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Research Article

Antiplasmodial and Organ-Protective Effects of Combined Aqueous Extracts of *Carica papaya* and *Tithonia diversifolia* Leaves in *Plasmodium berghei*-Infected Mice

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Keywords: Antiplasmodial; *Carica papaya*; *Tithonia diversifolia*; *Plasmodium berghei*; Combination therapy; Haematological parameters

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Abstract

Malaria remains a major global health burden, with rising *Plasmodium falciparum* resistance to conventional drugs such as chloroquine and artemisinin. This study evaluated the antiplasmodial and organ-related effects of combined aqueous leaf extracts of *Carica papaya* and *Tithonia diversifolia* in *Plasmodium berghei*-infected Swiss albino mice. Fifty-six mice were allocated to seven groups of eight: normal control, infected untreated control (INT), single extracts (Ca100, Ti100), and three combination ratios (CaTi75:25, CaTi50:50, CaTi25:75). Each mouse received 0.06 mL of extract orally once daily for seven days; blood films were taken on the third day after inoculation (four-day suppressive test) and the remaining endpoints after the seven-day course. Three INT mice died before terminal sampling, and five mice per group were used for every analysis so that group sizes were equal. We assessed parasitaemia, chemosuppression, body weight, haematological indices, liver and kidney biomarkers and tissue histology, and used gas chromatography-mass spectrometry (GC-MS) to profile the extracts. All treated groups had significantly lower parasitaemia than the INT group. Chemosuppression ranged from 51.86% to 88.78% and was numerically highest for CaTi25:75 (88.78 ± 6.91%), although the treated groups did not differ significantly from one another. Relative to INT, CaTi50:50 was associated with higher red blood cell counts and haemoglobin and lower ALT and AST; its creatinine was close to the normal control and it showed the mildest histopathological changes. However, urea, AST and ALP were still above normal-control values, so organ protection was partial. No standard antimalarial positive control or uninfected extract-treated group was included. GC-MS tentatively identified compounds including squalene, phytol and alpha-pinene. These preliminary findings indicate that the combined extracts merit further study using positive-control, toxicity-control and formal interaction (additivity/synergy) designs.

Abbreviations

ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; ANOVA: Analysis of Variance; AST: Aspartate Aminotransferase; Ca100: 100% *Carica Papaya* Aqueous Extract; CaTi25:75: Combination of *C. Papaya* and *T. Diversifolia* Extracts in a 25:75 Ratio; CaTi50:50: Combination of *C. Papaya* and *T. Diversifolia* extracts in a 50:50 Ratio; CaTi75:25: Combination of *C. Papaya* and *T. Diversifolia* extracts in a 75:25 Eatio; EDTA: Ethylenediaminetetraacetic Acid; FUTA: Federal University

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of Technology, Akure; GC-MS: Gas Chromatography–Mass Spectrometry; GRAN: Granulocyte Count; HGB: Haemoglobin Concentration; INT: Infected Untreated; LYM: Lymphocyte Count; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume; MID: Mid-Sized White Blood Cells; MPV: Mean Platelet Volume; PA: Percentage Peak Area; PCT: Plateletcrit; PLT: Platelet Count; RBC: Red Blood Cell Count; RDW-CV: Red Cell Distribution Width (coefficient of variation); RDW-SD: Red Cell Distribution Width (standard deviation); RI: Retention Index; RT: Retention Time; SEM: Standard Error of the Mean; Ti100: 100% *Tithonia Diversifolia* Aqueous Extract; WBC: White Blood Cell Count; WHO: World Health Organization; w/v: Weight Per Volume

Introduction

Malaria continues to rank among the most significant parasitic diseases of humans, accounting for an estimated 282 million cases and 610,000 deaths in 2024, with children under five disproportionately affected [1]. The disease is caused by protozoan parasites of the genus *Plasmodium*, transmitted through the bite of infected female *Anopheles* mosquitoes. Of the species that infect humans, *Plasmodium falciparum* is the most virulent and remains the dominant cause of severe disease across sub-Saharan Africa [2], while *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* contribute to chronic and relapsing infections in other regions [3–5], and the zoonotic *Plasmodium knowlesi* poses an emerging threat in Southeast Asia [6].

Beyond its direct parasitic burden, malaria frequently compromises the liver and kidneys, the two organs most often implicated in severe disease. Hepatic dysfunction manifests through jaundice and elevated transaminases, while renal involvement ranges from mild proteinuria to acute kidney injury requiring dialysis, a complication associated with substantial mortality [7]. Haematological derangement is also a near-universal feature of infection, and indices such as haemoglobin concentration, leukocyte counts, and platelet parameters have increasingly been explored as adjunct markers of diagnosis and severity.

The continued spread of *Plasmodium falciparum* resistance to frontline antimalarials across Africa [1,8] has renewed interest in plant-derived alternatives. Historical precedents, including quinine from *Cinchona* bark and artemisinin from *Artemisia annua*, illustrate the value of botanicals as templates for antimalarial drug development [9,10]. Aqueous leaf extracts of *Carica papaya* have shown suppression of parasitaemia in murine models [11,12], and *Tithonia diversifolia* has independently demonstrated antiplasmodial activity in vitro against *P. falciparum* and in vivo against *P. berghei* [13,14]; one study of a hot-infusion extract reported chemosuppression of about 66% at 400 mg/kg [15]. Combination therapy has been shown to improve efficacy and delay the emergence of resistance relative to monotherapy [16].

Despite the individually documented activity of *C. papaya* and *T. diversifolia*, their combined antiplasmodial and organ-related effects have not been systematically characterised. This study therefore evaluated the antiplasmodial activity of combined aqueous leaf extracts of *C. papaya* and *T. diversifolia* against *Plasmodium berghei* in mice, with specific objectives to compare the effect of different extract ratios on parasite suppression, evaluate haematological indices (including leukocyte counts), and determine the impact of the extracts on liver and kidney biomarkers and histoarchitecture.

Materials and methods

Study area and plant material

The study was carried out in the Department of Biology, Federal University of Technology, Akure (7.3037°N, 5.1388°E), Ondo State, Nigeria. Fresh leaves of *Carica papaya* and *Tithonia diversifolia* were collected on campus and identified at the Department of Forestry and Wood Technology, FUTA. Leaves were washed, air-dried at 20–25°C away from direct sunlight, and ground into powder. Powders were macerated in distilled water (*C. papaya*, 1:10 w/v; *T. diversifolia*, 1:15 w/v) for 72 h at room temperature with intermittent agitation, filtered through 8-µm Whatman paper, and concentrated in a water bath at 45°C [17].

GC-MS analysis

Phytocomponents of both aqueous extracts were profiled on an Agilent 8860 gas chromatograph coupled to a 5977B mass-selective detector fitted with an Elite-5MS capillary column (5% diphenyl/95% dimethyl polysiloxane; 30 m × 0.25 mm × 0.25 µm). Helium (99.99%) was used as the carrier gas at a constant flow of 1 mL/min; 1 µL was injected with a 10:1 split ratio. The injector was held at 300°C and the ion source at 250°C. The oven was programmed from 110°C (1 min hold) at 15°C/min to 310°C (2 min hold). Electron-impact ionisation was at 70 eV, with a scan interval of 0.5 s, a mass range of 45–450 Da and a solvent delay of 0–3 min. Compounds were identified by comparing mass spectra and retention indices with library data.

Experimental animals and grouping

Fifty-six female Swiss albino mice (13–20 g) obtained from the Institute of Medical Research and Training, University College Hospital, Ibadan, were acclimatised for one week under standard housing with ad libitum feed and water, in accordance with institutional ethical guidelines for animal use [18]. Mice were assigned to seven groups of eight (n = 8 per group; N = 56): normal control (uninfected, untreated), infected untreated control (INT), Ca100 (100% *C. papaya*, 400 mg/kg), Ti100 (100% *T. diversifolia*, 266.67 mg/kg), and three combinations: CaTi75:25 (366.67 mg/kg total), CaTi50:50 (333.33 mg/kg total) and CaTi25:75 (300 mg/kg total). Doses are nominal values for a 15 g mouse (see Dosage calculation); the same fixed volume was given to every group, so total dose differs between treatments (Table 1) and actual mg/kg varied with individual body weight. Each mouse received 0.06 mL of its assigned extract orally once daily for seven days. The normal and INT groups are the only controls in this study; no standard antimalarial drug group or

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uninfected extract-treated group was included. No mouse was excluded; three INT mice died before terminal sampling. All analyses (Tables 4–7) used five mice per group (n = 5): the five INT survivors and five mice from each of the other groups so that group sizes were equal.

Dosage calculation

The dosage (D, mg/kg) administered to each mouse was calculated from the concentration and volume of each extract component relative to body weight, using the formula:

Equation 1: Dosage Calculation in mg/kg

$$D = [(C_1 \times V_1) + (C_2 \times V_2)] / W$$

Table 1: Total dosage of *Carica papaya* and *Tithonia diversifolia* aqueous extracts administered per treatment group.

Treatment	*Carica papaya* (mg/kg)	*Tithonia diversifolia* (mg/kg)	Total dosage (mg/kg)
CaTi50:50	200	133.33	333.33
CaTi25:75	100	200	300
CaTi75:25	300	66.67	366.67
Ca100	400	0	400
Ti100	0	266.67	266.67

Each group received 0.06 mL of the respective treatment orally for 7 days, with an average mouse weight of 15 g.

Table 2: GC-MS analysis showing phytocomponents, molecular formula, retention time (RT) and percentage peak area (PA) of the aqueous extract of *Carica papaya* leaves.

S/N	Phytocomponent	Molecular formula	RT (min)	PA (%)
1	Cytosine	$C_4H_6N_4O$	4.614	2.27
2	Glycerol monoacetate	$C_5H_{10}O_4$	4.764	2.19
3	Maltol	$C_6H_8O_4$	5.517	4.25
4	Acetylmannose	$C_8H_{14}O_7$	6.451	3.31
5	Sorbic acid	$C_6H_6O_4$	8.588	4.06
6	Galactinol	$C_8H_{16}O_6$	13.05	3.19
7	Myristic acid	$C_{14}H_{28}O_2$	13.58	3.88
8	Syringic acid	$C_9H_{10}O_5$	15.52	1.13
9	Hexadecenoic acid, Z-11	$C_{16}H_{30}O_2$	16.269	19.17
10	Methyl palmitate	$C_{17}H_{34}O_2$	16.52	18.25
11	Palmitic acid	$C_{16}H_{32}O_2$	16.568	0.78
12	Cyclooctene, 3-ethenyl-	$C_{10}H_{16}$	18.176	5.74
13	Phytol	$C_{20}H_{40}O$	18.382	10.16
14	Isoheptadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	18.628	1.98
15	Methyl linoleate	$C_{19}H_{34}O_2$	18.709	5.38
16	Linoleic acid	$C_{18}H_{32}O_2$	19.04	2.16
17	Stearic acid	$C_{18}H_{36}O_2$	19.702	23.84
18	Ambrettolide	$C_{21}H_{40}O_2$	22.496	2
19	Glycerol monopalmitate	$C_{19}H_{38}O_4$	25.183	4.72
20	Glycerol monolinolenate, bis(trimethylsilyl) derivative	$C_{27}H_{52}O_4Si_2$	27.983	3.58
21	Squalene	$C_{30}H_{50}$	31.01	27.28
22	Delta-tocopherol	$C_{27}H_{46}O_2$	33.699	1.34

Table 3: GC-MS analysis showing phytocomponents, molecular formula, retention index (RI) and percentage peak area (PA) of the aqueous extract of *Tithonia diversifolia* leaves.

S/N	Phytocomponent	Molecular formula	RI	PA (%)
1	alpha-Pinene	$C_{10}H_{16}$	917.7	13.5
2	Phenol	C_6H_6O	968.9	0.4
3	p-Cymene	$C_{10}H_{14}$	1022.6	2.2
4	Eucalyptol (cineole)	$C_{10}H_{18}O$	1034.7	14.6
5	gamma-Terpinene	$C_{10}H_{16}$	1072.4	0.5
6	Epoxy linalool	$C_{10}H_{18}O_2$	1093.1	1.3
7	Linalool oxide	$C_{10}H_{18}O_2$	1111.9	1.5
8	Linalool hydrate	$C_{10}H_{18}O_2$	1121.8	0.7
9	alpha-Campholenaldehyde	$C_{10}H_{16}O$	1153.2	2.6
10	Pinocarveol	$C_{10}H_{16}O$	1168.4	5.1
11	Camphor	$C_{10}H_{16}O$	1194.7	14.3
12	Terpinen-4-ol	$C_{10}H_{18}O$	1203.5	1.8
13	alpha-Terpineol	$C_{10}H_{18}O$	1220.6	20.3
14	Myrtenol	$C_{10}H_{16}O$	1225.6	0.3
15	Carvacrol	$C_{10}H_{14}O$	1324.7	0.8
16	Spathulenol	$C_{15}H_{24}O$	1615.4	3.3
17	Globulol	$C_{15}H_{26}O$	1622.7	1.5
18	Ledol	$C_{15}H_{26}O$	1631.7	5.7
19	2-Naphthalenemethanol	$C_{11}H_{12}O$	1689.8	1.2

Table 4: Percentage parasitaemia and chemosuppressive activity of *Carica papaya* and *Tithonia diversifolia* aqueous extracts in *Plasmodium berghei*-infected mice.

Treatment	Parasitaemia (%)	Chemosuppression (%)
Ti100	30.28 ± 14.05^{abc}	66.95 ± 15.71^{b}
Ca100	45.19 ± 12.93^{c}	51.86 ± 14.27^{b}
CaTi75:25	25.79 ± 9.11^{abc}	72.45 ± 9.92^{b}
CaTi25:75	11.17 ± 6.93^{ab}	88.78 ± 6.91^{b}
CaTi50:50	35.76 ± 15.31^{bc}	60.88 ± 17.74^{b}
Normal	0.00 ± 0.00^{a}	No parasitaemia
Infected untreated	95.78 ± 2.93^{d}	0.00 ± 0.00^{a}

Values are mean ± SEM (n = 5 mice per group); means within a column bearing different superscripts differ significantly (Duncan's test, $p < 0.05$).

Table 5: Initial and final body weights of mice across treatment groups with *Carica papaya* and *Tithonia diversifolia* aqueous extracts in *Plasmodium berghei*-infected mice for 7 days.

Treatment	Initial weight (g)	Final weight (g)
Ti100	13.65 ± 0.32^{a}	14.38 ± 0.79^{a}
Ca100	14.49 ± 0.57^{a}	17.35 ± 0.90^{ab}
CaTi75:25	14.56 ± 0.36^{a}	17.07 ± 0.46^{ab}
CaTi25:75	13.99 ± 0.41^{a}	16.61 ± 0.71^{ab}
CaTi50:50	14.07 ± 0.57^{a}	14.39 ± 2.56^{a}
Normal	13.62 ± 0.45^{a}	23.47 ± 4.99^{b}
Infected untreated	14.33 ± 0.43^{a}	19.64 ± 0.56^{ab}

Values are mean ± SEM (n = 5 mice per group); means within a column bearing different superscripts differ significantly (Duncan's test, $p < 0.05$).

where C1 and C2 are the concentrations (mg/mL) of *Carica papaya* and *Tithonia diversifolia* extract, respectively; V1 and V2

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Table 6: Haematological parameters in mice treated with *Carica papaya* and *Tithonia diversifolia* aqueous extracts in *Plasmodium berghei*-infected mice for 7 days.

Parameter	CaTi25:75	CaTi75:25	CaTi50:50	Ti100	Ca100	Normal	INT	p-value
WBC (10^9/L)	15.25±0.78^{bc}	9.80±0.35^{abc}	8.70±0.00^{ab}	17.85±4.96^{c}	18.05±3.26^{c}	2.45±0.20^{a}	9.60±3.52^{abc}	0.008
LYM (10^9/L)	12.40±0.50^{bc}	7.55±0.80^{b}	7.70±0.00^{b}	15.15±4.01^{c}	14.25±2.28^{c}	1.70±0.35^{a}	12.50±0.46^{bc}	0.001
MID (10^9/L)	1.30±0.35^{ab}	1.05±0.32^{ab}	0.50±0.00^{a}	1.25±0.43^{ab}	2.20±0.75^{b}	0.50±0.058^{a}	1.25±0.14^{ab}	0.089
GRAN (10^9/L)	1.30±0.35^{bc}	1.20±0.17^{bc}	0.50±0.00^{a}	1.45±0.43^{bc}	1.60±0.23^{c}	0.25±0.09^{ab}	0.85±0.14^{ab}	0.005
RBC (10^{12}/L)	2.57±0.16^{a}	2.97±0.49^{a}	6.37±0.03^{b}	2.77±0.88^{a}	3.54±0.05^{b}	7.71±0.39^{b}	3.26±0.46^{a}	<0.001
HGB (g/dL)	5.50±0.40^{a}	5.50±0.58^{a}	11.23±0.09^{b}	5.55±1.184^{a}	6.50±1.06^{a}	12.70±0.35^{b}	5.85±0.36^{a}	<0.001
MCV (fL)	55.65±0.61^{b}	51.85±1.93^{ab}	49.40±0.36^{a}	55.45±2.92^{b}	51.05±1.99^{ab}	47.75±0.78^{a}	49.45±2.34^{a}	0.049
MCH (pg)	21.50±0.29^{bc}	19.15±1.24^{abc}	17.13±0.12^{ab}	23.10±3.06^{c}	18.40±0.40^{ab}	16.50±0.40^{a}	18.50±1.50^{ab}	0.0498
MCHC (g/L)	386±0.87^{ab}	369±9.53^{ab}	354±1.45^{a}	412±33.80^{b}	361±6.35^{a}	346±2.60^{a}	373±12.40^{ab}	0.084
RDW-CV	0.23±0.00^{c}	0.13±0.00^{a}	0.15±0.00^{a}	0.18±0.03^{b}	0.16±0.00^{ab}	0.156±0.00^{ab}	0.13±0.01^{a}	<0.001
RDW-SD (fL)	42.85±1.18^{c}	22.85±1.36^{a}	24.30±0.26^{a}	33.55±6.26^{b}	26.27±1.82^{ab}	24.45±0.89^{a}	20.55±0.20^{a}	<0.001
PLT (10^9/L)	80.00±16.17^{ab}	53.50±19.92^{a}	134.0±0.58^{c}	86.50±0.87^{ab}	81.50±17.61^{ab}	104.0±20.78^{ab}	79.00±25.17^{ab}	0.09
MPV (fL)	7.65±0.03^{a}	8.80±0.58^{b}	6.77±0.09^{a}	6.90±0.35^{a}	6.75±0.26^{a}	7.00±0.52^{a}	6.95±0.316^{a}	0.011
PCT (mL/L)	0.61±1.13^{a}	0.65±0.11^{a}	1.04±0.09^{b}	0.60±0.02^{a}	0.56±0.14^{a}	0.70±0.52^{ab}	0.57±0.18^{a}	0.12

WBC = white blood cell count, LYM = lymphocyte count, MID = mid-sized white blood cells, GRAN = granulocyte count, RBC = red blood cell count, HGB = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW-CV/SD = red cell distribution width (coefficient of variation/standard deviation), PLT = platelet count, MPV = mean platelet volume, PCT = plateletcrit, INT = infected untreated. Values are mean ± SEM; means within a row bearing different superscripts differ significantly (Duncan's test, $p < 0.05$). n = 5 mice per group.

Table 7: Plasma creatinine, urea, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in mice across treatment groups.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)
Normal	1.33 ± 0.03^{ab}	13.00 ± 6.75^{a}	54.40 ± 5.44^{b}	27.93 ± 1.75^{a}	29.10 ± 1.00^{a}
Ti100	3.15 ± 0.42^{c}	26.66 ± 1.37^{b}	68.00 ± 2.72^{c}	33.20 ± 0.00^{ab}	35.31 ± 6.10^{ab}
CaTi50:50	1.45 ± 0.56^{ab}	47.16 ± 2.26^{d}	68.00 ± 10.88^{c}	34.07 ± 2.62^{ab}	44.05 ± 7.86^{bc}
Ca100	2.31 ± 0.69^{bc}	36.37 ± 8.34^{bc}	76.16 ± 5.44^{cd}	44.55 ± 2.62^{c}	34.44 ± 0.00^{ab}
CaTi25:75	3.06 ± 0.61^{c}	51.76 ± 7.45^{d}	100.66 ± 0.00^{e}	55.89 ± 1.75^{d}	56.56 ± 1.00^{cd}
CaTi75:25	3.38 ± 0.35^{c}	41.77 ± 1.77^{cd}	100.64 ± 21.76^{de}	38.42 ± 7.00^{b}	33.57 ± 0.87^{ab}
Infected untreated	0.32 ± 0.01^{a}	43.24 ± 6.72^{cd}	23.12 ± 1.36^{a}	86.67 ± 3.54^{e}	63.35 ± 3.40^{d}

Values are mean ± SEM (n = 5 mice per group; an SEM of 0.00 means that the five values were identical); means within a column bearing different superscripts differ significantly (Duncan's test, $p < 0.05$).

are the corresponding volumes (mL) administered; and W is the body weight of the mouse (kg). Because a fixed 0.06 mL was given to every mouse, doses in Table 1 were calculated for a nominal body weight of 15 g (0.015 kg). Combinations were prepared by mixing the two stock solutions by volume (for example, CaTi75:25 = 0.045 mL *C. papaya* + 0.015 mL *T. diversifolia*), so that each group received the same total volume. For example, for a mouse weighing 0.015 kg dosed with *C. papaya* (C1 = 100 mg/mL, V1 = 0.03 mL) and *T. diversifolia* (C2 = 66.7 mg/mL, V2 = 0.03 mL): A1 = 100 × 0.03 = 3 mg; A2 = 66.7 × 0.03 = 2.001 mg; total dose = (3 + 2.001)/0.015 = 333.4 mg/kg. The resulting dosage of each treatment, each administered as 0.06 mL orally per mouse for seven days, is summarised in Table 1.

Parasite inoculation and chemosuppression

Plasmodium berghei, obtained from the Institute of Medical Research and Training, Ibadan, was passaged into donor mice, and parasitised erythrocytes were inoculated into experimental mice by the intraperitoneal route [19,20]. Parasite suppression was evaluated with a modified Peters' four-day suppressive test [19]: treatment commenced three hours post-infection and was given once daily for four days, and blood films were taken on the third day after inoculation. Dosing then continued to complete the seven-day course used for body weight, haematological, biochemical and histological endpoints. Thin blood films from tail blood were fixed in methanol, stained with 10% Giemsa, and examined by oil-immersion microscopy (×1000) to determine percentage parasitaemia. Percentage chemosuppression was calculated relative to the infected untreated group.

Equation 2: %Parasitaemia

$$\%\text{Parasitaemia} = \frac{\text{Number of parasitized red blood cell}}{\text{Number of total erythrocytes}} \times 100$$

Equation 3: Suppression Test

$$\text{Average percentage chemosuppression} = \frac{\text{Average percentage parasitaemia in the negative group} - \text{Average percentage parasitaemia in the test group}}{\text{Average percentage parasitaemia in the negative group}} \times 100$$

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Haematological and biochemical analyses

Mice were euthanised by cervical dislocation and blood was collected from the heart into EDTA bottles. Whole blood was analysed on a Mindray BC-30 auto-haematology analyser for white and red blood cell indices, haemoglobin, platelet parameters and derived indices. For biochemistry, the blood was centrifuged (3000 rpm, 20 min) and the plasma was assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as markers of liver function, and creatinine and urea as markers of kidney function, using commercial kits (AGD ClinicPak) following the manufacturer's protocols. EDTA plasma was used for all groups; because EDTA chelates divalent cations required by ALP, absolute ALP activities may be underestimated.

Histopathology

Liver and kidney samples were fixed in 10% formalin, dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, sectioned at 5–6 µm, and stained with haematoxylin and eosin [21,22]. Sections were examined by light microscopy at ×400 magnification, and photomicrographs were compared across groups under the guidance of a pathologist. Histological assessment was qualitative (representative fields); no semi-quantitative lesion scoring was performed.

Statistical analysis

Data were expressed as mean ± standard error of the mean and analysed by one-way analysis of variance (all comparisons) in SPSS version 21. Duncan's multiple range test was used to separate means across all seven groups and to display homogeneous subsets (Tables 4–7); groups sharing a superscript letter are not significantly different. Post hoc groupings are interpreted only for parameters with a significant overall ANOVA (Table 6). Each mean ± SEM is based on five mice per group. Treated groups were compared with two reference points: the infected untreated group (efficacy) and the normal control (closeness to normal physiology); conclusions on organ function are based on distance from the normal-control values rather than on improvement relative to INT alone. This study was not designed for formal interaction analysis (no combination index or isobologram was constructed), so combination effects are described only as observed differences between ratios. Differences were considered significant at $p < 0.05$.

Results

Phytochemical profile

GC-MS analysis of the *C. papaya* extract identified 22 compounds (Table 2), with the largest peak areas for squalene (27.28%), stearic acid (23.84%), Z-11-hexadecenoic acid (19.17%) and methyl palmitate (18.25%), alongside phytol (10.16%) and smaller proportions of glycerol derivatives and syringic acid. The *T. diversifolia* extract yielded 19 compounds (Table 3) dominated by monoterpenes, principally α-terpineol (20.3%), eucalyptol (14.6%), camphor (14.3%) and α-pinene (13.5%), together with sesquiterpenes such as ledol and spathulenol. Compounds were identified by library matching, and identities are considered tentative.

Parasitaemia and chemosuppressive activity

As shown in Table 4, the infected untreated group developed the highest parasitaemia (95.78 ± 2.93%), while parasitaemia was undetectable in the normal control. All treated groups had significantly lower parasitaemia than the infected untreated group ($p < 0.05$). Numerically, CaTi25:75 recorded the lowest parasitaemia (11.17 ± 6.93%) and the highest chemosuppression (88.78 ± 6.91%), followed by CaTi75:25 (72.45 ± 9.92%) and Ti100 (66.95 ± 15.71%); CaTi50:50 and Ca100 produced chemosuppression of 60.88 ± 17.74% and 51.86 ± 14.27%, respectively. The chemosuppression values of the five treated groups share the same superscript (Table 4), i.e. they did not differ significantly from one another, so the ranking among treatments is descriptive. No standard antimalarial reference drug was tested.

Body weight

Initial body weights did not differ significantly across groups (Table 5). The normal group gained the most weight (13.62 to 23.47 g, +9.85 g). The infected untreated group gained 5.31 g, more than any treated group: Ca100 +2.86 g, CaTi25:75 +2.62 g, CaTi75:25 +2.51 g, Ti100 +0.73 g and CaTi50:50 +0.32 g. Final weights differed significantly only between the normal group and the Ti100 and CaTi50:50 groups. The body-weight data therefore do not show a weight benefit of treatment relative to the infected untreated group.

Haematological indices

Haematological parameters differed significantly across groups for most indices measured (Table 6); MID, MCHC, PLT and PCT did not ($p > 0.05$). Red blood cell counts and haemoglobin concentration were markedly higher in the CaTi50:50 group ($6.37 \pm 0.03 \times 10^{12}$/L; 11.23 ± 0.09 g/dL) relative to the infected untreated group ($3.26 \pm 0.46 \times 10^{12}$/L; 5.85 ± 0.36 g/dL), falling in the same statistical subset as the normal group ($7.71 \pm 0.39 \times 10^{12}$/L; 12.70 ± 0.35 g/dL). Red cell count and haemoglobin of the other treated groups were not clearly improved relative to INT. White blood cell and lymphocyte counts were comparatively elevated in Ti100, Ca100 and CaTi25:75 (lymphocytes were also high in INT), which may reflect immune activation or inflammation; these data cannot distinguish the two. CaTi50:50 had the numerically lowest MID and GRAN counts among the treated groups (the overall MID difference was not significant, $p = 0.089$) and the numerically highest platelet count ($134.0 \pm 0.58 \times 10^{9}$/L), but the overall difference in platelet count was not significant ($p = 0.090$). Because group sizes were small, the haematological findings are preliminary.

Liver and kidney biomarkers

Values below are compared with the normal control (Table 7). The infected untreated group had the most deranged liver profile: ALT 86.67 ± 3.54 U/L (3.1 times the normal value of 27.93 ± 1.75 U/L), AST 63.35 ± 3.40 U/L (2.2 times normal, 29.10 ± 1.00 U/L) and ALP 23.12 ± 1.36 U/L (0.4 times normal, 54.40 ±

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5.44 U/L). Its creatinine was depressed (0.32 ± 0.01 mg/dL vs. 1.33 ± 0.03 mg/dL) whereas urea was 3.3 times normal (43.24 ± 6.72 vs. 13.00 ± 6.75 mg/dL). No treatment normalised the whole panel. Treatment with CaTi50:50 gave ALT of 34.07 ± 2.62 U/L (same subset as normal), AST of 44.05 ± 7.86 U/L (1.5 times normal; significantly above normal), ALP of 68.00 ± 10.88 U/L (1.25 times normal; significantly above normal) and creatinine of 1.45 ± 0.56 mg/dL (same subset as normal), but urea of 47.16 ± 2.26 mg/dL, 3.6 times the normal value and not lower than INT. CaTi50:50 was closest to normal for creatinine only: for ALT, Ti100 (33.20 U/L) was marginally closer, for AST, Ti100, Ca100 and CaTi75:25 (33.57–35.31 U/L) were closer, and for ALP, Ti100 had the same value (68.00 U/L). Urea was elevated in every treated group (26.66–51.76 mg/dL), creatinine was 1.7–2.5 times normal in the other four treated groups (2.31–3.38 mg/dL) and ALP in CaTi25:75 and CaTi75:25 was about 1.9 times normal (about 100 U/L). A standard error of 0.00 in some groups reflects identical values among the five mice (Table 7).

Histopathology

Liver and kidney sections from normal mice showed intact histoarchitecture, with well-defined hepatocytes, hepatic sinusoids and central veins in the liver, and normal glomeruli and tubules in the kidney. The infected untreated group showed severe hepatic necrosis with inflammation and renal glomerular degeneration with vacuolisation. Tissue damage was qualitatively least severe in the CaTi50:50 group, which showed only onset hepatocyte degeneration in the liver and mild renal tubule degeneration with no glomerular inflammation or oedema, whereas CaTi75:25 and Ca100 sections showed more pronounced sinusoidal enlargement, haemorrhage and renal vacuolisation. Ti100 and CaTi25:75 sections also showed inflammation, vacuolisation, necrosis and degenerative changes (Figures 2, 6, 11 and 12). Even in CaTi50:50, renal tubule degeneration was present, which may relate to its raised urea. Representative photomicrographs (×400, haematoxylin and eosin) are shown in Figures 1–12.

Discussion

All treated groups showed significantly lower parasitaemia than the infected untreated group, indicating antiplasmodial activity of the combined and single extracts under the conditions tested. The 66.95% chemosuppression of Ti100 at 266.67 mg/kg is of a similar magnitude to the roughly 66% reported for *T. diversifolia* at 400 mg/kg in another *P. berghei* study that used a hot-infusion extract [15]. Because no chloroquine or artemisinin-based combination group was included, the efficacy of the extracts relative to a standard drug cannot be stated. The GC-MS profiles contain compounds such as squalene, phytol and α-pinene, of which α-pinene has reported antimicrobial activity [23]; whether any of them contributes to the antiplasmodial effect was not tested here (extracts were not fractionated and no compound was assayed individually). The detection of lipophilic constituents in an aqueous extract was not investigated further. The monoterpene-rich profile of *T. diversifolia*, particularly eucalyptol and α-terpineol, is consistent with the reported anti-inflammatory activity of eucalyptol [24].

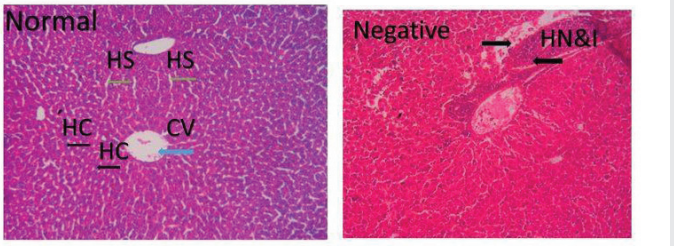


Figure 1: Normal and Negative control: Cross-section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV); cross-section of Negative control of liver showing hepatic necrosis associated with inflammation (HN and I) × 400.

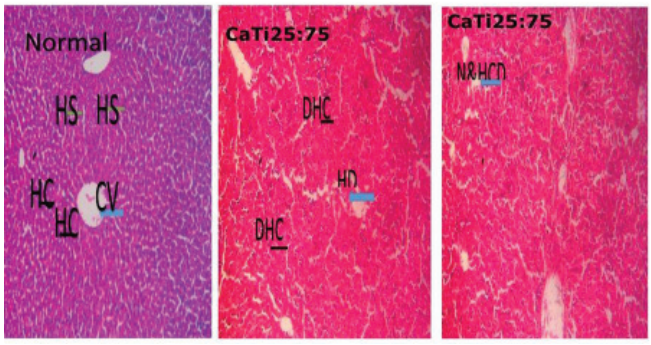


Figure 2: Normal and CaTi25:75: Cross section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV), with Cross section of CaTi25:75 of liver showing degenerated hepatocyte (DHC); hepatic degeneration (HD); necrosis (N) × 400.

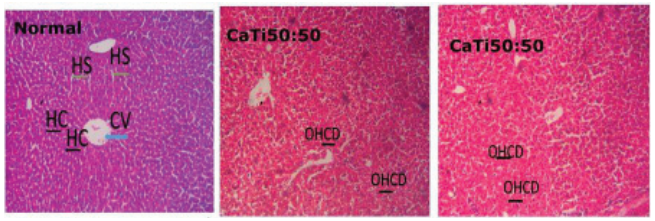


Figure 3: Normal and CaTi50:50: Cross section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV), with Cross section of CaTi50:50 of liver showing onset hepatocyte degeneration (OHCD) × 400.

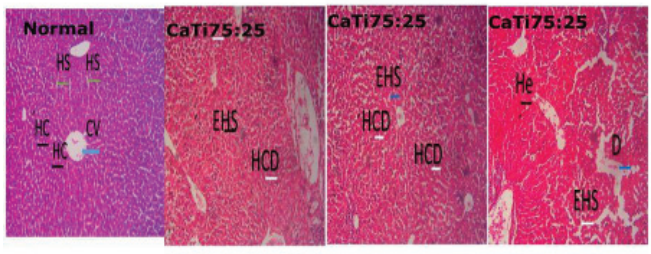


Figure 4: Normal and CaTi75:25: Cross section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV), with Cross section of CaTi75:25 of liver showing onset hepatocyte degeneration (OHCD); enlargement hepatic sinusoids (EHS); degenerated hepatocyte (HCD); hepatic degeneration (HD); degeneration (D); haemorrhage (He) × 400.

CaTi25:75 gave the numerically highest chemosuppression, but the treated groups did not differ significantly from one another (Table 4), so a superiority of any ratio cannot

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be concluded. Two of the three combinations (CaTi75:25 and CaTi25:75) numerically exceeded both single extracts, whereas CaTi50:50 (60.88%) lay between them; none of these differences was significant. In addition, the treatments received different total doses (266.67–400 mg/kg; Table 1), so ratio and dose are confounded, and no dose–response or combination-index analysis was performed. The present data therefore do not demonstrate synergy; they describe the effect of mixtures at fixed volumes. CaTi50:50 was associated with

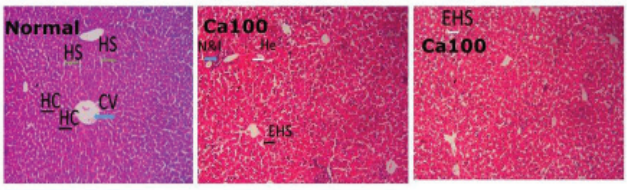


Figure 5: Normal and Ca100: Cross section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV) with Cross section of Ca100 of liver showing necrosis with inflammation (NandI); enlargement hepatic sinusoids (EHS); haemorrhage (He) × 400.

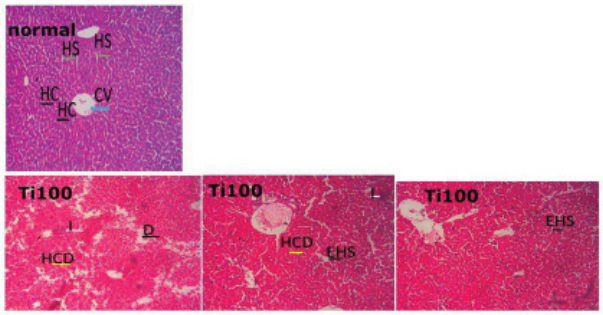


Figure 6: Normal and Ti100: Cross section of liver showing normal histoarchitecture; hepatocyte (HC); hepatic sinusoids (HS); central vein (CV) with Cross section of Ti100 of liver showing inflammation (I); enlargement hepatic sinusoids (EHS); haemorrhage (He); degeneration (D); degenerated hepatocyte (HCD) × 400.

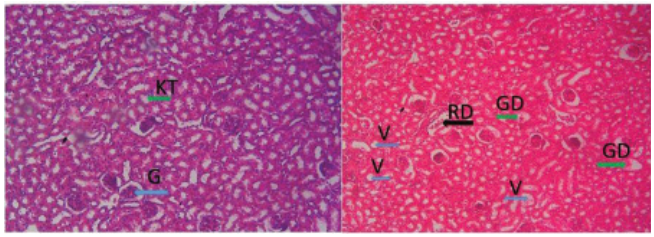


Figure 7: Normal and Negative control: Cross section of kidney showing normal histoarchitecture; glomerulus (G); kidney tubules (KT) with Cross section of Negative control of kidney showing vacuolisation (V); renal degeneration (RD); glomerulus degeneration (GD) × 400.

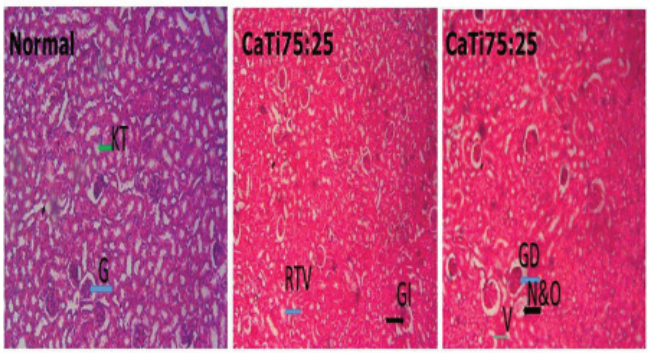


Figure 8: Normal and CaTi75:25: Cross section of kidney showing normal histoarchitecture of glomerulus within Bowman's capsule (G); kidney tubules (KT) with Cross section of CaTi75:25 of kidney showing vacuolisation (V); renal degeneration (RD); glomerulus degeneration (GD); glomerulus inflammation (GI); renal tubules vacuolisation (RTV); necrosis with oedema seen × 400.

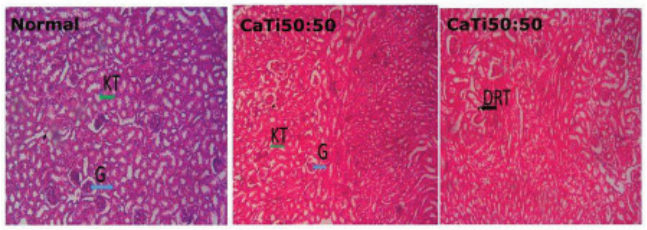


Figure 9: Normal and CaTi50:50: Cross section of kidney showing normal histoarchitecture of glomerulus within Bowman's capsule (G); kidney tubules (KT) with Cross section of CaTi50:50 of kidney showing degeneration of renal tubule (DRT); glomerulus (G); renal tubules (RT); no glomerulus inflammation (GI); no necrosis with oedema seen × 400.

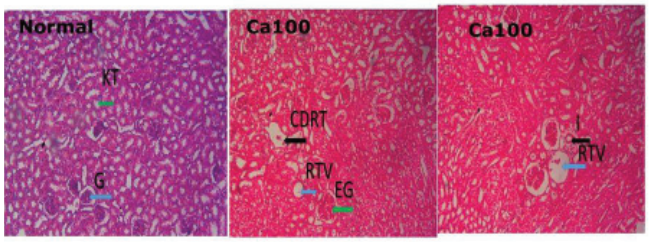


Figure 10: Normal and Ca100: Cross section of kidney showing normal histoarchitecture of glomerulus within Bowman's capsule (G); kidney tubules (KT) with Cross section of Ca100 of kidney showing; renal tubule vacuolisation (RTV); cystic renal tubule degeneration (GD); inflammation (I); glomerulus enlargement (GE) × 400.

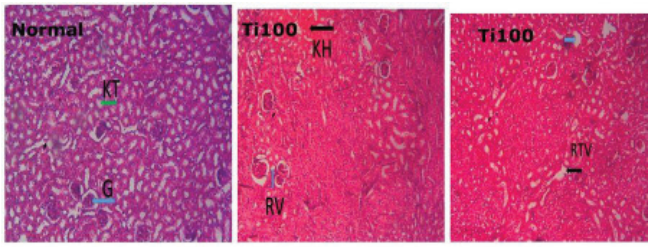


Figure 11: Normal and Ti100: Cross section of kidney showing normal histoarchitecture of glomerulus within Bowman's capsule (G); kidney tubules (KT) with Cross section of Ti100 of kidney showing renal vacuolisation (RV); inflammation (I) × 400.

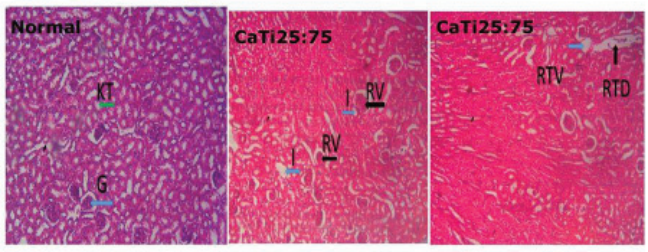


Figure 12: Normal and CaTi25:75: Cross section of kidney showing normal histoarchitecture of glomerulus within Bowman's capsule (G); kidney tubules (KT) with Cross section of CaTi25:75 of kidney showing renal tubule degeneration (RD); renal tubules vacuolisation (RTV); inflammation (I); renal vacuolisation (RV) × 400.

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the highest red cell and haemoglobin values among treated groups, creatinine and ALT close to the normal control, and the mildest histological changes, but not with a uniformly normal biochemical profile (see below). Maximal parasite suppression and the most favourable host indices were thus not achieved by the same ratio.

The elevated ALT and AST in the infected untreated group are consistent with hepatocellular injury during severe *Plasmodium* infection, whereas its markedly low ALP and creatinine are atypical and may reflect severe systemic illness (ALP measured in EDTA plasma may also be underestimated in all groups); their cause was not investigated and they should not be read as evidence of intact renal function. Relative to INT, CaTi50:50 lowered ALT and AST and brought creatinine to the normal-control range, in keeping with the reduced necrosis and inflammation seen in liver sections. However, AST (1.5 times normal), ALP (1.25 times normal) and especially urea (3.6 times normal) remained abnormal, and urea was raised in every treated group. The remaining treated groups also had creatinine 1.7–2.5 times normal and, for CaTi25:75 and CaTi75:25, ALP about 1.9 times normal. These data support, at most, a partial hepatic benefit of CaTi50:50 and give no evidence of renal protection. Because no uninfected extract-treated groups were included, we cannot separate residual infection effects from possible extract-related effects on liver and kidney, and the terms 'hepato-protective' and 'nephroprotective' are not justified by these data.

The improved red blood cell counts and haemoglobin concentration observed with CaTi50:50 suggest that the combined extracts may alleviate malaria-associated anaemia; the mechanism (reduced haemolysis, supported erythropoiesis or lower parasite burden) was not investigated, and the haematology sample sizes were small. Higher leukocyte counts in Ti100, Ca100 and CaTi25:75 may reflect immune activation or inflammation, which this study cannot distinguish. Taken together, the findings are preliminary: they show parasite suppression by all extract treatments and partial benefits for some host indices with CaTi50:50, but not a validated organ-protective effect.

Published safety data on the individual plants are mixed. In a 13-week study in rats, freeze-dried aqueous *C. papaya* leaf extract at up to 2 g/kg caused no mortality or histopathological change, although some biochemical values, including creatinine, differed from controls [25]. For *T. diversifolia*, repeated oral administration of aqueous leaf extract at 100 and 200 mg/kg for seven days altered serum electrolytes and tissue enzyme activities in rats, and the authors suggested possible adverse effects on the liver, heart and kidney [26]. The safety of the two extracts in combination has not been established, and the raised liver and kidney markers in several treated groups here (Table 7) support the need for toxicity testing; WHO guidelines on the research and evaluation of traditional medicine address safety assessment alongside efficacy [27].

Limitations. This study had no standard antimalarial positive control and no uninfected extract-treated (toxicity) groups; ratio and total dose were confounded and no dose–response or interaction analysis was done; haematological and biochemical results rest on small numbers of observations (five mice per group, chosen to equalise group sizes after three INT deaths) and EDTA plasma, which can lower measured ALP activity, with three deaths in the INT group; histology was qualitative; endpoints were measured at a single time point; and the post hoc test used (Duncan) is liberal. Future work should add chloroquine or artemisinin-based controls, uninfected extract-treated groups, a multi-dose matrix with combination-index or isobolographic analysis, acute and sub-acute toxicity testing, and bioassay-guided identification of the active constituents.

Conclusions

Combined aqueous leaf extracts of *Carica papaya* and *Tithonia diversifolia* significantly reduced parasitaemia in *Plasmodium berghei*-infected mice relative to the infected untreated group (chemosuppression 51.86–88.78%), with CaTi25:75 numerically highest but not significantly different from the other treated groups. CaTi50:50 was associated with improved red cell indices, lower ALT and AST than INT, creatinine close to normal and the mildest histological changes, but AST, ALP and urea remained above normal-control values, so organ protection was partial and renal protection was not demonstrated. Synergy was not established. GC-MS tentatively identified several phytocomponents, including squalene, phytol and α-pinene, whose contribution to the observed effects remains to be tested. These preliminary findings justify further development only after positive-control, toxicity-control and formal interaction studies, followed by pharmacokinetic and clinical validation.

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