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Anti-plasmodial effectiveness and Hematological effects of combined extracts of *Toddalia asiatica* and *Carica papaya*, in *Plasmodium berghei* infected mice

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Abstract

Background: Artemisinin-resistant parasites pose a danger to the efficacy of malaria treatment. For a very long time, pharmaceutical companies have relied on plants to provide the prototype molecules required to cure *Plasmodium* infections.

Objective: To evaluate the haematological effects and anti-plasmodial efficacy of combined extracts of *Toddalia asiatica* and *Carica papaya* in mice infected with *Plasmodium berghei*.

Methods: 30 mice were divided into 6 groups of 5 mice each. Each experimental mouse received an intraperitoneal injection of an infected blood containing $1x10^6$ red blood cells parasitized by *Plasmodium berghei*, followed by daily administration of the plant extracts. Group 1-3 received 500,250 and 100 mg/kg of the plant extracts respectively. Group 4 received 500mg/kg of a combination of the two plants extracts. Finally group 5 were give distilled water while group 6 was treated with chloroquine for four consecutive days. For toxicity test the animals received 500mg/kg of the plant extracts combination for14 consecutive days.

Results: At day three following infection, the data demonstrated a significant ($p < 0.05$) decrease in the percentage of parasite load between the infected treatment groups and the negative control group. This reduction persisted until the experiment's conclusion. The two plant extracts combined showed a reduction in parasites that was nearly identical to that of the positive control group. With the exception of MCH and Hb levels, the combination of study plants did not significantly change any of the mice's haematological indices ($P > 0.05$), suggesting that the combination is a reasonably safe treatment for malaria.

Keywords: *Anti-Plasmodium*; *Toddalia asiatica; Carica papaya*; Haematological*; In vivo*

1. Introduction

Malaria, a potentially fatal disease caused by *Plasmodium* parasites, is still a major public health concern in many tropical and sub-tropical regions of the world, especially in the countries of sub-Saharan Africa [1]. African Region continued its long-standing position as the principal carrier of the disease's burden in 2021, accounting for over 95% of all cases and 96% of all fatalities due to malaria [2]. Surprisingly, this disease still poses a serious risk for mortality, with young children particularly at risk especially in Africa [3]. While sub-Saharan Africa has the highest malaria endemicity, it can also be found in a few areas in Southeast Asia [4]. Malaria has a huge financial cost and detrimental consequences on national economy as well; in nations where significant transmission occurs, GDP per person can decline by up to 1.3 percent [5]. In Chad, Ghana, Nigeria, and Uganda, for example, the presence of malaria has a

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detrimental effect on overall national production. Sometimes referred to as the "malaria penalty," the decline in economic growth ranged from 0.41 percent in Ghana to as high as 3.8 percent in Nigeria and 8.9 percent in Chad [6,7]. Based on empirical data, malaria's effects on Kenya pose a serious threat to the country's efforts to reach sustainable development goals and have a negative economic impact. Malaria morbidity and mortality are linked to treatment, control, and prevention expenditures, which create a significant financial burden. However, the emergence of drug resistance and cross-resistance against the majority of antimalarials (including atovaquone, sulfadoxine, pyrimethamine, mefloquine, and more recently, the most effective artemisinin derivatives) as well as the declining efficacy of combinations in clinical practice present a significant challenge to malaria case management at the moment [8–11]. In developing countries, there is an urgent need to discover new therapeutic agents or drug combinations that can battle drug resistance and cure malaria more rapidly and efficiently [12]. Medicinal plants have played a major role in the discovery and development of anti-malarial drugs. And it is expected that medicinal plants will continue to provide new therapeutic leads because of their chemo-diversity [13]. Plant extracts analysis can not only lead to the discovery of novel anti-malarial compounds, but they may also enable researchers to reorient their focus from discovering new, potent chemicals to combining existing chemical agents with plant extracts in an effort to increase medication sensitivity [14,15]. Since plant extracts provide the most cost-effective way to find new anti-malarial drugs[16–18]. The anti-plasmodial efficacy and haematological effects of a combination of extract of *Toddalia asiatica* and *Carica papaya* were assessed in this study using mice infected with *Plasmodium* berghei.

2. Material and methods

2.1. Collection and Extraction of plant material

Toddalia asiatica and *Carica papaya* were collected from Homa-Bay County, Kenya. After being thoroughly washed with distilled water, the collected plant samples were allowed to dry in a shady area until they reached a constant weight. The plant samples were then finely ground using an electric blender. The plant samples were then extracted using polar organic solvents with increasing polarity (methanol, hexane, and ethyl acetate). As shown in Figure 1 below, this was accomplished by immersing 150 g of each sample in 400 mL of the organic solvent for 72 hours. A rotary evaporator was then used to dry the extract filtrate. Before being used, the dried plant extracts were stored at -20°C.

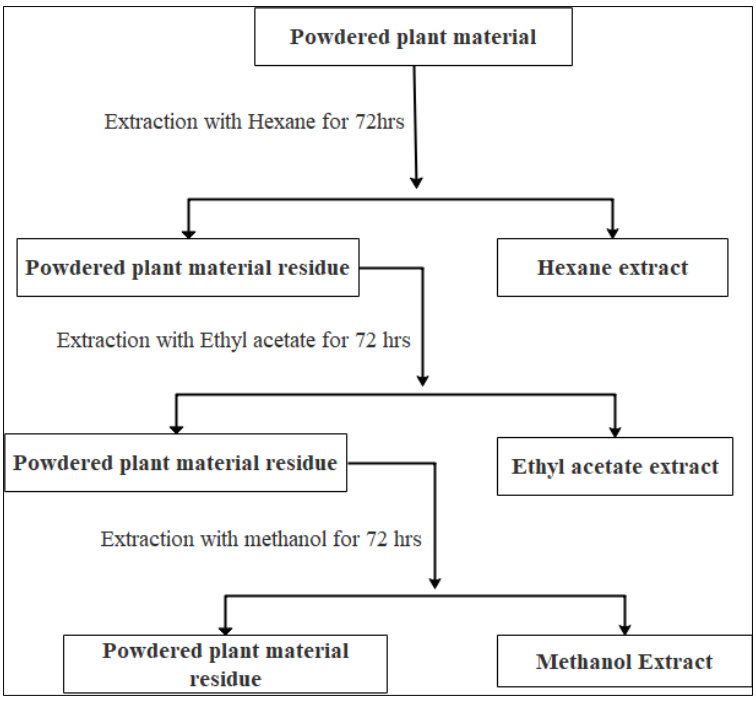

Figure 1 Schematic representation of the extraction procedure of medicinal plants using organic solvent of different polarity

2.2. *Plasmodium berghei* inoculation in mice

Inbred Swiss albino mice weighing around 20 grams were used in this study. The animals were kept in rodent cages and given access to unlimited water and mouse pellets for food. The animals were randomly allocated to four cages and

given a week to adjust to their new surroundings before being infected with *P. berghei*. Five mice were kept in each cage. When the parasitemia of the donor mice peaked (109 parasites/ml), the parasites were extracted from their blood and diluted with regular saline [19]. Every experimental animal received an intraperitoneal injection of 0.2 mL of an infected blood dilution containing 1x106 *P. berghei* parasitized red blood cells from donor mice. After a five-day incubation period, blood samples were obtained from the tail vein to assess the infection status of the mice. The plant extract's efficiency against malaria parasites was then assessed on the infected mice.

2.3. *In vivo* assay of single and combined plant extracts

The effectiveness of plant extracts was assessed using the four-day Peters suppressive test method. [19]. On day 0 (the day of infection), two hours after receiving the parasite injection, mice were given an oral dose of a test plant extract. 500 mg/kg of body weight was the starting dosage for the treatment. For four days, the same dosage was given twice a day. On the fifth day, a drop of blood was taken from each mouse's tail to make thin blood smears. After that, the slides were fixed with methanol and stained using a 10% Giemsa solution. Red blood cells (RBCs) infected with parasites were counted under a microscope in ten randomly chosen fields per slide [20]. The parasitaemia percentage was thus calculated as:

% Parasitemia= (Number of infected RBCs)/(Total number RBCs) X100

Using the controls as a benchmark, the mean percentage suppression of parasitaemia was calculated.

% Suppression of Parasitaemia =(A-b)/A X 100

A is the mean percentage parasitaemia in negative control group. B is the mean percentage parasitaemia in the test group [21,22]. Ethyl acetate and Methanol extracts of *Toddalia asiatica* and *Carica papaya* respective were used for this study.

2.4. Haematological Analysis

An automatic blood cells analyzer (Hemavet 950). was used to count the haemoglobin, hemocrit, red blood cells, and leucocytes in whole blood. The values of the animals' haemoglobin, haematocrit (packed cell volume), and red blood cell count were obtained before calculating their red cell indices. Thus, RBC indices such as mean corpuscular volume (MCV), MCH, and mean corpuscular heamoglobin concentration were calculated using the link between heamatocrit, haemoglobin, and red blood cells (MCHC). The haematocrit (Hct) was divided by the RBCs to get the mean corpuscular volume, or MCV. By dividing the total amount of haemoglobin (Hb) by the number of red blood cells (RBCs), the mean haemoglobin content (MCH) was determined. Lastly, by dividing haemoglobin by hemocrit, the mean concentration of haemoglobin per unit volume of red blood cells, or MCHC, was determined [23–25].

2.5. Data analysis

For data analysis, the raw data was first entered into Microsoft Excel and then imported into Minitab 19 software. One-way ANOVA was used to compare the differences in the parasite response to the plant extracts. The two sample t-test of the means was used to analyze the haematological parameters. P-values less than 0.05 were considered statistically significance.

3. Results

Based on the outcomes of the Peters four-day suppressive test, the extracts demonstrated strong anti-plasmodial activity against *P. berghei*, which is susceptible to chloroquine, at the early stages of infection. The best effectiveness of the extract in suppressing parasites was observed at 500 and 250 mg/kg body weight. Though, there was some suppression at 100 kg/body weight. In comparison to the negative control, the extract showed a decrease in the number of parasites at every dose level that was investigated. The study demonstrated the strong activity of *Toddalia asiatica* and *Carica papaya*, as demonstrated by their over 80% suppression at all dosages. *Carica papaya* and *Toddalia asiatica* achieved a percentage suppression range (>90%) at the maximum dose of the extract (500 mg/kg), which is noteworthy because it is similar to the dose of chloroquine used as a reference medication. In general, the two test plants each demonstrated a parasite suppression rate of more than 70% at 250 mg/kg body weight when administered individually to treat the malaria in mice . However, as indicated in Table 1, when the combination of the two plants extracts was assessed *in vivo* for its capacity to decrease parasite density on the fourth day, it revealed that the combination of *Carica papaya* and *Toddalia asiatica* extracts at a ratio 1:1 (250mg/kg *Carica* + 250 mg/kg *Toddalia*) had an over 90% suppression rate of *Plasmodium berghei* in mice. This indicated that higher effects witnessed by administering higher

dosage (500mg/kg) of the plant extract, can easily be achieved by administering a smaller dose (250mg/kg) of the two plant extracts in combinations.

Table 1 Plant extracts' effects on mice's early infection with malaria parasite

Extract	Dose mg/kg	PRBC	RBC	% Parasiteamia	Suppression
Carica	500	2.08	565.15	0.3651	95%
	250	7.55	643.05	1.1757	84.43%
	100	6.80	504.1	1.4728	80.36%
Toddalia	500	1.31	782.85	0.1779	98%
	250	7.4	537.80	1.4155	81%
	100	7.7	584.57	1.4656	80%
C. papaya + T. asiatica	100+100	6.0	584.57	1.2850	82%
	250+250	3.60	586.47	0.6275	91.7%
NC (Water)	1 ml	32.83	456.08	7.5409	N/A
CQ	10MG	0.39	465.78	0.0861	99%

3.1. Effects of *Toddalia asiatica* extracts on haematological parameter of mice

RBC count, total RBC indices, and HB estimate can provide an accurate indication of the plant extract's possible negative effects on an animal's hemopoietic activities after it has been administered. The changes in the mice's haematological parameters after a 14-day treatment with *Toddalia* are shown in Table 2. Extracts of Toddalia had no significant effect on any of the mouse haematological parameters (Hb, haemoglobin, MCV, MCH, MCHC, leucocytes, and platelets; $P > 0.05$), with the exception of RBC counts, which were significantly ($P = 0.014$) lower in the groups that received the extract at a dose of 500 mg/kg than in the control group.

Table 1 Haematological values of mice after treatment with Toddalia asiatica

Variable	Animal group	Mean	SE Mean	StDev	p- value
Hb Hb (g/dL)	Control	12.833	0.401	0.983	0.781
	Treatment	12.667	0.422	1.033	
Haematocrit (%)	Control	41.33	2.29	5.61	0.400
	Treatment	44.00	1.97	4.82	
RBC (x106/mm3)	Control	8.833	0.477	1.169	0.014
	Treatment	7.000	0.365	0.894	
MCV (fl)	Control	50.50	1.57	3.83	0.350
	Treatment	48.33	1.54	3.78	
MCH (pg)	Control	16.000	0.775	1.897	0.404
	Treatment	15.167	0.543	1.329	
MCHC (%)	Control	30.333	0.715	1.751	0.176
	Treatment	31.667	0.558	1.366	
WBC (X 103/mm3	Control	3.333	0.615	1.506	0.075
	Treatment	1.833	0.401	0.983	

Platelets (X 103/mm3)	Control	579.8	34.6	84.8	0.130
	Treatment	683.0	50.4	123.4	

3.2. Effects of *Carica papaya* extracts on haematological parameters of mice

As indicated in Table 3, following a 14-day administration of *C. papaya* extracts. There was a significant ($P < 0.05$) difference in the mean RBC count and Hb levels between the experimental and control groups after receiving *Carica papaya* extracts. Nevertheless, there was no significant difference in mean leucocyte and platelet counts between the experiment and control groups ($P > 0.05$). Mice given *Carica papaya* extracts did not also show a significant ($P > 0.05$) change in MCV, MCH, or MCHC levels, according to the study.

Table 3 Haematological values in mice after treatment with Carica papaya extract

Variable	Animal group	Mean	SE Mean	StDev	P-value
Hb (g/dL)	Control	12.833	0.401	0.983	0.032
	Treatment	11.500	0.342	0.837	
Haematocrit (%)	Control	41.33	2.29	5.61	0.306
	Treatment	37.83	2.27	5.56	
RBC (x106/mm3)	Control	8.833	0.477	1.169	0.049
	Treatment	7.500	0.342	0.837	
MCV (fl)	Control	50.50	1.57	3.83	0.65
	Treatment	46.67	1.99	4.89	
MCH (pg)	Control	16.000	0.775	1.897	0.489
	Treatment	16.667	0.494	1.211	
MCHC (%)	Control	30.333	0.715	1.751	0.176
	Treatment	31.667	0.558	1.366	
WBC (X 103/mm3)	Control	3.333	0.615	1.506	0.667
	Treatment	3.667	0.422	1.033	
Platelets (X 103/mm3)	Control	579.8	34.6	84.8	0.614
	Treatment	624.7	76.8	188.1	

3.3. Effects of Combined *Carica papaya* and *Toddalia asiatica* on haematological parameters of mice

All of the mouse haematological parameters (Hb, haemoglobin, MCV, MCH, MCHC, leucocytes, and platelets) were not significantly affected ($P > 0.05$), by extracts of *Toddalia asiatic*a and *Carica papaya* when administered in combination (Table 4). However, MCH and Hb were significantly ($P < 0.05$) lower in the groups that received the extract at a dose of 500 mg/kg than in the control group.

Table 2 Haematological parameters in mice treated with Toddalia asiatica and Carica papaya in combination

Variable	Animal group	Mean	StDev	SE Mean	P-VALUE
Hb (g/dL)	control	10.83	1.47	0.6	0.028
	treatment	12.83	1.17	0.48	
Haematocrit (%)	control	45.83	2.64	1.1	0.085

	Treatment	40.17	6.21	2.5	
RBC (x106/mm3)	Control	8.333	0.816	0.33	0.517
	Treatment	8	0.894	0.37	
MCV (fl)	Control	43.67	1.86	0.76	0.279
	Treatment	46.17	4.79	2	
MCH (pg)	Control	13.67	1.03	0.42	0.019
	Treatment	15.67	1.37	0.56	
MCHC (%)	Control	30.33	1.21	0.49	0.665
	Treatment	30.67	1.37	0.56	
WBC (X 103/mm3)	Control	3.833	0.983	0.4	0.298
	Treatment	3	1.55	0.63	
Platelets (X 103/mm3)	Control	648	137	56	0.228
	Treatment	562.5	84.1	34	

4. Discussion

The results of the four-day suppressive test showed that the extract exhibited strong anti-plasmodial activity against chloroquine-sensitive *P. berghei* on early infection. The highest levels of extract suppression of parasites were observed at 500 and 250 mg/kg body weight. There was also some suppression at 100 kg/body weight (Table 1). At every studied dose level, the extract exhibited a dose-dependent, statistically significant ($p<0.05$) reduction in parasite count as compared to the negative control. The combination of two or more plant extracts can have antagonistic or synergistic effects on *Plasmodium berghei* in mice. When the combination of ingredients improves each other's performance, they are said to have a synergistic effect [26]. When the two test plants were administered separately to treat the malaria parasite in mice, each had a parasite suppression rate of about 70% at 250 mg/kg body weight dosage. On the other hand, mice given extracts of *Carica papaya* and *Toddalia asiatic* combine at a ratio of 1:1 demonstrated at least an 80% reduction in *Plasmodium* berghei, which was equivalent to that of the positive control (chloroquine test group). This means that you can combine low doses of plant extracts to obtain the same therapeutic benefits and potency as higher dosages (500 mg/kg), which are usually associated with toxic symptoms. Similar research on the combined synergistic anti-plasmodial activity of plant extracts showed that there was no discernible synergistic toxicity associated with the plant extracts' synergistic anti-malarial effects on parasites [27]. Combinations of drugs improve the effectiveness of each one and enable dosage reductions. It is possible to prevent concentration-related toxicity by lowering the dosage of the combination drugs. Reducing toxicity (by lowering drug doses) and increasing efficacy (via drug interactions) are the primary objectives of employing pharmacological combinations in therapeutic settings. Results from this investigation showed that, rather than using a larger single dosage of 500 mg/kg, 250 mg/kg extracts of the two study plants should be combined in a 1:1 ratio to provide the best anti-plasmodial effects *in vivo*. This may reduce the harmful effects linked to larger dosages during chemotherapy. This is in line with other research that shown the plant's ability to inhibit the malaria parasite in mice was much enhanced when extracts from several plants were combined in a 1:1 ratio [26].

Following a 14-day period of 500 mg/kg plant extract administration to mice, changes in their haematological parameters were evaluated. The study's findings demonstrated that the leaf extract of *Toddalia* had no discernible effect on the haematological parameters in mice (Hb, haemoglobin, MCV, MCH, MCHC, leucocytes, and platelets) ($P > 0.05$). According to earlier studies, *T. asiatica* has a significant amount of easily obtainable iron that can be used to support the production of blood. The plant's copper facilitates the transformation of iron (II) into iron (III), which is needed to produce erythrocytes [28]. This may have offset any negative changes in blood parameters resulting from the toxicity of the herbal extract to red blood cells in this investigation. Giving mice *Carica papaya* extracts also had no discernible ($P>0.05$) effects on the mice's MCV, MCH, MCHC, or RBC count. This is consistent with other research showing that *Carica papaya* can raise haemoglobin and erythrocyte counts [29]. It has been shown in earlier studies that oral administration of *C. papaya* leaf extract has a statistically significant effect on platelet counts. Furthermore, compared to the control group of animals, the test group's RBC count increased considerably[30]. A dengue patient in Pakistan showed improvement in platelet count after receiving aqueous extract of *C. papaya* leaves (25 mL, twice daily for five

consecutive days). This supports the use of the plant extract as a blood booster in herbal medicine practices [31]. When administered together in ration of 1:1, extracts of *Toddalia* and *Carica papaya* did not substantially alter any of the mice haematological indices (Hb, haemoglobin, MCV, MCH, MCHC, leucocytes, and platelets; $P > 0.05$). However, the groups that were administered 500 mg/kg of the extract combination had considerably ($P < 0.05$) lower levels of MCH and Hb compared to the control group. This is likely owing to the lowered hemopoietic activities caused by the co-administration of the plant extracts.

5. Conclusion

When the three test plants were administered separately to treat the malaria parasite in mice, each had a parasite suppression rate of more than 70% at 250 mg/kg body weight. On the other hand, mice given a 1:1 ratio of 250 mg/kg of *Carica papaya* and *Toddalia asiatic* combined extracts had at least an 80% reduction in *Plasmodium berghei*, which was almost equivalent to that of the positive control. This means that you can use a mixture of low dosages of plant extracts to obtain the same therapeutic benefits and potency as larger dosages (500 mg/kg), which are typically linked to toxic symptoms. With the exception of MCH and Hb levels, the combination of study plants did not significantly change any of the mice's haematological indices (Hb, haemoglobin, MCV, MCH, MCHC, leucocytes, and platelets; $P > 0.05$), suggesting that the combination is a reasonably safe treatment for malaria.

Compliance with ethical standards

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Disclosure of Conflict of interest

No conflict of interest to be disclosed.

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