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## Evaluation of antihyperglycemic effect of dried Okra (*Abelmoschus esculentus*) seed infusion alone and in combination with antidiabetic drugs in Wistar rats

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### ABSTRACT

**Background:** Diabetes is a major health challenge, particularly in low-income countries. Okra (*Abelmoschus esculentus*), widely consumed in Côte d'Ivoire, is traditionally used to help reduce blood sugar. **Objective:** This study aimed to evaluate the antihyperglycemic effect of an infusion of dried Okra seeds in rats, both alone and in combination with conventional antidiabetics (metformin and gliclazide). **Materials and Methods:** A qualitative and quantitative phytochemical characterization was performed. The antihyperglycemic activity was tested *in vivo* on Wistar rats subjected to an oral glucose tolerance test (OGTT). Eight groups of rats with six (6) animals per experimental group were constituted: controls, treated with okra alone (195 mg/kg), metformin (10 mg/kg), gliclazide (100 mg/kg), and combinations of Okra 195 mg/kg + metformin 10 mg/kg and okra 195 mg/kg + gliclazide 100 mg/kg. **Results:** Administration of the Okra seed infusion (195 mg/kg) alone, which contained flavonoids, alkaloids, tannins, sterols, polyterpenes, and quinones, was not capable reducing orally induced hyperglycemia. Its combination with metformin (10 mg/kg) was safe, bringing blood glucose to 0.85 g/dL at T120, while with gliclazide (100 mg/kg), marked hypoglycemic effect, with glucose levels dropping to -31.15% below baseline at 120 min. **Conclusion:** The infusion of dried Okra grains lacks significant antihyperglycemic potential on its own. Its combination with metformin appears beneficial in a nutritional approach. Great caution is advised with concomitant use of sulfonylureas due to the risk of hypoglycemia. Further studies are recommended to explore molecular mechanisms and long-term effects.

**Keywords:** *Abelmoschus esculentus*, Diabetes mellitus, Antihyperglycemic activity, Oral glucose tolerance test (OGTT), Herb-drug interaction, Wistar rats.

### INTRODUCTION

Diabetes mellitus, a metabolic disorder characterized by chronic hyperglycemia, is no longer a disease exclusive to developed nations [1]. Today, over 500 million adults live with diabetes worldwide and three-quarters of them reside in low-income countries [2]. In Côte d'Ivoire, national prevalence of this disease was estimated at 5.19% [3]. A recent survey conducted by International Diabetes Federation (IDF) revealed that 77% of people with diabetes have developed anxiety, depression, and other mental health disorders [4]. Also, diabetes complications can affect vital organs such as heart, blood vessels and kidneys [5], and the cost of medication can be a barrier to effective disease control. Therefore, it is essential to effectively manage diabetes mellitus to significantly reduce risk of complications, by searching alternatives therapeutic like traditional medicines or functional foods.

Okra, *Abelmoschus esculentus* (L.) Moench (Malvaceae), is a widely consumed vegetable, particularly in Côte d'Ivoire. Popular claims attribute antidiabetic properties to Okra without Scientific's proofs, because of its low calorie (33 kcal/100 g) but high fiber content (10%) and its consumption could help reduce overall glycemic index of a meal [6], by slow digestion and delaying glucose absorption.

The general objective of this study was to evaluate impact of an infusion from dried Okra seed powder on blood glucose levels and also observed pharmacodynamics effects of combining this extract with conventional antidiabetic drugs.

## MATERIAL AND METHODS

### Chemicals and reagents

Okra grain powder infusion; Metformin hydrochloride (Glucophage®, Merck Santé, France); gliclazide (Diamicon®, Servier, France); D-glucose monohydrate (Pharmivoire, Côte d'Ivoire); sodium chloride 0.9% solution (Laborex Côte d'Ivoire) were used for pharmacodynamics effects evaluation, Folin-Ciocalteu reagent, gallic acid, quercetin, atropine sulphate, aluminium chloride, sodium carbonate, hydrochloric acid, Dragendorff reagent, Bouchardat reagent, ferric chloride, acetic anhydride and sulfuric acid were used for analytic evaluation and purchased from standard commercial suppliers.

### Experimental Animal

A total of 48 Wistar rats (24 males and 24 females) were used in this study, with six (6) animals per experimental group.

Male and female albino Wistar rats weighing between 160-200 grams were employed for antihyperglycemic potential evaluation. Animals were supplied from the animal facility of the Faculty of Pharmaceutical and Biological Sciences, Université Félix Houphouët-Boigny, Côte d'Ivoire. Rats were fed FACI® pellets (Manufacture of Ivorian Compound Feeds) and had free access to drinking water. Acclimatization occurred in hygienic, spacious plastic cages containing wood shavings at ambient temperature  $26 \pm 1$  °C,  $50 \pm 5\%$  humidity, and 12 h light-dark cycles.

### Ethical considerations

The study was conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (National Research Council, 8th Edition, 2011) and the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The study was approved under reference number UFHB/JAN/2025/28.

### Plant material and preparation

Dried Okra grains (*A. esculentus* variety GB1450) served as plant drug for this study. Fruit, fresh seeds, dried seeds are presented in Figure 1. Grains were purchased from National Center for Agronomic Research (CNRA) in Bouaké, Côte d'Ivoire [8]. In Pharmacology laboratory processing involved grinding grains using a mixer-grinder (Restsch GM 300 TM) to obtain fine powder with approximately 1 mm granulometry, stored in hermetically sealed plastic containers at  $23 \pm 2$  °C.

Okra infusion was prepared according to Choy *et al.* 2012 methodology [9]. Two hundred grams of Okra grain powder were introduced into two liters of distilled water for ten min. The infusion was filtered twice through hydrophilic cotton, then once through Whatman No.3 filter paper. The filtrate was dried in an oven at 50 °C for 72 h to obtain dry infusion presenting as brown powder weighing 27.57 g. Infusion powder was presented in Figure 2.

### Okra infusion dose determination

Dose determination was based on consultation with a naturopath from San-Pedro (Côte d'Ivoire) who recommends dried Okra grain powder to diabetic patients at usual dosage of two teaspoons mixed in hot water (90-95 °C) twice daily. One teaspoon weighed 4030.5 mg. A 70 kg patient would consume 16,122 mg powder in 60 mL hot water daily. After reconstitution and filtration, 7.5 mL filtrate was obtained. Water evaporation at  $50 \pm 5$  °C yielded 2,200 mg dry extract, corresponding to Human Daily Dose (HDD) for 70 kg adults.

For rat dose estimation, FDA conversion formula was used [10]: Human Equivalent Dose (HED) = HDD/70 = 31.42 mg/kg. Animal conversion factor = 6.2. Animal Equivalent Dose =  $31.42 \times 6.2 = 194.85$  mg/kg/day, rounded to 195 mg/kg/day.

### Phytochemical screening

Secondary metabolite identification involved characterization assays of major chemical compound groups in Okra grain infusion. Detection was based on specific chemical reactions with appropriate reagents. Tests were performed using analytical techniques described by various authors [11-13].

Test solution was prepared by homogenizing 5 g extract in 50 mL distilled water and screening included detection of: sterols and polyterpenes (acetic anhydride-sulfuric acid reaction), polyphenols (ferric chloride reaction), flavonoids (hydrochloric alcohol-magnesium reaction), catechic tannins (Stiasny reagent), gallic tannins (FeCl<sub>3</sub> reaction), quinone compounds (Borntraeger reaction), alkaloids (Dragendorff and Bouchardat reagents), and saponins (foam formation test).

### Secondary metabolites quantification

Quantitative measurements used spectrophotometry with standard curves using gallic acid, quercetin, and atropine as references for polyphenols, flavonoids, and alkaloids respectively.

Alkaloid assay: 100 mg plant powder dissolved in 4 mL ethanol (25 mg/mL concentration), pH maintained at 2-2.5 with HCl. Dragendorff reagent precipitation, centrifugation, sodium sulfate treatment, nitric acid dissolution, thiourea addition, absorbance measured at 435 nm against atropine standard curve ( $Y = 1.5306X$ ,  $R^2 = 0.8469$ ).

Polyphenol assay: Folin-Ciocalteu method with 1 mg/mL extract in methanol, reaction with FCR and sodium carbonate, incubation in darkness for 1 h, absorbance at 735 nm against gallic acid standard curve ( $Y = 0.01810X + 0.07179$ ,  $R^2 = 0.9911$ ).

Flavonoid assay was performed according to the aluminium chloride colorimetric method described by Zhishen *et al.*, 1999 [14].

### Oral glucose tolerance test (*in-vivo*)

Oral glucose tolerance test (OGTT) was performed according to Kambouche *et al.* 2011 [15]. Rats underwent short-term fasting (6-12 h), baseline glycemia measurement, then oral glucose administration (3 g/kg body weight) was conducted to induce hyperglycemia. Test extracts were administered and blood samples delicately collected from tail vein every 30 min until 120 min for glycemia measurement.

### Experimental design

After 12 h fasting, rats were divided into 8 homogeneous weight groups. Each experimental group consisted of six rats ( $n = 6$ ) received the following solutions by gavage at a rate of 1 mL/1kg:

- Group 1 (blank controls): Normoglycemic rats receiving physiological saline (1 mL/kg bw)
- Group 2 (test group 0): Normoglycemic rats receiving okra grain infusion (195 mg/kg bw)
- Group 3 (negative controls): Hyperglycemic rats receiving physiological saline (1 mL/kg po)
- Group 4 (test group 1): Hyperglycemic rats receiving okra grain infusion (195 mg/kg bw)
- Group 5 (positive controls 1): Hyperglycemic rats receiving metformin (10 mg/kg bw)
- Group 6 (positive controls 2): Hyperglycemic rats receiving gliclazide (100 mg/kg bw)
- Group 7 (test group 2): Hyperglycemic rats receiving okra infusion (195 mg/kg) + metformin (10 mg/kg)

- Group 8 (test group 3): Hyperglycemic rats receiving okra infusion (195 mg/kg) + gliclazide (100 mg/kg)

Thirty min after treatment, all rats received glucose overload via gavage (3 g/kg body weight solution) to create hyperglycemia. Glycemia was measured from caudal vein every 30 min at times T0, T30, T60, T90, and T120 min using a glucometer.

Glycemic variation amplitude was determined using the formula: Variation (%) = [(Gt - G0)/G0] × 100, where Gt = mean glycemia at time t, G0 = baseline mean glycemia.

To assess hypoglycemic risk, we compared Groups 1 and 2. Antihyperglycemic potential was determined by comparing Groups 3 and 4, and pharmacodynamic interactions were studied using Groups 5 through 8.

Glycemic variation was expressed as a percentage change using the formula [16]:

$$\text{Variation (\%)} = \frac{(Gt - G0)}{G0} \times 100$$

Gt = mean glycemia at time t

G0 = baseline mean glycemia.

A positive result indicates hyperglycemia; a negative result indicates hypoglycemia.

#### Data analysis

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 9.3.0. Comparisons among groups were carried out using the Kruskal-Wallis test followed by Dunn's multiple comparison test when appropriate. Differences were considered statistically significant at p < 0.05.

## RESULTS

### Chemical compounds

Phytochemical screening (Table 1) revealed presence of several bioactive secondary metabolites in dried okra seed infusion, including sterols and polyterpenes, polyphenols, flavonoids, quinone compounds, alkaloids and catechic tannins. However, saponins and gallic tannins were not detected. The predominance of phenolic compounds suggests a potential contribution to the biological properties traditionally attributed to okra.

**Table 1:** Qualitative composition of *A. esculentus* infusion

Secondary metabolites	Presence/Absence
Sterols and polyterpenes	+
Polyphenols	+
Flavonoids	+
Saponins	-
Quinone compounds	+
Alkaloids	+
Catechic tannins	+
Gallic tannins	-

(+): Compound present; (-): Compound absent

Quantitative analysis (Table 2) showed that polyphenols were the most abundant compounds (3.28 ± 0.22 mg GAE/g extract), followed by flavonoids (0.47 ± 0.05 mg QE/g extract) and alkaloids (0.30 ± 0.02 mg AE/g extract). These findings confirm the presence of phytochemicals metabolites to be involved in antioxidant and metabolic regulatory activities.

**Table 2:** Polyphenol, alkaloid, and flavonoid content

Compound	Content (mean ± SD)
Polyphenols	3.28 ± 0.22 mg GAE/g extract
Alkaloids	0.30 ± 0.02 mg AE/g extract
Flavonoids	0.47 ± 0.05 mg QE/g extract

GAE = Gallic acid equivalent; AE = Atropine equivalent; QE = Quercetin equivalent

### Hypoglycemic risk

At T30 (Figure 3), normoglycemic rats receiving physiological saline showed non-significant glycemic elevation (+12.60% vs. T0). Subsequently, glycemia varied little from T30 to T120, remaining slightly above initial values (+4.53%). In normoglycemic rats treated with okra grain infusion (195 mg/kg bw), the curve evolved similarly to NaCl 0.9% group. No statistically significant difference was observed between the saline-treated group and the okra-treated group throughout the experiment. Blood glucose levels remained close to baseline values, indicating that the infusion did not induce hyperglycemia under normoglycemic conditions.

### Antihyperglycemic activity

At T30 (Figure 4), hyperglycemic rats receiving physiological saline showed significant glycemic elevation (peak + 98.6% vs. T0). Subsequently, hyperglycemia decreased progressively from T30 to T120 toward normal values. In hyperglycemic rats treated with Okra grain infusion (195 mg/kg bw), glycemic evolution followed a similar curve to negative control group (NaCl 0.9%), but with slower decrease. In hyperglycemic rats, administration of okra infusion did not significantly modify glycemic response induced by oral glucose loading. The glucose profile remained comparable to that observed in negative control group, suggesting absence of a significant acute antihyperglycemic effect at the dose tested.

### Drug-Plant extract interactions

Effects of standard antidiabetic drugs alone and in combination with Okra infusion are shown in Figures 5 and 6. In hyperglycemic rats receiving metformin alone (10 mg/kg bw), significant glycemic increase was noted at T30 (peak +126.85% vs. T0). Subsequently, hyperglycemia decreased progressively from T30 to T120 toward normal values. In hyperglycemic rats treated with gliclazide alone (100 mg/kg bw), rapid hyperglycemia decrease was observed from T30. This blood sugar drop continued until T60, causing hypoglycemia with values of -16.37% at T90 and -30.22% at T120 compared to initial values.

Rats treated with combination metformin + Okra showed initial glycemic increase, then hyperglycemia rapid decrease stabilizing at normal levels of initial value. Conversely, rats treated with combination of gliclazide + Okra showed initial significant glycemic increase (peak +135.34%), then from T30, hyperglycemia dropped very rapidly until T60, evolving toward more pronounced hypoglycemia (-26.17% at T90 and -31.15% at T120).



Figure 1: Morphology of okra *A. esculentus*: whole fruit, fresh and dried seeds [7].



Figure 2: Powder from infusion of dry grains of *A. esculentus*

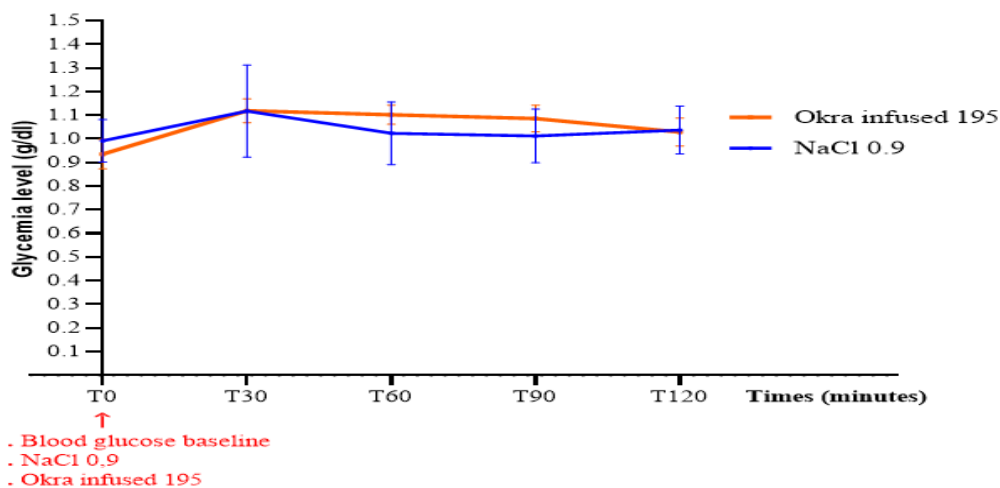


Figure 3: Blood glucose levels variation after administration of Okra dry seed infusion in normoglycemic rats

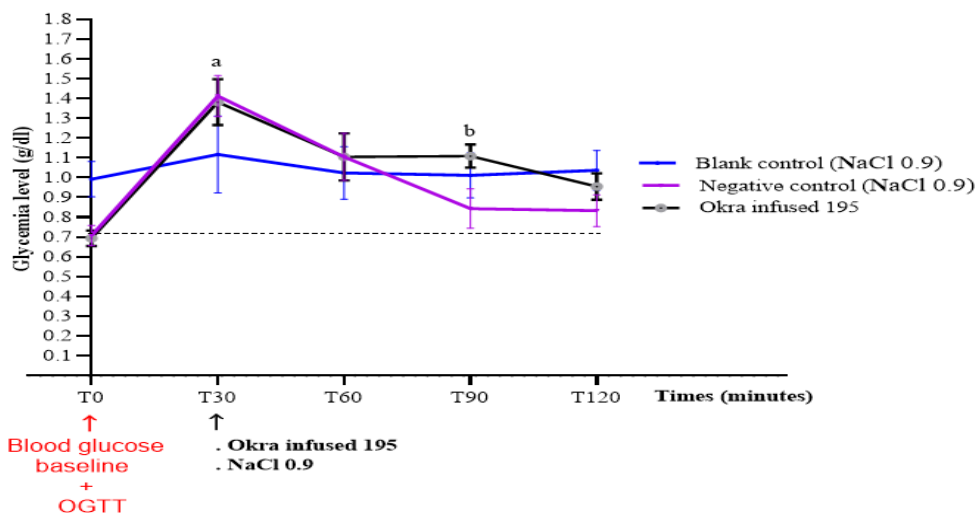
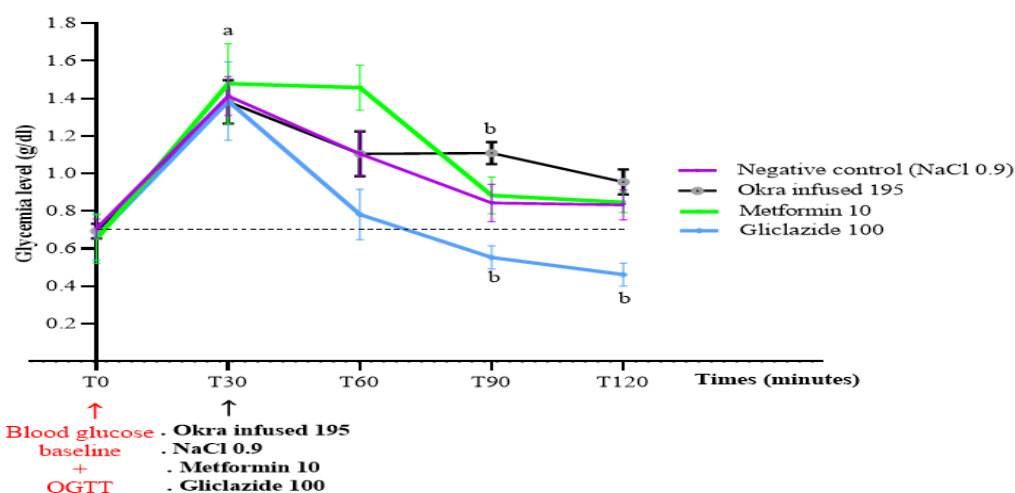
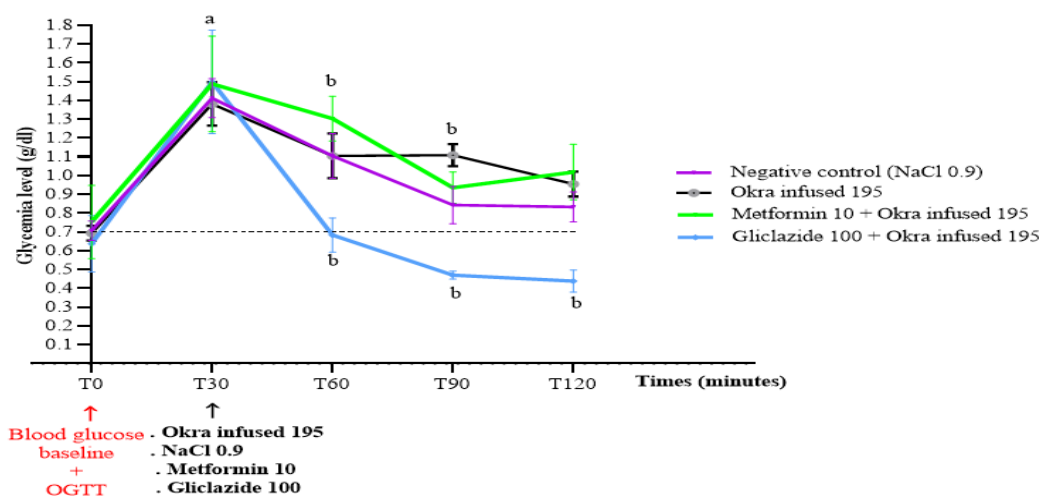


Figure 4: Blood glucose levels variation after administration of Okra dry seed infusion in hyperglycemic rats

- a. Significant difference compares to blood glucose baseline ( $p < 0,05$ )
- b. Significant difference compares to blank control ( $p < 0,05$ )



**Figure 5:** Blood glucose levels variation after administration of conventional antidiabetic drugs in hyperglycemic rats  
 a. Significant difference compares to blood glucose baseline ( $p < 0,05$ )  
 b. Significant difference compares to negative control ( $p < 0,05$ )



**Figure 6:** Blood glucose levels variation after administration of combining conventional antidiabetic drugs with Okra dry seed infusion in hyperglycemic rats  
 a. Significant difference compares to blood glucose baseline ( $p < 0,05$ )  
 b. Significant difference compares to negative control ( $p < 0,05$ )

## DISCUSSION

The present study evaluated antihyperglycemic potential of a dried seed infusion of *A. esculentus* and its pharmacodynamics interactions with two commonly prescribed antidiabetic drugs, metformin and gliclazide. Phytochemical analysis revealed the presence of several bioactive secondary metabolites, including polyphenols, flavonoids, alkaloids, catechic tannins, sterols, polyterpenes and quinone compounds. These findings are consistent with previous reports describing okra as a source of phytochemicals with antioxidant and metabolic regulatory properties.

These results align with Ademiluyi *et al.* 2018 [17] findings, demonstrating richness in phenolic compounds in Okra extracts, explaining their varied pharmacological properties including antidiabetic, antioxidant, and anti-inflammatory activities.

Quantitative analyses showed moderate concentrations of polyphenols ( $3.28 \pm 0.22$  mg GAE/g), flavonoids ( $0.47 \pm 0.05$  mg QE/g) and alkaloids ( $0.30 \pm 0.02$  mg EA/g) compared with hydroalcoholic or methanolic extracts reported in the literature by Sabitha *et al.* 2011 [18] (up to 10 mg GAE/g). This content difference could be explained by solvent choice. Water used in our study is generally less efficient for extracting phenolic compounds compared to hydroethanolic solvents. Nevertheless, even at these low concentrations, these components are biologically active as highlighted by Goyal *et al.* 2011 [19] in their work focus on antioxidant effects exerted by flavonoids. This is also the

case for the alkaloids ( $0.30 \pm 0.02$  mg EA/g); even at this low level, they also participate in properties of dried okra seeds, as Bamisaye *et al.* 2019 [20] so aptly revealed a synergistic effect between alkaloids and flavonoids in improving insulin sensitivity.

The oral glucose tolerance test produced the expected transient hyperglycemia in control animals, confirming the validity of the experimental model. Following glucose administration, blood glucose concentrations increased rapidly before gradually returning toward baseline values as a result of endogenous insulin-mediated regulation. This protocol is commonly used to evaluate substance capacity to regulate glycemia in response to significant glucose loads. An active extract or compound accelerates glycemic normalization by improving insulin secretion or action, or by inhibiting intestinal glucose absorption. After ingestion, glucose is rapidly absorbed in the small intestine, causing marked glycemic increase. This elevation stimulates insulin secretion by pancreatic  $\beta$ -cells, and insulin facilitates glucose uptake by peripheral tissues such as liver, muscles, and adipose tissue, where it is either used for energy production (glycolysis) or stored as glycogen (glycogenesis) [21,22]. In healthy rats, these mechanisms allow progressive glycemic normalization within two hours, demonstrating balanced glucose metabolism and functional insulin response.

When administered alone, dried okra seed infusion 195 mg/kg bw did not significantly alter blood glucose levels in either normoglycemic or glucose-loaded rats. These findings suggest that the infusion does not

possess a marked acute antihyperglycemic effect at dose tested under experimental conditions realized. Similar observations have been reported by Chukwuma *et al.* 2017<sup>[23]</sup> suggesting Okra acts primarily to delayed intestinal glucose absorption and inhibition of carbohydrate-digesting enzymes like  $\alpha$ -amylase and  $\alpha$ -glucosidase rather than direct stimulation of insulin secretion.

The combination of okra infusion 195 mg/kg b.w with metformin 10 mg/kg b.w., did not adversely affect glycemic regulation and appeared to maintain the antihyperglycemic profile of metformin. Since metformin primarily acts by reducing hepatic glucose production and improving insulin sensitivity without stimulating pancreatic insulin secretion, the absence of excessive glucose lowering in the combined treatment group is consistent with its known pharmacological profile. Mishra *et al.* 2015<sup>[24]</sup> findings consolidate our results, suggesting plant extracts like Okra can enhance oral antidiabetic effects, potentially reducing required conventional medication doses. Polyphenols and flavonoids in Okra would bind to enzyme active sites, slowing complex carbohydrate digestion into absorbable glucose without complete blockade, allowing glucose blood entry over extended periods, avoiding brutal peaks and preventing subsequent drops<sup>[25]</sup>.

In contrast, combination of okra infusion 195 mg/kg b.w and gliclazide 100 mg/kg b.w., produced a more pronounced reduction in blood glucose levels than gliclazide alone, resulting in marked hypoglycemia during the late phase of oral glucose tolerance test. Although the precise mechanism remains unclear, this observation suggests some possible pharmacodynamics interactions between okra constituents and sulfonylurea therapy. Because no insulin measurements or mechanistic investigations were performed, the present study cannot determine whether this interaction is mediated through enhanced insulin secretion, improved insulin sensitivity, delayed glucose absorption, or a combination of these mechanisms. Therefore, caution is warranted when interpreting these findings.

Conversely, gliclazide caused hypoglycemia, and okra-gliclazide combination did not prevent gliclazide hypoglycemia after glucose loading. Okra infusion at 195 mg/kg b.w., contains specific flavonoids and alkaloids that may suggest a pharmacodynamics interactions inducing weak stimulatory on pancreatic  $\beta$ -cells. Administered alone, this effect is negligible, but combined with gliclazide 100 mg/kg b.w., joint stimulation by both agents becomes possible, exceeding critical threshold and leading to excessive insulin release, basically blood glucose resorption causing observed severe hypoglycemia. Because no insulin measurements or mechanistic investigations were performed, the present study cannot determine whether this interaction is mediated through enhanced insulin secretion, improved insulin sensitivity, delayed glucose absorption, or a combination of these mechanisms.

Therefore, caution is warranted when interpreting these findings in possible additive pharmacodynamics interactions, which is unbeneficial and unpredictable. In this regard, the work of Chukwuma *et al.* 2017<sup>[23]</sup> and the American Diabetes Association, 2024<sup>[26]</sup> recommend increased clinical vigilance and rigorous glycemic monitoring when using medicinal plant extracts and insulin secretagogue drugs concomitantly.

Finally, absence of enzyme assays ( $\alpha$ -amylase,  $\alpha$ -glucosidase) prevents us from conclusion about the exact mechanism action of okra. However, as Kwon *et al.* (2008)<sup>[27]</sup> have shown, okra exerts inhibitory effects on these digestive enzymes, contributing to postprandial glycemic peak attenuation.

## CONCLUSION

The infusion of dried okra grains (*A. esculentus*) rich in fiber and low in calories content several bioactive compounds, particularly flavonoids, alkaloids, catechic tannins, sterols, polyterpenes, and quinones, could help reduce glycemia. A dose of 100 mg/kg bw did

not exhibit significant antihyperglycemic effect in this acute model. Its combination with metformin appears safe and could be explored in a dietary management context for type 2 diabetes. However, its combination with gliclazide poses a severe risk of hypoglycemia, highlighting the critical importance of understanding drug-plant interactions.

## Limitations and perspectives

The study presents some limitations. First, only an acute experimental model was used, preventing conclusions regarding long-term glycemic control. Second, biochemical markers such as insulin concentrations, glycated hemoglobin and digestive enzyme activities were not assessed. Finally, the use of a single dose of okra infusion does not allow establishment of a dose-response relationship.

Despite these limitations, the findings highlight the importance of investigating potential interactions between medicinal plants, functional foods and conventional antidiabetic medications. Further studies using diabetic animal models, mechanistic approaches and chronic administration protocols are required to clarify the therapeutic relevance of dried okra seed preparations and their interactions with oral antidiabetic drugs.

## Author Contributions

KSL, AYA, KA, and MKP contributed to the conceptualization and design of the study and to data acquisition. AYA and KM conducted the literature review, and the statistical analysis was performed by KSL and DATL. Kouakou SL and EKE took part in the interpretation of the results and the preparation of the manuscript. KSL acts as guarantor of the manuscript. KSG and INGG read and approved the final version submitted for publication.

## Data Availability Statement

The data that support the findings of this study are available and will be made available upon request.

## Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

## Conflict of Interest

The authors declared no conflict of interest.

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None declared.

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