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Full Length Research Paper

Evaluation of hypoglycemic, antihyperglycemic and antidiabetics properties of *Trilepisium madagascariense* D.C. Leeuwenberg (Moraceae)

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Study was carried out to investigate the anti-diabetics activity of *Trilepisium madagascariense* leaves used in traditional medicine to heal diabetes. Experiments were conducted on normoglycemic rats for hypoglycemia activity, on rats that were made hyperglycemic by orally loading (3 g/Kg) of glucose at 10% for anti-hyperglycemia activities and on rats made diabetics by injection of streptozotocin for the anti-diabetics activities. The aqueous decoction (400 and 800 mg/Kg, per os) and the macereted hydro-ethanolic of the same doses, per os produces a hypoglycemia from the second hour up the sixth hour when this activity became more significant (p<0.001) (n=5). All extract administered an hour ahead, the glucose overcharged has show a protective effect since they become, as well as glibenclamide, hyperglycemia which come upon half an hour after overloading the glucose, comparatively to control rats which have received distilled water with p<0.001 (n=5). From diabetics rats, in the two type of extracts, the total or row flavonoïdes, as well as insulatard (5 UI/Kg) decreases significantly the glycemia one hour ahead and the activity is maintained up to the 5th hour (p<0.001). These results suggest that the *Trilepisium madagascariense* leaves contains anti-diabetic compound and justify their traditional use in diabetes cure.

Key words: Diabetes, hypoglycemic, anti-hyperglycemic, antidiabetics, *Trilepisium madagascariense*.

INTRODUCTION

Trilepisium madagascariense is a spontaneous plant in tropical regions related to Moraceae family. Leaves cut into strips are domestically consumed as vegetable (Schnell, 1957, Raponda-Walker and Sillans, 1961) in the republic of Congo where their commercialization is a real source of income. Research work undertaken on the plant were more focus on botany and domestication: (Berg et al. 1985; Hauman, 1984; Makita-Madzou, 1985; Ngoliele, 2003; Tailfer, 1989; Schnell, 1951), a part from the work by Ngo Teke which have shown antidiarheatic and antimicrobial effects of the bark of that particular plant (Ngo Teke, 2010) on medico-traditional basis. Several curative virtues have been attributed to the plant: in-revigorate, antidiabetic (Bouquet, 1969), antirhumatism (Diafouka, 1997), anti-anemic, anti-ophthalmic, wound bandage, antibacterial (gonorrhea), anti-diarrhea, high blood pressure effect and antidiabetic according to traditherapi.

Future perspectives at 2025 horizons suppose a global prevalence of 300 millions of adults to contract diabetic (Werstuck, 2006). In the Republic of Congo, this has become a serious issue of public health (Monabeka et

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al., 2003). Our interest in this plant as reported in this work was born out of our desire to contribute to the improvement of the prognosis of the disease among those which have been registered from traditherapists, in order to investigate hypoglycemic, antihyperglycemic and antidiabetics' properties from rats.

MATERIALS AND METHODS

Materiel

Plant Materiel

Fresh leaves of *Trilepisium madagascariense* were collected in June 2011 a plot located in district 2, Bacongo (not far from the former Faculty of Sciences in Brazzaville)

Botanical identification of the plant was done by an herbiest from centre for vegetal resources studies in comparison to the sample N° 3640 collected by J.Koechlin march 1956 in Loudima. Leaves were dried on the bench floor in the cellular and Molecular Biology Laboratory, out of sun light during 3 weeks, then grinded into powder to allow the transport and to ready for use.

Animal material

The animals used for this study were male albino rats, the *Rattus norvegicus* (Faculty of Health Science of Marien Ngouabi University, Brazzaville) strain, matured at the animal unit in the faculty of Sciences where food and drinking water were abundant .Rats was between 2 to 3 months age and selected for this study those weighing 250 to 300 g.

Methods

Diabetes type1 induction Method

Diabetes type1 induction was done by injecting in each animal, the streptozotocin dissolved in 0, 9% sodium chloride (NaCl) in the dorsal vein of penis under anesthetic, the ether diethyl for the dose of 55mg/kg .The diagnosis was done the third day, and to be selected in the experimental process, animals with empty stomach presenting the glycemia level greater or equal to 270 mg/dl. Animals use as control has received injection of NaCl at 0, 9% iv corresponding to an ml/kg at the induction time. The glycemia was dertermined using Accu-chek Softclix. A very light incision of the distal tail using razor blade was use to enable get a drop of blood which was immediately put on a large reactive beach of a strip type Accu-chek.

Extracts Preparation

*Preparation of aqueous decoctio*n

Leaves freshly collected were air dried and grinded, 200g of resulting powder were mixed with 800ml of ethanol 90° and 200ml of distilled water. After filtration with wathman paper, the filtrate was evaporated with rotative evaporator. 18,08g of dried extract was obtained with the yield of 9, 04%.

Preparation of hydro-ethanolique Macerate

Leaves freshly collected were air dried and grinded; 100 g of resulting powder were mixed with boiled distilled water. The mixture was left for 72 hours and shaked all the time. After filtration with wathman paper, macerated was evaporated with rotative evaporator. 12g of dried extract was obtained with the yield of 12%.

Preparation of extract for raw or total flavonoids (Bruneton, 2009)

In the following protocol all steps were done on ice at 4°C.
50g of fresh leaves lightly grinded were put in 500 ml glassware, 375 ml of acetone 80% were added, and the pH was adjusted at 2 by adding concentrated HCl. After 30 min of sonication, we spined at 10000 g for 10 min. The supernatant containing flavonoïds compound was collected and evaporated in a rotative evaporator. The result was stored dark and cold place in order to avoid oxidation and des photo-oxidation.

Phytochimical Screening

Staining tests in tubes were used to screen the presence or not of chemical compound [9]. The following appropriate chemicals regents : Mayer reagent, ninhydrin reagent, ammoniac, Shinoda reagent, liqueur de fehling, producing mouss-test, acetic anhydride, Stiany reagent, sulfuric acid have been use to screen respectively alkaloids, amino acids, antraquinones, flavonoïdes, glycosides, saponin, steroids, tannins and triterpenoïdes.

Evaluation of hypoglycemic activity

Five groups of 5 rats males normo-glycemic each was constituted as follow: after 16 hours, all animals receive per os, either distilled water (control group), or the extract at fixed time. Glycemia was done after 2, 4 and 6 hours.

- The group receiving especially distilled water;
- The group receiving the aqueous decoction at 400 mg/kg;
- The group receiving the aqueous decoction at 800 mg/kg;
- The group receiving the hydro-ethanolic macerate at 400 mg/kg;
- The group receiving hydro-ethanolic macerate at 800 mg/kg.

Evaluation of anti-hyperglycemic activity

This activity was evaluated by provoking high blood pressure (hyperglycemia) per os or glucose tolerance test (OGTT = Oral Glucose Tolerance Test). Different extracts was administered orally, an hour before overloading 10% glucose for 3 g/kg. This test will evaluate the capability of the extract to prevent hyperglycemia and normalize glycemia, comparing with

Table 1. photochemical analysis of the two *Trilepisium madagascariense.* Leaves extract

	Aqueous Decoction	Macerate water-ethanol
Alcaloides	+	+
Amino Acid	+	+
Anthraquinones	-	-
Flavonoïde	+	++
Glycosides	++	++
Saponines	±	±
Steroides	+	+
Tannins	+	+
Triterpenoïdes	-	+

+: presence; ++: abundant presence; ±: presence lightly remarquable; - : absence.

control animals. Five groups of the 5 rats each have been constituted:

-The group receiving distilled water or the negative control;
-The group receiving the glibenclamide at 10 mg/kg (dose therapeutiqually indiquated) or the positive control;
-2 groups receiving the aqueous decoction respectively at 200 and 400 mg/kg doses;
-The group receiving the hydro-ethanolic macerate at 400 mg/kg

The glycemia were recorded:

-At the beginning of the experiment when administrating water (control), glibenclamide and extracts;
-An hour later, during the glucose overload;
-Then, half an hour, one, two, three and five hours after the glucose overload.

Evaluation of antidiabetics activity

Male rats weighing more than 250g to 300g were first of all emptied stomach approximately 16 hours and the glycemia was evaluated in all animals before the experiment. A 60 mg of streptozotocine powder (sigma chemical Co.) was dissolved in 0, 9% saline solution. The rats were first anesthetized using ether has receive through penis veine with a dose of 60 mg/kg streptozotocine solution. 72 hours after streptozotocin injection, rats with empty stomach for 16 hours which show sincere high glycemia permanent (200 and 400 mg/dl) associated with glucosuria and a body weight loss, has been selected for study. Rats were distributed in seven groups of five each:

- The group receiving distilled water or negative control;
- The group receiving glibenclamide at 10 mg/kg or positive control;
- The group receiving the insulatard at 5 UI/Kg [18];
- The group receiving the aqueous decoction at 400 mg/kg;
- 2 groups receiving the hydro-ethanolic macerate for doses of 400 and 800mg/kg;
- Group receiving an extract of flavonoids in gross or total 400 mg/kg.

Statistical analysis

Glycemia values are express in means ± standard error for the mean Standard Error (MSE) in the table below. The Student test was use for statistical analysis of results. A p value of $p< 0.05$ was use as significance criteria of; n=5 representing experiences number.

*=P<0.05; **=P<0.01; ***=P<0.001: significance differences compare to the initial the value.

RESULTS

Staining tests in tubes have shown the presence of several chemicals substances in those extract (Table 1).

In Comparison to control rats which have receiveived distilled water, the two types of extract with chosen doses ,show a significative hypoglycemia already from the 2nd hour (P<0.05) for the aqueous decoction at 800mg/kg and P<0.01 for the two extract for 400 mg/kg and macerate at 800 mg/kg. The dose of 400 mg/kg of the aqueous decoction seem to be more efficient (p<0.001) than the dose of 800 mg/kg of the same extract (p<0.05) until the 4th hour.

The dose of 400 mg/kg of hydro-ethanolic macerate seem lightly to be more efficient (PRG=40.35%) than the same dose of the aqueous decoction (PRG = 38.91%) from the 6th hour. At that time, the doses of 400 mg/kg have show the efficiency than the doses of 800 mg/kg, but in all case, the hypoglycemia activity of each extract remain for all doses (Table 2).

Hyperglycemia treatment provoked by oral route has show that the two extracts intake, even the aqueous decoction at 200 mg/kg, provokes a significative

Table 2. Hypoglycemia activity of the aqueous decoction and of the hydro-ethanolic macerate of *Trilepisium madagascariense* leaves.

Glycemia (in mg/ dl)					
Rat Groups		**0hour**	**2hours**	**4hours**	**6hours**
Control (distilled water)	Glycemia	104.80±1.98	106.80±1.74	90.60±14.62	89.60±3.47
	PGR		-1.90%	13.55%	14.50%
Aqueous decoction at 400 mg/kg	Glycemia	95.60±6.98	84.40±5.20	68.60±3.26	58.40±2.50
	PGR		11.72% **	28.24% ***	38.91% ***
Aqueous decoction at 800 mg/kg	Glycemia	95.00±5.50	88.00±5.02	82.20±4.97	69.80±3.97
	PGR		7.36% *	13.47% *	26.52% **
hydro-ethanolic Macerate at 400mg/kg	Glycemia	102.60±5.49	89.40±2.69	79.20±3.13	61.20±2.90
	PGR		14.15% **	22.80% **	40.35% ***
hydro-ethanolic Macerate at 800 mg/kg	Glycemia	98.53±4.76	79.55±3.57	73.88±5.71	54.89±1.45
	PGR		19.26% **	25.01% ***	44.29% ***

*=P<0.05; **=P<0.01; ***=P<0.001: significance differences compare to the initial the value.
P. G .R. = percentage of glycemia reduction.

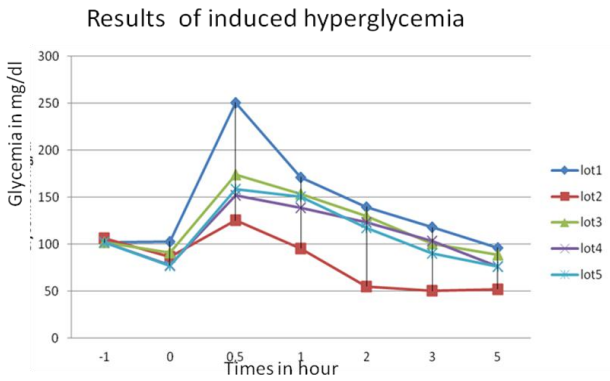

Figure 1. Results of glucose tolerance test with animals which have receive plant extracts.

decrease of glycemia. The two extracts for the dose of 400 mg/kg has better reduced the glycemia, an hour later than the glibenclamide which is the pure product: from 106,2±1,69 to 86,2±2,08 (p< 0.01) for the glibenclamide; from 102.2±1.68 to 77.6±2.76 (p< 0.001) for the aqueous decoction at 400 mg/kg and that of 102±2.91 to 77±2.05 (p< 0.001) for the hydro-ethanolic macerate.

Half an hour later after overloading the glucose, glycemia pics for animals which have received the plant extract and also the glibenclamide are less than glycemia pic shown by control animals (Figure 1). From Diabetics, an hour later after extracts intake, the hydro-ethanolic macerate at the dose of 400 mg/kg seem to be efficient after the insulator which a pure product. Flavonoïdes in absence of other chemical compound of the plant reduces significantly the glycemia and their activities persist a growing manner until 5th hour.

Plant extracts have an activity which persist afler 5 hour, when the insulator activities which reach the pic after 2 hours tend to decrease (Table 3).

DISCUSSION

Problems in diabetes treatment in modern medicine include, the treatment cost, lack of medical support and also cell resistance to insulin. This investigation was carried out in order to find naturals hypoglycemic substances by evaluating hypoglycemic anti- hyperglycemic and antidiabetic activities of *Trilepisium madagascariense* leaves.

The results obtained showed that leaves extracts of *Trilepisium madagascariense* contained active substances

Table 3. Antidiabetic activity of different extracts.

	O hour	1 hour	2 hours	3 hours	4 hours	5 hours
Control negative (distilled water)	333±11.38	347.40±18.23	312.20±24	311.60±13	306.40±14.50	296.80±13.33
P. G. R.		-4. 32	6.24	6.42	7.98	10.87
Positive Control (glibenclamide 5 mg /Kg)	328.60±12.90	308.20±23	296.60±18.52	286.20±21	279.60±14.	266.80±20
P.G .R		6.21	8.09	12.90	14.91	18.81
positif Control (insulatard 5 UI/kg)	297.80±13.92	***75.60±07.08	***51.80±07.13	***57.20±07.39	***61.40±07.80	***74±09.82
P.R.G.		74.61	82.60	80.79	79.38	75.15
aqueous Decoction 400 mg/kg	279.60±08.22	**159.60±14	***142.40±02.01	***140.80±07.01	***131.80±04.10	***120.60±07.70
P. G. R.		42.92	49.07	49.64	59.29	59.50
Macerate hydro-alcoolique 400mg/kg	357.80±05.14	***120.20±14.76	***112.80±13.62	***108±13.52	***113.6±11.01	***115.40±09.05
P.R.G.		66.40	68.47	69.82	68.25	67.74
hydro-alcoholic Macerate 800mg/kg	356.2±06.57	***196.40±14.21	***132.80±16.25	***118.60±09.60	***112.6±06.72	***100.60±08.50
P. G. R.		44.86	62.72	66.87	68. 39	71.76
Total Flavonoïdes or raw	366±5.68	***186±5.09	***164.80±3.84	***149±5.75	***128.20±4.75	***111±3.32
P.R.G.		49.18	54.97	59.29	64.97	69.67

*=P<0.05; **=P<0.01; ***=P<0.001: significance differences compare to the initial the value.
P. G .R= Percentage of Glycemia Reduction (in percentage)

with hypoglycemic, anti-hyperglycemic and antidiabetic activities. Aqueous decoction, the hydro-ethanolic macerate, as well as glibenclamide reduced significantly the glycemia in normal rats from the 2nd hour until the 6th hour. The dose of 400 mg/kg appear to be more efficient than the 800mg/kg aqueous extracts (38.91% of percentage of glycemia decrease against 26.52% from the 6th hour). At the same dose and the same time, the hydro-ethanolic macerate was found to be slightly more efficient (40.35%) than the aqueous extract, probably caused by the ethanol which more polar than the aqueous extract.

The tolerance test of glucose showed that the two extract have a protective effect (Figure 1). This finding agreed with the work of Kamtchouing (Kamtchouing et al., 1998) *Anacardium occidentale* extract. In this regards, the administration of glucose an hour later, before overloading the two extract at respective doses of 200 and 400 mg/kg for the aqueous decoction and 400 mg/kg for the hydro-ethanolic macerate, protect animals from hyperglycemia compare to control rats. In other word, these extracts and glibenclamide prevent animal from a very high hyperglycemia (250,60±10,6 of glycemia half an hour later for control rats against: 125.2±10.57 for rats treated with glibenclamide, 173.8±5.61 and 151.6±12.08 respectively for rats treated with the decoction for respective dose of 200 and 400 mg/kg, 158.8±2.92 for rats treated with the macerate.

In diabetics rats, the two extracts, as well as the insulatard are revealed to be active on diabetic rats from the first hour, as far as the pure product activity to be higher than that of the others: from 297.80±13.92 to 75.60±7.08 ($p<0.001$), either P.G.R. of 74.61% for the insulatard against P.G.R. of 42.92% (aqueous decoction at 400 mg/kg), 66.40% (hydro-ethanolic macerate at 400 mg/kg), 44.86% (hydro-ethanolic macerate) and 49.18% (flavonoides).

From the 5th hour, all extract remains more active, but the insulatard remain the more active product.

Regarding the result given by the two types of data from the two plant extracts on diabetics rats in one hand and those given by the two reference products on the other hand, it could be concluded that the mode of action of the extract are similar to that of the insulatard. The glibenclamide use for the type II diabetes was not able to reduce the glycemia in a very significative manner, whereby the insulatard prescribed for the type I diabete has well reduce the glycemia. The bioactive substances contain in this leaves has stimulated the insulino-secretion by acting on the β cell of the pancreas. We are reserved to say at the current stage of the work, that these substances regenerate the destroyed β cells by streptozotocine.

Photochemical analysis of the extracts revealed the presence of alkaloids, amino acid, flavonoïdes, glycosides, steroids, tannins, the triterpenoïdes and the saponin in a very small quantity. The flavonoïdes, substances with polar ring, are more present in the hydro-ethanolic macerate due to the higher extractability of ethanol compare to water (N'Diaye et al., 2008).

Active principles isolated and purified from plants belong mostly to the saponin and flavonoïd group (Badila, 2003; Koumar et al., 2009; Olivier, 1980; Ragunathan and Sulochana, 1994). The two putative chemical groups have anti diabetics' effect, saponins has not really revealed their presence in the extracts, that's why flavonoïds extraction was privileged.

Total or raw Flavonoïd extracts tested on diabetic gave a very significant reduction of glycemia at the 1st hour: from 336±5.68 to186±5, 09 ($p<0.001$), either a P. G. R of 49.18%. Their activity was maintained up to the 5th hour ($p<0.001$). (see results in Table)

The result given by the raw flavonoïds extract earlier from the 1st hour is less important than that of the hydro-ethanolic macerate. This let to think that there is other molecule non flavonic of the raw extract which contribute to the improvement of the antidiabetic power observed with the raw extract. We can suppose that saponins, as well as in low quantity, can contribute to the activity. Even alkaloid present in the raw extract can also improve the hypoglycemic power. This last hypothesis is in agreement with Benzi *et al*. (Benzi et al*.,* 1984) work which shows that the alkaloids isolated from *Catharanthus roseus* (Apocynaceae) are able to destroy and reduce the glucose rate in cephalic tissue of diabetics, reestablishing therefore the sensibility in animals

Conclusion

The aqueous decoction and the hydro-ethanolic macerate contain substances with hypoglycemic, antihyperglycemic and antidiabetic properties. These properties are attributed to raw flavonoïds which might act in stimulating insulino-secretion. Further work will allow knowing the flavonoïde portion which contains the antidiabetics power.

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