

(RESEARCH ARTICLE)



EFFECTS OF *ANNONA SENEGALENSIS* AND *SECURIDACA LONGEPEDUNCULATA* EXTRACTS ON THE PROTEINS IN *ECHIS OCELLATUS* VENOM, WHICH CAUSES ARTERIAL HYPOTENSION.

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ABSTRACT

Some victims die, others survive with disabilities following the ophidian envenomations. Among the clinical signs of snake bites is hypotension. Most of snake bites victims live in countryside use medicinal plants to treat themselves in case of snakes bite. This study is part of research on antivenom effects of *Annona senegalensis* and *Securidaca longepedunculata*, specifically, the mechanisms by which both plants control blood pressure variation caused by *Echis ocellatus* venom. Venom toxicity tests determined LD₅₀ of *E. ocellatus* venom in Sprague-Dawley rats, which is equal to 0.235 mg/kg. *E. ocellatus* venom decreases mean arterial blood pressure in rats to 27.16 ± 3.04 % and decreased heart rate to 49.51 ± 3.16 beats/min. Hydroethanolic extracts of *A. senegalensis* and *S. longepedunculata* slow down the decrease in blood pressure caused by *E. ocellatus* venom significantly ($p > 0.05$) the mean arterial pressure at doses of 20 mg/kg. These results show that hydroethanolic extract of *A. senegalensis* roots and hydroethanolic extract of *S. longepedunculata* roots have antivenom properties against *E. ocellatus* venom.

Keywords: Hypotension, *Echis ocellatus*, *Securidaca longepedunculata*, *Annona senegalensis*, Venom, Snake, Bite

1 INTRODUCTION

Ophidian envenomation results from convergence between human occupations and snake activities. According to WHO, world snakebite victims per year is estimated between 1.8 to 2.7 million cases of envenomings and around 81 410 to 137 880 people die each year because of snake bites. A household survey in Africa found that few victim use modern treatment after being bitten. So statistics provided by health services on snake bites largely underestimate actual proportion cases that occur especially in rural areas due to victims constraints face. Viperid family envenomation causes more victims in Togo rural area [1]. Viperine bite is characterized by : hemorrhage, edema, necrosis, fever, pain, nervous disorders and sometimes renal failure and arterial hypotension.

Envenomation makes victims inactive by the loss of their limbs and causes deaths among the rural population. Majority of these populations whom live in the countryside use medicinal plants to treat themselves after snakes bites [2]. Therefore, there is a serious problem of effective care for victims of snake bites that lead to disability and loss of life in rural world. All these factors lead snake bite's victims to use traditional pharmacopoeia for their care. Surveys conducted in Togo show that *E. ocellatus* is responsible for more cases of bites and plant species such as *A. senegalensis* and *S. longepedunculata* are used in snake bites treatment [1].

A. senegalensis is traditionally used in several diseases management. Stem bark has been used in hernias treatment [3]. The root is used in the following cases: difficulty swallowing, gastritis, erectile dysfunction, tuberculosis, and as an antidote for necrotizing toxins; the root bark is effective in infectious diseases [4-6]. Tree juice is used in chickenpox treatment [7]. Many parts of the plant are used as antidotes for venomous and in diabetes management [8] [9]. In Guinea, *A. senegalensis* is also used to treat malaria [10]. Phytochemical tests showed that *A. senegalensis* contains tannins, flavonoids, saponosides, alkaloids, carbohydrates, sterols, volatile oils, anthraquinones and amino acids [11-17].

S. longepedunculata is traditionally used in diabetes and hypertension management [18]. It is used in dementia and epilepsy treatment [19-20]. It is also used in envenomations treatment [1]. It is used in the management of gastrointestinal disorders and malaria by the Tem people of the central region in Togo [21-22]. In Nigeria, the plant is used in the management of constipation, fever, erectile dysfunction, tuberculosis and rheumatism [23-24]. It is also used in the same country for infections related to the nervous and circulatory systems management [25]. Plant roots are used in Zimbabwe to treat fever, epilepsy, tuberculosis and syphilis [26]. *S. longepedunculata* phytochemical studies show that this plant contains saponids, carbohydrates, tannins, flavonoids, sterols, phenols and volatile oils [27-32].

The use of extracts from *A. senegalensis* and *S. longepedunculata* in traditional treatment of snake bites directs our research to antivenom properties of hydroethanolic extracts from their roots on arterial pressure in rats. To scientifically prove not only the antivenom properties of these two plants but also, understand the mechanisms by which the hydroethanolic extracts of these two plants neutralize *E. ocellatus* venom.

2 MATERIALS AND METHODS

2.1 Plants materials collection

Plant material was carried out in two different localities. *A. senegalensis* roots, a plant from the family Annonaceae were collected in the locality of Gblainvié located in Zio 1 commune in Togo. This plant has been identified by Laboratory of Botany and Plant Ecology of Lomé University (Togo) and a specimen was deposited in herbarium of this laboratory under voucher number TG 15673. *S. longepedunculata* roots were taken from locality of Anié located Anié 1 commune in Togo. Identification of this plant is made by the same laboratory and specimen was also deposited in herbarium of this laboratory under voucher number TG 15676.

2.2 Plant extract preparation

A. senegalensis and *S. longepedunculata* roots were washed, then dried under air conditioning at 22 °C for 15 days and then reduced into powder using THOMAS-Wiley, LABORATORY MILL, Model 4 Mill. 800 g of powder from each of two plants were macerated in 8 L water/ethanol mixture (50 : 50 ; V/V) for 72 hours under intermittent manual agitation. Macerates were filtered on Whatman paper and evaporated in vacuum at 45°C using a Rotavapor (Heidolph2, Germany). The extracts are obtained were in the form of crystals and were stored at 4 °C for experimentation.

2.3 Animals materials

Animal material consists of rats of Sprague-Dawley strain of both sexes with a weight varying between 150 and 210 g, which come from the pet store of Animal Physiology Department at Lomé University. They have free access to water, food, are kept in cages and are subject to a regular cycle of 12 hours of light and 12 hours of darkness.

2.4 *Echis ocellatus* venom

E. ocellatus lyophilised venom was obtained from LATOXAN Laboratory in France under reference number L1114. This venom was stored at 4 °C.

2.5 LD₅₀ determination of *Echis ocellatus* venom

LD₅₀ of *E. ocellatus* venom was determined by dividing twenty Sprague-Dawley rats of both sexes into four groups. These rats received increasing volumes of venom at a rate of 2 mL/kg body weight intraperitoneally and 24 hours after the injection, deceased rats were counted in each batch and mortality rates were used to graphically determine the LD₅₀. *E. ocellatus* venom was diluted in a 9 g/L NaCl solution [33].

2.6 Neutralization of *Echis ocellatus* venom hypotensive effects

2.6.1 Animals preparation

Bloody practice was used blood pressure study. The rat was anaesthetized with 80 % ethyl carbamate (1.5 g/kg, i.v.) at a rate of 1 mL/100 g body weight [34]. Jugular vein and carotid artery were exposed and intubated using a thin polyethylene catheter attached to a syringe [35]. Heparinized Ringer's solution 10% was injected at a rate of 0.1 mL/100 g body weight through jugular vein to prevent blood clotting. Pressure measurements were carried out using a pressure sensor coupled to an acquisition system (PowerLab; ADInstruments, Castle Hill, NSW, Australia) connected to a computer controlled by LabChart 6.0 software (ADInstruments).

2.6.2 Venom and extracts administration

For control, each rat received intra venous Ringer physiological solution, then *E. ocellatus* venom at 0.235 mg/kg body weight. Five minutes after the rats received intra venous extracts at increasing volumes corresponding to doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg. Venom and the extracts were administered to rats through the jugular vein at a rate of 0.1 mL/100 g after a blood pressure stabilisation time of approximately 30 minutes. Effects of *E. ocellatus* venom and extracts from *S. longepedunculata* and *A. senegalensis* on mean blood pressure were recorded continuously throughout the experiment.

2.7 Data analysis

Data was processed using GraphPad Prism 6.05 software (GraphPad Software Inc, California). Results are expressed as a mean value with the standard error on the mean ($M \pm SEM$). The significance level was set at $p > 0.05$.

3 RESULTS

3.1 *Echis ocellatus* venom LD₅₀

LD₅₀ was determined by counting rat deaths number in each group. The results allowed us to plot the curve of the mortality rate in rats as a function of the venom dose log. This curve was used to determine the LD₅₀ of *E. ocellatus* venom in Sprague-Dawley rats, which is 0.235 mg/kg.

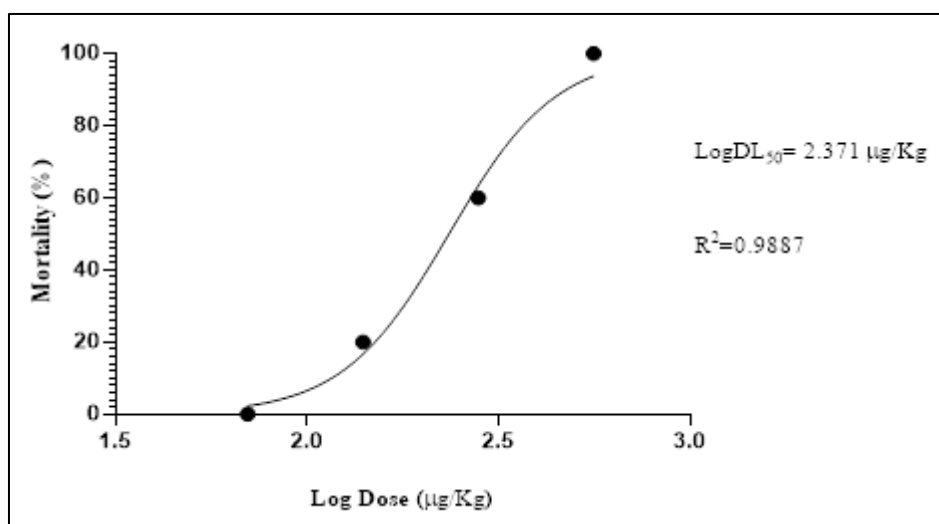


Figure 1: LD₅₀ determination

3.2 Extracts effects on venom proteins responsible for arterial hypotension

E. ocellatus venom decreases MAP to 27.16 ± 3.04 %. Extracts at 10 mg/kg and 20 mg/kg significantly raise MAP decreased by *E. ocellatus* venom. Extract dose at 20 mg/kg raises MAP to 81.49 ± 6.87 % with *A. senegalensis* extract, whereas the same dose of *S. longepeduncula* extract raises MAP to 84.59 ± 8.36 %.

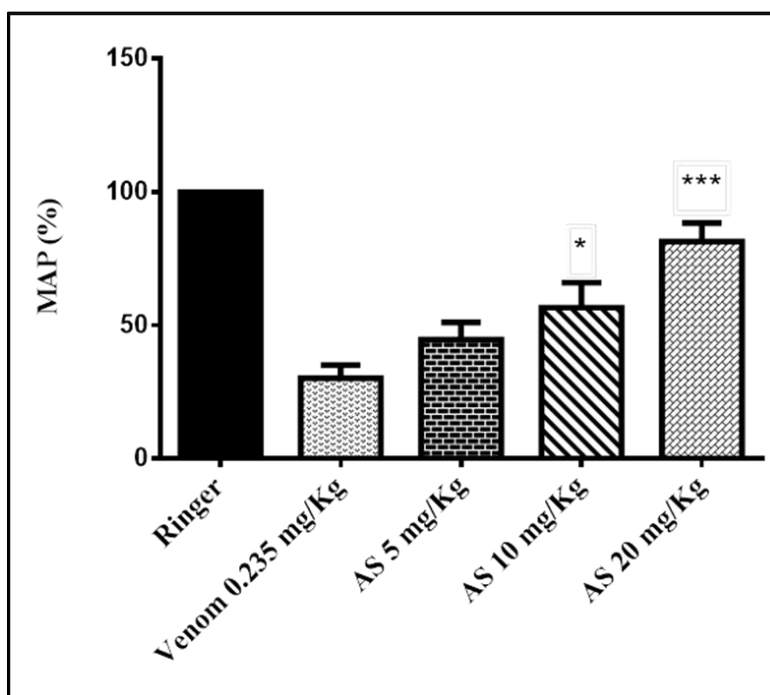


Figure 2: Venom and *Annona senegalensis* extracts effects on mean arterial blood pressure.

Data are expressed as an $M \pm SEM$. * $p < 0.05$; *** $p < 0.001$ (compared to venom). AS = *Annona senegalensis*.

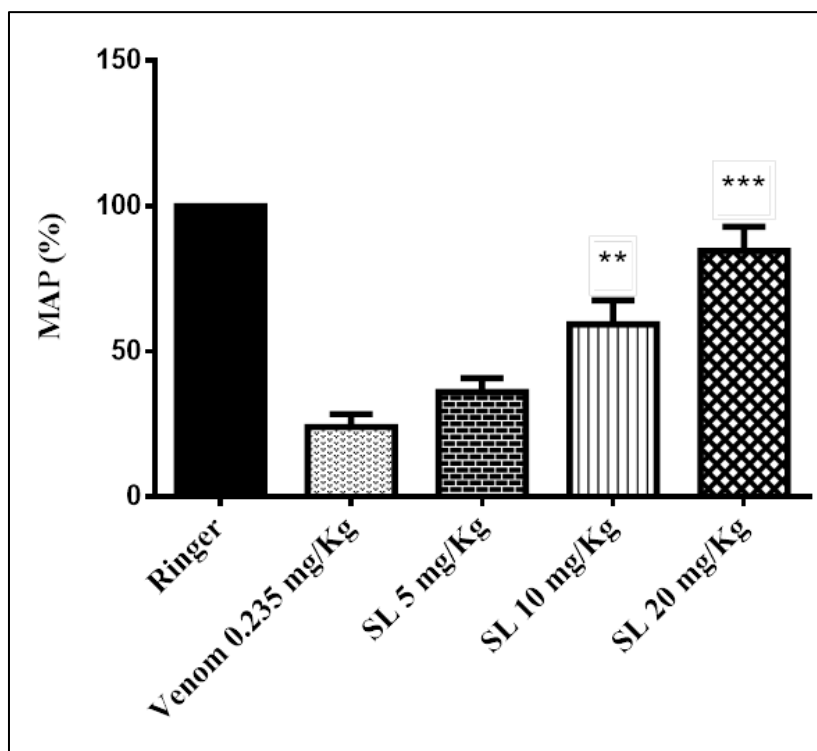


Figure 3: Venom and *Securidaca longepedunculata* extracts effects on mean arterial blood pressure.

Data are presented as the $M \pm SEM$. ** $p < 0.01$; *** $p < 0.001$ (compared to venom). SL = *Securidaca longepedunculata*.

3.3 Venom plus hydroethanolic extracts of *Annona senegalensis* and *Securidaca longepedunculata* effects on heart rate

E. ocellatus venom decreased heart rate to 49.51 ± 3.16 beats/min. *A. senegalensis* and *S. longepedunculata* root hydro-ethanolic extracts at 10 mg/kg and 20 mg/kg significant increase heart rate value. For this, *A. senegalensis* at 20 mg/kg increases heart rate to 79 ± 5.57 beats/min while *S. longepedunculata* at 20 mg/kg increases heart rate to 80 ± 1.16 beats/min.

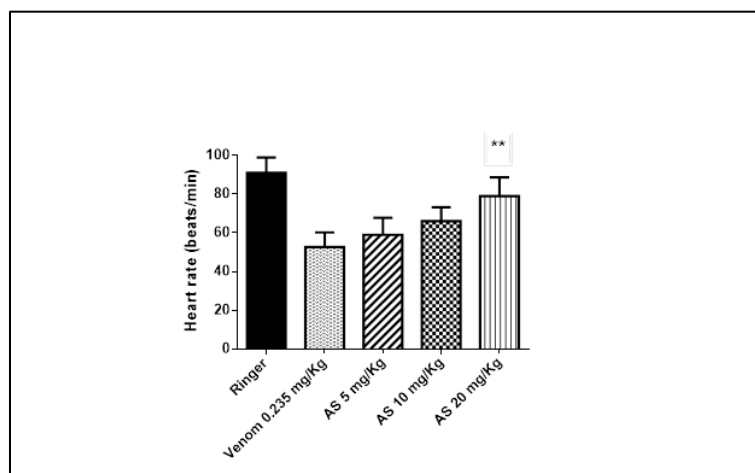


Figure 4: Venom and *Annona senegalensis* effects on heart rate.

Data are presented as the $M \pm SEM$. ** $p < 0.01$ (compared to venom). AS = *Annona senegalensis*.

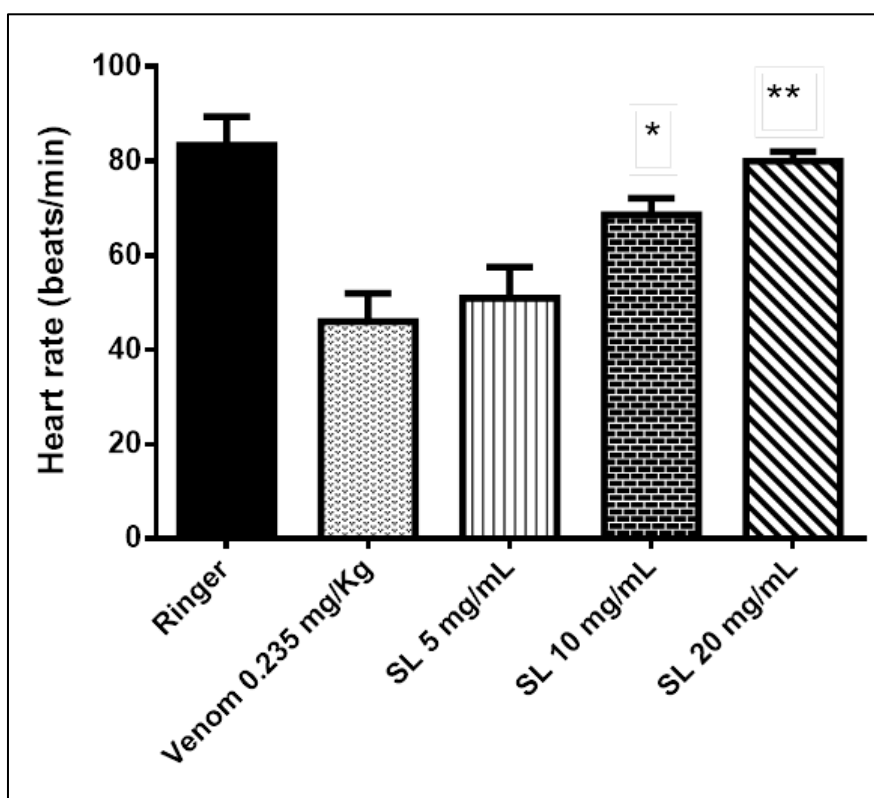


Figure 5: Venom and *Securidaca longepedunculata* effects on heart rate.

Data are presented as the $M \pm SEM$. * $p < 0.05$; ** $p < 0.01$ (compared to venom). SL = *Securidaca longepedunculata*.

4 DISCUSSION

Snake bites are real public health problem that remains little known, due to the lack of dedicated observatory. In some parts of Africa, depending on the time of year, up to 10% of hospital beds may be occupied by snakebite victims [36]. One third of patients subsequently have deformities or amputations of limbs following bites from vipers. Viperid venom causes hemostatic and local disorders with severe pain, sometimes extensive edema, phlyctene and skin necrosis and hypotension [37]. *A. senegalensis* and *S. longepedunculata* roots are used in ophidian envenomations traditional treatment in Togo [1,38]. Our study aims to determine the mechanisms by which these two plants control arterial hypotension disorders caused by viper envenomation.

Our study shows that intraperitoneal LD₅₀ of *E. ocellatus* is 0.235 mg/kg in rats. This low LD₅₀ shows that *E. ocellatus* venom is highly toxic substance. A similar study conducted in Nigeria gave an LD₅₀ of 0.22 mg/kg [39] which is approximately to our value. This can be explained by same proteins presence in both venoms. On the other hand, another study conducted in Nigeria but in Borno state gave an LD₅₀ of 0.35 mg/kg for the same species [40]. This LD₅₀ difference can be explained by the climate and environment, which are certainly not the same for the two snakes.

MAP obtained with *E. ocellatus* venom is significantly low compared to that obtained with the reference solution, which is the Ringer. This result implies that *E. ocellatus* venom has a hypotensive action on rat blood pressure. The fact that venom also increases heart rate confirms *E. ocellatus* venom hypotensive action because blood pressure depends on blood flow and vessel resistance. Blood flow is the product of blood ejected volume at each beat and heart rate. If heart rate is low then blood flow is also low and consequently there will be hypotension.

Some Elapidae venom cause cardiovascular depression, with sweating, cooling of the extremities, tachycardia, and hypotension [41]. Angiotensin converting enzyme increases blood pressure by increasing angiotensin II concentration. The same enzyme converts bradykinin into an inactive metabolite [42]. Angiotensin II Type 1 receptor stimulation generates systemic arterioles vasoconstriction, Na⁺ reabsorption and water retention, as well as increased aldosterone secretion by adrenal glands [43-44]. Bradykinin-potentiating peptides inhibit bradykinin degradation and the synthesis of bradykinin by serine proteases from snake venom increases bradykinin concentration. Therefore, stimulation bradykinin receptor B₂ generates to release of NO and prostacyclin, which all cause vasodilation that leads hypotension.

Hypotensive molecules have been found in snake venom, such as phospholipases A₂ present in venom of Colubridae, Elapidae and Viperidae. Phospholipases A₂ proteins capable to reduce blood pressure through arachidonic acid production, a precursor of cyclooxygenase metabolites (prostaglandins or prostacyclins), and interaction with platelets and leukocytes [45]. Bradykinin-potentiating peptides were also found in *Bothrops jararaca* venoms [46]. Bradykinin-potentiating peptides inhibit angiotensin converting enzyme. Latter converts angiotensin I to angiotensin II, which is a vasoconstrictor hormone that indirectly increases blood pressure. Therefore, inhibition of angiotensin converting enzyme increases bradykinin hypotensive capacity. Hypotensive effect and mechanism of action of bradykinin-potentiating peptides contained in snake venom have been studied in genera such as : *Bitis*, *Bothrops* and *Crotalus* [47-49]. Natriuretic peptides, which are vasoactive hormones that act through two main mechanisms, have also been found in snake venom. Two main mechanisms by which they act are vasodilation and renal blood flow regulation. Natriuretic peptides cause effects such as inhibition of renin-angiotensin and aldosterone, diuresis, natriuresis and vascular permeability regulation [50]. Serine proteases also isolated from snake venom are trypsin-like enzymes that act on coagulation cascade, fibrinolytic systems and cells, causing an imbalance in haemostatic system. A subset of serine proteases lower blood pressure by releasing bradykinin from kininogen [51].

E. ocellatus venom decreases MAP in rats up to 27.16 ± 3.04 %. Both extracts slow down blood pressure decrease caused by venom. The dose of *A. senegalensis* and *S. longepeduncula* extracts at 20 mg/kg were most effect, with an increase in MAP of 81.49 ± 6.87 % with *A. senegalensis* and an increase in MAP of 84.59 ± 8.36 % with *S. longepeduncula*. We can deduce that our two extracts action mechanism could be explained by fact that they act by neutralizing venom, which will allow the angiotensin converting enzyme to convert angiotensin I into angiotensin II, which will cause arteriolar vasoconstriction and also stimulate aldosterone secretion by adrenal cortex. Aldosterone promotes Na⁺ retention and water reabsorption by kidneys, thus increasing blood volume. Arteriolar vasoconstriction and blood volume increase leads to increased blood pressure.

5 CONCLUSION

Snakebite is real public health problem. Its management must be effective and must be done within a short period of time in order to avoid serious complications that result. This work allowed us to show that *E. ocellatus* venom causes arterial hypotension. Hydro-ethanolic extract of *A. senegalensis* and *S. longepedunculata* roots contains molecules that neutralize *E. ocellatus* venom proteins, which are responsible for arterial hypotension. Our results show that hydro-

ethanolic extract of *A. senegalensis* roots and the hydro-ethanolic extract of *S. longepedunculata* roots have antivenom properties and can be used in ophidian envenomations. However, further studies will be needed.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

Authors declare that they have no conflicts of interests.

Statement of ethical approval

All animal procedures were approved by Physiology-Pharmacology Laboratory of Faculty of Sciences at University of Lomé.

Authors contributions

DATAGNI G, conceptualization, methodology, software, formal analysis, data curation, writing original draft preparation. METOWOGO K, validation. MOUZOU AP, validation, writing – review & editing. ASSIH M, methodology. PAKOUSSI T, visualization. EKLUGADAGBEKU K, Supervision.

Availability of data and materials

The authors of this article declare that all data used in writing of said article are available and will be made available to the journal committee from the corresponding author upon request, in accordance with Journal's policy.

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