Anti-Hyperglycemic Activity of *Raphia gentiliana* De Wild. (*Arecaceae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author PTM designed the study and wrote the first draft of the manuscript, author TAM performed the experimental work, author BFL performed the statistical analysis, author DSTT wrote the protocol and managed the literature searches; author KNN managed biological analyses of the study. All authors red and approved the final manuscript.

Research Article

ABSTRACT

Aims: *Raphia gentiliana* De Wild fruit is edible. The aim of this work is to evaluate the anti-hyperglycemic activity of this fruit for its use as medicinal food.
Study Design: Phytochemical analysis; extraction by lyophilzation process; measure of blood glucose level; calculation of glycemic load and index.
Place and Duration of Study: National Institute of Biomedical research (DR Congo), between October 2011 and June 2012.
Methodology: The study was performed *in vivo* (mice and humans). A Dose of 0.2g/Kg of *Raphia gentiliana* fruit extracts was administered to fasting (18 hours) hyperglycemic induced NMRI mice by oral application. The kinetics profile of the blood sugar level of the hyperglycemic induced mice was evaluated using a glucometer after one to two hours of administration. For humans, the *Raphia gentiliana* fruit was taken by 45 consenting individuals. Glycemia was measured by spectrophometry and the triangle surface area ratio's method was used to calculate the glycemic and load index.
Results: The results about the anti-hyperglycemic activity in NMRI's mice showed a significant decrease in blood sugar level. After oral application of aqueous *Raphia gentiliana* fruit extracts, the decrease of 27% and 56% were observed after respectively

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one and two hours. For human subjects, observed values of glycemic index and load were -3.1% and -1.36% respectively. These negative glycemic index and glycemic load values are excepted standard.

Conclusion: The obtained results indicate that *Raphia gentiliana* fruits have an anti-hyperglycemic activity and justify the use of this plant in Congolese traditional medicine.

1. INTRODUCTION

Diabetes mellitus or simply diabetes is a metabolic disorder in which a person has abnormally high blood sugar concentration. This is due to the fact that the body does not produce enough insulin (Type 1) or cells do not respond to the produced insulin (Type 2). This high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. Both Type 1 and Type 2 are chronic and cannot be cured [1,2]. This disease remains today among dreaded diseases to mankind. The Type 2 diabetes is the most common type and is typically contacted over the age of 40 years and can also be controlled by diet and physical exercises alone. This type is due primarily to the lifestyle factors and genetics [3-5].

Nowadays the diabetes of type 1 or type 2 is a major public health problem. Its treatment represents a high cost especially for African countries. An estimated U.S. $ 194 billion worldwide cost per year for diabetes care is reported [6].

About 800 plant species have been reported to possess hypoglycemic properties. Several plant species have been used for prevention or managing diabetes by many people in the world [1,7-11].

A limited number of medicinal plant species have been studied and validated for their hypoglycemic properties using laboratory diabetic animal models and in clinical studies using human subjects. . Several medicinal plants and their products (active, natural principles, and crude extracts) have been reported in the literature as having been used to control diabetes in the African traditional system of medicine [1,9,11,12].

Raphia gentiliana, commonly known in Democratic Republic of Congo (DR Congo) as Makeke or Nteke (Equateur), Masende (Kikongo), Balempâ Bakulu (Lingala) and Libondo (Swahili) is a plant species belonging to the *Arecaceae* family [13].

Raphia gentiliana is from African origin, it is mainly found in the central basin in the province of the "Equateur" and in the province of Bandundu. In Kinshasa city, it is found especially along Congo River [14].

This plant is used in DR Congo as medicinal food for its antidiabetic and anti-inflammatory properties. Its fruits are known to be rich in edible oil [13].

The aim of this work is to perform the phytochemical study and to test the anti-hyperglicemic and glucose-lowering activity of fruit extracts of this plant using respectively mice and humans subjects as models.

2. MATERIALS AND METHODS

2.1 Plant and Biological Material

Plant materials (fruits) used in this study were collected in Bandundu (DR Congo). Botanical identification was made by Mr. Nlandu of the "Institut National d'Etudes et Recherches Agronomiques" (INERA), Science Faculty, University of Kinshasa. Voucher specimens are kept at the "INERA" herbarium service, n° Hombert 291.

2.2 Extraction and Chemical Screening

The lipids of the dried and powdered plant material (100 g) were removed by reflux heating using n-hexane. This material was divided in two parts. The first part (50g) was extracted during 48 hours in distilled water (400 ml). The aqueous filtrate was then lyophilized. The second part was submitted to ethanol 95% (100 ml) extraction.

A chemical screening was then performed on the aqueous and ethanol crude extracts. Several classes of compounds were screened, including alkaloids, polyphenols (tannins, flavonoids, anthocyanins and leucoanthocyanins), terpenoids, carbohydrates, fibbers and lipids.

2.3 Anti-Hyperglycemic Activity

Forty five fasting mice of NMRI races (males, range 1-2 months old) randomly divided in three groups of 15 mice each were used to test anti-hyperglycemic activity of the extracts. Oral glucose tolerance test (OGTT) was used to evaluate the anti-hyperglycemic activity. Briefly, temporary hyperglycemia was induced by gavage using glucose solution (200mg/ml) to fasting mice during 18 hours. Blood samples were collected from the mice's tail. The induced anti-hyperglycemic activity was measured from one to two hours after administration, in the presence of the extract of *Raphia gentiliana* (0.2 g/Kg) with glibenclamide (10 mg/Kg) (glycophage) drug as positive control and physiological solution as negative control, according to a standard protocol [6,14]. Blood glucose values were measured using a glucometer. All animals received human care in compliance with the institution's guideline and criteria for human care as outlined in the National Institute of Biomedical Research Guidelines for the Care and Use of Laboratory Animals.

2.4 Determination of the Glycemic Index and Glycemic Load

Human venous blood samples were collected by medical biologists, in the hemolysis tubes, in the presence of NaF (0.5%) solution as anticoagulant. A written consent for each patient was obtained before the experiment. The protocol was approved by the national ethic committee (N°BE117).

Fourty five persons (25 males and 20 females, age range 18-50 years old) with normal blood sugar levels were selected for this study. Thirty persons were submitted to fruits of *Raphia gentiliana* as food (0.14g/Kg), while fifteen were submitted to the glucose solution (0.07g/Kg) (standard). The glycemic values were measured in fasting, after one to two hours of administration and the calculation of the glycemic index was obtained according to the method of area ratio between the sample and the standard [15].

The glycemic index provides only the measurement of the blood absorbed carbohydrate quantity after food digestion, while the glycemic load considers the total quantity of the glucose in the normal portion, taking into account the anti-glycemic effect of the fibbers food [15,16].

The determination of blood glucose values requires tracing a calibration curve. Glucose solutions were freshly prepared and plated at various dilutions. The glycemia was carried out by enzymatic method (glucose oxidase and peroxidase) at 505 nm using the UV-Visible spectrophotometer 320SAFAS Monaco and data were analyzed using the Microcal Origin 7.5 software.

The glycemic index (GI) and glycemic load (GL) were calculated as follows [15-17]:

$$GI = \frac{S\ Food\ area}{S\ glucose\ area} \times 100 \quad or \quad GI = \frac{T1\text{-}T0}{T0} \times 100$$

S= surface   T1= value after 1 h   T0 = value in fasting

Calculation of glycemic load:

$$GL = GI \times \frac{m\ carbohydrates}{m\ food\ (in\ 100g)}$$

2.5 Statistical Analysis

Data obtained were analyzed using: One way analysis of variance, SPSS version 12 and Origin version 7.5 software. Results were expressed as mean ± SD. Differences between means were regarded at P=0.05.

3. RESULTS AND DISCUSSION

The chemical screening was carried out from aqueous and ethanolic extracts. Several classes of compounds were identified including polyphenols (tannins, flavonoids, anthocyanins, Leucoanthocyanes and quinones), alkaloids, saponins and terpenoids.

The lipids, water, proteins and minerals rates were evaluated in order to determine the carbohydrates in the fruits. This led us to determine the food quantity for the calculation of glycemic index.

Fig. 1 gives the *R. gentiliana* fruits composition in lipids, carbohydrates, humidity, proteins and minerals.
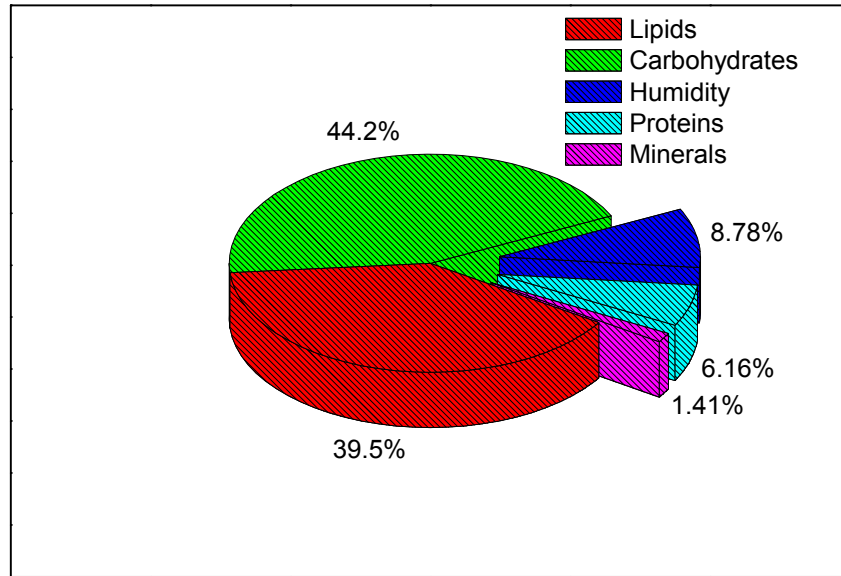
Fig. 1. Yield of *R. gentiliana* fruits composition in lipids, carbohydrates, humidity, proteins and minerals

As it can be seen in the Fig. 1, carbohydrates constitute the main part of the fruit with, 44% (35 % of fibbers and 9 % digestible carbohydrates).

Anti-hyperglycemic activity of *R. gentiliana* fruits was evaluated on mice. The mean values of blood glucose levels in fasting NMRI mice observed after glucose tolerance and *Raphia gentiliana* extract dose are listed in the following table. The dose, the average weights and blood glucose level of in fasting mice were respectively: 0.2 g/Kg, 22 g and 133 mg/dl.

Table 1. Mean values of blood glucose levels in fasting NMRI mice and *Raphia gentiliana* extract dose

Groups (mg/dl)	GT0 (mg/dl)	GT60 (mg/dl)	GT90 (mg/dl)	GT120 (mg/dl)
Negative CG	133.1± 0.2	214.4 ± 0.3	201.5 ± 0.2	201.2 ± 0.2
Group with extract	133.1± 0.2	156.6 ± 0.1	119.2 ± 0.1	95.3 ± 0.1
Positive CG	133.1 ± 0.2	155.8 ± 0.1	117.4 ± 0. 2	95.6 ± 0.2

Legend: GT0: blood glucose level measured in fasting
GT60: blood glucose level measured after 60 minutes
GT90: blood glucose level measured after 90 minutes
GT120: blood glucose level measured after 120 minutes

It can be observed from this table that there is not significative difference ($p = 0.05$) between the glycemic mean values of positive control and that of the group of mice that takes *Raphia gentiliana* fruit extract.

The Table 1 shows also a drastic decrease of glycemia for NMRI mice under the *R. gentiliana* fruits extract compared to those under the physiologic solution (NaCl 0.9%, negative control). This means the decrease rate of 27% after one hour and 56% after 2 hours.

It can be seen that there is not a significant difference between the glycemia of the positive control and the fruit extract. This indicates that the extract has interesting anti-hyperglycemic activity potential.

As the *R. gentiliana* fruits are edible, it was necessary in this work to evaluate the glycemic index and the glycemic load of these fruits used as food in human (Table 2).

Table 2. Glycemia mean values in fasting after 1h and 2h of food consumption

Glycemia (g/l)			
	00 min.	60 min.	120 min.
Control glucose	1.00 ± 0.21	1.99 ± 0.30	1.03 ± 0.12
R. gentiliana fruits	1.01 ± 0.10	0.98 ± 0.10	1.03 ± 0.11

The value of the glycemic index is obtained by the scheme of the area ratio of the Fig. 2 (Table 3).
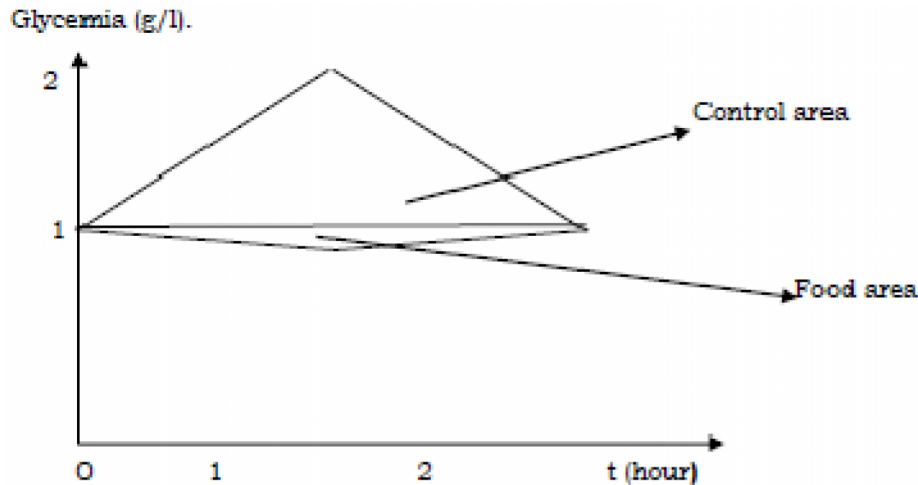

Fig. 2. Glycemic variation verses time

Table 3. Glycemic index and glycemic load values

	Area of control (cm^2)	Area of food (cm^2)	GI (%)	GL (%)
Values	10	0.31	- 3.1	- 1.36

Area is calculated as: base x height/2

The glycemic index is a parameter which measures the body absorption rate of glucose before and after taking an amount of food compared to the same amount of glucose. Its value scale ranges between 0% and 100% as a classification index food: low (0-30%), medium (30-55%) and high (55-100%) [16-18]. This index is used to measure the hyperglycemic effect of the eaten food.

According to Table 3, it can be seen that the consumption of a quantity of *R. gentiliana* containing 4.3% of carbohydrates (for 10g of fruits) does not contribute to the hyperglycemic

effect. The glycemia increase appears to be inhibited by the presence of substances contained in the fruit.

Index values and glycemic load of fruits of *R. gentiliana,* which are respectively -3.1% and 1, 36%, are excepted standard. This suggests the presence in the fruit of substances that prevent the absorption of glucose. Indeed, Thompson [19] reported that high proportion of polyphenols generally induce low glycemic index of foods. The presence of polyphenols in *R. gentiliana* fruits could explain the low glycemic index and the glucose-lowering activity. These results confirm the use of *R. gentiliana* fruits by traditional healers in DR Congo against diabetes.

4. CONCLUSION

Data obtained both in animals (fruits aqueous extract) and humans (fruits) showed that *R. gentiliana* fruits have an interesting potential to decrease blood glucose level. This anti-hyperglycemic activity could be due to the secondary metabolites such as polyphenols. This effect should be investigated in experimental diabetic subjects and bioguided studies undertaken in order to determine the active chemical groups and to isolate and elucidate structures of active compounds.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

All authors also declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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