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Research Article

Phytochemical Analysis, Acute Toxicity and Glycemic Regulation of the Aqueous Extract of *Moringa oleifera* Leaves in Normoglycemic and Hyperglycemic Rats

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Abstract

Moringa oleifera is generally considered to have numerous therapeutic properties in traditional Ivorian medicine. The objective of this study was to evaluate the acute toxicity and blood glucose regulation in rats of an aqueous extract of *Moringa oleifera* leaves. Phytochemical analysis of the aqueous extract of *Moringa oleifera* leaves was performed using the standard staining and precipitation method. The toxicity study was conducted in accordance with OECD 423, by administering a single dose of the extract at 5,000 mg/kg body weight. The antihyperglycemic and hypoglycemic activities of *Moringa oleifera* extract were evaluated by administering a single dose of 75 mg/kg µg, 150 mg/kg µg, 300 mg/kg µg, 600 mg/kg µg, and 1200 mg/kg µg of aqueous *Moringa oleifera* extract to normoglycemic and hyperglycemic animals. The effects of the different *Moringa oleifera* extracts on rats were monitored for 120 minutes. Blood glucose levels were measured at 0, 30, 60, 90, 120, and 180 minutes. Phytochemical analysis showed that the extract is rich in secondary compounds such as polyphenols, flavonoids, sterols, tannins, and saponins. *Moringa oleifera* extract is non-toxic, with an LD_{50} greater than 5,000 mg/kg body weight. Aqueous *Moringa oleifera* extract administered to normoglycemic rats did not induce hypoglycemia or hyperglycemia. Aqueous *Moringa oleifera* extract significantly reduced anhydrous glucose-induced hyperglycemia in hyperglycemic rats, from 170 mg/dL to approximately 78 mg/dL. These results confirm the regulatory properties of *Moringa oleifera* on blood glucose levels in Wistar rats.

Keywords

Moringa oleifera, Rattus Norvegicus, Hypoglycemic, Anti-hyperglycemic

1. Introduction

Diabetes is a metabolic disease characterized by chronic hyperglycemia linked to a deficiency or insufficiency of insulin secretion, abnormalities in insulin action, or a combination of these two mechanisms [1]. For some time now, diabetes has represented a fundamental threat to human health and development. More than 80% of diabetes-related deaths occur in low- and middle-income countries. Thus, in 2013, nearly 20 million people in sub-Saharan Africa had diabetes, representing a prevalence of 4.9% [2]. The cur-

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rent global prevalence of diabetes is approximately 347 million people [3]. Estimates for 2030 project that it will affect approximately 4.7% of the world's population [4]. In Côte d'Ivoire, the International Diabetes Federation (IDF) estimated the prevalence of diabetes at 9.6% in 2014. Diabetes clearly appears to be a major public health problem [5]. The concern surrounding the morbidity caused by hyperglycemia is a concern for several countries, including Côte d'Ivoire [6]. In this country, the therapeutic management of diabetes currently relies on strict diets and the use of oral antidiabetic drugs such as sulfonylureas, biguanides, alpha-glucosidase inhibitors, glinides, and thiazolidinediones [7].

Generally, chemical antidiabetic agents cause various side effects that vary depending on the class and generation of the drug. Specifically, sulfonamides, insulin secretagogues, cause hypoglycemia. This effect leads to hematological disorders, possible dermatological reactions, and weight gain due to hyperinsulinemia. Due to these adverse side effects, some biguanides, inhibitors of gluconeogenesis and intestinal glucose absorption, have been withdrawn from the market [8]. Faced with the failures of conventional medicine, several studies have been conducted on traditional plants to treat these chronic conditions [9]. Among these plants, *Moringa oleifera* appears to be one of the most promising species based on its nutrient content, antioxidant activity, phytochemical compounds, ease of cultivation and processing, and organoleptic qualities [10]. This plant is used in traditional medicine for the treatment of inflammatory, infectious, parasitic, and tumorous diseases, and for the prevention of oxidative damage [11]. This study aims to verify the anti-hypoglycemic and anti-hyperglycemic effects of the aqueous extract of *Moringa oleifera leaves* in rats.

2. Material and Methods

2.1. Material

The animal material consists of thirty adult rats (Muridae), of the *Wistar strain*, weighing between 180 and 200 grams. The animals are fed daily with pellets from the company FACI (Manufacturing of Ivorian Compound Feed) and have free access to water.

The plant material consisted of *Moringa oleifera leaves*, collected in the commune of Cocody, in the Abidjan District in southern Côte d'Ivoire. A sample of this plant was identified at the National Floristic Center (CNF) of the Felix Houphouët-Boigny University.

2.2. Methods

2.2.1. Method for Extracting the Aqueous Extract from *Moringa oleifera* Leaves

The harvested leaves are rinsed with water, dried on benches in the laboratory in the shade, at a temperature of 30 ±2 °C. The dried leaves were ground into a powder.

The resulting powder is macerated by mixing 50 g with 1.5 L of distilled water and stirring for 24 hours using a magnetic stirrer. After five filtrations through poplin and then five more through absorbent cotton, the filtrate is placed in an oven at 50 °C until a dry extract is obtained.

2.2.2. Phytochemical Screening Study

The phytochemical screening test allows for the identification of certain chemical constituents of the studied plant that have therapeutic potential. To this end, several physicochemical reactions requiring characterization reagents were carried out according to the method of [12]. The phytochemical tests were performed on the aqueous extract of *Moringa* leaves. *oleifera* by qualitative characterization techniques.

2.2.3. Acute Oral Toxicity

This study was conducted according to OECD Guideline 423 for Chemical Testing [13]. Six (6) healthy, nulliparous, non-pregnant young female rats, weighing between 100 g and 120 g and aged 8 to 10 weeks, were used. The animals were divided into two groups and fasted overnight prior to the administration of the total aqueous extract of *Moringa. oleifera*, but they had access to water. This test consisted of administering orally to selected rats a dose of 5000 mg/kg of BW from the aqueous extract of *Moringa oleifera*. The animals were deprived of food for 24 hours before and 3 hours after administration of the extract, but had access to water. The animals were observed individually and regularly over 24 hours, then daily for 14 days. Observations included fur, eye color, mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhea, morbidity, mortality, heart rate, food intake, and water intake.

2.2.4. Demonstration of the Effect of Aqueous Extract of *Moringa oleifera* on Blood Glucose Levels in Normoglycemic Rats

The effects of aqueous extracts of *Moringa oleifera* on blood glucose levels in normoglycemic rats were evaluated for two hours after the animals were gagged with different doses of the extract. Thirty Wistar rats weighing between 200 and 210 g were used for this experiment. These animals were divided into six groups of five rats and fasted for 14 hours. Before administration of the test substances, baseline blood glucose was measured in all animals at time T0. Rats in group 1 (negative control group) received 2 ml of distilled water. Rats in groups 2, 3, 4, 5, and 6 (test groups) received 2 ml of 75 mg/kg MC, 150 mg/kg MC, 300 mg/kg BW, 600 mg/kg BW, and 1200 mg/kg BW of aqueous *Moringa oleifera extract, respectively*. Blood glucose levels were then measured at regular time intervals of 30, 60, 90 and 120 minutes after administration of the test substances.

2.2.5. Demonstration of the Effect of Aqueous Extract of *Moringa oleifera* on Blood Glucose Levels in Hyperglycemic Rats

(i). Demonstration of the Effect of Aqueous Extract of *Moringa oleifera* on Blood Glucose Levels in Pre-treated Hyperglycemic Rats

Normal rats, weighing between 200 g and 210 g, were fasted for 14 hours before the start of the experiment. Six groups of five (5) rats were formed: group 1 (negative control) received 2 ml of distilled water, and group 2 (positive control) consisted of hyperglycemic control rats. The rats in this group received distilled water and, 30 minutes later, 4 g/kg body weight of anhydrous glucose. Group 3 consisted of rats that received glibenclamide at a dose of 10^{-2} g/kg of BW and, 30 minutes later, 4 g/kg of BW of anhydrous glucose. Group 4 consisted of rats that received 75 mg/kg of BW of an aqueous extract of *Moringa oleifera* and, 30 minutes later, 4 g/kg of BW of anhydrous glucose. Group 5 consisted of rats that received 1200 mg/kg of BW of an aqueous extract of *Moringa oleifera* and, 30 minutes later, 4 g/kg of BW of anhydrous glucose.

Moringa oleifera extracts on rats were evaluated over a 120-minute period. Blood glucose levels were measured in these rats at 0 min, 30 min, 60 min, 90 min, 120 min, and 180 min.

(ii). Demonstration of the Effect of the Extract on Blood Glucose Levels in Post-treated Hyperglycemic Rats

Six (6) groups of five (5) rats were formed. Group 1 was the negative control, where the rats received 2 ml of distilled water, and Group 2 was the positive control. This group consisted of hyperglycemic rats that received 4 g/kg BW of anhydrous glucose. Group 3 consisted of rats that received 4 g/kg BW of anhydrous glucose and then, 30 minutes later, glibenclamide at a dose of 10^{-2} g/kg BW. In Group 4, the rats received 4 g/kg BW of anhydrous glucose and then, 30 minutes later, 75 mg/kg BW of aqueous extract of *Moringa oleifera*. Group 5 consisted of rats that received 4 g/kg BW of anhydrous glucose and then, 30 minutes later, 1200 mg/kg BW of aqueous extract of *Moringa oleifera*. The evaluation of the effects of different doses of the aqueous extract of *Moringa oleifera* on the blood glucose of rats is monitored for 120 minutes at regular time intervals (0, 30, 60, 90, 120 and 180 min).

3. Statistical Analyses

The results were analyzed using GraphPad Prism 8 software with a significance threshold of $p < 0.05$. All values are presented as mean ± SEM (Standard Error of the Mean). Means were compared using ANOVA followed by the Tukey-Kramer comparison test.

4. Results

4.1. Chemical Compounds Present in the Aqueous Extract

The results of the phytochemical analysis revealed the presence or absence of certains groups of chemical compounds of therapeutic interest (Table 1). Polyterpenes, polyphenols, flavonoids, and tannins were the chemical compounds identified by these analyses. Their presence in the aqueous extract of *Moringa oleifera* is relatively abundant. However, this extract does not contain alkaloids or quinonic substances.

4.2. Effect of the Aqueous Extract of *Moringa oleifera* on the Body Mass of Rats Treated with a Single Dose of 5000 mg/kg of BW

Oral administration of the aqueous extract of the plant at a dose of 5000 mg/kg BW to rats caused no signs of toxicity. Furthermore, the rats exhibited no changes in physical appearance, nor did they experience tremors or convulsions. Also, no mortality or morbidity was observed during the 14-day observation period. During the experiment, no growth disturbances were observed in the rats. However, statistical analysis showed no significant difference ($p > 0.05$) between the body mass of the rats receiving the single dose of extract and that of the control rats (Figure 1).

4.3. Effects of Aqueous Extract of *Moringa oleifera* on Rat Blood Glucose

4.3.1. Effects of *Moringa oleifera* on Blood Glucose Levels in Normoglycemic Rats

The blood glucose level of the control rats, which received only distilled water, did not vary significantly ($p > 0.05$) throughout the duration of this study (2 hours). It remained at 110 ± 7.662 mg/dl. Blood glucose levels in normoglycemic rats treated with aqueous extract of *Moringa oleifera leaves* decreased from 97.40 ±4.39 mg/dL to 98.60 ±6.90 mg/dL (75 mg/kg BW dose), from 101.20 ±4.39 mg/dL to 94.80 ±6.30 mg/dL (150 mg/kg BW dose), from 94.40 ±10.95 mg/dL to 82 ±5.29 mg/dL (300 mg/kg BW dose), from 103.40 ±9.27 mg/dL to 79.80 ±3.94 mg/dL (600 mg/kg BW dose), and from 99.40 ±5.10 mg/dl to 82 ±3.64 mg/dl (1200 mg/kg BW dose). The aqueous extract of *Moringa oleifera* did not cause a significant change ($p > 0.05$) in blood glucose levels in treated rats compared to controls (Figure 2).

4.3.2. Effects of Aqueous Extract of *Moringa oleifera on Blood Glucose Levels in* Pre-treated Hyperglycemic Rats

Thirty (30) minutes after administration of glucose at 4 mg/kg of BW to rats pretreated with the different substances,

a significant increase ($p < 0.05$) in blood glucose levels was observed in the test groups and the positive control group compared to the negative control group. Blood glucose levels in the rats of the different groups increased from 76 ± 4.58 to 165.3 ± 13.90 mg/dL of BW (positive controls), from 80.33 ± 9.83 to 136 ± 9.53 mg/dL of BW (glibenclamide group), and from 83.67 ± 2.43 to 153.3 ± 6.89 mg/dL of BW in the *Moringa extract group. oleifera* and from 78.67 ± 6.38 to 213 ± 44.10 respectively for the groups receiving the aqueous extract at doses of 75 and 1200 mg/kg of BW. At the sixtieth (60th) minute, blood glucose levels increased to 170 ± 10.67 mg/dl, 132.3 ± 5.45 mg/dl, 169.3 ± 15.37 mg/dl and 167 ± 9.54 mg/dl respectively for the positive control group, the groups receiving glibenclamide and the extract at doses of 75 and 1200 mg/kg of BW compared to those of the negative control (88.67 ± 4.70 mg/dl of BW).

From the 120th minute onwards, a decrease in blood glucose levels was observed in all groups receiving glucose. However, these values remained significantly different compared to rats that did not receive glucose solution. The glucose levels recorded at this time were 148.3 ± 5.67, 113.7 ± 7.21, 120 ± 14.7, and 124.7 ± 5.6 mg/dL, representing reduction rates of 14.19 ± 9.89%, 16.91 ± 3.21%, 24.1 ± 14.7%, and 59.03 ± 3%, respectively, for the positive control group, the groups receiving glibenclamide, and the extract at doses of 75 and 1200 mg/kg of body mass index (BMI). At the end of the experiment (180 min), these percentage reductions in blood glucose levels were 34.27 ± 3.9%, 64.85 ± 14%, 42.13 ± 14%, and 73.39 ± 23%, respectively. It should be noted that the aqueous extract of *M. oleifera* at a dose of 1200 mg/kg of BW significantly reduced blood glucose levels compared to glibenclamide (Figure 3).

4.3.3. Effects of Aqueous Extract of *Moringa oleifera on Blood Glucose Levels in* Post-treatment Hyperglycemic Rats

Thirty (30) minutes after administration of glucose, peaks of hyperglycemia were observed in all groups of animals that received glucose at 4 mg/kg of BW with an average of 172.333 ± 15.962 mg/dl.

Following administration of the test substances (glibenclamide or plant extract), a significant decrease in blood glucose levels was observed in treated rats starting at 60 minutes.

In the presence of glibenclamide, the rats' blood glucose levels decreased from 139 ± 9.074 mg/dL at 60 minutes to 78.67 ± 8 mg/dL (120 minutes) and then to 55.33 mg/dL at 180 minutes. In hyperglycemic rats treated with the extract, blood glucose levels decreased from 135 ± 9.20 to 126 ± 15.71 at 60 minutes, from 107 ± 5.68 to 108.3 ± 12.38 at 120 minutes, and from 80.3 ± 13.12 to 105.3 ± 12.44 at 180 minutes. The extract at doses of 75 and 1200 mg/kg of BW resulted in a decrease in blood glucose levels. Blood glucose levels increased from 135 ± 9.20 to 126 ± 15.71 at the 60th minutes, from 107 ± 5.68 to 108.3 ± 12.38 at the 120th minutes, and from 80.3 ± 13.12 to 105.3 ± 12.44 at the 180th minutes.

At the end of the experiment, glibenclamide significantly reduced blood glucose compared to the aqueous extract of *M.* oleifera (Figure 4).

Table 1. *Phytochemical screening of the aqueous extract of Moringa oleifera.*

Sterols	Polyphenols	Flavonoids	Tannins	Quinonic substances	Alkaloids	Saponosides
+	+	+	+	-	-	+

+: presence of chemical compounds,
-: absence of chemical compounds

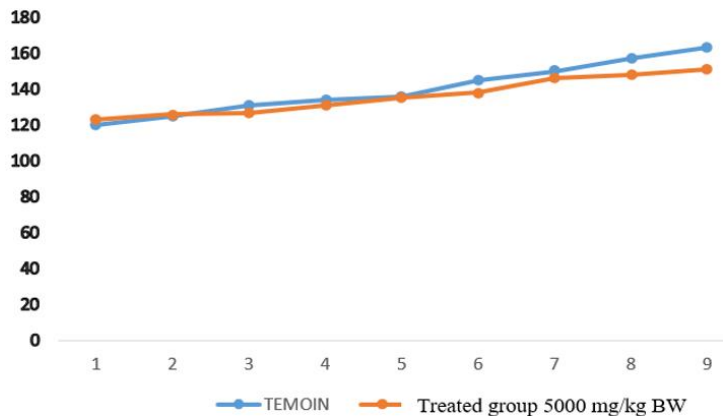

Figure 1. *Variation in body mass of rats.*

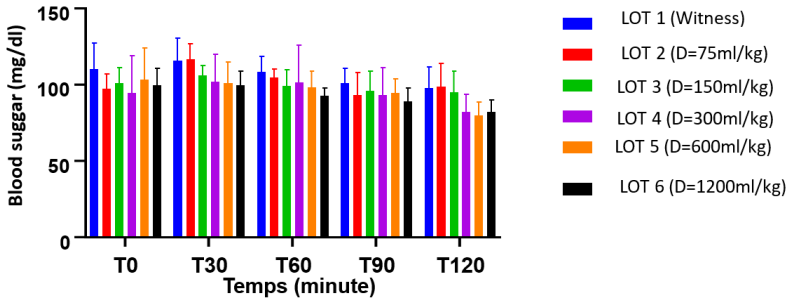

Figure 2. *Effects of aqueous extract of Moringa oleifera on blood glucose levels in normoglycemic rats.*

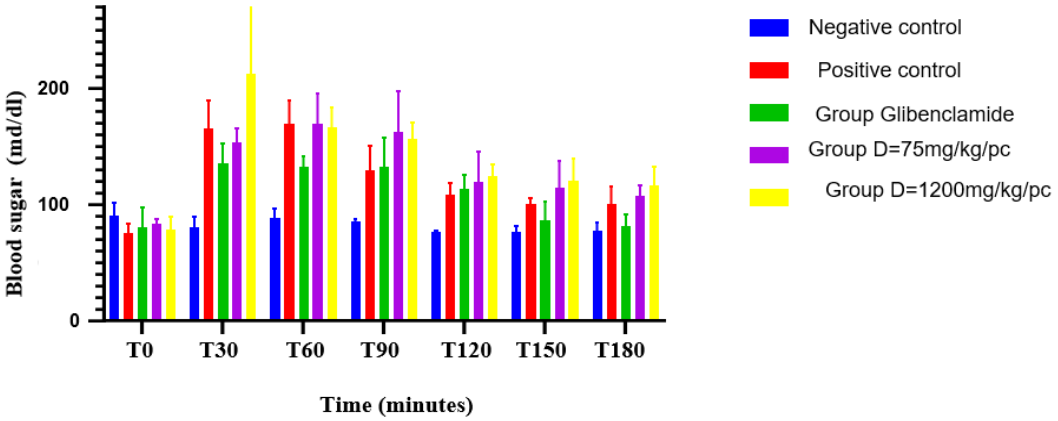

Figure 3. *Effects of aqueous extract of Moringa oleifera on blood glucose levels in pre-treated hyperglycemic rats.*

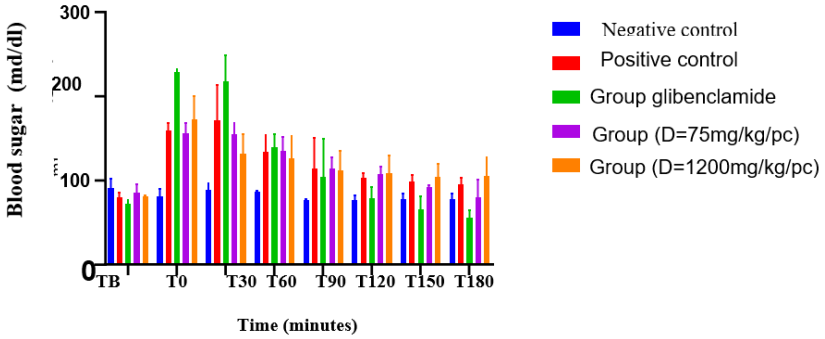

Figure 4. *Effects of aqueous extract of Moringa oleifera on blood glucose levels in post-treated hyperglycemic rats.*

5. Discussion

Phytochemical screening of the aqueous extract *of Moringa oleifera* revealed the presence of polyterpenes, polyphenols, alkaloids, and catecholic tannins. However, the aqueous extract of *Moringa oleifera* did not contain gallic tannins, flavonoids, saponins, or quinonic substances.

The presence of these various chemical compounds in the aqueous extract of *M. oleifera* would explain the properties of this plant and its common use in traditional medicine. Furthermore, studies have shown that the plant's leaves contain numerous molecules with pharmacological properties, including antioxidant, anti-inflammatory, and immune-regulating properties [14-16].

The acute toxicity study of the aqueous extract of *M. oleifera* in rats showed that this extract, administered orally, caused no mortality or clinical signs at a single dose of 5000 mg/kg of BW. These results demonstrate the non-toxicity of the aqueous extract of *Moringa oleifera.* Nevertheless, based on these results, according to OECD guideline 423, *Moringa oleifera leaves* would have an LD_{50} greater than 5000 mg/kg of BW. These findings are consistent with the studies by [17]. which also showed that the acetic extract of *Moringa oleifera seeds* was non-toxic. Kouakou and Tahiri showed that the aqueous extract of *Moringa oleifera* was not toxic orally at a

dose of 5000 mg/kg DM [18]. Similar results were also obtained with the aqueous extract of *Amaranthus viridis* by Affy and collaborateurs [19]. Pierre and collaborateur [20] showed that the LD_{50} of the plant-based extract was greater than 5000 mg/kg body weight. Aqueous extract of *Moringa oleifera* administered to rats with normal blood glucose levels did not alter blood glucose levels compared to control rats. However, when blood glucose levels were elevated, the extract lowered blood glucose, as did glibenclamide. Furthermore, glibenclamide lowered blood glucose levels more than aqueous extract of *Moringa oleifera.*

The effects of the aqueous extract of *Moringa oleifera* on blood glucose levels showed significant antihyperglycemic activity in treated hyperglycemic rats. Specifically, thirty minutes after the peak of hyperglycemia observed in post-treated rats, blood glucose levels decreased. Glibenclamide administered to the rats significantly reduced blood glucose levels compared to the aqueous extract of *Moringa oleifera.* These properties of the *M. oleifera extract* are similar to those of many medicinal plants from the African pharmacopoeia. Indeed [21], having studied the activity of the extract of *Piper longum* (Piperaceae) roots in rats, showed that this extract had an antihyperglycemic effect. Similar results were obtained with extracts of *Nauclea latifolia* (Rubiaceae) [22], Boscia *senegalensis* (Capparaceae), and *Colocynthis vulgaris* (Cucurbitaceae) [23], which exhibited antihyperglycemic effects in rats. These results suggest that aqueous extract of *Moringa oleifera* and glibenclamide, a hypoglycemic sulfonylurea, act similarly to regulate blood glucose levels. These findings indicate that *Moringa oleifera*, in addition to regulating blood glucose, may play a protective role, and therefore regular consumption could prevent cases of diabetes.

6. Conclusion

M. oleifera leaves on blood glucose regulation in rats. The study showed that **M. oleifera* leaves* are not orally toxic. This study also demonstrated that **M. oleifera* leaves* possess hypoglycemic properties, exhibiting antihyperglycemic effects. The polyterpenes, polyphenols, and alkaloids present in the aqueous extract of **M. oleifera* * could be responsible for these pharmacological effects.

This experimental study will only have full scientific value if it is conducted over a relatively long period. Since the treatment of diabetes mellitus is individualized, the mechanism of action of *M. oleifera* must be elucidated in order to optimize its use.

Abbreviations

IDF	International Diabetes Federation
CNF	National Floristic Center
FACI	Manufacturing of Ivorian Compound Feed
OECD	Organization for Economic Co-operation and Development
BW	Boby Weight
ANOVA	Analysis of Variance
LD_{50}	Median Lethal Dose

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Author Contributions

Kouakou Koffi Roger: Conceptualization, Resources, Data curation, Investigation, Writing – review & editing

Kouadio Kouakou John: Data curation, Methodology, Software, Writing – original draft

Affi Mataphouet Emmanuel Guy Joslin: Supervision

Tahiri Annick: Validation

Conflicts of Interest

The authors declare no conflict of interest.

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