Available online on 15.04.2026 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Polyphenolic extract from *Entada africana* Guill. Pers is a potential antioxidant and anti-Tumor Necrosis Factor alpha (TNFα) ingredient

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Article Info:

Article History:

Received 11 Jan 2026
Reviewed 20 Feb 2026
Accepted 16 March 2026
Published 15 April 2026

Cite this article as:

Njami RM, Kouoh Elombo F, Pechangou Nsangou S, Bolling B, Njayou Nico F, Moundipa Fewou P, Polyphenolic extract from *Entada africana Guill. Pers is a potential* antioxidant and anti-Tumor Necrosis Factor alpha (TNFα) *ingredient*, Journal of Drug Delivery and Therapeutics. 2026; 16(4):32-38 *DOI: http://dx.doi.org/10.22270/jddt.v16i4.7643*

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Abstract

Entada africana Polyphenolic Extract (PEE) revealed higher contents of total polyphenols (264.91±1.51mg EAG/g of extract), flavonoids (14.26 ±0.26mg EQ.g of extract) and flavonols (2.1±0.03mg EQ/g of extract). Concentration of Scavenging 50% (CS_{50}) of hydroxyl radical (HO^{o}) radicals with PEE (20.65 ± 0.35 µg/mL) compared to that of vitamin C (7.90 ± 0.00 µg/mL) showed a significant difference at $p< 0.05$ for HO^{o} scavenging activities. The PEE extract inhibited lipidic peroxidation with IC_{50} of 9.70 ± 0.21 µg/mL but not significantly different from vitamin C (8.70 ± 0.14 µg/mL). PEE extract have inhibited the production of TNFα with IC_{50} (71.05 ± 1.20 µg/mL) significantly ($p< 0.05$) when compared to 25% of methylene chloride fraction of *Khaya* grandifoliola (32.10 ± 1.13 µg/mL) considered as a standard. For the activation of erythrocytes plasma membrane redox system (PMRS), PEE had an Effective Concentration 50% (EC_{50}) of 8.4 ± 0.00 µg/mL relatively less than that of quercetine 4.6 ± 0.00 µg/mL. Moreover, PEE inhibits proteins oxidation in a dose-concentration manner with an IC_{50} of 14.60 ± 1.13 µg/mL significantly different ($p< 0.05$) from that of quercetin (4.60 ± 0.00 µg/mL) considered as a standard. Altogether, PEE could be used as potential antioxidant and anti-Tumor Necrosis Factor alpha (TNFα) ingredient for food and nutrition.

Keywords: *Entada africana*, polyphenols, antioxidant, anti-TNFα, erythrocytes PMRS.

INTRODUCTION

Oxidative stress and inflammation are involved in the physiopathology of oxidative diseases such as anemia, neurodegenerative diseases, type II diabetes, edema, cardiovascular diseases, obesity and tumors. On the one hand, the erythrocytes plasma membrane redox system (PMRS) plays an essential role in maintaining the level of plasma ascorbic acid, the primary antioxidant and the main reducing equivalent of this system[1]. When erythrocytes are subjected to an excessive level of reactive oxygen species (ROS), the activity of PMRS decreases and consequently the concentration of extracellular ascorbic acid and the antioxidant potential of blood plasma decreases. Erythrocytes become weak and decrease in number throughout hemolysis. On the other hand, inflammation is the main protective response of the organism in case of tissue injuries. It becomes chronic when this process is abnormally regulated and is involved in pathologies such as type II diabetes, edema, cardiovascular diseases, obesity and tumors[2]. ROS can contribute to extend this process by inducing transcription factors such as nuclear factor kappa-b (NF-Kb)[3]. To solve health problems associated with oxidative stress and inflammation, one of the treatment approaches is the use of medicinal plants. They are privileged sources of panoply of bioactive molecules such as polyphenols and particularly flavonoids that are endowed with several beneficial biological activities including antioxidant and anti-inflammatory activities[4]. In African traditional medicine, Entada africana Guill. Pers is used to treat different ailments in which oxidative stress and inflammation are underlining physiopathological mechanisms[5]. To appreciate this ancestral use, we had extract and concentrated polyphenols of Entada africana Guill. Pers stem barck, and evaluated it's antioxidant and anti-inflammatory properties in vitro as well as their ability to activate the erythrocytes PMRS.

It's antioxidant and anti-inflammatory properties were evaluated in vitro as well as their ability to activate the erythrocytes PMRS.

MATERIAL AND METHODS

Extraction of polyphenols. Fresh barks of *Entada africana* was collected, washed and rinsed with distilled water and dried at laboratory temperature. After crushing, 350 g of powder was placed into 2L of hexane. The polyphenolic extracts were obtained according to Singleton and collaborator[6]**.** The hexane extracts were weighed and stored at 4° C. The extraction yields were calculated from the following formula:

yield = (mass of extract)/ (mass of bark powder) ×100

Qualitative analysis of polyphenolic extract. Colorimetric analysis was performed as previously described[7,8] for screening of polyphenols, flavonoids, tannins, anthraquinone, alkaloids, saponins, terpenes, triterpenes and sterols. High performance liquid chromatography (HPLC) analysis was monitored as previously described[9] and the compounds were eluted by a gradient for 20 min followed by re-equilibration of the column, using a flow rate of 0.5 mL/min at 25°C.

Quantitative analysis of PEE. In 5 mL of methanol, 50 mg of PEE was dissolved for analyses according to Dhar and collaborators[10].

Total polyphenols content was determined by Folin-Ciocalteux method and the absorbance read at 765 nm.

Flavonoid and Flavonols contents were measured as previously described[9]. The optical densities were read respectively at 420 nm and 440 nm; content was determinate as previously described [9]. Absorbance was read at 440 nm.

Determination of the antioxidant properties of the PEE

For testing, PEE was dissolved in DMSO at final concentrations of 0.1; 1; 10 and 100µg/mL. Vitamin C was used as standard and treated under the same conditions as the extracts.

DPPH° radical scavenging activity was determined as previously described[11]. Absorbance was read at 517 nm and the fifty percent scavenging concentrations (SC_{50}), the fifty percent efficacy concentrations (EC_{50}) and finally the anti-radical powers (AP) were determinate.

Hydroxyl radical (OH°) scavenging activity was measured as previously described[12]. The Mixtures were incubated at 37°C for 1 hour and absorbances read at 562 nm.

Reduction activities

Potassium ferricyanide reducing test was conducted as previously described[13]. Absorbances were read at 700nm.

Molybdate reducing assay. As previously described1[4, 9], after incubated (95°C, 90 minutes) the mixture was cooled. Absorbances were read at 695 nm. The antioxidant capacity was estimated in mg ascorbic acid equivalent per gram of extract (mg EAA/g of extract).

Lipid and proteins peroxidation inhibition assays

Lipid peroxidation inhibition assay was conducted as previously described[9]. Following boiling (15 min), cooling and centrifugation (1620g, 5 min, 4°C), the supernatants were collected and the absorbance read at 532 nm. The percentages of inhibition were calculated.

Protein oxidation inhibition assay[15]. Briefly, after incubation of the mixture and addition of TCA the tubes were centrifuged and the pellets washed and suspended in urea. Absorbance was read at 372 nm and the percentage of inhibition was calculated.

The effects of PEE extracts on the Erythrocyte Plasma Membrane Redox System (PMRS)[1]

PMRS activity assay was conducted as previously described[9]. In briefly, to erythrocytes suspension was added: 1.7mL PBS-glucose and 100 µl of 20 mM potassium ferrocyanide 20 mM. All the tubes were incubated and centrifuged. A mixture was prepared with the supernatant prior to incubation for 10 minutes at room temperature and the absorbance was read at 535nm. The percentage of inhibition was calculated.

Effect of PEE on Tumor Necrosis Factor alpha (TNFα) production

Jurkat T cells (Cone E6-1, ATCC® TIB-152™) were used in the presence of PEE at different concentrations as previously described[9]. TNF-α production was assayed using Human TNF-α ELISA MAX™ Deluxe kit (Biolegend, San Diego, CA, USA) according to the manufacturer protocol.

Data analysis

GraphPad Prism 8.0.1 software was used for analyses. Results were expressed as mean ± standard deviation. The different values were compared using the analysis of variance test "one-way ANOVA" followed by the multiple comparison test of Tukey with a p-value $p<0.05$.

RESULTS AND DISCUSSION

Polyphenols were extracted from *E. africana* with an extraction yield of 11.22%. PEE was measured by colorimetric test using screening methods. The presence of polyphenols, flavonoids, tannins and leuco-anthocyanins has been revealed (Table 1).

Table 1: Phytochemical profile of PEE

Phytochemicals	Presence in PEE
Sugars	-
Polyphenols	+
Flavonoids	+
Tannins	+
Leuco anthracyanes	+
Free quinones	-
Anthraquinones	-
Alkaloids	-
Terpenes and sterols	-
Saponines	-

PEE: polyphénolic extract of *Entada africana* ; (+) = present ; (-) = absent

The presence of those polyphenols such as flavonoids was shown by HPLC at 254nm and 370nm (Fig. 1A, 1B); with a confirmation of pictures observed by UV analysis (Fig. 1C, 1D).

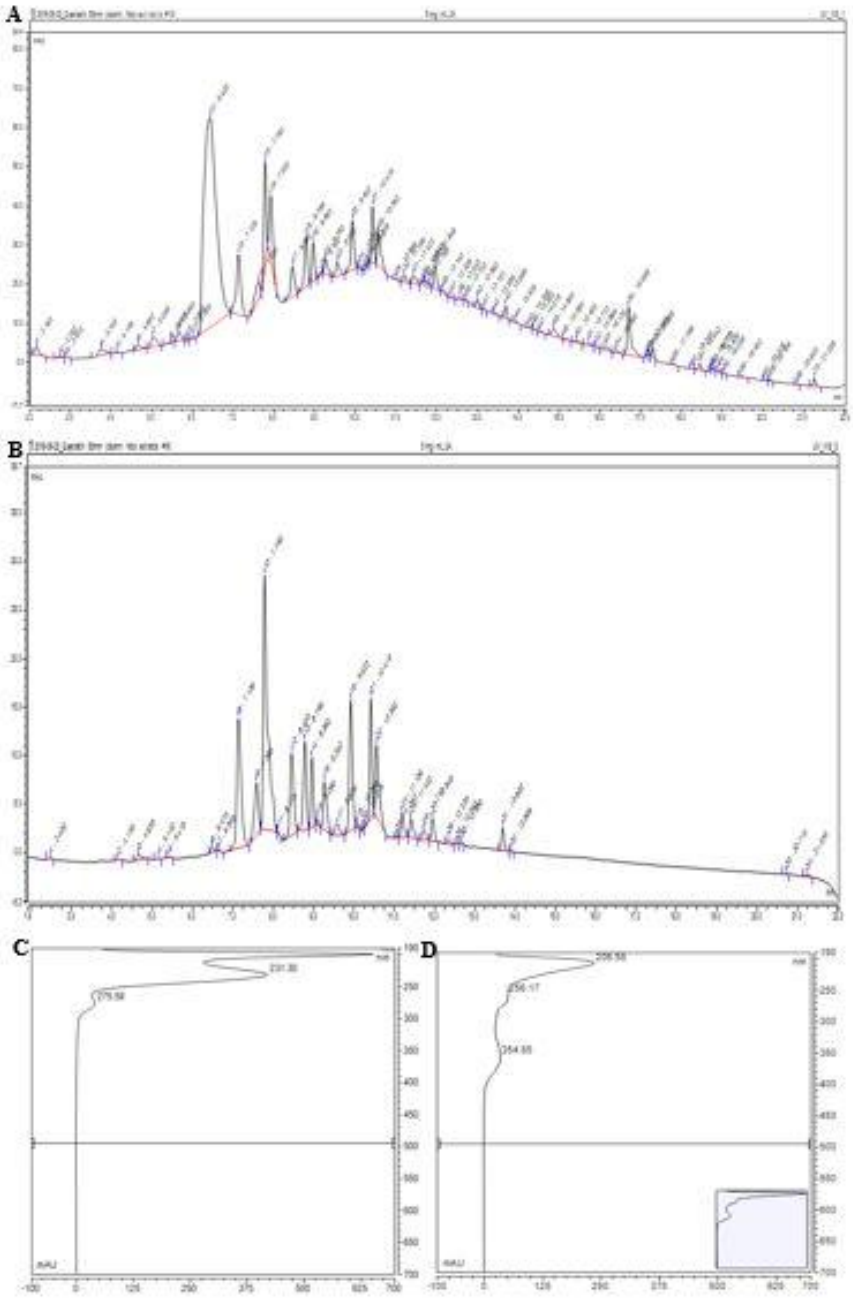

Figure 1. HPLC profile of PEE

A: PEE extract chromatogram at 254 nm as function of retention time; **B:** PEE extract chromatogram at 370 nm as function of retention time; **C :** Absorption band in UV of the 13th pic at 254 nm for PEE; **D :** Absorption band in UV of the 13th pic at 370 nm for PEE.

The two wavelengths corresponded to the absorption zones of flavonoids[16]. UV analysis of the main peaks at these two wavelengths was carried out and the results revealed that PEE shows several major peaks at those two wavelengths. The quantification of the studied phytochemical previously highlighted revealed the presence of polyphenols (264.91±1,51mg EAG/g of extract), flavonoids (14.26 ±0,26mg EQ.g of extract) and flavonols (2.1±0,03mg EQ/g of extract) in PEE (Table 2).

Table 2: Total polyphenols, flavonoids and flavonols content of PEE

Class of compound		Values
Total polyphenols	mg EAG/g of extract	264.91 ± 1.51
	mg EQ/g of DM	27.71 ± 0.13
Flavonoids	mg EQ/g of extract	14.26 ± 0.26
	mg EAG/g of DM	1.49 ± 0.02
Flavonols	mg EQ/g of extract	2.10 ± 0.03
	mg EQ/g of DM	0.34 ± 0.03
TAC	mg EAA/g of extract	412.96 ± 2.34
	mg EAA/g of DM	68.96 ± 0.39

PEE: polyphénolic extract of *Entada africana;* EAG: equivalent gallic acid; DM: Dried material; EQ: equivalent quercetine; AA: ascorbic acid

On the one hand, all those Polyphenolic compounds are bioactive molecules with anti-inflammatory and antioxidant activities [4, 17]. On the other hand, it is kwon that an overproduction of reactive oxygen species (ROS) causes oxidative stress. This leads to several pathologies[18, 19]. ROS are underlining physiopathological mechanism of inflammation[20]. In many diseases associated with inflammation or oxidative stress, anti-inflammatories or antioxidants play respectively an important role in the body's defense system. With antioxidants, we could have free radical scavenging, reduction of oxidative compounds, chelation of transition metals or protection of biomolecules[21]. For scavenging activities, PEE antiradical activity was determinate throughout the scavenging of the DPPH and HO° radicals. *E. africana* Polyphenolic Extract effectively trap those two radicals in a concentration-dependent manner between 0.1 and 100µg/mL (Fig. 2A). The respective IC_{50} values are 6.95 ± 0.07µg/mLand 20.65 ± 0.35µg/mL for DPPH and HO°. The corresponding activities for ascorbic acid (AA) were 5.00 ± 0.00 µg/mL and 7.9 ± 0.00µg /mL (Table 2). From the results obtained, PEE scavenging DPPH radical was not significantly different from that of vitamin C and could be explained by the high flavonoids contain in the extracts.

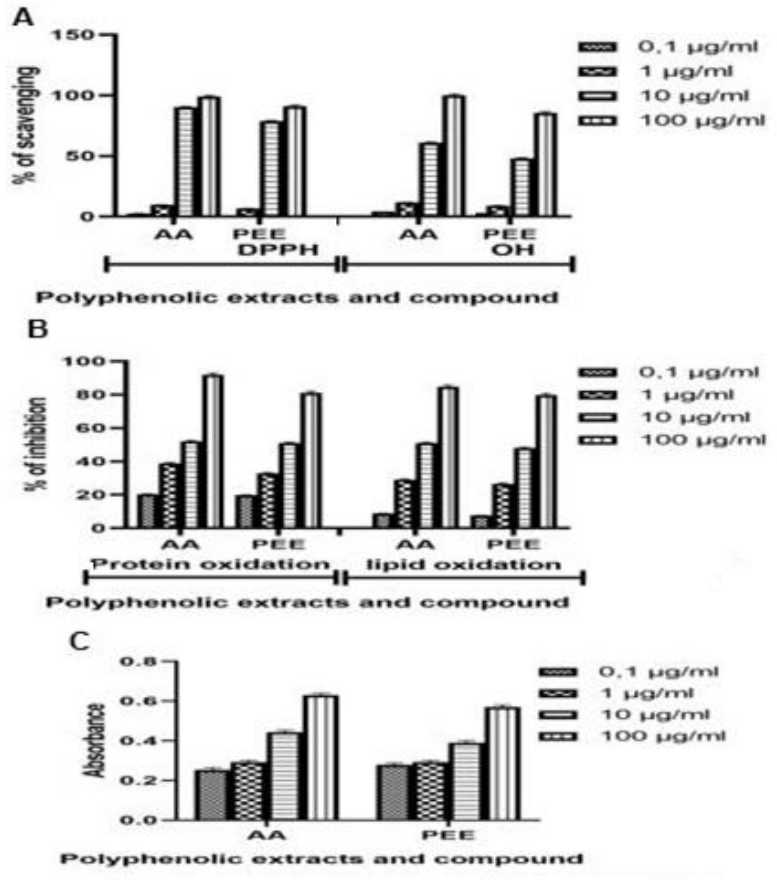

Figure 2: Antioxidant potential of PEE.

(A): DPPH and OH radical scavenging activities; **(B):** Inhibition of protein oxidation and lipid peroxidation; **(C):** Ferrous reducing agent power. PEE: polyphénolic extract of *Entada africana.*

However, DPPH free radicals are not found in biological systems. Among the radicals likely to be formed in cells, HO• radical is the most reactive and dangerous, especially since it is the initiator of the macromolecule damages [22]. Therefore, the scavenging of the HO• radical can be an effective means of defense against several diseases associated with oxidative stress. This radical generated in vitro by the $FeSO_4/H_2O_2$ system, was

effectively scavenged by PEE and its activity was higher than that obtained with the methanolic extract of *Zingiber officinale* (IC_{50}: 22.36 µg/mL) [23]. This difference may be due to the fact that the PEE flavonoids release protons more easily to stabilize the HO• radical. However, this activity remained less effective compared to vitamin C (IC_{50}: 7.90±0.00). It's similar to what was observed with Khaya grandifoliola phenolic compounds[9]. This shows the extent of involvement of these extracts in iron metabolism, particularly in the reduction of Fe III provided by plant diet to Fe II, which is important for hemoglobin porphyrin synthesis[9]. PEE showed strong reducing powers not significantly different from that of vitamin C at the concentrations tested (Figure 2).

The total antioxidant capacity of PEE is 68.96 ± 0.39 mgEAA/g of dry material. Furthermore, the evaluation of the capacity to protect cellular macromolecules is a good parameter to investigate the antioxidant potential of a polyphenolic extract. However, it remains limited due to low in vivo bioavailability of polyphenols. As far as lipid and protein oxidation inhibitory activities were concerned, PEE exhibited antioxidant activities throughout inhibition of membrane lipid peroxidation and protein oxidation evolving in a concentration-dependent manner (Fig. 2B). The respective IC_{50} values are 9.70 ± 0.21µg/mL and 14.60 ± 0.21µg/mL for the inhibition of membrane lipid peroxidation and protein oxidation. The corresponding activities, in Table 3 for ascorbic acid (AA) and quercetine are respectively 8.70 ± 0.14 µg/mL for membrane lipid peroxidation inhibition and 9.40 ± 0.14µg /mL for protein oxidation inhibition. In addition, PEE as well as quercetine were active on erythrocyte PMRS.

Table 3: IC_{50} and EC_{50} of PEE activities

Assays	Concentration	Standard	PEE
DPPH radical scavenging assay	IC_{50} (µg/mL)	AA : 5.00 ± 0.00	6.95 ± 0.07
HO radical scavenging assay	IC_{50} (µg/mL)	AA : 7.90 ± 0.00	20.65 ± 0.35[a]
Inhibition of lipid peroxidation	IC_{50} (µg/mL)	AA 8.70 ± 0.14	9.70 ± 0.21
Inhibition of protein oxidation	IC_{50} (µg/mL)	Quercetine : 9.40 ± 0.14	14.60 ± 0.21 [a]
Activation of the membrane redox system	EC_{50} (µg/mL)	Quercetine : 4.06 ± 0.00	8.40 ± 0.00 [a]
TNFα inhibition assay	IC_{50} (µg/mL)	F25 : 32.10 ± 1.13	71.00 ± 1.27 [a]

PEE: polyphenolic extract of *Entada africana*; EC_{50} : the fifty percent efficacy concentration IC_{50}: the fifty percent Inhibitory concentration; [a]: significantly different from ascorbic acid at $p < 0.05$.

PEE effectively activated the plasma membrane redox system of red blood cells in a concentration-dependent manner as shown on figure 3.

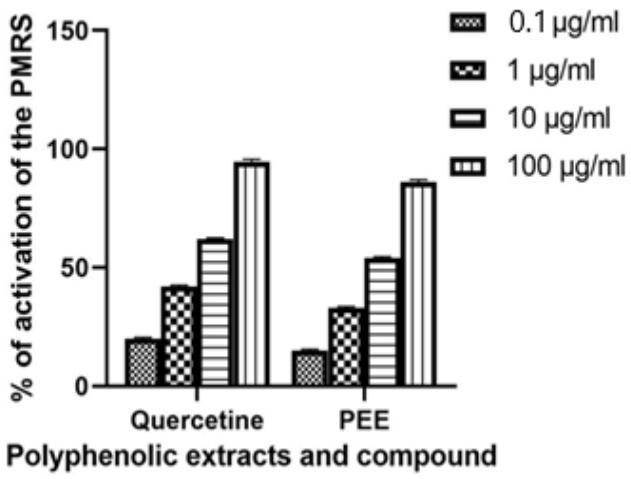

Figure 3. Effect of PEE on the erythrocyte PMRS.

PEE: polyphénolic extract of *Entada africana.*

The corresponding IC_{50} is 8.40 ± 0.00 µg/mL, lower compared to quercetine used as standard (4.6 ± 0.00 µg/mL). These activities were similar to those exhibited on erythrocyte PMRS by curcumin extracted from *curcuma longa* [24] or Khaya grandifoliola phenolic compounds[9]. Polyphenols by acting through protection of proteins from denaturation, regulation of pro-inflammatory mediators and transcription factors involved in the inflammatory process[25] are considered as anti-inflammatory agents. In the process of inflammation, NF-KB factor plays a very important role as it induces the transcription of a wide range of genes that code for pro-inflammatory mediators such as TNFα [26]. PEE effectively inhibited the production of TNFα (IC_{50} is 71 ± 1.27 µg/Ml), significantly different at $p < 0.05$ from the 25% fraction of *K. grandifoliola* considered as reference (IC_{50} is 32.10 ± 1.13 µg/Ml) with regard to Table 3. To explain this anti-inflammatory activity we could suggest the presence of flavonoids which act either by inhibiting the nuclear translocation of NF-KB or by inhibiting transcription or translation.

CONCLUSION

Entada africana Guill et Perr polyphenolic extracts exhibited antioxidant properties throughout the inhibition of: protein oxidation, the activation of erythrocyte PMRS and lipid peroxidation. Furthermore, PEE inhibited the production of TNF-α. All those health benefits may be used to propose PEE as a potential ingredient of functional foods. PEE could be used to combat health conditions involving oxidative stress and inflammation.

Acknowledgements: We would like to thank the staff of the National Herbarium of Cameroon for helping us locate and identify the Entada africana Guill. Pers plants.

Funding: This work received funding from the Cameroonian Ministry of Higher Education throughout the special allowance for the modernization of research

Disclosure: The study was independently designed by the authors and the funding body had no role in Lab experiments, analysis and interpretation of the data

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author's contributions:

Ridel Mbiandou Njami: performed the analyses, processed the data, and drafted the manuscript.

Bradley Bolling

Frederic Nico Njayou: conceived the study, collected the samples

Ferdinand Kouoh Elombo contributed to data processing, analyses and manuscript writing.

Sylvain Nsangou Pechangou performed the analyses

Paul Fewou Moundipa contributed to manuscript writing.

All authors read and approved the final manuscript.

Ethical statement: All procedures in this study followed the Cameroon National Veterinary Laboratory guidelines and were approved by the Animal Ethical Committee of the Laboratory of Animal Physiology of the Faculty of Sciences, University of Yaoundé I–Cameroon.

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