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Structure-Based Design Synthesis of Functionalized 3-(5-(s-Phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one Motifs and Indigenous Plant Extracts and Their Antimalarial Potential

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Abstract. Resistance of the malaria parasite to conventional therapeutic agents calls for increased efforts in antimalarial drug discovery. Current efforts should be targeted at developing safe and affordable new agents to counter the spread of malaria parasites that are resistant to existing therapy. In this study, toxicological and *in vivo* antiplasmodial properties of 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*42H*-chromen-2, *Mangifera indica* and *Tithonia diversifolia* in swiss albino mice models, *Musmusculus* were investigated. *2H*-Chromen-2-one also known as coumarin is highly privileged oxygen-containing heterocyclic entity which are present in plant kingdom as secondary metabolites. The maceration technique of crude drug extraction was employed using cold water extraction. Toxicological analysis was carried out using Lorke's method for acute toxicity testing while the chemosuppressive activity was carried out using Peter's four day test on early infection. We also report the synthesis of functionalized 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs *via* microwave assisted synthetic approach and isolation of indigenous plant extract in order to investigate their antimalarial efficacy. The condensation reaction of 3-acetylcoumarin with various benzaldehyde derivatives resulted in the formation of 3-[3-acryloyl]-*2H*-chromen-2-one which was subsequently reaction the hydrazine hydrate *via* microwave assisted hydrazinolysis to afford the targeted 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs. The chemical structures were confirmed by analytical data and spectroscopic means such as FT-IR, UV, ^{1}H NMR, ^{13}C NMR and DEPT-135. The microwave assisted reaction was remarkably successful and gave targeted 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs in higher yields at lesser reaction time compared to conventional heating method. The LD_{50} of the aqueous extracts of the leaves and stem bark *Mangifera indica* was established to be ± 707.11 mg/kg b.w., p.o. (body weight, administered orally) in mice. *Tithonia diversifolia* aqueous leaf extracts is non-toxic at doses as high as 1000 mg/kg while the LD_{50} of the ethanolic leaf extracts was established to be ± 707.11 mg/kg b.w., p.o. in mice. The *in vivo* antiplasmodial activity was studied in chloroquine-sensitive *Plasmodium berghei berghei* - NK65 infected mice. All the plant extracts, at the doses (100, 200 and 400 mg/kg b.w., p.o.) used, produced significant ($p < 0.05$), dose dependent activity (> 80% inhibition of parasitaemia at maximum dose) against the parasite in the suppressive tests. The *in vitro* antimalarial screening of the synthesized compounds is presently on-going and the finding will be reported in due course.

INTRODUCTION

Malaria is a leading cause of death globally. Although this past year has recorded some success in the fight against the disease, malaria still continues to take its toll in the WHO African Region with over 88% of reported cases occurring in this region [1]. A major challenge encountered in the control of malaria today is antimalarial drug resistance. This has resulted in the spread of malaria to new areas and re-emergence of malaria in areas where the

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disease had been eradicated [2]. In 2015, malaria was the fourth leading cause of death, accounting for 10% of child deaths in sub-Saharan Africa [1]. For several generations, plants have served as a source of various kinds of remedies for medicinal purposes to treat different types of ailments. They are still being used as remedies especially in rural areas in developing countries. In most parts of sub-Saharan Africa, traditional medicine has been acclaimed to be vital in preventing and curing various diseases, thereby playing an important role in health and well-being [3]. In tandem with the rising increase in antimalarial drug resistance, experimental research should be carried out on the rich biodiversity of natural flora that could possibly translate to meaningful drug discovery and synthesis of important pharmacologically active antimalarial compounds. New antimalarial drugs are expected to exhibit rapid efficacy, minimal toxicity and low cost.

MATERIALS AND METHODS

Functionalized 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs was synthesized via microwave assisted synthetic approach. Ethanolic Extraction Procedure for *Tithonia diversifolia* and *Mangifera indica* was carried out as described by Olasehinde *et al.* [4]. Antiplasmodial activities of synthesized drug candidate and plant extracts were examined using the 4-day Peter's test method. Swiss albino mice, Mus musculus of either sex weighing between 18-25g of either sex were obtained from the Nigerian Institute of Medical Research (NIMR). Ethical approval was obtained from the Covenant University Research Ethics Committee (CUREC).The *Plasmodium berghei berghei* NK65 rodent parasite used to assess the antimalarial potential of the extracts was obtained from National Institute for Medical Research (NIMR), Lagos, Nigeria. Parasite inoculation was carried out according to standard methods. Acute assessment of toxicity was performed to determine the lethality and potency of the extracts. In order to determine LD_{50} (Lethal Dose 50), the acute toxicity of the crude extracts of the plant material was evaluated using the Lorke's method [5] with a slight modification. The chemo-suppressive activity of the aqueous extracts of the plant material was assessed by the 4-day suppressive test of acute infection developed by [6]. Percentage chemo-suppression is taken as inhibition of parasite growth/multiplication by the extract relative to the control expressed in percentage.

$$\text{Percentage chemo-suppression} = \frac{(A-B)}{A} \times 100 \quad (1)$$

Where A is the average parasitaemia in the negative control group, B is the average parasitaemia in the test group.

$$\text{That is } \frac{(\text{control mean-dose mean})}{\text{control mean}} \times 100 \quad (2)$$

The means were calculated as mean ± standard error of mean.

Statistical Analysis

Results obtained were expressed as mean ± standard error of mean (S.E.M.). The significance of difference between the controls and treated groups was determined using the student t-test one way analysis of variance (ANOVA) at $p < 0.05$ were considered to be statistically significant. The results were analysed using IBM SPSS 22.0.

RESULTS AND DISCUSSION

The microwave assisted reaction was remarkably successful and gave targeted 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs in higher yields at lesser reaction time compared to conventional heating method. The strategic synthesis of the targeted pyrazole-based coumarin motifs was successful. Microwave assisted synthesis was more efficient and ecofriendly as compared with conventional heating method under reflux and the targeted compounds are good candidates for further study for effective future drug design (Figure 1 and Figure 2). The microwave assisted reaction was remarkably successful and gave targeted 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs in higher yields at lesser reaction time compared to conventional heating method (Table 1). The strategic synthesis of the targeted pyrazole-based coumarin motifswas successful. microwave assisted Synthesis

was more efficient and ecofriendly as compared with conventional heating method under reflux and the targeted compounds are good candidates for further study for effective future drug design. The LD_{50} of the aqueous extracts of the leaves and stem bark *Mangifera indica* was established to be ± 707.11 mg/kg b.w., p.o. (body weight, administered orally) in mice. *Tithonia diversifolia* aqueous leaf extracts is non-toxic at doses as high as 1000 mg/kg while the LD_{50} of the ethanolic leaf extracts was established to be ± 707.11 mg/kg b.w., p.o. in mice. The *in vivo* antiplasmodial activity was studied in chloroquine-sensitive *Plasmodium berghei berghei* -NK65 infected mice. All the plant extracts, at the doses (100, 200 and 400 mg/kg b.w., p.o.) used, produced significant ($p < 0.05$), dose dependent activity (> 80% inhibition of parasitaemia at maximum dose) against the parasite in the suppressive tests. The antiplasmodial activity of synthesized 3-(5-(s-phenyl)-*4H*-pyrazol-3-yl)-*2H*-chromen-2-one motifs will be reported.

TABLE 1. Result of physico-chemical properties of the products 3a-h

Com No	Molecular formula	Mol. Wt.	Melting Pt (°C)	Colour	Elem. Analysis: %Cald (%Found)		
					C	H	N
3a	$C_{18}H_{14}O_2N_2$	290	111	Orange	74.48 (74.61)	4.83 (5.01)	9.66 (9.75)
3b	$C_{20}H_{19}O_2N_3$	333	152-154	Orange	72.07 (71.96)	5.71 (5.87)	12.61 (12.80)
3c	$C_{17}H_{16}O_4N_2$	311	143-145	Orange	69.15 (69.29)	5.42 (5.33)	9.49 (9.59)
3d	$C_{20}H_{17}O_3N_2$	333	120-121	Yellow	72.07 (71.88)	5.11 (4.98)	8.41 (8.56)
3e	$C_{18}H_{13}O_2N_2Cl$	322	150-153	Yellow	67.08 (66.81)	4.04 (3.97)	8.70 (8.61)
3f	$C_{18}H_{14}O_3N_2$	306	129-131	Brown	70.59 (70.42)	4.58 (4.44)	9.15 (9.29)
3g	$C_{18}H_{14}O_3N_2$	306	140-141	Orange	70.59 (70.49)	4.58 (4.43)	9.15 (9.08)
3h	$C_{19}H_{14}O_3N_2$	295	120-121	Orange	69.15 (69.05)	5.08 (4.98)	9.49 (9.69)

TABLE 2. Percentage chemo-suppression of the aqueous extracts of *Mangifera indica* leaves

Drug	Dose	Mean Parasitaemia density (D3)	Percentage chemo suppression (%)
Physiological saline	0.5 ml	334.00±10.00	0
M. indica	100 mg/kg	113.75±7.78	65.94*
Leaves	200 mg/kg	104.33±6.34	68.76*
	400 mg/kg	50.25±5.45	84.95*
Chloroquine phosphate	10 mg/kg	5.50±1.32	98.35*

D3= Day 4, *significantly different from negative control at $p < 0.05$ (n=4)

TABLE 3. Percentage chemo-suppression of the aqueous extracts of *Mangifera indica* bark

Drug	Dose	Mean Parasitaemia density (D3)	Percentage chemo suppression (%)
Physiological saline	0.5 ml	359±4.50	
M. indica	100 mg/kg	110.20±4.56	69.30*
Bark	200 mg/kg	92.75±4.63	74.16*
	400 mg/kg	71.0±4.49	80.22*
Chloroquine phosphate	10 mg/kg	9.00±1.55	97.49*

D3= Day 4, *significantly different from negative control at $p < 0.05$ (n=4)

TABLE 4. Percentage chemo-suppression of the aqueous extracts of *T. diversifolia* leaves

Drug	Dose	Mean Parasitaemia density (D3)	Percentage chemo suppression (%)
Physiological saline	0.5 ml	359±4.50	-
T. diversifolia	100 mg/kg	96.33±17.95	73.54*
Leaves	200 mg/kg	78.67±13.84	78.39*
	400 mg/kg	48.25±9.67	86.74*
Chloroquine phosphate	10 mg/kg	10.00±1.29	97.25*

D3= Day 4, *significantly different from negative control at $p < 0.05$ (n=4)

TABLE 5. Percentage chemo-suppression of the ethanolic extracts of *T. diversifolia* leaves

Drug	Dose	Mean Parasitaemia density (D3)	Percentage chemo suppression (%)
Physiological saline	0.5 ml	334±10.00	-
T. diversifolia	100 mg/kg	83.00±9.51	75.15*
Leaves	200 mg/kg	60.00±6.47	82.04*
	400 mg/kg	48.50±9.06	85.48*
Chloroquine phosphate	10 mg/kg	2.75±1.80	99.40*

D3= Day 4, *significantly different from negative control at $p < 0.05$ (n=4)

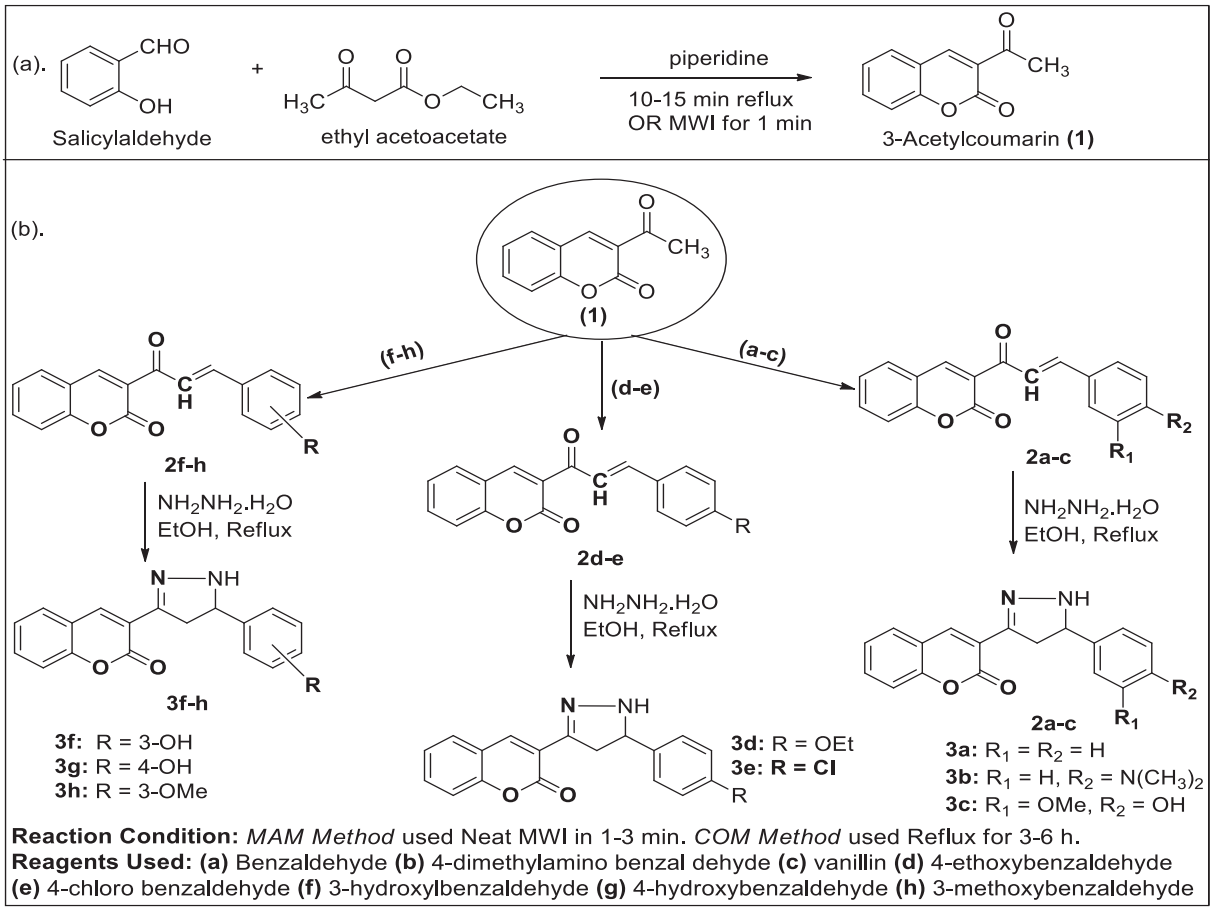

FIGURE 1. Synthetic pathway to (a) precursor and (b) Pyrazole-based coumarin products **3a-h**

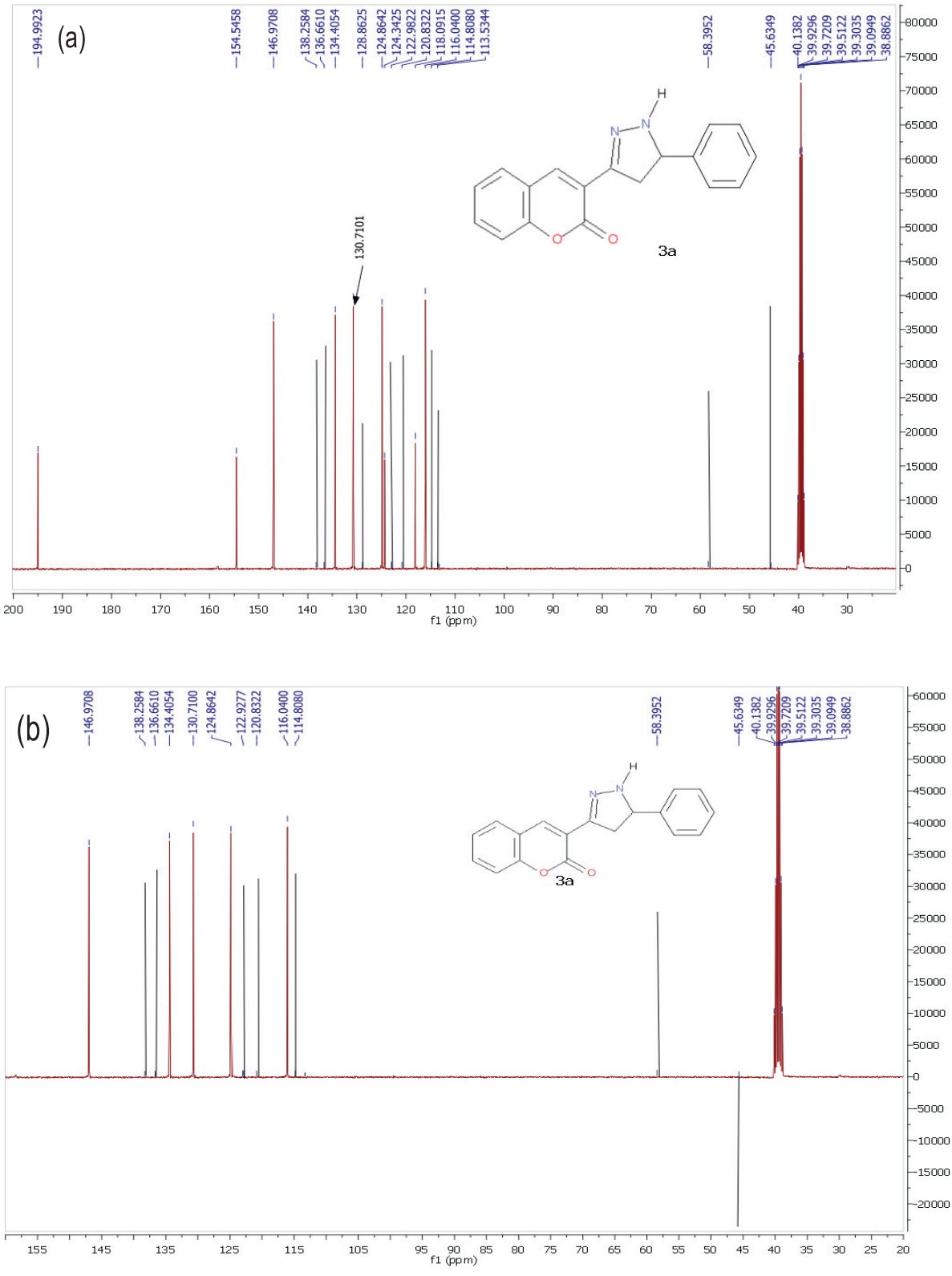

FIGURE 2. a) ^{13}C-NMR and b) DEPT-135 Spectrum of 3a

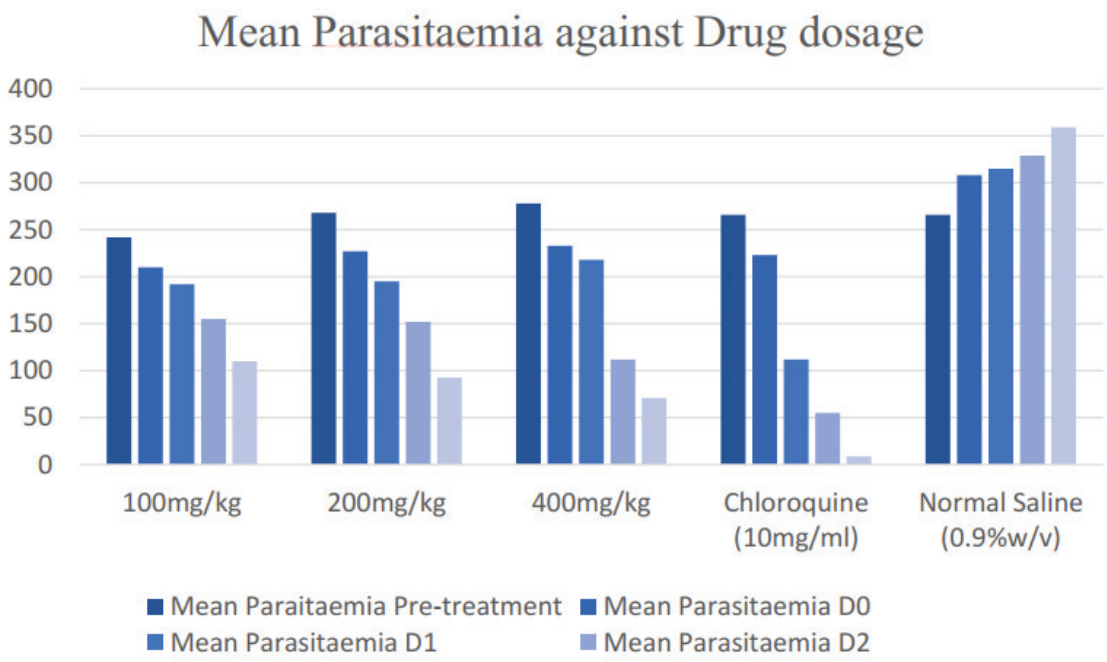

FIGURE 3. Graphical representation of rate of parasitaemia n aqueous extracts of *M. indica* leaves

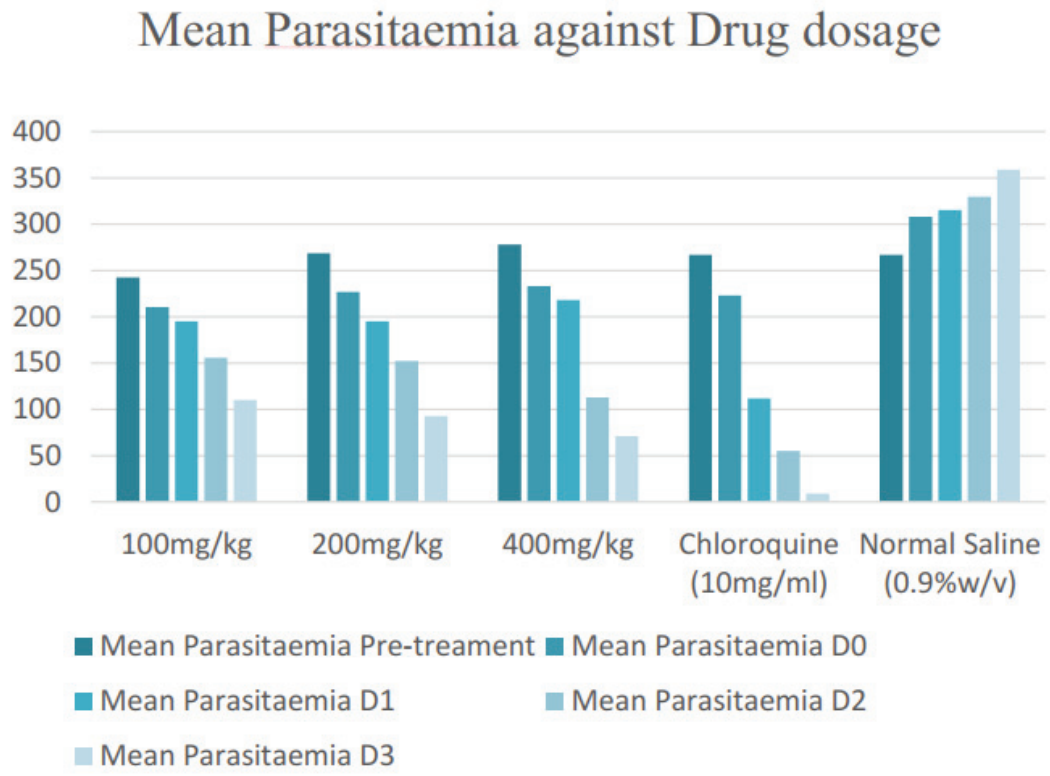

FIGURE 4. Graphical representation of the trend in the burden of parasitaemia for the aqueous extracts of *M. indica* stem bark

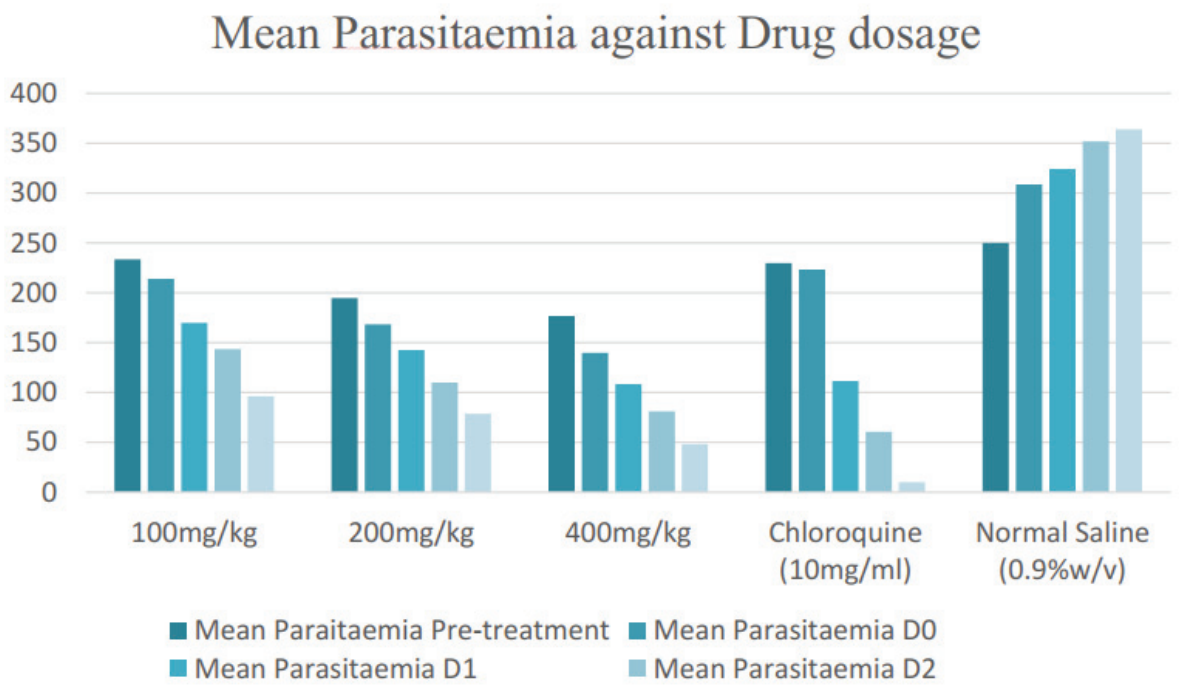

FIGURE 5. Graphical representation of the trend in the burden of parasitaemia for the aqueous extracts of *T. diversifolia* leaves

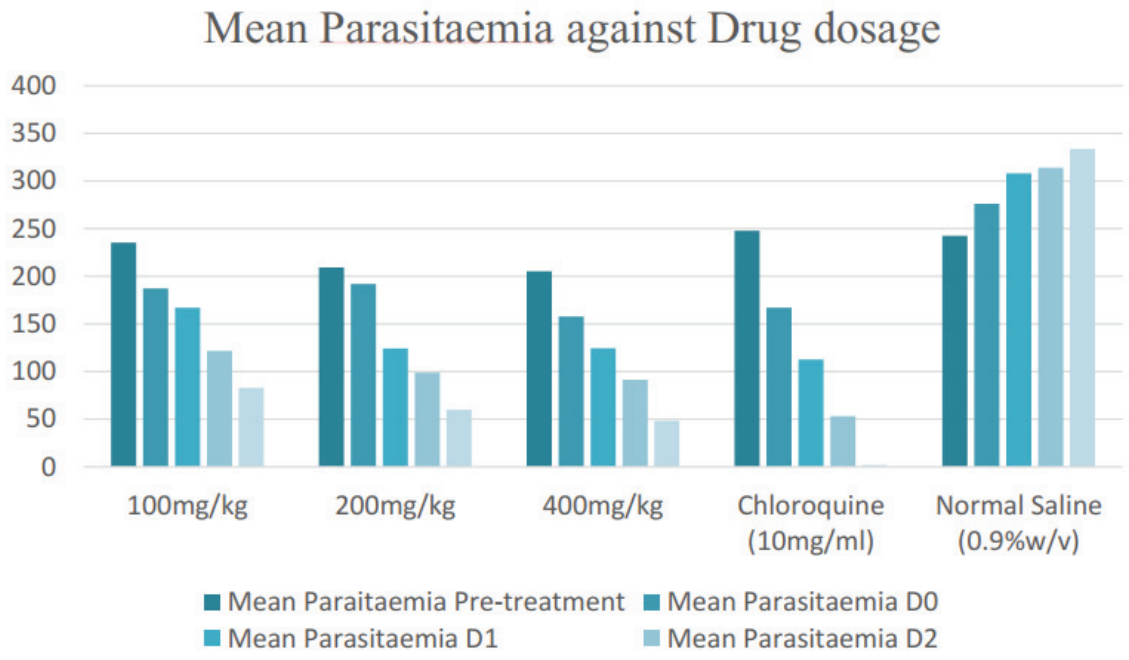

FIGURE 6. Graphical representation of the trend in the burden of parasitaemia for the ethanolic extracts of *T. diversifolia* leaves

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