



(RESEARCH ARTICLE)



## Prophylactic antimalarial effects of *Cymbopogon citratus* (DC.) Stapf (Poaceae) in a mouse model of *Plasmodium berghei* ANKA infection: normalisation of haematological and serum biochemical status

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### Abstract

**Ethnopharmacological relevance:** *Cymbopogon citratus* (DC.) Stapf (Poaceae) is a medicinal plant known for its antimalarial, antipyretic and antifatigue activities in Cameroonian folk medicine.

**Aim of the study:** The aim of this work was to evaluate the prophylactic antimalarial effects of the decoction prepared from the leaves of *Cymbopogon citratus* on *Plasmodium berghei* ANKA infection in mice and investigate its action on haematological and serum biochemical status.

**Materials and methods:** Swiss mice were treated with *Cymbopogon citratus* leaf decoction (25, 50, 100 and 200 mg/kg) and later inoculated with *Plasmodium berghei* ANKA. The prophylactic antimalarial activity of the decoction was evaluated by determining the parasitaemia, percentage chemosuppression, body weight, body temperature, food and water intake in pretreated parasitised mice. The possible ameliorative effects of the decoction on malaria associated haematological and serum biochemical changes were also assessed.

**Results:** The decoction exhibited a prophylactic activity of 85.32% and its chemotherapeutic effects ranged from 56.88 – 85.32% with maximum effect observed at the highest experimental dose. It significantly inhibited parasitaemia ( $P < 0.001$ ) compared to the negative control group. Interestingly, treatment of parasitised mice with the decoction significantly restored the malaria modified haematological and biochemical status compared with distilled water-treated parasitised mice.

**Conclusion:** The results of this prophylactic assay indicated that *Cymbopogon citratus* decoction has antimalarial effects and normalised haematological and serum biochemical aberrations generated by malaria. Hence, *Cymbopogon citratus* represents a promising source of new antimalarial agents.

**Keywords:** *Cymbopogon citratus*; malaria prophylaxis; haematological and biochemical; mouse model.

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## 1. Introduction

Malaria remains one of the deadliest infectious diseases in the world today, causing high rate of morbidity and mortality annually. Malaria, caused by *Plasmodium* parasite, is a leading poverty associated disease that undermines the development of countries. It is endemic in tropical and sub-tropical regions including parts of Africa, Asia, and the Americas. In 2015, there were 212 million cases of malaria, leading to 429,000 deaths, most of which were children less than 5 years old [1]. These figures rose to 216 million cases in 2016, resulting in 445,000 deaths, most of which occurred in Sub-Saharan Africa and India [2]. World Health Organization (2018) estimated that about 3.2 billion people across 91 countries are still at risk of malaria thereby necessitating efficient control measures against the disease. Global Technical Strategy for Malaria sets a target to reduce the case of malaria incidence and mortality rates by at least 40% by the year 2020. The promising vaccine RTS, S/AS01 is still currently undergoing a phase 4 clinical trial [3]. Vector control measures involving the use of insecticides treated bed nets and indoor residual spraying amongst others are already experiencing problem of insecticide resistance [4]. Drug overuse and misuse including fake drugs in circulation have been reported as the main drivers of drug resistance in parasites (including the malaria parasite) [5]. Consequently, there is a dire need to develop new antimalarial therapeutic agents from natural products or treatment approaches that will help in reducing further increase in malaria associated morbidity and mortality.

Since malaria parasites are blood parasites, haematological changes are the most common complications encountered. Anaemia is a common symptom associated with malaria. In anaemic conditions, the total amount of red blood cells or the haemoglobin concentration in whole blood decreases, hence oxygen carrying capacity of the blood is lowered [6]. Haemolytic anaemia is witnessed in malaria infection due to the destruction of red blood cells. Following this, there is an increased in the level of bilirubin in the blood, and also liver enzymes like aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels [7].

*Cymbopogon citratus* is a tropical plant belonging to the Poaceae family used by the traditional healers as an anti-inflammatory, antipyretic, antiprotozoal and particularly antimalarial agent [8-11]. The essential oils isolated from *Cymbopogon citratus* have been reported to exhibit antimalarial activity in mice [12, 13], and the whole *Cymbopogon citratus* plant elicited higher anti-malarial activity than the herbal infusion when used as a prophylactic treatment. However, the decoction prepared from the leaves of *Cymbopogon citratus* has not been experimentally assessed for prophylactic effects against malaria infection [13]. Therefore, the test of prophylactic antimalarial activities of the decoction prepared from the leaves of *Cymbopogon citratus* was used to examine the possible preventive effects in mice infected by *Plasmodium berghei* ANKA. In addition, a relationship between the reduction of parasitaemia was correlated with the haematological and serum biochemical status in *Plasmodium berghei* ANKA infected mice.

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## 2. Material and methods

### 2.1. Plant material

The leaves of *Cymbopogon citratus* used in this study were harvested in the Mount Cameroon area, locality of Buea (South West Region of Cameroon, harvesting coordinates 9°25'17" N and 13°27'2" E). The plant collection was carried out on a private land, following permission by the owner (Mrs Ndibabonga Solange, resident of Bunduma quarter, Buea), to conduct the study on this site. The field studies did not involve endangered or protected species. The species was identified by a botanist, Dr Andrew Enow Egbe from the Department of Botany and Plant Physiology; authenticated and deposited at the National Herbarium of Yaoundé (Cameroon), where a voucher was deposited (Sample Number 106592/HNC).

### 2.2. Preparation of *Cymbopogon citratus* aqueous extract

The leaves of *Cymbopogon citratus* were cut into pieces and allowed to air dry at room temperature (25°C). The dried leaves were then reduced to fine particles. The powder (500 g) was boiled in 5000 ml of distilled water for 20 minutes. After it cooled, the supernatant (concoction) was collected and filtered with Whatman No. 1 filter paper and dried using an oven. The yield of the extraction was 11.72% (w/w). The decoction (aqueous extract) was prepared 45 minutes to 2 hours before its oral administration at the doses of 25, 50, 100 and 200 mg/kg, to mice using a volume of 10 mL/kg of body weight.

### 2.3. Preliminary phytochemical study

The decoction of *Cymbopogon citratus* was examined for its phytochemical contents as described previously by Taiwe et al. [14] and several families of compounds (alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones,

saponins, phenols) were screened. A comparative thin layer chromatographic study was also performed to screen the presence of bufadienolides in the decoction of *Cymbopogon citratus* using anisaldehyde sulphuric acid reagent under UV (254 – 365 nm) [14].

## 2.4. Chemicals

Chloroquine (nivaquine®) and pyrimethamine (malocide®) were obtained from SANOFI AVENTIS, France.

All the used reagents for phytochemical characterisation and biochemical analyses were obtained respectively from Sigma Chemical, USA and Randox, UK.

## 2.5. Animals

Adult male BALB/c mice (*Mus musculus* Swiss, weighing 23 - 30 g) were used in this study. Animals were housed in standard cages at 25°C, 12/12 hours light-dark cycle, with free access to food and water. Each animal was used only once. All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised in 1996) and received an approval from the University of Buea - Institutional Animal Care and Use Committee (UB-IACUC N° 002/2019).

## 2.6. Malaria parasites (*Plasmodium berghei* ANKA)

The malaria parasite *Plasmodium berghei* ANKA was obtained from the Malaria Research and Reference Reagent Resource Centre (MR4, MRA-865, Manassas, Virginia), were stored at -80°C until used. Donors *Plasmodium berghei* infected *Mus musculus* Swiss mice (with a maximum of 30% parasitemia) were sacrificed by cervical decapitation. Immediately blood was collected through cardiac puncture. The blood was diluted with sterile normal saline (0.9% NaCl). After dilution, 0.2 mL of blood containing about 10<sup>7</sup> infected red blood cells was obtained. Each mouse was infected by intraperitoneal injection of 0.2 mL blood suspension, and was expected to develop steadily rising consistent infection of the required intensity in mice [15].

## 2.7. Pharmacological testing

### 2.7.1. Test for prophylactic antimalarial activity

The antimalarial study used in this study was a prophylactic model, where mice were randomly divided into seven groups of six mice each. The animal grouping consisted of one normal group (NoG), one negative control group (NCG), two positive control groups, and four test groups. The activity of *Cymbopogon citratus* was assessed using the method described by Peters [16, 17]. After random grouping of experimental animals, group 1 (NoG) was treated with 10 mL/kg distilled water and was not parasitised, group 2 (NCG) was treated with distilled water, groups 3 and 4 (positive controls) were treated respectively with chloroquine (CQ; 10 mg/kg) and pyrimethamine (PYR; 30 mg/kg), and finally groups 5 to 8 (decoction test groups) were respectively treated orally with 25, 50, 100 and 200 mg/kg of *Cymbopogon citratus* aqueous extracts. Administration of the plant extracts and reference standard drugs continued for three consecutive days (D0 - D2), and on the fourth day (D3), the mice were inoculated with approximately 10<sup>7</sup> *Plasmodium berghei* ANKA infected red blood cells. Only the normal group (group 1) of mice was not inoculated with *Plasmodium berghei* ANKA.

The level of parasitaemia was assessed by blood smear 72 hours later (after the inoculation of *Plasmodium berghei* ANKA to mice). A thin blood film was prepared from the tail blood of each experimental animal, fixed in absolute methanol and stained with Giemsa to reveal parasitized erythrocytes. Parasitaemia was determined by light microscopy using the 100× (oil immersion) objective lens and the following equation was used to calculate parasitaemia:

$$\% \text{ parasitaemia} = \frac{\text{Number of parasitised red blood cells}}{\text{Total number of red blood cells counted}} \times 100$$

Average percentage chemosuppression of *Plasmodium berghei* ANKA was also calculated in this experiment as:

$$100 \left[ \frac{A-B}{A} \right]$$

Where A is the average percentage parasitaemia in the negative control group and B is the average percentage parasitaemia in the test group. The body weight, food intake and water intake for each mouse were also evaluate.

### 2.7.2. Evaluation of haematological parameters

At the end of the evaluation of parasitaemia, animals were anesthetized with ether and blood collected with and without anticoagulant (ethylene diamine tetra acetate) by retro-orbital puncture [18], using capillary tubes for haematological and biochemical studies respectively. Haematological analysis was performed using an automatic haematological analyser (Sysmex KX-21N). The parameters included: red blood cell (RBC) count, leukocyte (WBC) count, haemoglobin (Hb), haematocrit (Hct), package corpuscular volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, lymphocyte, monocyte, neutrophil, basophil and eosinophil counts [19].

### 2.7.3. Determination of biochemical parameters

For biochemical analysis, blood was centrifuged at 3000rpm for 10 min. Serum was separated and stored at -20°C until determination of biochemical parameters using Randox commercial kits. The quantification methods were described on the manufacturer's instructions and the following parameters were estimated: total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase ( $\gamma$ -GT), total bilirubin, lactate dehydrogenase (LDH), and glucose [19].

## 2.8. Statistical analysis

The results were presented as mean  $\pm$  S.E.M in tables or histograms. For the evaluation of antimalarial efficacy tests, statistical differences were tested by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to compare the level of parasitaemia, body weight, body temperature, food intake, water intake, haematological parameters and serum biochemical status measured between of distilled water-treated *Plasmodium berghei* ANKA infected mice and *Cymbopogon citratus*-treated *Plasmodium berghei* ANKA infected mice. Mean values with  $p < 0.05$  were considered significant.

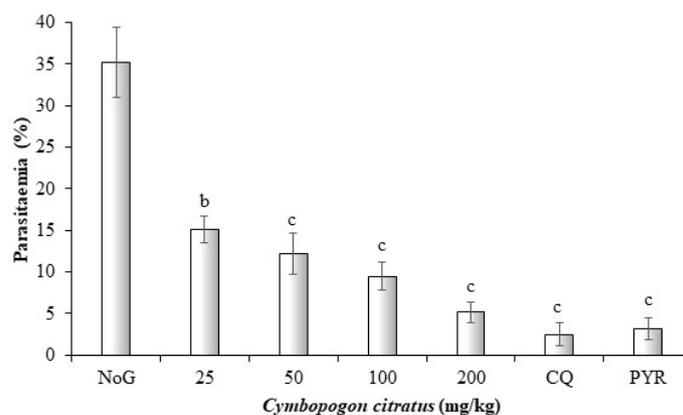
## 3. Results

### 3.1. Phytochemical constituents of *Cymbopogon citratus* aqueous extract

The phytochemical analysis of the decoction of *Cymbopogon citratus* showed the presence of flavonoids, triterpenoids, phenols, and alkaloids in high concentration. However, the presence of glycosides and tannins were detected in moderate concentrations, while anthraquinones and saponins were detected in low concentrations. Bufadienolides were absent.

### 3.2. Effects of *Cymbopogon citratus* aqueous extracts on the level of parasitaemia

From the results obtained, there were significant decreases ( $P < 0.001$ ) in the level of parasitaemia in a dose (25, 50, 100 and 200 mg/kg) dependent manner ( $15.16 \pm 1.61$ ,  $12.16 \pm 2.5$ ,  $9.5 \pm 1.66$  and  $5.16 \pm 1.27\%$ ; respectively), when compared with the negative control group or distilled water-treated parasitised mice ( $35.16 \pm 4.22\%$ ). A similar decrease in the level of parasitaemia was also recorded with the positive control groups, chloroquine-treated parasitised mice ( $2.5 \pm 1.33\%$ ) and pyrimethamine-treated parasitised mice ( $3.16 \pm 1.27\%$ ), respectively (Figure 1).

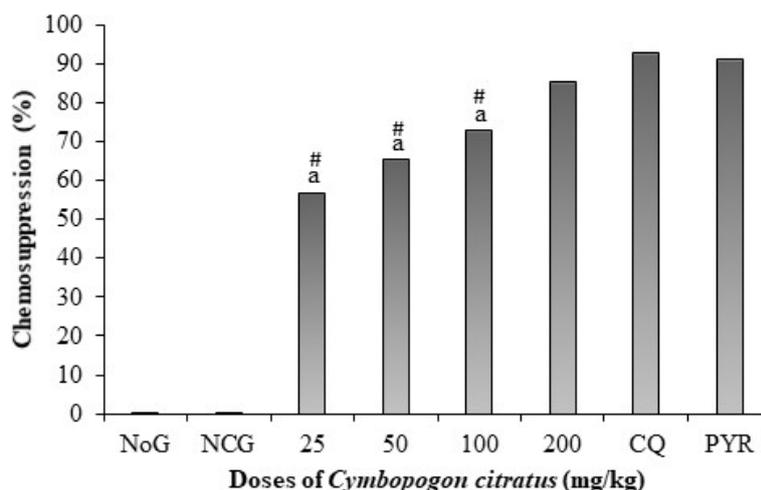


**Figure 1** Effects of *Cymbopogon citratus* aqueous extracts on the level of parasitaemia (%)

Results are expressed as percentages.  $n = 6$  animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ , significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine.

### 3.3. Effects of *Cymbopogon citratus* aqueous extracts on the average percentage chemosuppression

As demonstrated previously, following the evaluation of the level of parasitaemia with respect to treatment with the decoction of *Cymbopogon citratus* and the standard antimalarial drugs; the average percentage chemosuppression was however calculated. One way ANOVA revealed that the average percentage chemosuppression obtained from the group of mice treated with 200 mg/kg *Cymbopogon citratus* aqueous versus chloroquine [ $F(4, 24) = 108.14$ ,  $p > 0.05$ ] and 200 mg/kg *Cymbopogon citratus* aqueous versus pyrimethamine [ $F(4, 24) = 96.08$ ,  $p > 0.05$ ] were not statistically different (Figure 2). The percentage chemosuppression was 85.32% in the group of mice administered 200 mg/kg *Cymbopogon citratus* aqueous, and respectively 92.88 and 91.01% were recorded with the positive control groups chloroquine and pyrimethamine.

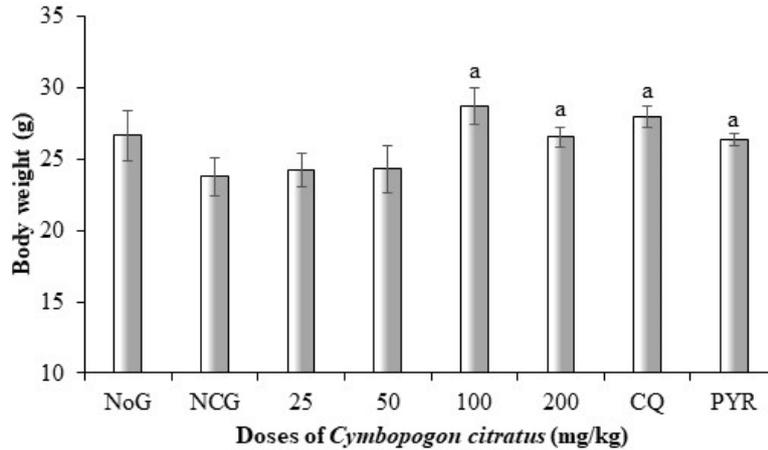


**Figure 2** Effects of *Cymbopogon citratus* aqueous extracts on the average percentage chemo-suppression

Results are expressed as mean  $\pm$  S.E.M.  $n = 6$  animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup> $P < 0.05$ , significantly different compared to chloroquine-treated parasitised mice. <sup>#</sup> $P < 0.05$ , significantly different compared to pyrimethamine-treated parasitised mice. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine.

### 3.4. Effects of *Cymbopogon citratus* aqueous extracts on body weight

Body weight of the different groups of mice was equally taken and recorded accordingly and the obtained results are presented in Figure 3. From the result recorded, it indicated that there were significant increases within the group of mice treated with the plant decoction at the doses of 100 mg/kg ( $P < 0.05$ ) and 200 mg/kg ( $P < 0.05$ ), when compared with the negative control. A comparable increase was registered within mice treated with the standard antimalarial drug chloroquine and pyrimethamine, respectively.

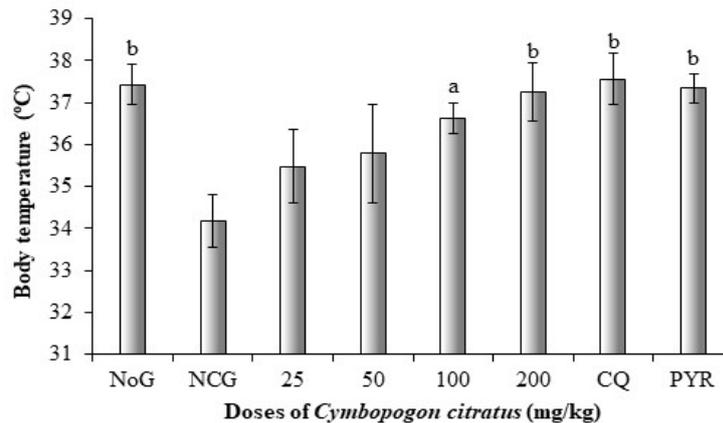


**Figure 3** Effects of *Cymbopogon citratus* aqueous extracts on body weight

Results are expressed as mean  $\pm$  S.E.M.  $n = 6$  animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup> $P < 0.05$ , significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine.

### 3.5. Effects of *Cymbopogon citratus* aqueous extracts on body temperature

The body temperature of each animal was also recorded at the end of this experiment (72 hours after the inoculation of *Plasmodium berghei* ANKA to different groups of mice and the result is depicted in Figure 4. There was a significant reduction in body temperature from  $37.43 \pm 1.08^\circ\text{C}$  in distilled water-treated non-parasitised mice (normal group) to  $34.16 \pm 1.66^\circ\text{C}$  in distilled water-treated parasitised mice. Interestingly, there was a significant increase in body temperature recorded in the group of mice administered 100 and 200 mg/kg *Cymbopogon citratus*, to  $36.63 \pm 0.37^\circ\text{C}$  and  $37.25 \pm 0.68^\circ\text{C}$ , respectively. A significant increase in body temperature was registered amongst the positive control groups treated with chloroquine and pyrimethamine, respectively.



**Figure 4** Effects of *Cymbopogon citratus* aqueous extracts on body temperature

Results are expressed as mean  $\pm$  S.E.M.  $n = 6$  animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine.

### 3.6. Effects of *Cymbopogon citratus* aqueous extracts on food intake and water intake

The quantity of food intake and water intake were assessed 72 hours after the inoculation of *Plasmodium berghei* ANKA to different groups of mice. From the results obtained it was observed that there was a significant decrease ( $P < 0.01$ ) in the quantity of food intake from  $5.08 \pm 0.61$  g/group/day in the normal group of mice to  $2.23 \pm 0.31$  g/group/day in

the negative control group of animals. However, there was a significant increase in the quantity of food intake in a dose (25, 50, 100 and 200 mg/kg) dependent manner ( $3.83 \pm 0.83$  g/group/day ( $P < 0.05$ ),  $4.03 \pm 0.71$  g/group/day ( $P < 0.01$ ),  $5.31 \pm 0.48$  g/group/day ( $P < 0.01$ ) and  $5.16 \pm 0.55$  g/group/day ( $P < 0.01$ ); respectively), when compared to the negative control group ( $2.23 \pm 0.31$  g/group/day). A similar increase in food intake was recorded within the positive control groups treated with chloroquine ( $5.56 \pm 0.43$  g/group/day ( $P < 0.01$ )) and pyrimethamine ( $5.36 \pm 0.48$  g/group/day ( $P < 0.01$ )), respectively (Table 1).

**Table 1** Effects of *Cymbopogon citratus* aqueous extracts on food intake and water intake

Parameters	NoG	NCG	Doses of <i>Cymbopogon citratus</i> (mg/kg)				CQ (mg/kg)	PYR (mg/kg)
			25	50	100	200		
Food intake								
Day 1	$5.08 \pm 0.61^b$	$2.23 \pm 0.31$	$3.83 \pm 0.83^a$	$4.03 \pm 0.71^a$	$5.31 \pm 0.48^b$	$5.16 \pm 0.55^b$	$5.56 \pm 0.43^b$	$5.36 \pm 0.48^b$
Water intake								
Day 1	$5.33 \pm 0.33^b$	$3.33 \pm 0.44$	$4.11 \pm 0.45$	$4.26 \pm 0.35$	$5.33 \pm 0.24^b$	$5.25 \pm 0.25^b$	$5.58 \pm 0.58^b$	$5.36 \pm 0.63^b$

Results are expressed as mean  $\pm$  S.E.M. n = 6 animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine.

The level of water intake significantly increased from  $3.33 \pm 0.44$  mL/group/day in the distilled water-treated parasitised mice to  $5.33 \pm 0.24$  mL/group/day ( $P < 0.01$ ) and  $5.25 \pm 0.25$  mL/group/day ( $P < 0.01$ ), across the group treated with the doses of 100 and 200 mg/kg *Cymbopogon citratus*, respectively. As shown in Table 1, a similar increase was recorded amongst the positive control group treated with chloroquine ( $5.58 \pm 0.58$  mL/group/day ( $P < 0.01$ )) and pyrimethamine ( $5.36 \pm 0.63$  mL/group/day ( $P < 0.01$ )), respectively as well as within the normal control group ( $5.33 \pm 0.33$  mL/group/day ( $P < 0.01$ )).

### 3.7. Effects of *Cymbopogon citratus* aqueous extracts on haematological parameters

The haematological parameters for prophylactic antimalarial activity of *Cymbopogon citratus* decoction on *Plasmodium berghei* ANKA infection in mice are shown in Table 2. As indicated in the results obtained, it was realised that there was a significant decrease in the level of RBC from  $6.51 \pm 0.06 \times 10^6/\mu\text{L}$  in the normal control group of mice to  $3.87 \pm 0.49 \times 10^6/\mu\text{L}$  ( $P < 0.001$ ) in the distilled water-treated parasitised mice (negative control group). Oral administration of 100 and 200 mg/kg *Cymbopogon citratus* significantly increased the level of RBC to  $5.83 \pm 1.06 \times 10^6/\mu\text{L}$  ( $P < 0.01$ ) and  $5.37 \pm 0.11 \times 10^6/\mu\text{L}$  ( $P < 0.01$ ), respectively, when compared to the negative control group. The two way ANOVA indicated a main difference in the level of haemoglobin [F(7, 24) = 85.31,  $p < 0.001$ ], haematocrit [F(7, 24) = 105.49,  $p < 0.001$ ], MCV [F(7, 24) = 81.22,  $p < 0.001$ ], MCH [F(7, 24) = 104.52,  $p < 0.001$ ], MCHC [F(7, 24) = 72.41.28,  $p < 0.01$ ], platelets [F(7, 24) = 88.75,  $p < 0.05$ ], WBC [F(7, 24) = 97.21,  $p < 0.001$ ], neutrophils [F(7, 24) = 102.58,  $p < 0.001$ ], eosinophils [F(7, 24) = 97.01,  $p < 0.001$ ], lymphocytes [F(7, 24) = 102.91,  $p < 0.001$ ], and monocytes [F(7, 24) = 74.81,  $p < 0.001$ ], counts were significantly different between the decoction-treated parasitised mice and distilled water-treated parasitised mice.

### 3.8. Effects of *Cymbopogon citratus* aqueous extracts on serum biochemical parameters

It was nevertheless noticed from Table 3 that there was a significant increase ( $P < 0.01$ ) in the level of total protein from  $54.17 \pm 6.78$  g/L in the normal control group to  $76.53 \pm 4.04$  g/L in the negative control group. However, there was a significant decrease in the level of total protein to  $58.49 \pm 4.88$ g/L ( $P < 0.05$ ) and  $54.68 \pm 4.97$ g/L ( $P < 0.01$ ) for the respective doses of 100 mg/kg and 200 mg/kg as well as with the respective positive control groups chloroquine ( $54.45 \pm 3.55$ g/L) and pyrimethamine ( $54.64 \pm 9.56$ g/L), when compared with the negative control group ( $76.53 \pm 4.04$  g/L).

Table 3 illustrates that the levels of albumin and glucose significantly increased in the groups of mice administered 100 and 200 mg/kg *Cymbopogon citratus* aqueous extracts, respectively as compared with the distilled water-treated parasitised mice. Statistical analysis indicated that there was a significant decrease in the level of globulin [F(7, 28) = 42.73.28,  $p < 0.001$ ], AST [F(7, 28) = 83.78.28,  $p < 0.05$ ], ALT [F(7, 24) = 102.39,  $p < 0.001$ ], ALP [F(7, 28) = 98.51,  $p < 0.001$ ],  $\gamma$ -GT [F(7, 28) = 49.93,  $p < 0.001$ ], total bilirubin [F(7, 28) = 102.71,  $p < 0.001$ ], and LDH [F(7, 28) = 105.72,  $p < 0.001$ ] in the decoction-treated parasitised mice when compared to the distilled water-treated parasitised animals.

**Table 2** Effects of *Cymbopogon citratus* aqueous extracts on haematological parameters

Parameters	NOG	NCG	Doses of <i>Cymbopogon citratus</i> (mg/kg)				CQ (mg/kg)	PYR (mg/kg)
			25	50	100	200		
RBC ( $\times 10^6/\mu\text{L}$ )	6.51 $\pm$ 0.06 <sup>c</sup>	3.87 $\pm$ 0.49	3.74 $\pm$ 0.44	4.57 $\pm$ 0.85	5.83 $\pm$ 1.06 <sup>b</sup>	5.37 $\pm$ 0.11 <sup>b</sup>	6.51 $\pm$ 0.20 <sup>c</sup>	6.48 $\pm$ 0.16 <sup>c</sup>
Haemoglobin (g/dL)	11.6 $\pm$ 0.41 <sup>c</sup>	6.07 $\pm$ 1.12	9.27 $\pm$ 0.22 <sup>c</sup>	10.79 $\pm$ 0.62 <sup>c</sup>	10.77 $\pm$ 0.78 <sup>c</sup>	11.33 $\pm$ 0.60 <sup>c</sup>	11.40 $\pm$ 0.42 <sup>c</sup>	11.66 $\pm$ 0.67 <sup>c</sup>
Haematocrit (%)	37.04 $\pm$ 0.19 <sup>c</sup>	28.67 $\pm$ 0.27	31.10 $\pm$ 2.38	34.32 $\pm$ 2.83 <sup>b</sup>	37.88 $\pm$ 2.11 <sup>c</sup>	37.87 $\pm$ 0.93 <sup>c</sup>	36.85 $\pm$ 0.27 <sup>c</sup>	37.69 $\pm$ 0.64 <sup>c</sup>
PCV (%)	41.26 $\pm$ 6.02 <sup>c</sup>	23.55 $\pm$ 4.05	28.04 $\pm$ 3.44	30.26 $\pm$ 4.37	36.14 $\pm$ 2.14 <sup>b</sup>	41.61 $\pm$ 2.81 <sup>c</sup>	41.64 $\pm$ 4.86 <sup>c</sup>	41.54 $\pm$ 5.17 <sup>c</sup>
MCV (fL)	58.25 $\pm$ 0.21 <sup>c</sup>	38.70 $\pm$ 0.59	39.03 $\pm$ 3.47	42.08 $\pm$ 6.70	47.13 $\pm$ 8.41	55.93 $\pm$ 2.79 <sup>c</sup>	55.96 $\pm$ 2.28 <sup>c</sup>	53.36 $\pm$ 4.01 <sup>b</sup>
MCH (pg)	17.61 $\pm$ 0.23 <sup>c</sup>	12.51 $\pm$ 0.24	13.53 $\pm$ 0.92	15.92 $\pm$ 1.35 <sup>b</sup>	16.59 $\pm$ 1.23 <sup>c</sup>	17.39 $\pm$ 0.59 <sup>c</sup>	17.50 $\pm$ 0.84 <sup>c</sup>	17.38 $\pm$ 0.73 <sup>c</sup>
MCHC (g/dL)	29.56 $\pm$ 0.16 <sup>b</sup>	24.71 $\pm$ 0.87	26.67 $\pm$ 1.16	29.20 $\pm$ 1.42 <sup>b</sup>	29.34 $\pm$ 2.23 <sup>c</sup>	30.61 $\pm$ 0.15 <sup>c</sup>	30.58 $\pm$ 0.25 <sup>c</sup>	30.94 $\pm$ 0.28 <sup>c</sup>
Platelets ( $\times 10^3/\mu\text{L}$ )	482.17 $\pm$ 8.22 <sup>a</sup>	464.83 $\pm$ 7.11	478.16 $\pm$ 7.11	475.33 $\pm$ 14.89	481.83 $\pm$ 6.78 <sup>a</sup>	480.67 $\pm$ 1.77 <sup>a</sup>	480.67 $\pm$ 1.77 <sup>a</sup>	480.16 $\pm$ 10.17 <sup>a</sup>
WBC ( $\times 10^3/\mu\text{L}$ )	12.50 $\pm$ 1.01 <sup>c</sup>	16.69 $\pm$ 0.38	15.21 $\pm$ 0.41	14.10 $\pm$ 0.89 <sup>a</sup>	13.49 $\pm$ 0.75 <sup>b</sup>	12.58 $\pm$ 1.38 <sup>c</sup>	12.56 $\pm$ 1.49 <sup>c</sup>	12.96 $\pm$ 0.88 <sup>b</sup>
Neutrophils (%)	11.98 $\pm$ 0.63 <sup>b</sup>	15.09 $\pm$ 0.22	14.85 $\pm$ 0.91	13.35 $\pm$ 1.14	12.78 $\pm$ 0.57 <sup>b</sup>	12.77 $\pm$ 0.33 <sup>b</sup>	11.91 $\pm$ 1.05 <sup>c</sup>	11.67 $\pm$ 0.87 <sup>c</sup>
Eosinophils (%)	2.41 $\pm$ 0.08 <sup>a</sup>	1.77 $\pm$ 0.20	1.90 $\pm$ 0.34	2.06 $\pm$ 0.30	2.11 $\pm$ 0.06	2.68 $\pm$ 0.09 <sup>c</sup>	2.45 $\pm$ 0.06 <sup>b</sup>	2.48 $\pm$ 0.16 <sup>b</sup>
Basophils (%)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.0 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Lymphocytes (%)	74.26 $\pm$ 6.00 <sup>c</sup>	54.13 $\pm$ 5.09	60.96 $\pm$ 4.01	68.6 $\pm$ 3.21 <sup>b</sup>	70.43 $\pm$ 3.18 <sup>b</sup>	71.33 $\pm$ 5.12 <sup>b</sup>	72.16 $\pm$ 5.77 <sup>b</sup>	72.66 $\pm$ 7.5 <sup>b</sup>
Monocytes (%)	5.86 $\pm$ 0.24 <sup>c</sup>	13.31 $\pm$ 0.87	9.93 $\pm$ 1.05 <sup>b</sup>	7.36 $\pm$ 0.66 <sup>c</sup>	8.26 $\pm$ 1.64 <sup>c</sup>	6.78 $\pm$ 1.27 <sup>c</sup>	6.45 $\pm$ 0.36 <sup>c</sup>	6.06 $\pm$ 0.82 <sup>c</sup>

Results are expressed as mean  $\pm$  S.E.M. n = 6 animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001, significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine, RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; PCV: packed cell volume.

**Table 3** Effects of *Cymbopogon citratus* aqueous extracts on serum biochemical parameters

Parameters	NoG	NCG	Doses of <i>Cymbopogon citratus</i> (mg/kg)				CQ (mg/kg)	PYR (mg/kg)
			25	50	100	200		
Total Protein (g/L)	54.17±6.78 <sup>b</sup>	76.53±4.04	70.10±6.04	63.12±6.42	58.49±4.88 <sup>a</sup>	54.68±4.97 <sup>b</sup>	54.45±3.55 <sup>c</sup>	54.64±9.56 <sup>b</sup>
Albumin (g/L)	39.70±1.61 <sup>a</sup>	36.28±1.40	37.20±1.10	37.76±1.33	38.00±0.85	39.84±0.45 <sup>b</sup>	39.71±1.21 <sup>a</sup>	39.89±1.23 <sup>b</sup>
Globulin (g/L)	28.08±4.23 <sup>c</sup>	45.08±1.19	36.26±4.87	31.74±2.99 <sup>b</sup>	30.92±4.20 <sup>c</sup>	28.64±2.80 <sup>c</sup>	8.71±5.49 <sup>c</sup>	28.48±4.24 <sup>c</sup>
AST (U/L)	26.03±1.30	31.19±1.41	30.76±2.21	28.62±3.43	27.74±3.71	26.54±2.98	26.24±2.10	26.24±2.42
ALT (U/L)	25.13±1.38 <sup>a</sup>	32.78±2.12	31.96±3.64	30.03±4.19	28.41±4.40	25.12±1.63 <sup>a</sup>	25.12±2.11 <sup>a</sup>	25.55±2.59 <sup>a</sup>
ALP (U/L)	116.78±11.23 <sup>c</sup>	156.79±7.08	143.64±6.44	133.15±4.70 <sup>c</sup>	121.89±4.36 <sup>c</sup>	118.04±3.22 <sup>c</sup>	117.69±6.15 <sup>c</sup>	116.72±4.66 <sup>c</sup>
γ-GT (U/L)	13.19±1.64 <sup>c</sup>	22.22±1.31	20.22±1.61	18.49±2.65	15.20±1.82 <sup>c</sup>	13.42±0.44 <sup>c</sup>	14.00±1.90 <sup>c</sup>	13.55±0.55 <sup>c</sup>
T Bilirubin (mg/dL)	0.15±0.02 <sup>c</sup>	0.79±0.06	0.67±0.11	0.45±0.13 <sup>c</sup>	0.27±0.05 <sup>c</sup>	0.18±0.05 <sup>c</sup>	0.17±0.04 <sup>c</sup>	0.17±0.05 <sup>c</sup>
LDH (U/L)	3.90±0.46 <sup>c</sup>	13.38±0.31	9.89±0.70 <sup>c</sup>	7.56±1.26 <sup>c</sup>	4.70±0.93 <sup>c</sup>	3.67±0.31 <sup>c</sup>	3.60±0.75 <sup>c</sup>	3.35±0.57 <sup>c</sup>
Glycemia(mg/dL)	152.62±8.54 <sup>c</sup>	50.75±7.79	72.50±10.88	91.99±12.77 <sup>c</sup>	126.09±7.54 <sup>c</sup>	151.39±9.24 <sup>c</sup>	152.34±8.30 <sup>c</sup>	151.33±4.33 <sup>c</sup>

Results are expressed as mean ± S.E.M. n = 6 animals. Data were analysed by ANOVA, followed by Tukey's (HSD) multiple comparison test, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001, significantly different compared to negative control group. NoG, normal group treated with 10 mL/kg distilled water and not parasitised; NCG, negative control group constituted with distilled water-treated parasitised mice; CQ, 10 mg/kg chloroquine; PYR, 30 mg/kg pyrimethamine; ALT, alanine amino transferase; AST, aspartate amino transferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GT, gamma glutamyl transferase.

#### 4. Discussion

Based on the sustained and potent antimalarial activity of the whole *Cymbopogon citratus* plant infusion in other studies [12, 13, 20], we assessed the prophylactic antimalarial activity of the decoction prepared from the leaves of the plant. The leaf decoction also exhibited a significant prophylactic effect on stages of infection comparable to that of the standard drugs, chloroquine or pyrimethamine as demonstrated in the levels of parasitaemia of animals in the decoction test groups and the positive control groups. These results indicate that *Cymbopogon citratus* possesses a significant anti-plasmodial activity as evident from the chemosuppression obtained during the 72-hours early infection stage, as well as during the other stages of the malaria parasite. Interestingly, these results indicate the non-selectivity of *Cymbopogon citratus* decoction on the stages of malaria parasite. It is not clear how *Cymbopogon citratus* decoction exerts prophylactic activity on *Plasmodium berghei* ANKA infection but it may be inhibiting the multiplication of malaria parasites and may also have a direct cytotoxic effect on the parasites [21]. It may modulate the membrane properties of the erythrocytes preventing parasite invasion [22]. Pyrimethamine used as a positive control in this study exerts prophylactic activities via the inhibition of dihydropteroate synthetase and dihydrofolate reductase enzymes of the parasites [23]. Generally, prophylactic antimalarial drugs work by disrupting the initial development of malaria parasites in the liver (causal activity). They may act by suppressing the emergent asexual blood stages of the parasite (suppressive activity) or by preventing the relapses induced by the latent liver forms (hypnozoites) [24]. *Cymbopogon citratus* can therefore be used for malaria prophylaxis as well as a curative agent such as sulfadoxine pyrimethamine [25].

The preliminary phytochemical analysis of the decoction prepared from the leaves of *Cymbopogon citratus* showed the presence of flavonoids, triterpenoids, phenols, alkaloids, glycosides, tannins, anthraquinones and saponins. As earlier reported, many antimalarial herbal remedies may exert their antiparasitic effects not only by directly affecting the pathogen, but also by indirectly stimulating natural and adaptive defense mechanisms of the host by other mechanisms. Therefore, extracts that can stimulate innate and/or adaptive immunity may be able to contribute to prophylaxis and treatment not only for malaria but for other diseases as well [26, 27]. This suggests that a combination of the biological activities of the active compounds of the decoction prepared from the leaves of *Cymbopogon citratus* results in an enhanced overall antimalarial activity of this plant.

The results from this study demonstrated that *Cymbopogon citratus*, chloroquine and pyrimethamine successfully prevented body weight loss and antagonised the loss of body temperature in mice during the prophylactic study. Unlike in humans, increase in parasitaemia levels in rodent models usually results in decreased metabolic rates and a consequent decrease in body temperatures [28], which might result in death. An ideal anti-malarial agent would, therefore, prevent this occurrence, an effect observed in *Cymbopogon citratus*-treated parasitised animals. Taken together, these results confirm that *Cymbopogon citratus* has therapeutic activity against early or established infection. Chaniad et al. [29] reported that body weight loss is one of the general symptoms of malaria infection in humans and rodents. The decrease in body weight in malaria cases has been associated with decreased food intake, disturbed metabolic function and hypoglycemia (Basir et al., 2012). Therefore, a potential antimalarial is expected to ameliorate anaemia, prevent body weight loss and stabilize body temperature in infected mice [30]. These effects can be supported by the normalisation of haematological parameters and serum biochemical status induced the oral administration of the decoction of *Cymbopogon citratus* to mice. This could be due to the ability of this decoction to prevent loss of appetite, increase food intake and prevent disturbed metabolic function associated with malaria. These observations are in agreement with those reported by Chukwuocha et al. [13]. In this study, we demonstrated that the whole *Cymbopogon citratus* plant displays higher prophylactic antimalarial activity and promotes the maintenance of a more stable temperature, body weight, haematological parameters as well as serum biochemical status when compared with chloroquine and pyrimethamine.

The results obtained in our study indicated that the oral administration of the decoction prepared from the leaf extract of *Cymbopogon citratus* antagonised the disturbance in haematological parameters by normalisation of several haematological indices. Haematological indices such as platelet counts, total white blood cell count, red blood cell count, packed cell volume and haemoglobin level are common biomarkers of malarial infection and are frequently monitored as indicators of drug efficacy against plasmodial infection. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for the oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body [31, 32]. Similarly, packed cell volume is an indicator of the body's ability to transport oxygen and absorbed nutrients. An increased packed cell volume shows a better transportation capacity of the red blood cells [32]. Packed cell volume is used to assess anaemia, erythrocytosis, haemodilution, and haemoconcentration [33]. A decrease in packed cell volume indicates anaemia [33, 34]. Changes in red blood cell count are the most typical features of malarial infections and anaemia is the most common complication associated with malaria infection [35]. The ability of the decoction of *Cymbopogon citratus* to restore haemoglobin level, packed cell

volume and red blood cell count in decoction-treated parasitised mice when compared with *Plasmodium berghei* infected mice suggests that the extract possess erythropoietic activity. In the same vein, the ability of the decoction of *Cymbopogon citratus* to normalise the reduction in white blood cell in decoction-treated parasitised mice when compared with *Plasmodium berghei* infected mice showed that the extract has immunostimulatory effects. This may explain why there was a reduction percentage in malaria parasitaemia in decoction-treated parasitised mice. Malaria has been reported as the major cause of thrombocytopenia in malaria endemic areas [36]. Platelets engulf malaria parasites and get damaged in the process and thus are removed from circulation, leading to a reduction in platelet count during malaria [36]. The oral administration of *Cymbopogon citratus* significantly increased the platelet count of decoction-treated parasitised mice when compared to distilled water parasitised mice. The haematopoietic effects observed in this study could be attributed to the phytoconstituents such as alkaloids, tannins, glycosides, and terpenoids. These phytoconstituents have been shown to increase the release of erythropoietin, the hormone that boosts red blood cells production as well as stimulates stem cells to divide into blood cells. Koffur et al. demonstrated that plants rich in alkaloids, tannins, glycosides, and terpenoids exhibited haematopoietic effects in rabbits [37].

After evaluation of the effects of *Cymbopogon citratus* on serum biochemical status, the results obtained indicated that there is a significant reduction in the activities of liver marker enzymes (AST, ALP, and ALT) in the serum of decoction-treated parasitised mice when compared with the distilled water-treated parasitised mice. Several studies demonstrated that in parasitised mice, liver enzyme activities in the serum increase due to disruption of the liver membrane by malaria parasite during exo-erythrocytic stage as well as the products of their damage to red blood cells during erythrocytic stage which affect the liver. The normalization of liver function enzymes observed may be linked with the membrane stabilisation and maintenance of hepatocyte integrity potentials [38], by preventing the leakage of liver enzymes into circulation. Reduction in red blood cell destruction by malaria parasite due to the antiplasmodial effects of *Cymbopogon citratus* might be responsible for the reduction in serum levels of total serum bilirubin in the decoction-treated parasitised mice when compared to distilled water-treated parasitised mice [39]. Interestingly, the oral administration of *Cymbopogon citratus* significantly decreased the elevated total protein, albumin, globulin,  $\gamma$ -GT and total bilirubin. Malaria parasite infections is accompanied by cellular mobilization of T lymphocytes and its complements with a resultant synthesis and secretion of antibody molecules leading to elevated globulin in parasitised mice [40]. Therefore, the increased serum total protein, globulins, ALT, AST, ALP and  $\gamma$ -GT in *Plasmodium berghei* infected mice was suggested be due to cellular response to hyper parasitaemia [40]. The increased activities of serum AST, ALT, ALP and  $\gamma$ -GT in the liver and blood of *Plasmodium berghei* infected mice may be due to hepatic dysfunction [41, 42] or hepatic damage. However, the oral administration of *Cymbopogon citratus* extracts to mice resulted in the near normalisation of total protein, globulin and the activities of AST, ALT, ALP and  $\gamma$ -GT in the serum of infected treated mice. The activity of the plant decoction is dose dependant with strongest activity at a dose of 200 mg/kg. The results also suggested that the extract of *Cymbopogon citratus* may possess hepatoprotective compounds.

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## 5. Conclusion

The decoction prepared from the leaves of *Cymbopogon citratus* possesses prophylactic antimalarial properties on *Plasmodium berghei* ANKA-induced malaria in Swiss mice as well as antipyretic effects. The antimalarial properties could be combined with the normalisation of haematological and serum biochemical status. It is therefore an ideal antimalarial drug candidate.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

### *Statement of ethical approval*

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and received an approval from the University of Buea - Institutional Animal Care and Use Committee (UB-IACUC N° 002/2019).

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