ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 13;2 http://apjmt.mums.ac.ir June 2024

ORIGINAL ARTICLE

Histopathological Analysis and Antiplasmodial Efficacy of *Momordica balsamina* Linn Leaf: Extracts against Mice Infected with *Plasmodium berghei*

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Abstract

Background: In the midst of transient conquests by medications against malaria, *Plasmodium* parasites are the foremost public health problems globally. The emergence of drug-resistant parasites has worsened the condition, emphasizing the need for effective and inexpensive antimalarial. In line with this necessity, the study aimed at evaluating the toxicity and antiplasmodial activity of *Momordica balsamina* extracts against *Plasmodium berghei*.
Methods: Successive extraction technique via maceration was employed. Acute oral toxicity study was conducted as per the standard protocol. *In vivo* antiplasmodial activity of *M. balsamina* extracts against early infection was assessed in *P. berghei*-infected mice at different doses of 100, 200, and 400 mg/kg b.w. Data were analyzed using GraphPad Prism version 8.0.2 using analysis of variance (ANOVA) followed by Tukey's post hoc test with $p<0.05$ considered statistically significant.
Results: The extracts of *M. balsamina* did not cause any significant toxicological changes and the lethal dose estimated to be greater than 5000 mg/kg. Ethyl acetate extract demonstrated highest chemosuppression (52.8%), followed by methanol extract (44.9%), and n-hexane extracts (30.3%).
Conclusion: The study ascertained the safety of *M. balsamina* leaf and emphasized caution regarding higher doses and studies that could advance scientific knowledge are highly encouraged. The findings demonstrated moderate suppressive antiplasmodial capacities of *M. balsamina* leaf extracts.

Keywords: Antiplasmodial activity, Toxicity, Antimalarial activity, Malaria, *Plasmodium berghei*

How to cite this article: Igiri BE, Doko MHI, Inabo HI, Balogun EO. Histopathological Analysis and Antiplasmodial Efficacy of *Momordica balsamina* Linn Leaf: Extracts against Mice Infected with *Plasmodium berghei*. *Asia Pac J Med Toxicol* 2024; 13(2): 49-55.

INTRODUCTION

Malaria is an infectious and life threatening ailment caused by *Plasmodium* parasites, which affects millions of individuals in tropical as well as subtropical regions [1]. In 2021, according to the World Malaria Report, there was a projected 247 million malaria cases in 84 countries with 619,000 deaths [2].

The foremost challenge jeopardizing efforts in the management of malaria is the rapid development and spread of *Plasmodium* resistance to existing and emerging antimalarial drugs. A noteworthy approach in the control of malaria is the use of plant-based remedies for the treatment of malaria.

The expansion of resistance developed by *P. falciparum* to the existing antimalarial has intensified the quest for drugs with a new mechanism of action [3]. The growing threat of drug toxicity and drug resistance to ACTs and other currently administered drugs necessitates the development of novel products for malaria treatment [4].

A considerable portion of the world population depends on medicinal plants and their extracts for primary healthcare needs [5, 6]. *Momordica balsamina* (*M. balsamina*) also known as Balsam apple (African pumpkin) is an important medicinal and nutritional plant of the Cucurbitaceae family, comprising health-promoting secondary metabolites [7]. Few studies have reported biological activities of leaf extracts of *M. balsamina* such as anti-malaria [8] and anti-diabetic properties [9].

Previous toxicity studies reported extremely weak, inactive and no toxicity of *M. balsamina* [7, 10]. To the best of our knowledge, there exists little or no study regarding the toxicity and antiplasmodial activity of *M. balsamina*, even though it is used in Nigerian folkloric medicine. To that purpose, this study focused on toxicity and therapeutic effects, which is a major challenge. Premised on this fact, this study was conducted to explore the scientific prove of *M. balsamina* in the treatment of malaria to probably establish new anti-malarial agent.

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METHODS

Study Design

Experimental study design was used and simple random sampling technique employed for grouping of animals and assigning of treatments. The study conforms to the declaration of Helsinki (2000) [11]. The protocol follows the standard W.H.O protocol for the surveillance of antimalarial treatment efficacy [12].

Ethical Approval

The study protocol was reviewed and approved by Ahmadu Bello University Animal Research Ethics Committee (approval no. ABUCAUC/2023/035) in conformity with guidelines that are in compliance with national and international laws and guidelines.

Collection, Identification and Preparation of Plant Samples

The fresh leaves of *M. balsamina* were harvested in Zaria, Kaduna State. The plant was identified by a botanist, authenticated and assigned voucher specimen number (ABU01255) at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria. The leaves were rinsed under running tap water to remove dirt and air-dried at room temperature and pulverized to coarse powder using mortar and pestle, then sealed and kept in a dry area till use for further analysis [13].

Preparation of M. Balsamina Leaf Extracts

One (1) kg pulverized leaves sample was macerated sequentially in n-hexane for 72 h using aspirator bottle and shaken at interval. The marc obtained was further extracted using ethyl acetate (EtOAc) and then methanol (MeOH). The extracts were filtered using muslin cloth and then with Whatman filters paper No. 1 and the filtrates were concentrated at room temperature.

Preparation of Stock Solutions of Extracts and Drug

For the acute toxicity assay, 1g of each extract was dissolved in 2ml (500mg/ml) of 1% tween 80 to serve as the stock solution for the acute toxicity test and was administered according to their body weight. For suppressive model, 0.4g of the extracts was dissolved in 20ml (20mg/ml) of 1% tween 80 as stock solution and administered according to their body weight, while the stock solution of the negative control is 1% tween 80 and the chloroquine was 5 mg/kg

Grouping and Dosing of Mice

Mice were randomly assigned to 9 extract-treated groups and 2 controls with 5 mice per group. The cages were given numerical designation and the treatment was as followed: *Group 01* (*P. berghei* + 5 mg/kg b.w of chloroquine); *Group 02* (*P. berghei* + 1% tween 80) (negative control); *Group 03, 04, 05* (*P. berghei*-infected mice + 100, 200, and 400 mg/kg b.w MeOH extract) respectively. *Group 6, Group 7,* and *Group 8* were *P. berghei*-infected mice + 100, 200, and 400 mg/kg b.w EtOAc extract respectively. *Group 9, Group 10,* and *Group 11* were *P. berghei*-infected mice + 100, 200, and 400 mg/kg b.w n-Hex extract respectively.

Maintenance of P. Berghei in Mice

Plasmodium berghei NK-65 was obtained in animal house, Department of Pharmacology and therapeutics, Ahmadu Bello University Zaria. The parasite was maintained in a naive mouse by serial passaging. The blood of a donor mouse with parasitemia level of 28% was utilized to infect the experimental mice. The blood from the donor mice was drawn by retro orbital puncture into a tube that contained 2% trisodium citrate as an anticoagulant and diluted in physiological saline to 1 x 10^7 infected RBCs.

Four Day Chemosuppressive Model

The protocol for a 4-day suppressive test was utilized according to previous study [14]. First, mice were arbitrarily divided into (11) groups of (5) mice each. Every mouse in each of the mouse groups for the experimental was coded and intraperitoneally infected with 0.2 mL of blood carrying around 1 x 10^7 RBCs infected with *P. berghei* [15]. Treatment began 4 h after the inoculation and continued every day for 4 days. The mice in each group were administered with an oral dosage of each extracts of *M. balsamina*.

Determination of Safe and Lethal Dose

The methanol extract, ethyl acetate extract and n-hexane extract of *M. balsamina* leaf were evaluated for toxicity in mice according to the standard guidelines of the Organization for Economic Cooperation and Development [16]. Twenty five (25) mice were randomly divided into five (5) groups of five mice (5) each.

Mice treated with 5000 mg/kg body weight of methanol extract, ethyl acetate extract and n-hexane extract of *M. balsamina* as group I, II and III respectively. Negative control (group IV) was given 1% of tween 80 solution and the untreated (group V) was given water and feeds only. The doses of the extract were 1/12.5th, 1/25th, and 1/50th of the LD_{50} (lethal dose 50%) value from the acute oral toxicity study.

Histopathological Examination

Histopathological investigation was performed using a standard laboratory procedure as previously reported [17]. Briefly, the tissues of the mice were fixed in 10% (v/v) formalin at room temperature, dehydrated with series of alcohol concentrations, cleared with xylene, and embedded in paraffin. After tissue processing, the liver and kidney tissues were cut into 5 μm thickness using a microtome, stained with hematoxylin and eosin solution, and evaluated under a light microscope by a histopathologist blinded to the condition groups. The microscopic features and photographs of organs of *M. balsamina* extracts treated mice were compared to those of the control group.

Data Analysis

Data generated were presented as mean of three replicates standard error of mean (SEM) (*in-vitro*; n=3) and analysis was done using GraphPad Prism® 8.02 windows software. The *p*-value $p < 0.05$ was taken as statistically significant.

RESULTS

Acute Oral Toxicity Assays

The results of the *in vivo* acute toxicity assay of *M. balsamina* leaf at a dose of 5000 mg/kg bodyweight during the 14 days study period are depicted in Table 1. Physical and behavioral observations of the experimental mice revealed no visible signs of acute toxicity such as salivation, lacrimation, sweating, diarrhea, and loss of appetite. Nevertheless, mice treated with methanol extract recorded one death at Day 14 with percentage mortality of 20%.

Relative Liver and Kidney Weights of Mice in Acute

Toxicity Assay of M. Balsamina Leaf

The liver and kidney weights of mice in the acute toxicity assay are shown in Table 2. The relative weights of the liver in mice fed with the methanol extract, ethyl acetate extract, and n-hexane extract were 4.67%, 3.67%, and 4.55%, while the relative weights of the kidney were 1.00%, 0.87%, and 1.07% respectively. Moreover, the relative weights of the liver and kidney of the untreated group were 5.86% and 1.89% respectively.

The weights of the liver of mice that received the 5000mg/kg extracts of methanol, ethyl acetate and n-hexane revealed no statistically significant difference when compared with those of the mice in the untreated group at Day 14. The weight of the kidney of mice treated with methanol extract and ethyl acetate extract showed no significant ($p > 0.05$) difference when compared with those of mice in the untreated group while that of mice treated with n-hexane extract showed a significant ($p = 0.026$) difference when compared with the untreated group.

Histopathological Analysis of the Liver and Kidney Tissues in Acute Toxicity Assay

The kidneys and livers of mice in each group of methanol extract, ethyl acetate extract, and n-hexane extract were removed and histopathological data are shown in figure 1. The histopathology of kidney section showed normal architecture of cells in untreated mice and slight tubular adhesion (Figure 1a and 1c). The kidney histology of methanol extract, ethyl acetate extract, and n-hexane extract treated mice revealed moderate tubular adhesion, slight tubular necrosis, and slight glomerular necrosis respectively (Figure 1e, 1g and 1i).

The liver tissues showed normal hepatocytes in untreated mice and slight hepatic necrosis in negative control mice (Figure 1b and 1d). The liver histology of the mice treated with a single 5,000 mg/kg dose of methanol extract, ethyl acetate extract, and n-hexane extract revealed normal hepatocyte morphology and normal structures (Figure 1 f, h, and j). Decisively, these findings imply that there was no obvious toxicity in the livers and kidneys of treated mice for all *M. balsamina* crude leaf extracts.

Antiplasmodial Activities of M. Balsamina Crude Leaf Extracts in Suppressive Assay

Treatment of *P. berghei* infected mice with methanol **extract**, ethyl acetate extract, and n-hexane **extract** of *M. balsamina* leaf in suppressive model resulted in suppressive activities and showed that all extracts (100-400 mg/kg) caused a dose-dependent decrease in parasitaemia (Figure 2).

The mice treated with 100, 200, and 400 mg/kgb.w of the methanol extract revealed parasitemia of 14%, 13.4%, and 9.8% with a chemosuppression of 21.3%, 24.7%, and 44.9% respectively, while those treated with the ethyl acetate extract showed a parasitemia of 13.8%, 12.2%, and 8.4% with a chemosuppression of 22.4%, 31.5%, and 52.8%, respectively.

As for the mice treated with the n-hexane extract at those concentrations, they showed parasitemia of 14.2%, 13.4%, and 12.4% with suppression of 20.2%, 24.7%, and 30.3% respectively. The mice in the negative control group had a parasitemia of 17.8% while those in the positive control had a parasitemia of 4.6% and suppression of 74.2%.

The observed reductions in parasitemia were statistically

Table 1. Acute toxicity (LD_{50}) of *M. balsamina* crude leaf extracts of mice up to 14 days

Type of extract	Number of mice	Dosage (mg/kg/bw)	Behavioral change	Change in physical appearance	No. of death	% Mortality
MeOH extract	5	5000	none	None	1	20
EtOAc extract	5	5000	none	None	0	0
n-Hex extract	5	5000	none	None	0	0
Neg. control	5	10ml/kg	none	None	0	0
Untreated	5	-	none	None	0	0

MeOH; methanol, EtOAc; ethyl acetate, n-Hex; n-hexane, 10ml/kg of 1% tween 80

Table 2. Relative liver and kidney weight of mice in acute toxicity test of crude extracts of *M. balsamina* leaf

Group	Concentration of extracts mg/kg/bw	Weight (Mean ± SEM) (g)			Relative organ weight (%)	
		Bodyweight	Liver	Kidney	Liver	Kidney
MeOH extract	5000	27.3 ± 1.250	1.28±0.13d[4]	0.275 ± 0.08a[1]	4.67	1.00
EtOAc extract	5000	30.0 ± 1.140	1.10±0.07e[5]	0.260 ± 0.02b[2]	3.67	0.87
n-Hex extract	5000	22.4 ± 1.208	1.02±0.04f[6]	0.240 ± 0.02c[3]	4.55	1.07
Neg. control	10ml/kg	22.1 ± 1.030	1.00 ± 0.06	0.300 ± 0.03	4.52	1.36
Untreaed	-	22.2 ± 0.374	1.24 ± 0.07	0.4200 ± 0.04	5.86	1.89

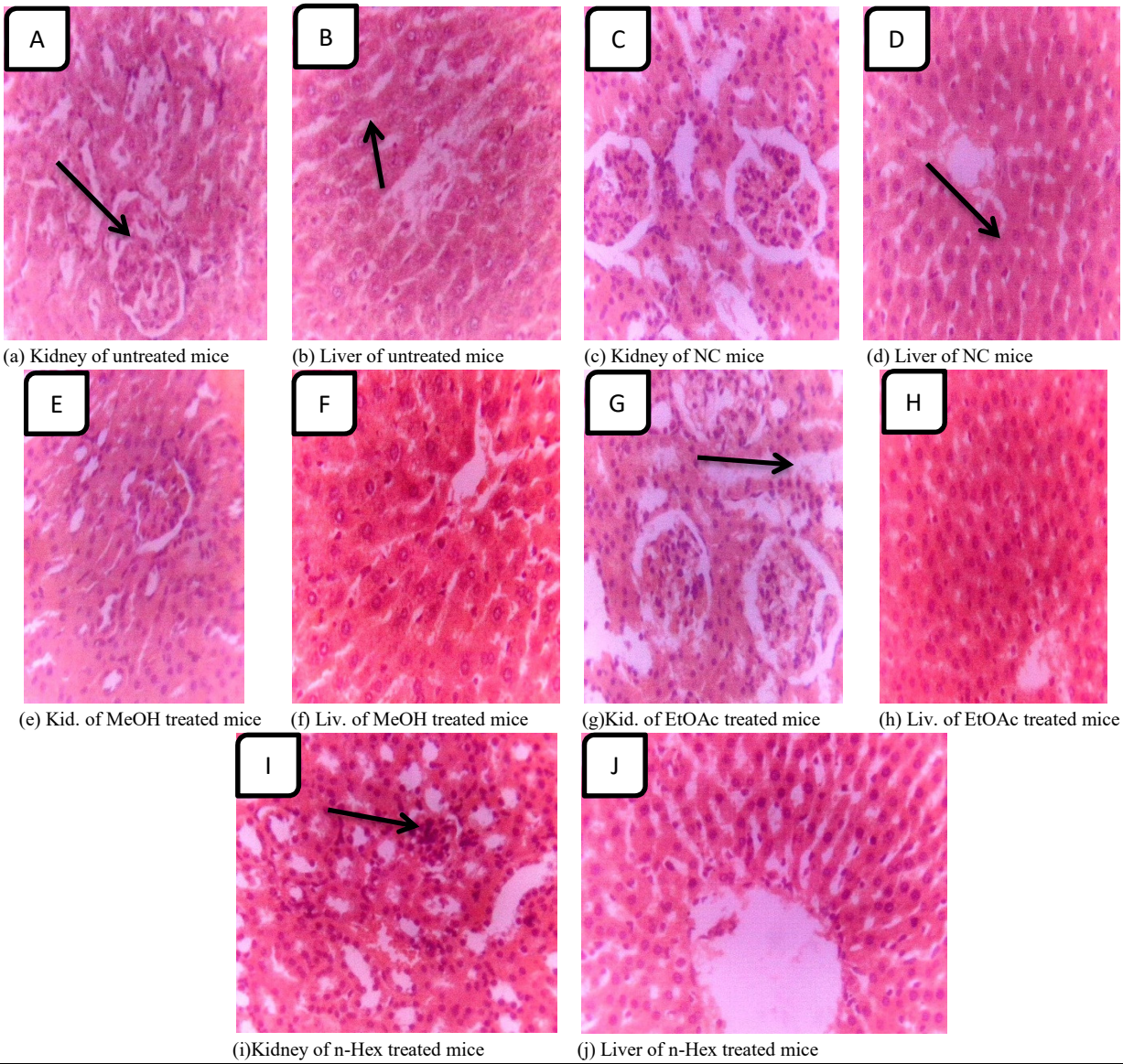
All values are expressed as the mean ± SEM ($n = 5$ per group), MeOH = methanol, EtoAc = ethyl acetate, n-Hex = n-hexane. [c] compared with the untreated kidney, [3] $p = 0.026$.

significant when compared to the negative control (1% tween 80) group ($p < 0.0001$). The reference drug revealed significant suppressive activity when compared with methanol extract ($p = 0.0007$), ethyl acetate extract ($p = 0.0323$) and n-hexane extract ($p = 0.0001$)) at 400mg/kgb.w. when compared with the positive control.

Effect of M. Balsamina Extract on Bodyweight and Survival Time in Suppressive Assay

The effect of extract of *M. balsamina* leaf on bodyweight and survival time of mice infected with *P. berghei* is shown in Figure 3. Bodyweight in the methanol extract treated group increased slightly between Day 0 and Day 4 at the higher doses (200 mg/kgb.w) from 25.8 to 27.0 g, and 400 mg/kgb.w (26.2 to 26.6 g). The bodyweight in the ethyl acetate extract group increased (22.6 to 23.0 g), while n-hexane extract group (22.6 to 23.6 g) at 400 mg/kgb.w. The bodyweight in the reference drug (chloroquine) group increased (25 to 26 g) at 5 mg/kgb.w and there was a decrease in the bodyweight of the negative control group (20.3 to 19.8 g).

The ethyl acetate treated group at 100, 200, and 400 mg/kgbw extended the survival time of *P. berghei* infected mice for a period of 30 days. The methanol extract treated group and n-hexane extract treated group had a mean survival time of 30 days at doses 200 mg/kgb.w and 400 mg/kgb.w, while the 100 mg/kgb.w group had mean survival time of 28.6 days. Also, there was a prolonged mean survival time of the chloroquine treated group for 30 days while the negative control group survived for a period of 24.2 days.



(a) Kidney of untreated mice (b) Liver of untreated mice (c) Kidney of NC mice (d) Liver of NC mice

(e) Kid. of MeOH treated mice (f) Liv. of MeOH treated mice (g)Kid. of EtOAc treated mice (h) Liv. of EtOAc treated mice

(i)Kidney of n-Hex treated mice (j) Liver of n-Hex treated mice

Figure 1. Photomicrograph of kidney and liver tissues of mice administered with methanol extract, ethyl acetate extract, and n-hexane extract of M. balsamina leaf in acute toxicity test

(a) Nornal glomerular necrosis, (b) Normal hepatocytes, (c) Normal features (d) Normal feature (e) Moderate tubular adhesion, (f) Normal features, (g) Slight tubular necrosis, (h) Normal features (i) Slight glomerular necrosis, (j) Normal features.
MeOH, methanol; EtOAc, ethyl acetate; n-Hex, n-hexane; NC, negative control mice; Liv, liver; Kid, kidney.

ica balsamina leaf extracts (mg/kg)

Figure 2. Chemosuppression effect of M. balsamina leaf extracts in suppressive model compared to positive control (CQ). 1p <0.000, 2p = 0.001, 3p = 0.032.

Figure 3. Bodyweight and mean survival time of mice treated with extract in suppressive model

DISCUSSION

Malaria remains the major parasitic illness in a global scale and is responsible for the death of more persons than most other infectious ailments [18]. There is emergence of *Plasmodium* resistance to all available antimalarial drugs. Hence, the urgent need for innovation and the development of novel antiplasmodial drugs to resolve this challenge [19]. Natural antimalarial products obtained from medicinal plants are considered a leading strategy to solve this problem [20]. Thus, assessing the antimalarial activity of plant constituents is very significant to contribute to the finding of lead chemical(s) [21].

Acute toxicity test results of the extracts of the leaves of *M. balsamina* showed no sign of toxicity in all exposed mice. It is therefore possible to conclude that the LD_{50} of the extracts is beyond 5000 mg/kg [OECD] and as per WHO hazard classification, *M. balsamina* is designated as "unlikely to be hazardous" [22].

Pharmaceutical drugs with an oral LD_{50} higher than 5000 mg/kg are usually considered as safe and display low toxicity [23]. Consequently, *M. balsamina* appear almost non-toxic at 5000 mg/kg single dose and this finding agrees with the OECD protocol for acute toxicity studies. The acute toxicity status of *M. balsamina* in this current study is in agreement with the findings of Koonrungsesomboon *et al.* [24], Mabasa *et al.* [7], Anaduaka *et al.* [10], who also reported extremely weak, inactive and no toxicity elsewhere. The toxicity status is also in agreement with Kadiri *et al.* [25] who reported no toxicity. This indicates a wide safety margin as well as justifying the safety of *M. balsamina* in traditional medicine as per OECD guideline no. 425 [16]

The relative organ weights of vital organs including the liver and kidney were within normal with no significant differences observed ($p > 0.05$). The administration of *M. balsamina* leaf extracts did not induce any changes in the relative organ weights of the treatment groups compared to the control groups. The absence of significant changes in the liver and kidney supports the safety of *M. balsamina*.

Histopathology of *M. balsamina* extract-treated at 5000 mg/kg single dose of the liver section did not show substantial pathological changes in hepatic cells compared with untreated mice. This finding proposes that the extracts of *M. balsamina* leaf have slight acute toxicity after oral administration under the experimental conditions. The liver and kidney histology in this study revealed normal and slight tissue architecture in some experimental groups relative to control, affirming slight signs of toxicity from the extracts administered.

The antiplasmodial result, as revealed in this study, demonstrated that *M. balsamina* leaf extracts inhibits malaria parasites growth in *in vivo* suppressive model. The leaf extracts of *M. balsamina* reduced parasitemia in early infection, indicating possible suppressive and therapeutic benefits in malaria infection.

To be considered very good, good, or medium in terms of antiplasmodial efficacy, plant extract must inhibit parasitemia by at least 50% at doses of 100, 250, or 500 mg/kg/day [26-28]. This criterion places *M. balsamina* in the medium level of antiplasmodial drug candidates and augments several findings that have demonstrated antiplasmodial potency of plant extracts [29, 30]. These findings revealed that the antiplasmodial activity of *M. balsamina* leafs is polarity dependent with extracts from polar solvents displaying higher bioactivity than non-polar solvent.

The highest percentage of parasitemia suppression was exhibited by the ethyl acetate extract at 400 mg/kg (52.8% in the 4-day suppressive test ($p = 0.0323$.). The extracts of *M. balsamina* showed considerable antiplasmodial properties and suggest that the activity of *M. balsamina* may arise from a single or combination of active phytochemical ingredients. This result is in agreement with that of Bature *et al.,* [8] with 65.1% chemosuppression using ethanol extract of *M. balsamina* against *P. berghei*.

LIMITATION

The main drawback in this study is that information on long-term effects of *M. balsamina* extract were not ascertained. Regardless of this limitation, several strengths associated to the study can be delineated. Mainly, this study examined the acute toxicity effect of *M. balsamina* leaf extracts and the effect of different extracts was explored at varied concentrations in order to reveal the existence or the absence of a concentration-dependent outcome.

CONCLUSION

The result of the toxicity revealed that the approximate lethal dose and no-observed-adverse effect level of leaf extracts of *M. balsamina* had a higher safety margin greater than 5000 mg/kg.b.w with negligible adverse effects. This study encourages applied patronage of *M. balsamina* by traditional folks as they do not pose any threat to life. Nevertheless, abusive usage should be discouraged as further studies on long-term effects were not ascertained. The study displayed suitable antiplasmodial activity of *M. momordica* leaf against *P. berghei* infected mice.

Conflict of Interest: The authors declare no conflict of interest.

Funding and Support: None

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