.39±0.09	1.19±0.10	1.02±0.12	0.85±0.18	1.39±0.09	1.19±0.10
	/	(14.36 %) *	(26.58%) ***	(38.64%) ***	(40.80%) ***	(40.94%) ***
EHE 200 mg/kg	1.53±0.12	1.43±0.16	1.22±0.12	1.21±0.19	1.09±0.13	1.02±0.11
	/	(6.52 %) ns	(19.97 %) **	(20.62 %) *	(28.85%) ***	(33.28%) ***
EHE 400 mg/kg	1.36±0.07	1.13±0.14	1.12±0.12	1.06±0.18	0.99±0.14	0.97±0.13
	/	(16.71%) **	(17.74 %) **	(22.28 %) **	(26.97%) ***	(28.73%) ***

EHE: Hydroethanolic extract; Glib: Glibenclamide; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Significant difference, the hydroethanolic extract compared to the control; ns: not significant.

4. DISCUSSION

Administration of hydroethanolic maceration of Persea americana kernels (200 and 400 mg / Kg) to normoglycemic rats resulted in a decrease in baseline blood sugar. These results are similar to those obtained by Mamadou et al[8] who worked on the aqueous extract of the leaves of Persea americana. These results show that extracts from the kernels and leaves of Persea americana contain bioactive substances with hypoglycemic properties.

Oral hyperglycemia test shows that rats given glibenclamide and both doses of the extract had lower mean peaks in blood sugar than peak mean blood glucose levels in rats treated with distilled water. These plant extracts therefore contain bioactive substances capable of protecting animals against the onset of hyperglycemia: this is the protective effect. These results are similar to those obtained by other researchers who have worked on extracts of Manilkara multinervis[9], Trilepisuim madagascariense D.C. Leuwenberg.[10] They suggest stimulation of the very active use of glucose by peripheral tissues. The increased tolerance of tissues to glucose has been reported by other researchers who have worked on extracts of: Cogniauxia podolaena[11], Ceiba pentandra.[12]

Our results show that the activity of the hydro-ethanolic extract of Persea americana kernels varies with the doses administered: for this reason, the effect of Persea americana extract can be said to be dose-dependent. This observation was also made with extracts from Bridelia ferruginea[13] and Icacina senegalensis.[14]

Administration of this extract at doses of 200 and 400 to diabetic rats resulted in a reduction in the mean blood sugar levels in all the diabetic rats one hour later compared to the mean starting blood sugar levels; but this reduction was only significant for diabetic rats that received glibenclamide and the hydro-ethanolic extract of Persea americana nuclei at 400 mg / kg ($p < 0.01$), compared to diabetic rats that received only distilled water. The reduction in blood sugar in the case of the hydro-ethanolic extract of the stones of Persea americana at a dose of 200 mg / Kg does not become significant ($p<0.01$) until 2 hours later. It can therefore be deduced from these results that the extracts of the nuclei of Persea americana have antidiabetic activity in rats. The 400 mg / Kg dose acts quickly by reducing blood sugar and its activity increases over time, while the hydro-ethanolic extract of Persea americana kernels at a dose of 200 mg / Kg acts slowly on blood sugar. This result confirms the dose-dependent effect of this extract.

The hypoglycemic, antihyperglycemic and antidiabetic activities of the hydro-ethanolic extract of Persea americana kernels can be attributed to flavonoids which work by improving the sensitivity of the body's cells to insulin, thereby reducing the incidence of the disease. Diabetes.[18] Indeed, the antihyperglycemic power of flavonoids has been reported by other researchers who have worked on extracts of: Zizyphus mauritiana[15], Bridelia ferruginea.[13] Jung's work[16] on Citrus aurantifolia has also shown that flavonoids may have antihyperglycemic properties by acting on the enzymatic activity involved in hepatic glucose metabolism (activation of glycolysis and inhibition of gluconeogenesis). Flavonoids have also been shown to increase the activity of adipocyte glucotransporter 4 (GLUT4) for glycogen synthesis in the liver.[17]

Flavonoids with antidiabetic activity have been identified in extracts from some plants.[19]; [10]; [20] In addition, other chemicals in Persea americana kernel extract such as

alkaloids may also be responsible for this activity. Previous work on leaf decocts of Vernonia colorata and extracts of Zizyphus mauritania have shown that alkaloids, flavonoids, saponosides contain anti-diabetic properties.[21] It can also be a combined activity of these (3) substances. It may be a combination of flavonoids-alkaloids, flavonoids-terpenoids, as has been observed with the bark and leaves of Gnidia glauca Lin[22], the leaves of Lycium shawil.[23]

5. CONCLUSION

The hydroethanolic extract of Persea americana kernels contains bioactive substances capable of lowering blood sugar levels in normal, hyperglycemic and diabetic rats. These results justify the traditional use of the powder of these nuclei in traditional medicine for various conditions including diabetes.

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