Assessment of Garlic Oil and Bitter Leaf Extract in Malaria Model: *Plasmodium berghei*-Induced Wistar Rats

A Presentation at the International Research & Development Conference (IRDec), 2023 held at Kampala International university, Kampala, Uganda

by

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INTRODUCTION

- Plasmodium parasites are the present known causative agents of a tropical disease called “malaria.”

- It is recognized as one of the major health menaces in Africa and is accountable for the demise of approximately 4,000,000 individuals every year, most of whom are pregnant women and children (WH0, 2020).

- Transmission of the malaria parasites into the mammalian host begins with the injection of infectious motile sporozoite into the skin through the bite of an infected mosquito (Vaughan, 2021; Matsuoka *et al*., 2002).

STATEMENT OF RESEARCH PROBLEM

- Plasmodium species are the main causes of malaria, implicated in increased mortality in children, pregnant women, and immune-suppressed people in disease-endemic countries (Aditya *et al*., 2013).

- Statistics have shown that about 60% of malaria death occurs in under-developed and developing countries, including Nigeria and Sub-Saharan regions (Snow *et al*., 2005).

Significance of the Study

- Plants, generally for years, have been widely used as a medicine to reduce various risk factors associated with several diseases (Tom-Otu *et al*., 2022).

- They contain bioactive components that have proven beneficial in the treatment of infections, inflammation, and ulceration, amongst other disease conditions (Tom-Otu *et al*., 2023; Amagase, 2006).

AIM OF THE STUDY

- This study aimed at assessing the antimalarial properties of garlic oil and bitter leaf extract in malaria model: *Plasmodium berghei*-induced Wistar rats.

Objectives of the study

The objectives of this study were to:

i. determine the *in-vitro* antimalarial potentials of garlic oil and bitter leaf ethanol extract in *Plamodium berghei*-induced rats against a standard drug (Lokmal).

ii. determine the anti-anaemic and antipyretic potentials of garlic oil and bitter leaf ethanol extract in *Plamodium berghei*-induced rats against a standard drug (Lokmal).

METHODOLOGY

Study area

- This research work was carried out in the Advanced Microbiology and Biochemistry Laboratories, Salem University, Lokoja, Kogi State, Nigeria.

Collection of plant samples

- Fresh leaves of bitter leaf were harvested from a residential house in Ganaja village, Kogi State, Nigeria. The botanical identification and authentication of the plants (SU-H-0056) were done by a Botanist in the Biological Department of Salem University, Lokoja. The leaves were carefully detached and airdried until crispy to touch and then pulverized into a fine powder using a sterile electrical blender (Euro premium: 1500-1799 W). Pure garlic oil was purchased in a local shop at Ganaja village, Lokoja, Kogi State.

Preparation of extracts

- The bitter leaf powder was weighed using a Camry automatic scale, model No: Ek3250 (A & D Company Limited, Japan) into a clean dry bottom flask. Twenty-five grams (25 g) of the pulverized sample was cold macerated in 250 ml ethanol (1: 10 w/v) for 48 hrs with constant rocking, using an electronic shaker, model No: 001 (AOAC, 2010). The ethanol extract was filtered using Whatman No. 1 filter paper, a pore size of 100 (195 mm by 195 mm). The filtrate was concentrated using a rotary evaporator and placed on a water bath at 60^{o}C to allow evaporation of the solvents as described by AOAC (2010). The garlic oil used was purchased from a local supermarket in Lokoja metropolis.

METHODOLOGY CONT'D

Experimental animals

- Twenty-four (24) healthy male Wistar rats, weighing 110-120 g were purchased from Nigerian Institute of Malaria Research (NIMR), Jos, Plateau State, Nigeria. The rats were randomly distributed into four groups of six rats each.

- I_0 group (Negative control) was not infected, group 2 was infected and treated with 140 mg/kgbwt of garlic oil (GO), group 3 was infected and treated with 140 mg/kgbwt of bitter leaf ethanol extract (BLEE), and group 4 was infected and treated with 140 mg/kgbw of a standard drug (Lokmal).

- The rats were kept in the animal house of Salem University, Lokoja, Nigeria, and maintained under 12 hrs (light/dark) cycle. The experimental rats were allowed free access to a pellet diet and clean water *ad libitum*.

METHODOLOGY CONT'D

Plasmodium berghei inoculation and treatment

- *Plasmodium-berghei* inoculation of the experimental rats was carried out by injecting 0.2 ml of blood containing approximately 1.0×10^5 *Plasmodium berghei* per unit, intraperitoneally into each rat in the infected groups as described by Ndungu *et al*. (2019).

Determination of _Plamodium berghei_ level

- *Plamodium berghei* level was determined according to the method described by Mergial *et al*. (2014), with a slight modification.

- The *Plasmodium berghei* level was monitored daily by microscopic examination of blood obtained from the tail of each rat.

- The droplet was placed on a clean, dry, and grease-free glass slide, and covered with a copper slip. Microscopic examination of this preparation was done under ×400 objective magnification.

METHODOLOGY CONT'D

Determination of haemoglobin level and packed cell volume

- Haemoglobin (Hb) and packed cell volume (PCV) were determined by measuring the Hb and PCV of each experimental animal, before infection, during infection and during treatment periods, using the method of "Rule of Three" by Tallquist method as described by Olalekan and Emmanuel (2016).

Determination of change in body temperature

- The body temperature of each experimental rat in all the groups was taken using small laboratory thermometer before infection, during infection and every day of the treatment.

Data analysis

- Data were analyzed using graph prism (V: 20) and one-way analysis of variance together with post hoc Duncan's multiple range tests. A statistically significant association between variables was said to exist if the p value < 0.05 at 95% confidence level

RESULTS

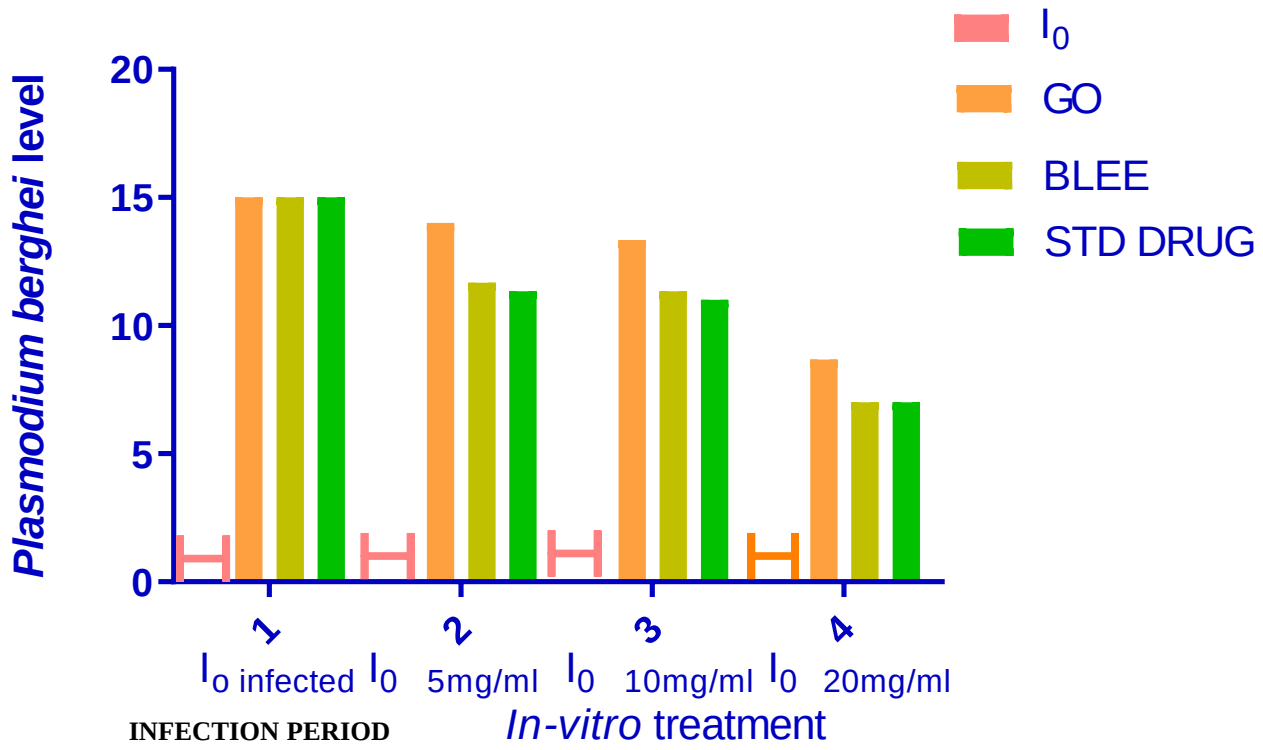

Figure 1: *Plasmodium berghei* levels of experimental rats

RESULTS CONT'D

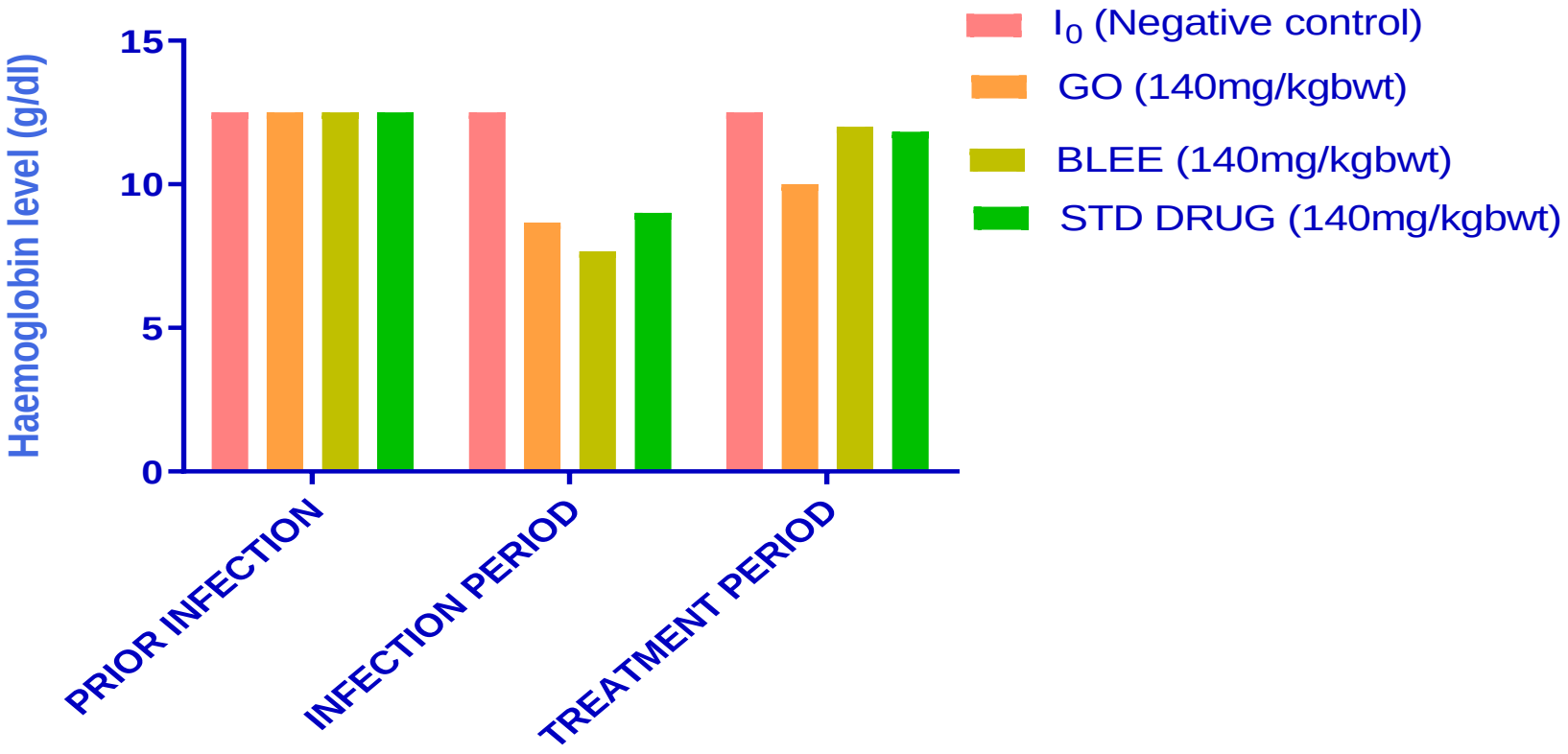

Figure 2: Haemoglobin levels of experimental rats

RESULTS CONT'D

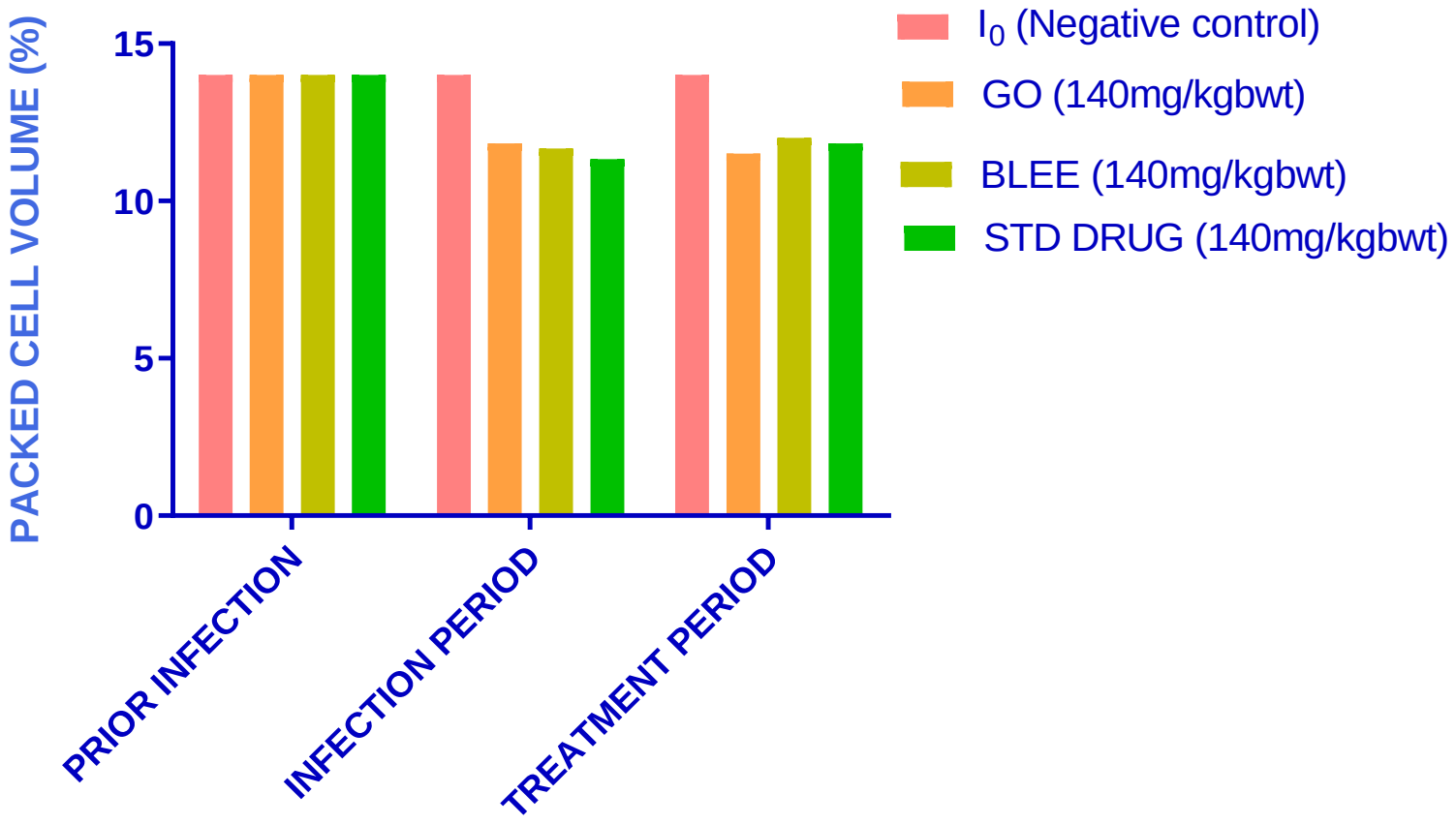

Figure 3: Packed cell volume of experimental rats

RESULTS CONT'D

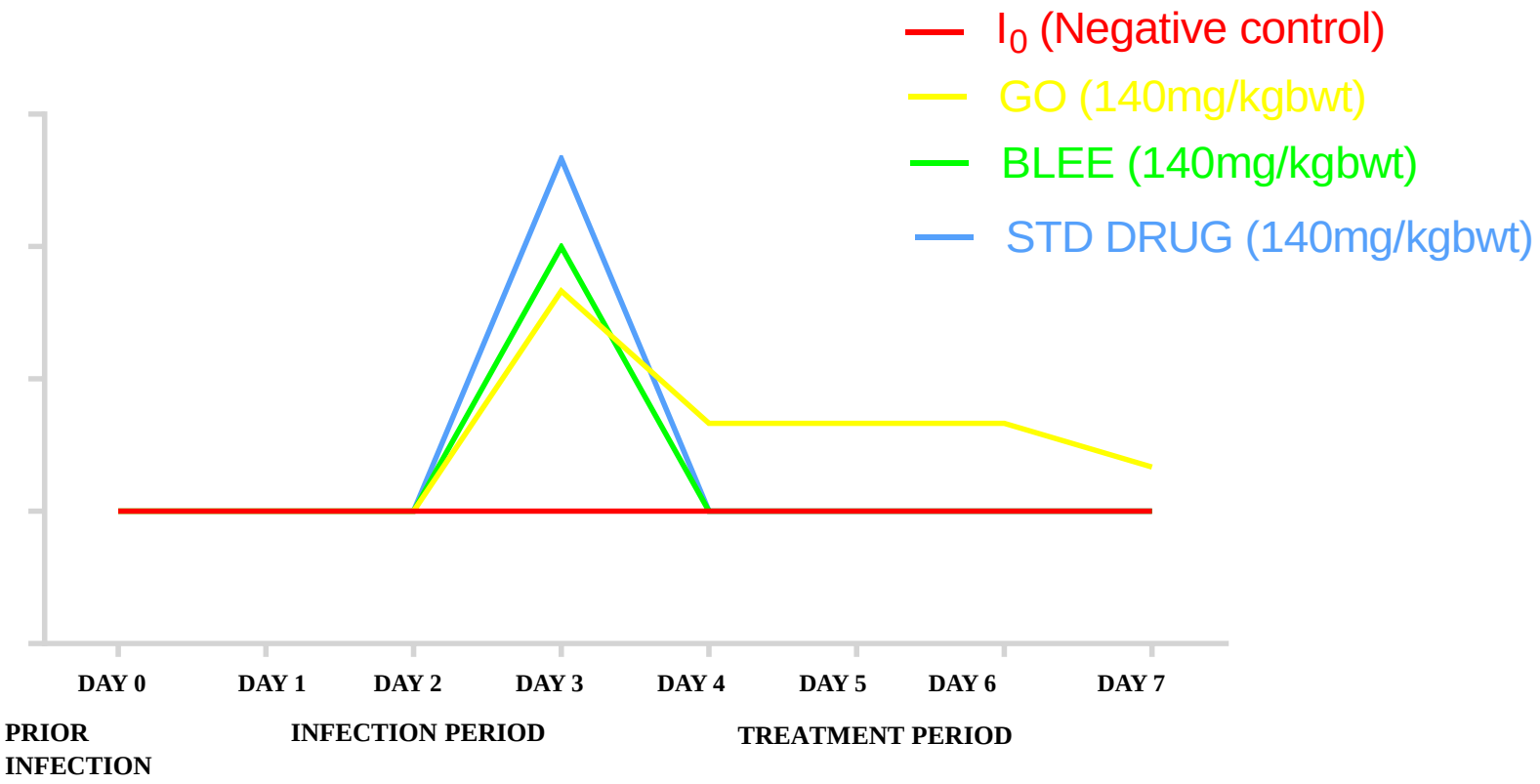

Figure 4: Temperature change of experimental rats

CONCLUSION

- The use of bitter leaf could be employed in the formulation of malarial drug. Garlic oil could be used on a combination therapy, or at a higher dose than its concentration in this study.

CONTRIBUTION TO KNOWLEDGE

The antiplasmodial activities demonstrated by GO and BLEE against *Plasmodium berghei*-infected rats in this study could be a potential indication of an active antiplasmodial effect against the common *Plasmodium falciparum*.

Selected References

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