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Opposite Effects of F1 and F5 Fractions of Total Methanol Leaf-extract of *Dialium guineense* (Cesalpiniaceae) on Blood Glucose in Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Author FSB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MS and DD managed the analyses of the study. Authors MB and AW managed the literature searches. Final manuscript review was done by author SGY. All authors have read and approved the final manuscript.

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ABSTRACT

The purpose of this study is to investigate the effects of total methanol leaf-extract of *Dialium guineense* (Cesalpiniaceae) and its fractions on blood glucose in rat.
The fractions of methanol extract were obtained by chromatography Sephadex LH 20 gel, numbered F1 to F5. Experiments were performed in normoglycemic, glucose tolerance test, and type 2 diabetic rats.
The total methanol leaf-extract (300 mg/kg, *per os*), induced a significant increase of blood glucose level in normoglycemic rats (2.27±0.12 vs 0.94±0.03 g/L) ($p<0.05$, n=5). F1 and F2 fractions (100

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mg/kg, *per os*) increased the blood glucose level. Glycaemia respectively varied from 0.90 ± 0.03 to 2. 28 ± 0.22 g/L and 0.91 ± 0.03 to 1.43 ± 0.04 g /L ($p<0.05$, n=5). However, F5 fraction (300 mg/kg, per os), induced hypoglycemia (0.61 ± 0.01 vs 0. 80 ± 0.03 g/L) ($p<0.05$, n=5). F5 fraction prevented the pic of hyperglycemia caused by glucose (4 g/kg, *per os*). In type 2 diabetic rats, the daily oral administration of F5 fraction (300 mg/kg) induced an anti-hyperglycemic effect (1.28 ± 0.15 vs 4.48 ± 0.08 g/L). Fractionation by gel permeation chromatography allowed to highlight the presence of compounds both hyper-and hypoglycemic in total methanol leaf-extract of *D. guineense*. The lack of hypoglycemic effect in normoglycemic rats of total methanol leaf-extract, could respectively be related to the presence of both hyper- and hypoglycemic compounds in F1 and F5 fractions, which induced opposite effects.

Keywords: Dialium guineense; leaves; blood glucose; normoglycemic rats; type 2 diabetes.

1. INTRODUCTION

The type 2 diabetes, which is an insidious form, usually appears in middle age, representing about 85-90% of the diabetic population. It is treated with biguanides, sulfonylureas, α-glucosidase inhibitors, thiazolidinediones, glinides and incretinomimetics, which are used as monotherapy or in combination for better management of diabetes [1,2].

The recent development of herbal medicine offers an opportunity to find molecules in plants and that may have beneficial effects on the regulation of glucose metabolism, while avoiding side effects of synthetic substances [3,4].

The use of herbal extracts remains a common practice in many regions of the world. According to the World Health Organization (WHO), nearly 80 % of people in developing countries have used traditional medicine [5]. Anti-diabetic phyto-therapy has seen renewed interest to date, with the discovery of extracts or molecules effective in the treatment of diabetes [6]. In Senegal, ethno-pharmacological surveys have revealed the use of several plants with anti-diabetic properties by traditional healers. A study at the Marc SANKHALE Center of ABASS NDAO Hospital of Dakar, had shown that many diabetic patients have resorted to the use of plant extracts for their anti-diabetic activity [7].

Dialium guineense (Cesalpinaceae) is widespread in Africa especially in Guinea, Gambia, Sierra Leone, Ivory Coast, Nigeria and Togo. In Senegal, it is found in the humid forests of Casamance but also around the area of Dakar including the forest of Mbao (Dakar). Phytochemically, it was shown that *D. guineense* contains anthraquinones, alkaloids, flavonoids, tannins and saponins [8].

D. guineense, is a useful plant in traditional medicine which is known as vernacular name "Solom" in Senegal. Its leaves are used by the traditional healers to treat type 2 diabetes.

Preliminary study had shown that the aqueous leaf-extract of *D. guineense* had no effect on blood glucose in normoglycemic rats. However, its chronic administration in type 2 diabetic rats had shown an anti-hyperglycemic effect [9].

The objective of this study was to separate the phytochemical groups of methanol leaf-extract of *D. guineense* by gel permeation chromate-graphy, and to investigate the effects of different fractions in normo- and hyperglycemic rats.

2. MATERIALS AND METHODS

2.1 Animal Material

The Wistar Kyoto rats were used in the experiments. They were raised at the department of Pharmacy at 25°C and fed to the feed mills Poulette SENTENAC Dakar. The experiments were performed with rats, weighing between 125 and 200 g.

2.2 Plant Material

D. guineense leaves were harvested in October in the forest of Mbao (Dakar). They were identified in the Pharmacognosy and Botany laboratory of Medicine and Pharmacy faculty of the Cheikh Anta Diop University (CADU) of Dakar. The leaves (specimen DPB-12-02) were dried in the shade at room temperature of about 25°C for 1 month. They were then pulverized.

2.3 Extraction

A sample of leaves of *D. guineense* was finely pulverized. The resulting powder (200 g) was

subjected to a decoction in 2000 ml of methanol for 2 hours with refrigerant system. After cooling, the total methanol extract was filtered. The filtrate was then evaporated.

2.4 Fractionation

The compounds of the total methanol leaf-extract evaporated to dryness, were isolated by Sephadex gel permeation chromatography LH 20, a technique which separates molecules according to their size. The dry residue was dissolved in methanol and deposited in small amounts on the surface of Sephadex gel LH20 contained in the column. The column was eluted with the methanol mobile phase. The fractions were collected into tubes numbered 1 to 19. A volume of about 10 ml elute was collected in each tube. This operation was repeated until all the sample were exhausted. Between two rounds of chromatography, the column was rinsed with methanol to remove the residue of the extract on the gel. A thin layer chromatography (TLC) was performed for each elute. After development, the elutes with a similar chromatogram were grouped in order to obtain a total of 5 fractions F1, F2, F3, F4 and F5.

For the TLC, 10 mg of the total extract were dissolved in 1 ml of methanol. The deposits were made using micropipettes on a microplate silica gel, 1 cm from the bottom edge of the base line, at different locations. The deposits were for total methanol extract, fractions and the various witnesses of tannins, alkaloids and flavonoids. The plate was dried in an oven for 10 seconds, and then introduced into a tank which contained the chromatographic migration solvent (methanol / hexane / water (4v/5v/1v)). After migration, the plate was removed from the tank and dried at 100°C during 5 min. The disclosure of chemical constituents was carried out by spraying potassium iodide if necessary, a UV lamp was used to identify the nature of the constituents at 366 nm. Fractions were grouped according to the report frontal and color tasks.

Consecutive spots of the same *Rf* (relative front) which have the same color were grouped in the same bottle, the spots with two different colors were collected separately [10].

2.5 Tests in Normo-glycemic Rats

The rats were fasted for 12 hours. They were divided in groups of 5 rats. At T_0, blood sampling was performed at the retro-orbital sinus. Rats were treated with physiological serum 0.9% (10 ml/kg, *per os)*, methanol leaf-extract (100 mg, 300 mg/kg, *per os*), or fractions (100, 300 mg/kg, *per os*). Blood samples were taken every hour for 4 hours [11].

2.6 Oral Glucose Tolerance Test

Two groups of five rats were first fasted for 12 hours. Blood samples were performed at T-90. Immediately after, rats were treated with physiological serum 0.9% (10 ml/kg, *per os*) or F5 fraction (300 mg/kg). Glucose (4 g/kg, *per os*) was administered to rats at T_0. Blood samples were taken every 30 min during 90 min [12].

2.7 Tests in Type 2 Diabetic Rats

Norrmoglycemic rats were treated by intra-peritoneal injection of alloxan monohydrate (150 mg/kg) dissolved in physiological serum 0.9%. After 3 days, all rats showing a positive glucosuria were assessed by the use of Keto-Diastix test strips [11]. A blood sample was taken to determine glucose. The type 2 diabetic rats were divided in groups of 5 rats:

- **Lot 1:** treated with physiological serum 0.9% (10 ml/kg/day, *per os*).
- **Lot 2:** treated with glibenclamide (0.3 mg / kg/day, *per os*).
- **Lot 3:** treated with F5 fraction (300 mg / kg/day, *per os*).

The rats were daily treated, samples were taken every two days.

2.8 Determination of Blood Glucose

Blood glucose was measured by the glucose oxidase method.

3. RESULTS

3.1 Effects of Total Methanol Leaf-extract and Its Fractions in Normoglycemic Rats

In control group, the vehicle (10 ml/kg physiological serum 0.9%) administered by oral route did not change the blood glucose in normoglycemic rats (0.98 ± 0.05 vs 0.89 ± 0.05 g / L). The methanol leaf -extract (100 mg/kg, *per os*) induced a transient decrease of blood glucose at T2h (0.72±0.02 vs 0.84±0.04 g/L) ($p<0.05$, n=5). However, at 300 mg/kg *per os*, it significantly increased glycaemia (2.27±0.12 vs 0.94±0.03 g/L, $p<0.05$, n=5) (Fig. 1). The F1 fraction (100 mg/kg, *per os*), also induced a

significant hyperglycemia (2.28± 0.22 vs 0.90 ± 0.03 g/L) (p<0.05, n=5). The hyperglycemic effect was also observed with F2 fraction (100 mg/kg, *per os*) (1.43±0.04 vs 0.91±0.03 g/L) (p<0.05, n=5). In contrast, F3 (100 mg/kg, *per os*) and F4 (100 mg/kg, *per os*) fractions administered by oral route in normoglycemic rats, did not induce hyperglycemia. The blood glucose varied from 0.88±0.08 to 0.97±0.04 and 0.91±0.04 to 0.99±0.06 g/L (Fig. 2). The F5 fraction of methanol leaf-extract dose dependently decreased glycaemia. At 100 mg/kg *per os*, the blood glucose varied from 1.00 ± 0.01 to 0.72 ± 0.03 g/l) (p<0.05, n=5). The hypoglycemic effect of F1 fraction is more important at 300 mg/kg, *per os* (0.61 ± 0.01 vs 0.80 ± 0.03 g/l) (p<0.05, n=5) (Fig. 3).

3.2 Anti-hyperglycemic Effect of F5 Fraction in Glucose Tolerance Test

The control group, pretreated with vehicle, showed a significant pic of hyperglycemia after oral administration of glucose (4 g/kg). The blood glucose varied from 0.73 ± 0.02 to 2.00 ± 0.03 g/l. Pretreatment with F5 fraction of methanol

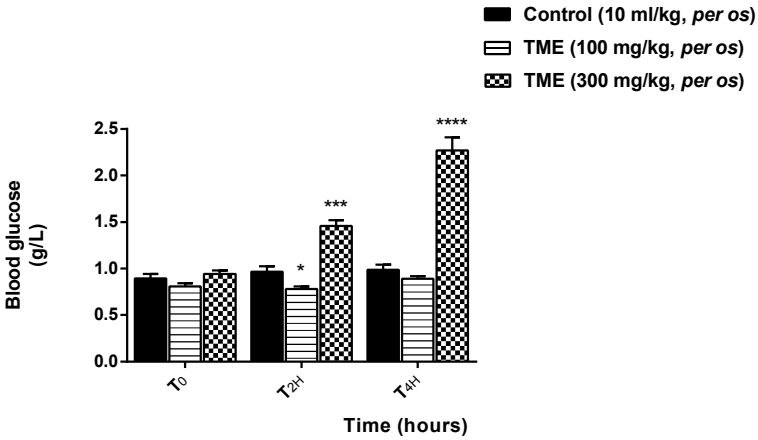

Fig. 1. Hyperglycemic effect of Total Methanol Leaf-extract (TME) of *Dialium guineense* in normoglycemic rats

**p<0.05 vs baseline, ***p<0.0001 vs baseline, ****p<0.00001 vs baseline (n=5)*

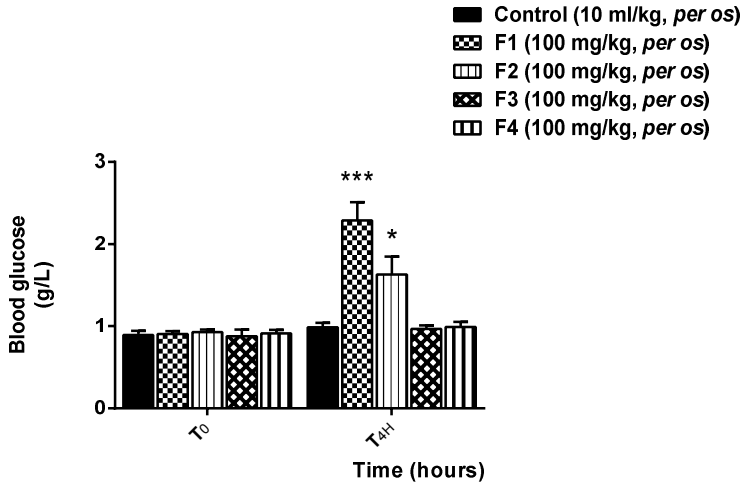

Fig. 2. Hyperglycemic effect of F1 fraction of total methanol leaf-extract in normoglycemic rats

*F1: F1 fraction, F2: F2 fraction, F3: F3 fraction, F4: F4 fraction. *p<0.05 vs baseline, ***p<0.0001 vs baseline (n=5)*

extract significantly prevented the pic of hyperglycemia. The blood glucose varied from 0.81 ± 0.02 to 1.45 ± 0.13. The prevention of hyperglycemia is significantly different to group control (Fig. 4).

3.3 Anti-hyperglycemic Effect of F5 Fraction (300 mg/kg, per os) and Glibenclamide (0.3 mg/kg, per os) in Alloxan Induced Type 2 Diabetic Rats

In control group, the hyperglycemia is permanent (3.43±0.05 vs. 3.76±0.22 g/L) after 8 days of observation. The daily oral administration of F5 fraction (300 mg/kg) induced an anti-hyperglycemic effect. The blood glucose varied from 4.48±0.08 to 1.28±0.15 g/L ($p<0.05$, n=5). In the same way, glibenclamide (0.3 mg/kg/day, *per os*) decreased the blood glucose of type 2 diabetic rats (0.89±0.05 vs 3.93±0.14 g/L, $p<0.05$, n=5) (Fig. 5).

4. DISCUSSION

Preliminary studies performed in our laboratory had shown the absence of hypoglycemic effect of aqueous leaf extract of *D. guineense* in normoglycemic rats. However, the daily administration of this extract induced an anti-hyperglycemic effect in type 2 diabetic rats. Those results suggested, either the aqueous leaf extract of *D. guineense* contained exclusively anti-hyperglycemic compounds, or a low concentration of hypoglycemic molecules.

The present study attempts to separate the phytochemical compounds of total methanol leaf-extract by gel permeation chromatography. The different fractions were tested in normoglycemic and the type2 diabetic rats induced with alloxane. The total methanol leaf- extract did not induce hypoglycemia in normoglycemic rats. The results are similar to those previously obtained with the aqueous extract of the leaves of *D. guineense* [9].

Several studies had already shown the possible co-existence in a same fraction or a plant part, of compounds that could cause opposite effects [13]. In normoglycemic rats, the absence of hypoglycemic effect of methanol extract of *D. guineense* leaves, could be probably associated to the presence of hypo- and hyperglycemic molecules in the extracts.

The different fractions of methanol leaf-extract of *D. guineense*, were tested in diabetic models. The F5 fraction significantly induced a dose dependent hypoglycemia. In contrast, the F1 and F2 fractions had hyperglycemic effect in normoglycemic rats. These results insinuate the presence of both hypo- and hyperglycemic compounds in the total methanol extract.

Glibenclamide decreased blood glucose by insulin- secretion underlying the potassium channel of β-pancreatic membrane cells. Also, the sulfonyl (thio) urea derivative induction of insulin secretion is at least mediated by channel

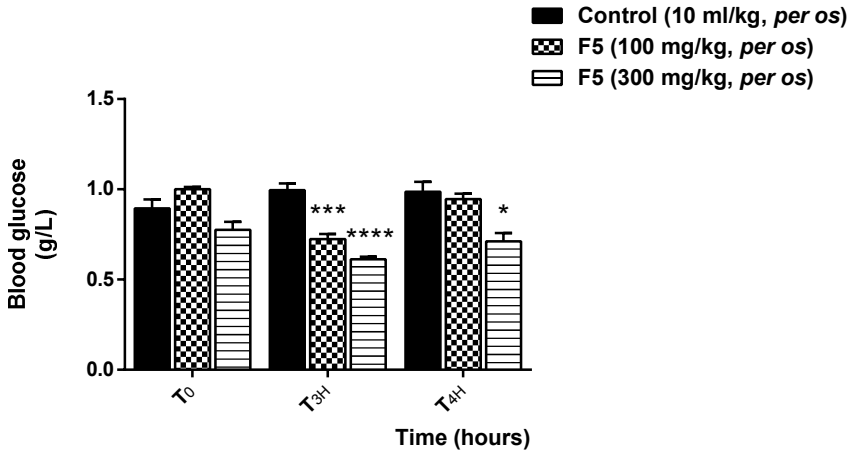

Fig. 3. Hypoglycemic effect of F5 fraction of total methanol leaf-extract in normoglycemic rats
*F5: F5 fraction. *p<0.05 vs baseline, ***p<0.0001 vs baseline, ****p<0.00001 vs baseline (n=5)*

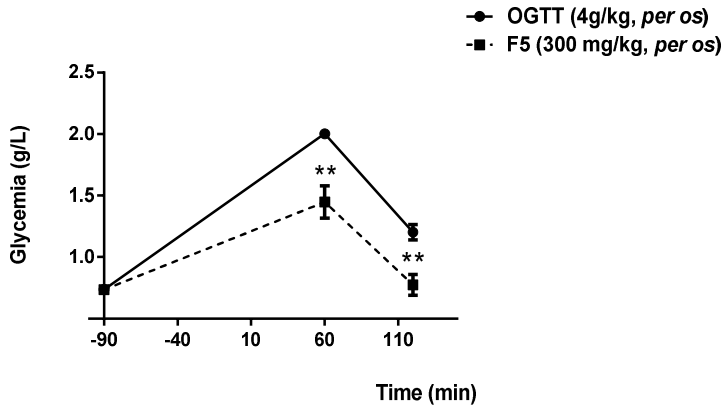

Fig. 4. Anti-hyperglycemic effect of F5 fraction of total methanol leaf-extract in glucose tolerance test

*OGTT: oral glucose tolerance test. F5: F5 fraction. **p<0.001 vs control (n=5)*

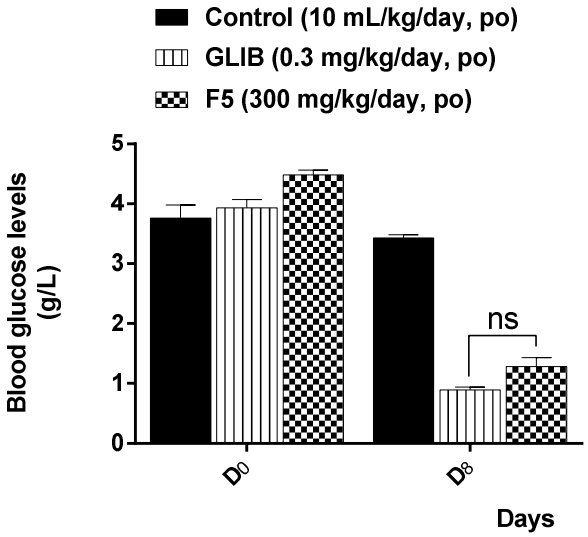

Fig. 5. Anti-hyperglycemic effect of F5 fraction of total methanol leaf-extract in type 2 diabetic rats

F5: F5 fraction. GLIB: glibenclamide. n=5. ns: not significant vs glibenclamide

transduction [14]. The compounds of F5 fraction induces its hypoglycemic effect in normoglycemic rats probably by insulin release, such as a traditional antidiabetic plant *Ficus deltoidea* var. *trengganuensis* which triggers insulin secretion from pancreatic beta cells [15].

Several plant extracts produced an anti-hyperglycemic effect through a mechanism involving insulin secretion. In fact, *abrus precatorius* leaves methanol extract showed a significant anti-hyperglycemic effect and insulin secretagogue activity in streptozotocin-induced diabetic rats [16]. It was also reported that *Lupinus mutabilis* extract exerts an anti-diabetic effect by improving insulin release in type 2 diabetic Goto-kakizaki rats [17].

In the present study, compared to control group, the F5 fraction induced an anti-hyperglycemic effect in both glucose tolerance test and alloxane-induced type 2 diabetic rats. F5 fraction would induce its anti-hyperglycemic effect through a mechanism involving insulin secretion. The opposite effects of F1 and F5 fractions on blood glucose level, could explain the lack of

hypoglycemic effect in normoglycemic rats of total methanol leaf-extract of *D. guineense.*

5. CONCLUSION

F1 and F5 fractions of total methanol leaf-extract of *D. guineense* induce opposite effects on blood glucose level in normoglycemic rats, explaining the lack of hypoglycemic effect of total aqueous and methanol leaf-extracts in acute administration. F5 fraction-decreased blood glucose level in both normoglycemic and type 2 diabetic rats, might justify the anti-hyperglycemic effect of total methanol leaf-extract of *D. guineense* in chronic administration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were examined and approved by the appropriate ethics committee (0373/2019/ CER/UCAD).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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