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Effects on Blood Glucose of Ethyl Acetate-Butanol Fraction of *Dialium guineense* Aqueous Leaf Extract (Cesalpiniaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: The overall objective of the study was to evaluate the effect on blood glucose of the ethyl acetate-butanol fraction (EABF) compounds of the aqueous leaf extract of *Dialium guineense* (Cesalpiniaceae).
Materials and Methods: The powder of *D. guineense* leaves was subjected to decoction. The aqueous solution was respectively fractionated with ethyl acetate and butanol. The sub-fractions were grouped to form the ethyl acetate-butanol fraction (EABF), enriched in flavonoids and tannins.

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The condensed tannins of EABF were eliminated under the action of casein, resulting in a condensed tannin-free fraction (EAB-TFF). EABF and EAB-TFF were phytochemically characterized and tested in normo-glycemic rats, a glucose tolerance test and type 2 diabetic rats.
Results, Analysis and Discussion: EABF (300 mg/kg, *per os*) has no significant effect on blood glucose in normo-glycemic rats (0.80±0.12 vs 0.81±0.01 g/L). Under the same conditions, EABF without tannins (EAB-TFF: 300 mg/kg, *per os*) is hypoglycemic (0.57±0.05 vs 0.82±0.05 g/L). These results suggest the existence of an antagonism, in the effects on blood glucose, between tannins and probably flavonoid-like compounds. In type 2 diabetic rats, the daily administration of EABF (300 mg/kg/day, *per os*) varied blood glucose from 2.73±0.39 to 1.14±0.58 g/L (n=5, p<0.05). Similar effects were observed with EAB-TFF (300 mg/kg/day, per os), administered under the same conditions (1.12±0.04 g/L vs 3.01±0.5 g/L) (p<0.05, n=5). EABF has no effect on blood glucose in normo-glycemic rats, whereas under the same conditions, EAB-TFF induces an hypoglycemic effect. These results suggest the existence of compounds acting in opposite directions, in the regulation of blood glucose.

Keywords: D. guineense; leaves; tannins; flavonoids; blood glucose; normoglycemic rats; type 2 diabetes.

1. INTRODUCTION

Diabetes is a metabolic condition characterized by chronic hyperglycemia resulting from a lack of secretion or action of insulin or these two associated abnormalities [1]. The global prevalence of diabetics was estimated at 246 million. It is predicted that Africa alone will attain 15 million diabetics by 2025 [2]. Type 1 diabetes is linked to a drying up of insulin secretion, and usually occurs among children and adolescents, although few cases are diagnosed among the adult population. Its treatment is based on insulin therapy [3]. Type 2 diabetes, which is an insidious form, usually manifests in the 40s and represents 85-90 % of the diabetic population. It is treated with biguanides, hypoglycemic sulfonamides, α-glucosidase inhibitors, thiazolidinediones, glinides and incretiomimetics, that are used as monotherapy or in combination for a better management of diabetes [4].

The rise of herbal medicine offers an opportunity to find in the plant kingdom molecules which are likely to exert beneficial effects on the regulation of carbohydrate metabolism, while avoiding the adverse effects of some drugs from the existing therapeutic arsenal.

According to the World Health Organization (WHO), nearly 80 % of people in developing countries and in Africa region have used traditional medicine at least once [5].

In Africa, herbal medicine is often the first remedy for some patients. In Senegal, several plants with antidiabetic properties are used in traditional medicine [6]. A study conducted at the Marc Sankhalé Centre of Abass Ndao Hospital, showed that many patients used plant extracts with antidiabetic activity [7]. This enthusiasm can be probably explained by the decline of purchasing power, the high cost of conventional medicines, and mistrust of synthetic products [8].

Dialium guineense (Cesalpiniaceae) is a plant of the traditional Senegalese pharmacopoeia commonly called «Solom». In Senegal, *D. guineense* is found in maritime Casamance in the forests of *Parinari excelsa*. It is usually common in wet soils, along the brackish bolons of Casamance, from the Saloum Islands to the north of Dakar. It exists in the shady ravines of the hills of the region of Tambacounda [8]. In traditional environments, the decoction of leaves is used as an antidiabetic, antipyretic and revitalizing. The juice of the leaves is used by pregnant women against stomach aches. In the treatment of generalized edema, incorporate leaf powder into all foods and rub vigorously with a “cap” of fresh leaves [8].

Phytochemically, a previous study had shown the presence of alkaloids, anthraquinones, flavonoids, saponins and tannins in the leaves of *D. guineense* [9]. The aqueous extract of *D. guineense* leaves containing tannins and flavonoids has no hypoglycemic effect in normoglycemic rats in acute administration, whereas it is antihyperglycemic in daily administration in diabetic type 2 rats [6]. However, the F5 fraction of the methanol extract of *D. guineense* leaves containing flavonoids and tannins is both hypoglycemic in normoglycemic rats and anti-hyperglycemic on a glucose tolerance test [10].

The purpose of that study was to assess the possible involvement of flavonoids and/or tannins in the regulation of blood glucose in various diabetes studies models.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *D. guineense* were harvested in Cabrousse in the region of Ziguinchor (Senegal) in December 2018. They were identified at the Botanical Laboratory of the Faculty of Medicine, Pharmacy and Odontology of Cheikh Anta Diop University (CADU) of Dakar, then dried at the Laboratory of Pharmacology and Pharmacodynamics of the same Faculty. After being dried in the shade for two weeks at room temperature (25 °C), the leaves were pulverized using a Brabender® electric crusher. The powder obtained has a bitter taste and green colour. It has a sternutatory power.

2.2 Animal Material

It consists of normoglycemic rats of the Wistar Kyoto strain. The rats are bred at the Laboratory of Pharmacology and Pharmacodynamics pet store, at 25 °C under light during the day and darkness at night. They were fed with « Poulette » of SENTENAC® mills from Dakar and had free access to tap water.

2.3 Experimental Procedures

2.3.1 Extraction

A sample of dried leaves of *D. guineense* was finely ground. The powder obtained (200 g) has been subjected to decoction in 2 L of distilled water for 30 min under reflux. After cooling, the mixture was filtered into an Erlenmeyer.

2.3.2 Preparation of the Ethyl Acetate-Butanol Fraction (EABF)

The aqueous solution obtained previously was subjected to a liquid-liquid separation successively with ethyl acetate and n-butanol [11].

2.3.2.1 Ethyl acetate separation

In the aqueous extract (300 ml) of *D. guineense* leaf powder, 300 ml of ethyl acetate (v/v) were added. After agitation, the mixture has been slept during 45 mn. The aqueous and ethyl acetate phases were then separated. The residual aqueous phase has been further fractionated with 100 ml of ethyl acetate for 15 min. This process was repeated twice. The different organic phases were combined and evaporated at Rotavapor®.

2.3.2.2 n-butanol separation

The previously depleted aqueous phase with ethyl acetate was fractionated with n-butanol under the same conditions as separation with ethyl acetate. The ethyl acetate and butanolic phases were combined and evaporated with Rotavapor®, resulting in an ethyl acetate-butanol fraction (EABF).

2.3.3 Fixation of tannins of the ethyl acetate-Butanol fraction (EABF)

EABF was dissolved in 300 ml of distilled water. Tannins have been complexed by the addition of 5 g of casein [12]. The mixture has been stirred for 3 hours and filtered. The absence of tannins in the filtrate was confirmed by the Stiasny reaction of mixing 4 ml of filtrate and 2 ml of reagent (4: 2 v/v). An absence of pink precipitate indicates that the EABF is devoid of tannins [13]. This process resulted in a tannin-free EABF (EAB-TFF).

2.3.4 Thin Layer Chromatography (TLC) of flavonoids and tannins

2.3.4.1 Preparation of extracts

Three milligrams of EABF were dissolved in 10 ml of ethanol. After filtration, the extract collected was deposited on chromatography plates.

2.3.4.2 Phytochemical screening

The freshly prepared EABF was qualitatively tested for the presence of flavonoids and tannins through established methods [14].

2.3.4.3 Tannin Thin Layer Chromatography (TLC)

Support: silica
Migration solvent: ethyl acetate/methanol/water (40v/8v/5v)
Deposits:
• Control: gallic acid
• EABF
• Developer: Ferric chloride solution (FeCl3) [15].

2.3.4.4 Flavonoid Thin Layer Chromatography (TLC)

Support: cellulose
Migration solvent: 15 % acetic acid in water
Deposits: Witness: Rutin, EABF
• Revealers: 5 % aluminum chloride (AlCl3) solution in water-methanol mixture (1v/1v) and UV lamp at 366 nm [16].

2.3.5 Pharmacological Testing

2.3.5.1 Normoglycemic rat tests

The rats have been fasted during 12 hours. They were divided into batches of 5 rats. At T0 time, a blood sample was taken from the retro-orbital sinus. Physiological water (10 ml/kg, *per os*), EABF and EAB-TFF (100 and 300 mg/kg *per os*) were administered. Blood samples have been taken away every hour during 4 hours.

2.3.5.2 Glucose tolerance test

Five batches of 5 rats have been fasted during 12 hours, blood samples have been taken away at T-90 min, that is 90 min before glucose *per os* (4 g/kg). Immediately thereafter, rats were fed with physiological water (10 ml/kg), EABF (100 and 300 mg/kg) and EAB-TFF (100 and 300 mg/kg). At T0 time, a blood sample has been taken away at the retro-orbital sinus, followed by gavage of rats with a glucose solution at 4 g/kg. Blood samples have been taken away every 30 min during 120 min.

2.3.5.3 Tests in type 2 diabetic rats

Type 2 diabetes was induced in normoglycemic rats by intra-peritoneal (IP) injection of alloxan monohydrate at 150 mg/kg body weight, in solution in physiological serum. After 72 h, the rats developed positive glycosuria, which was appreciated with Keto-Diastix test strips. A blood sample was taken away to determine zero day's blood glucose.

Rats with hyperglycemia between 2 and 3 g/L were selected and divided into 5 lots of 5 rats:

Lot 1: Physiological serum (10 mg/kg, *per os*);
Lot 2: EABF (100 mg/kg, *per os*);
Lot 3: EABF (300 mg/kg, *per os*);
Lot 4: EAB-TFF (100 mg/kg, *per os*);
Lot 5: EAB-TFF (300 mg/kg per os);

Rats were daily fed and blood samples have been taken away every other day during 8 days of observation.

2.3.6 Determination of blood glucose

Blood glucose levels were determined by the Accu-chek glucose monitor.

2.3.7 Analysis and expression of results

The results were expressed as mean ± standard error of the mean (mean±sem). The homogeneity of the different groups was verified by variance analysis (ANOVA). The statistical comparison was done with the Student test. A value of $p<0.05$ has been set as the significance threshold, n = 5 is the number of experiments in each group.

3. RESULTS

EABF thin-layer chromatography (TLC) revealed the presence of major constituents such as flavonoids and tannins.

3.1 Flavonoid TLC

The TLC results for EABF show the presence of flavonoids (Table 1, Fig. 1).

Table 1. Flavonoids specific characterization by TLC

Deposits	Front report	Spots
Witness : rutine	0.76	Yellow
EABF		
Spot 1	0.76	Yellow
Spot 2	0.94	Yellow

3.2 Tannin TLC

The migration of the EABF has revealed the presence of tannins, which are characterized by black spots (Table 2, Fig. 2).

Table 2. Tannins specific characterization by TLC

Deposits	Front report	Spots
Witness: gallic acid (GA)	0.79	Black
EABF		
Spot 1	0.52	Black
Spot 2	0.63	Black
Spot 3	0.68	Black
Spot 4	0.74	Black

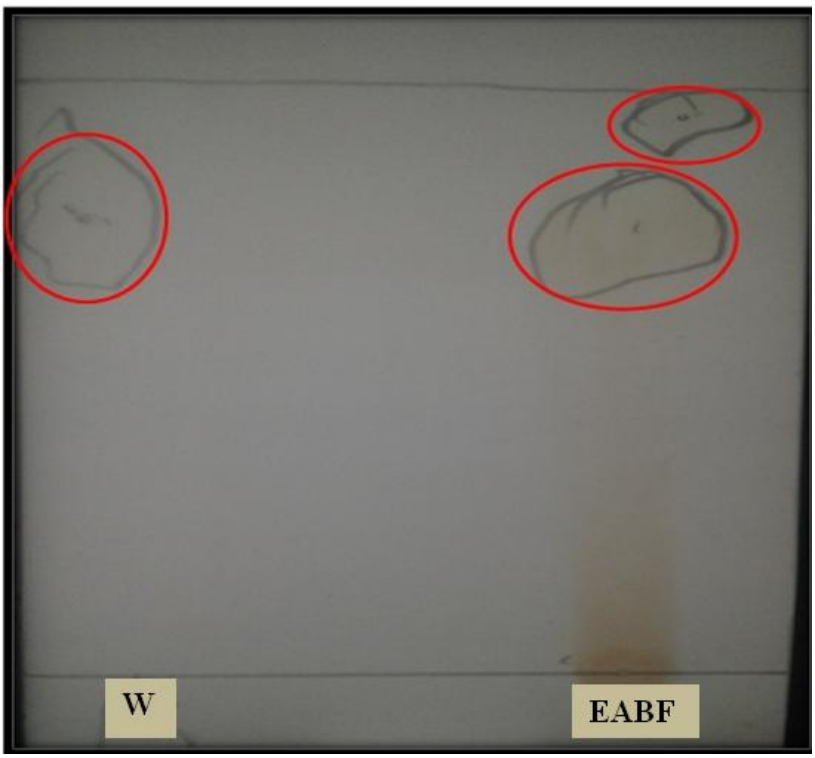


Fig. 1. TLC Migration of EABF flavonoids
W (Witness) = Rutin

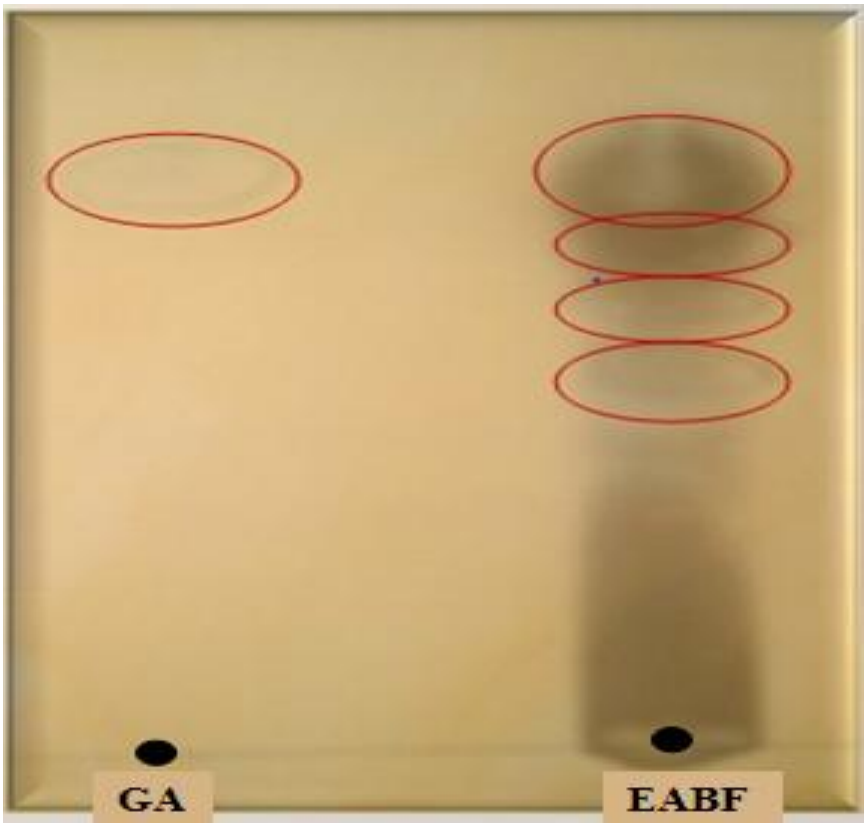


Fig. 2. TLC Migration of EABF tannins
GA = Gallic acid

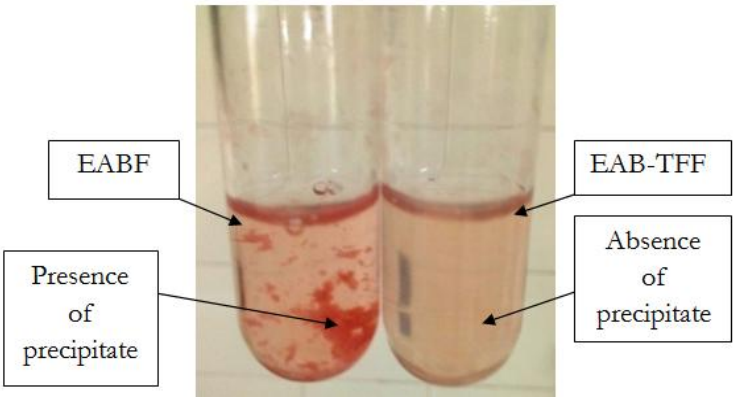


Fig. 3. EABF tannin complexation

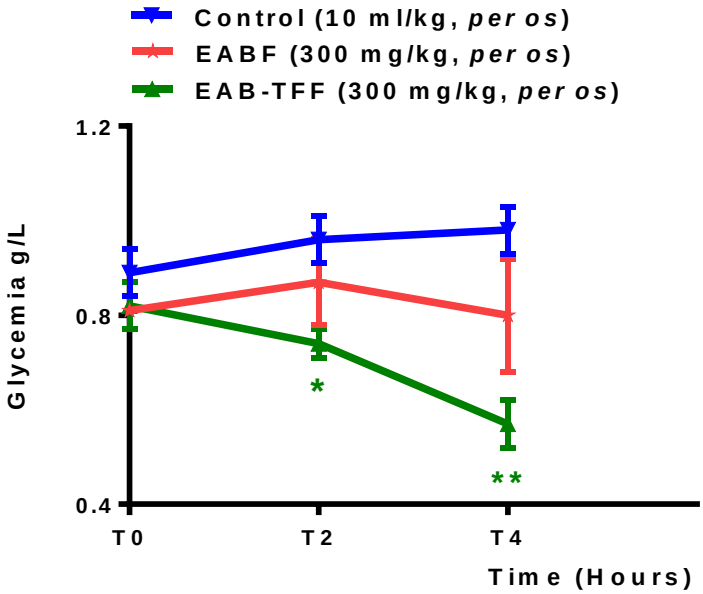


Fig. 4. Hypoglycemic effect of EAB-TFF of *D. guineense* aqueous leaf extract
**p<0.05 vs baseline, n=5*

EAB-TFF does not show precipitates after the Stiasny reaction, suggesting the absence of tannins (Fig. 3).

3.3 Pharmacological Results

3.3.1 Normoglycemic rat tests

The administration of physiological water (10 ml/kg, *per os*) does not modify the baseline blood glucose in rats (0.98±0.05 vs 0.89±0.05) (ns, n=5). Administration of EABF at 300 mg/kg *per o,s* does not significantly affect blood glucose (0.77± 0.10 vs 0.81±0.01) (ns, n=5). However, administration of EAB-TFF at 300 mg/kg *per os,* induces a significant hypoglycemia (0.57 0.05 vs 0.82 0.05 g/L) (p<0.05, n=5) (Fig. 4).

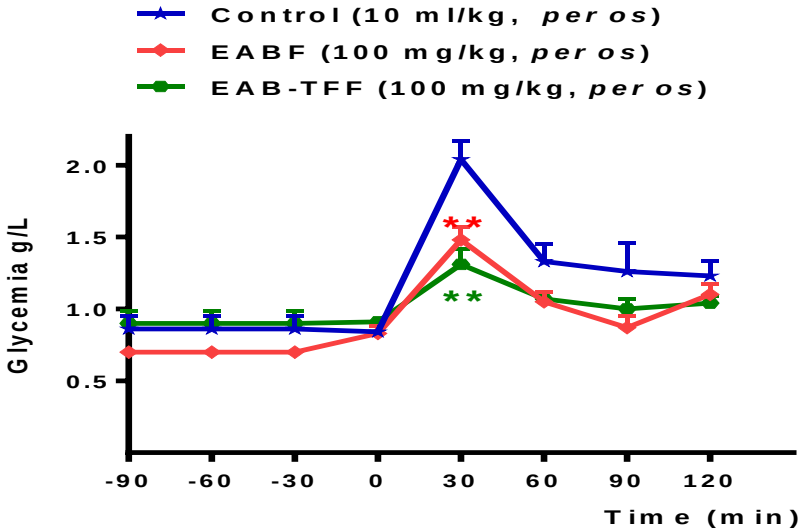
3.3.2 Glucose tolerance tests

In the control group, rats previously treated with physiological water (10 ml/kg, *per os*), glucose administration (4 g/kg, *per os*) induces a frank hyperglycemia whose peak appears after 30 min (2.04±0.13 vs 0.81±0.06 g/L) (p < 0.05, n=5). In a glucose tolerance test, the pretreatment of rats with EABF (100, 300 mg/kg, *per os*) dose-dependently prevents the peak of hyperglycemia. At 100 mg/kg *per os*, blood glucose varies from 0.83±0.05 to 1.48±0.09 g/L. This variation was significantly different from the control group (2.04±0.1 vs 1.48±0.09 g/L) (p<0.05, n=5). Similar results were observed with EAB-TFF (300 mg/kg, *per os*), administered under the

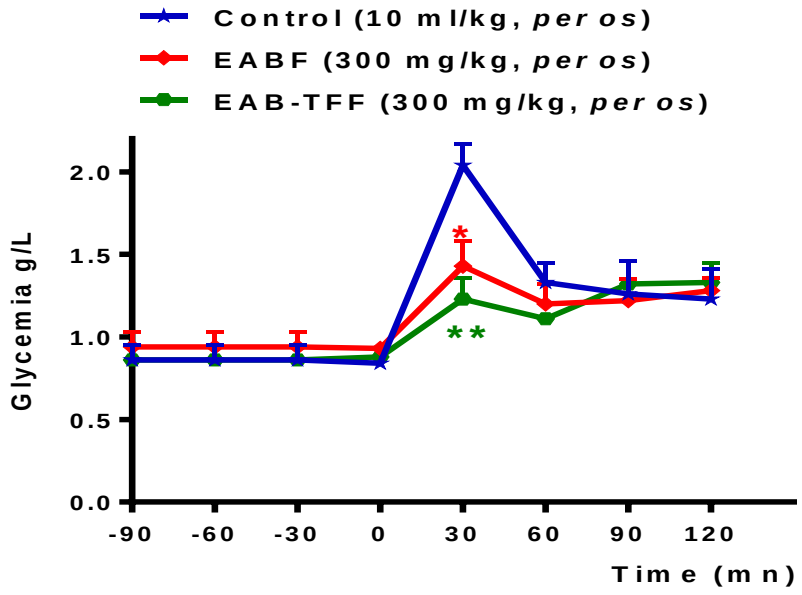
same conditions (1.23±0.13 vs 0.86±0.04 g/L) (Fig. 5).

3.3.3 Tests in Type 2 Diabetic Rats

Daily administration of EABF (100 mg/kg, *per os*) varies blood glucose from 3.26±0.61 to 1.14±0.13 g/L (p<0.05; n=5). An identical anti-hyperglycemic effect was observed at 300 mg/kg *per os* (1.14±0.58 vs 2.73±0.39 g/L) (P<0.05, n=5). Daily administration of the EAB-TFF fraction (100 mg/kg, *per os*) reduces blood glucose to 3.05±0.2 to 1.26±0.1 g/L (p<0.05; n=5). At the dose of 300 mg/kg *per os*, the variation in blood glucose decrease is 1.12±0.04 g/L vs 3.01±0.5 (p<0.05, n=2) (Fig. 6).



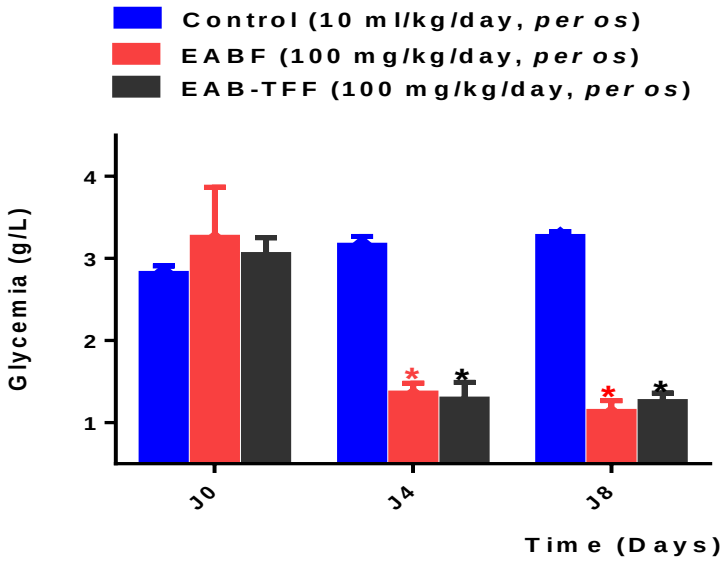
a- EABF et EAB-TFF (100 mg/kg, *per os*)



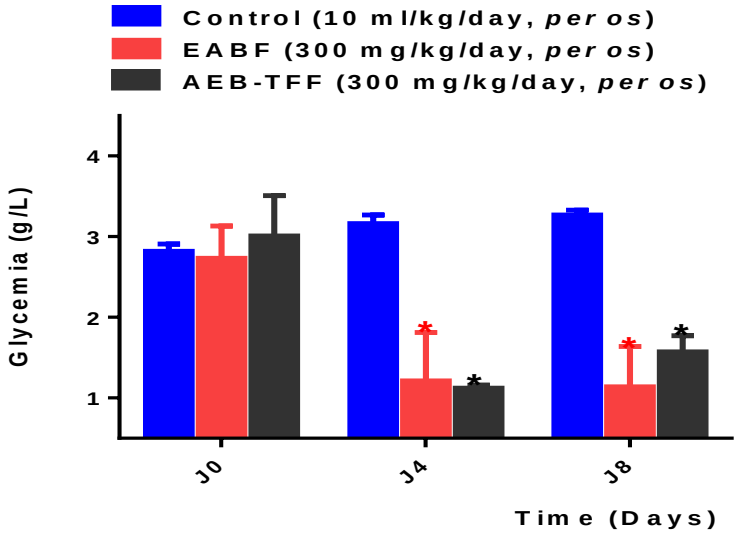
b- EABF et EAB-TFF (300 mg/kg, *per os*).

Fig. 5 (a and b). Anti-hyperglycemic effect of EABF and EAB-TFF of *D. guineense* aqueous leaf extract in glucose tolerance test

**p<0.05 vs control, n=5*



a- EABF and EAB-TFF (100 mg/kg/day, *per os*).



b- EABF and EAB-TFF (300 mg/kg/day, *per os*).

Fig. 6 (a and b). Anti-hyperglycemic effect of EABF and EAB-TFF of *D. guineense* aqueous leaf extract in type 2 diabetic rats

**p<0.05 vs baseline, n=5*

4. DISCUSSION

Previous work had shown the absence of hypoglycemic effect of the aqueous extract of *D. guineense* leaves, whereas under the same conditions, this extract is anti-hyperglycemic, in chronic administration in type 2 diabetic rats [5]. In addition, studies to fractionate cephadex gel from the methanol extract of *D. guineense* leaves had shown the existence of an hyperglycemic F1 and hypoglycemic F5 fractions. The latter contains flavonoids [17]. Therefore, these various works suggest the presence of phytochemicals probably different and resulting in opposite effects on blood glucose in the aqueous and methanol extracts.

The objective of that study was to evaluate the effect of *D. guineense* leaves containing flavonoids and tannins on blood glucose. Indeed,

in normoglycemic rats, EABF has no effect on basic blood glucose, whereas under the same conditions it induces an anti-hyperglycemic effect on the glucose tolerance test. This result is reminiscent of the profile of aqueous and methanol extracts of *D. guineense* leaves, in normoglycemic rats and on hyperglycemic models [5,17].

Previous studies had reported the possibility of coexistence in the same extract or part of a plant of compounds that may have opposite effects [18]. It had also been shown that there are compounds in the methanol extract of *D. guineense* leaves that have opposite effects on blood glucose regulation [19]. In this study, EABF containing flavonoids and tannins has no effect on the basic blood glucose of normoglycemic rats and anti-hyperglycemic agents in the glucose tolerance test and in type 2 diabetic rats. The absence of hypoglycemic effect of EABF in normoglycemic rats, may be caused by the presence of compounds with opposite effects on basic blood glucose in the extract. To support this hypothesis, the tannins of the ethyl acetate-butanol fraction were fixed with casein.

EAB-TFF is hypoglycemic in normoglycemic rats. These results suggest that tannins in *D. guineense* leaves have a hyperglycemic action, which would also explain the hyperglycemic effect of the F1 fraction of *D. guineense*, observed in previous studies of Barboza et al. [19].

The absence of hypoglycemic effect of total aqueous and methanol extracts, reported by previous work, could be attributed to the presence of tannins in these extracts.

It is recognized that the hypoglycemic effect of sulfonylurea such as glibenclamide involves insulin secretion in normoglycemic rats. In this study, the tannins of *D. guineense* leaves could oppose the functional effect, the insulin-secretory effect of the hypoglycemic compounds of EABF.

On the glucose tolerance test, the variation in the anti-hyperglycemic effect of EABF is identical, in the presence and absence of tannins. These observations suggest that the anti-hyperglycemic effect of EABF on the glucose tolerance test and in type 2 diabetes could involve a compound, different from that responsible for the hypoglycemic effect in normoglycemic rats.

The isolation of the EABF compounds from *D. guineense* leaves, could allow to highlight the molecules responsible for the hypo- and anti-hyperglycemic effects of the leaves of this plant.

5. CONCLUSION

EABF of *D. guineense* leaf extract, containing flavonoids, tannins and free of alkaloids, has no effect on the basic blood glucose levels of normoglycemic rats, whereas EAB-TFF is hypoglycemic under the same conditions. These results suggest the existence of a functional antagonism between tannins and other compounds, probably flavonoid type. The anti-hyperglycemic effect of EABF and EAB-TFF is linked to the presence of molecules different from those responsible for the hypoglycemic effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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