Evaluation of the Toxic And Synergetic Effects of Decoction Of Citrus Aurantifolia and Carica Papaya on Mice Infected with Plasmodium Berghei

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Abstract. In endemic regions such as Nigeria, malaria persists as a significant public health issue. Researchers may enhance malaria treatment by using natural remedies and synergistic botanical extracts. This study explored synergistic and toxic effects of *Carica papaya* and *Citrus aurantifolia* decoction in mice infected with *Plasmodium berghei*. Researchers used the Rane's test to evaluate the decoction's efficacy against *P. berghei* in mice: 40 mice inoculated with *P. berghei* were randomly grouped into five different groups (A – E); following infection with the protozoan, mice in groups B – E were treated with 0.2 ml decoctions of *C. papaya, C. aurantifolia, C. papaya + C. aurantifolia + Artemether, or C. papaya + C. aurantifolia*, respectively.

Researchers estimated the level of parasitised red cells using a microscope (Giemsa stain, thin smear), determined the packed cell volume with the microhematocrit method, and examined the stained liver sections. The findings showed that all treatment groups significantly reduced parasitemia, and mice given *C. papaya* alone achieved the most significant suppression. Nevertheless, the treatment produced no noticeable change in haematological indices. Histopathological examination revealed the hepatoprotective effects of the single treatment with *C. papaya* decoction. From the findings of this study, a single treatment with *C. papaya* decoction demonstrated significant anti-plasmodial effects against *P. berghei*-induced parasitemia and liver damage compared with the combination of decoctions.

Keywords: *Citrus aurantifolia; Carica papaya;* Mice; *Plasmodium berghei;* Synergy.

INTRODUCTION

Eukaryotic parasites of the genus *Plasmodium* cause malaria. Mosquitoes transmit the infectious disease caused by *Plasmodium,* which affects both humans and other animals. These genera, *P. falciparum, P. vivax, P. ovale*, and *P. malaria* are the primary human illness-causing agents, and an infected female Anopheles mosquito typically spreads the disease during a blood meal [1, 2]. Researchers have viewed malaria as among the leading causes of morbidity and mortality globally [3]. A large portion of the world's population remains vulnerable to malaria infection, according to the report issued by the World Health Organisation, 2017; in sub-Saharan Africa alone, malaria-related illnesses claimed the lives of over 400,000 individuals in 2019 [4]. The primary ways of treating and controlling the disease have been chemotherapy and insecticide-treated nets. Notable achievements in malaria control have been documented, with a 44% decrease in malaria-related mortality between 2010 and 2019 [5].

A significant obstacle to the control and eradication of malaria has resulted from the emergence of Plasmodium strains resistant to antimalarial drugs, such as artemisinin and chloroquine [6]. In addition to drug resistance, severe side effects have also been linked to chemotherapy. Given these obstacles, there has been an urgent need to continue searching for novel antimalarial medicines and strategies that are safer, more effective, more accessible, and more affordable [7]. In the hunt for antimalarial drugs, medicinal plants may be a valuable resource due to their diverse secondary metabolite profiles. WHO has recommended a combination therapy in the treatment of infectious diseases, including HIV/AIDS, malaria, tuberculosis, and gastrointestinal ulcers.

METHODS

Sample Collection. Researchers commercially obtained tea (yellow label), *Citrus aurantifolia*, and *Carica papaya* from Samaru Market in Zaria, Kaduna State, Nigeria. They then took the *Citrus aurantifolia* and *Carica papaya* samples to the Department of Botany at Ahmadu Bello University, Zaria, for authentication and voucher preparation.

Sample Preparation. The researchers prepared the tea by infusing a tea bag in 100 ml of distilled water and then cooling it. *Citrus aurantifolia* and *Carica papaya* decoctions were prepared by thoroughly rinsing ripe fruits of each plant in distilled water to remove debris, then chopping them into small pieces. The researchers added 200 mL of distilled water to the beaker, added the chopped fruit pieces, and allowed the mixture to boil for about 12 minutes. To improve the mixture's palatability, one teaspoon of honey was added and allowed to cool. The mice were given the tea blends prepared from the decoctions of *Citrus aurantifolia* and *Carica papaya* by measuring out 0.2 ml of tea and 0.2 ml of the decoction. The researchers administered the mixture to the mice twice daily, preparing a fresh mixture for each administration.

Source of Experimental Animals and Plasmodium Species. The researchers purchased mice weighing 13-18 g from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

The researchers obtained a chloroquine-sensitive strain of *Plasmodium berghei* from the same source for the investigation. They kept the mice in well-ventilated plastic cages and fed and watered them ad libitum. During the trial, they changed the bedding twice a week.

Inoculation of Experimental Animals with P. berghei. Donor mice exhibiting a parasitemia level of 20% were sacrificed under chloroform anaesthesia. Blood samples were obtained through cardiac puncture, immediately transferred into heparinised tubes, and diluted with normal saline (0.9% NaCl) to prepare a standard inoculum containing 1×10^7 parasites. Subsequently, 0.2 ml of this suspension—equivalent to 2×10^7 *Plasmodium berghei*-infected erythrocytes—was administered to healthy mice via intradermal injection [8].

Rane's Test (Curative). After a five-day acclimatisation period, the mice were inoculated intradermally with 2×10^7 infected red blood cells obtained from the donor mice. The researchers assessed parasitemia in each mouse 72 hours post-inoculation and randomly assigned infected mice to five groups, each consisting of 8 animals.

Group 1 – Negative control (infected but untreated)

Group 2 – Infected and treated with 0.2 ml *Carica papaya* decoction

Group 3 – Infected and treated with 0.2 ml *Citrus aurantifolia* decoction

Group 4 – Infected and treated with 0.2 ml combined *Citrus aurantifolia* and *Carica papaya* decoction plus Artemether

Group 5 – Infected and treated with 0.2 ml combined *Carica papaya* and *Citrus aurantifolia* decoction

Estimation of Parasitemia. A tiny smear of Giemsa-stained blood from the mice's tail was examined under a microscope to ascertain the amount of parasite present in the blood. The stained slides were examined under a light microscope at 1000× magnification using oil immersion. Parasitemia was evaluated by calculating the percentage of red blood cells (RBCs) infected with parasites in relation to the total number of RBCs observed in the field.
The researchers then calculated the percentage parasitemia for each mouse using the following formula:

$$\% \text{ parasitemia} = (\text{No. of parasitised RBC})/(\text{No. of total RBC}) \times 100$$

Percentage malaria suppression was calculated as follows:

$$\% \text{ Suppression} = (\text{Average \% parasitemia in negative control} - \text{Average \% parasitemia in test group})/(\text{Average \% parasitemia in negative control})$$

The researchers administered the treatment twice daily for 96 hours (4 days) and then redetermined parasitemia in the mice to assess the level of parasite clearance [8].

Statistical Analysis. The mean ± standard error of the mean (SEM; n = 5) was used to present the data. The researchers used SPSS version 21 (SPSS Inc., Chicago, Illinois, USA) to analyse the data. They compared the differences in the means of the measured parameters using a one-way ANOVA followed by a post hoc test. P-values < 0.05 at the 95% confidence level were considered statistically significant.

RESULTS AND DISCUSSION

Patency of *Plasmodium berghei* in Infected mice before treatment: Following inoculation, all mice across the experimental groups developed parasitemia, with mean parasitemia levels ranging from 8.02 ± 0.7% to 19.82 ± 1.4%.

Effect of *C. aurantifolia* and *C. papaya* on parasitemia in *Plasmodium berghei* Infected mice: The mean parasitemia in the untreated (negative) control was significantly higher ($p < 0.05$) than that recorded in the treated mice; in the negative control, the mean parasitemia was, while in mice in group 3 (treated with 0.2 ml *Citrus aurantifolia* decoction), group 4 (treated with 0.2ml *Citrus aurantifolia* and *Carica papaya* + Artemether), and group 5 (treated with 0.2 ml *Citrus aurantifolia* and *Carica papaya*), the mean parasitemia was 6.48% each; the researchers recorded the lowest parasitemia level (3.91%) in mice treated with 0.2 ml of *Carica papaya* decoction. (Group 2). The variation in parasitemia levels among the treated groups was statistically significant ($p < 0.05$).

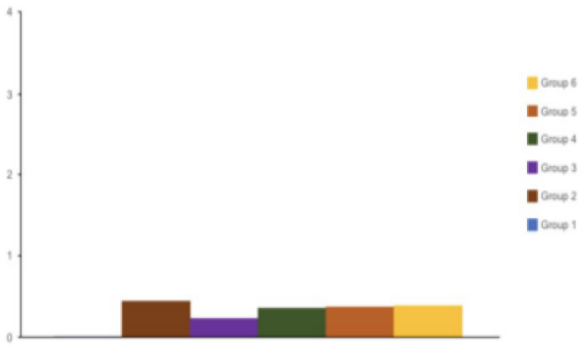


Figure 1 – Mean of parasitemia of infected mice after treatment

Mean ± standard deviation of the animals in each group. Keys:

Group 1 – Negative control (infected but untreated)

Group 2 – Infected and treated with *Carica papaya* decoction

Group 3 – Infected and treated with *Citrus aurantifolia* decoction

Group 4 – Infected and treated with a combined *Citrus aurantifolia* and *Carica papaya* decoction plus Artemether

Group 5 – Infected and treated with a combined *Carica papaya* and *Citrus aurantifolia* decoction

Effect of *C. aurantifolia* and *C. papaya* on haematological indices in *Plasmodium berghei*-infected mice: in untreated *P. berghei*-infected mice, packed cell volume declined from before to after infection; however, the difference was not statistically significant ($p > 0.05$). Treatment with *C. papaya* decoction resulted in the amelioration of

P. berghei-associated PCV loss before treatment. However, the PCV value in mice treated with C. aurantifolia did not return to the pretreatment level, and the difference was not statistically significant ($p > 0.05$). Combined treatment with *C. papaya* and *C. aurantifolia*, supplemented with Artemether, maintained PCV values close to pre-inoculation levels. In contrast, the removal of Artemether from the combination of *C. papaya* and *C. aurantifolia* elevated PCV levels above pre-infection levels (Figure 2).

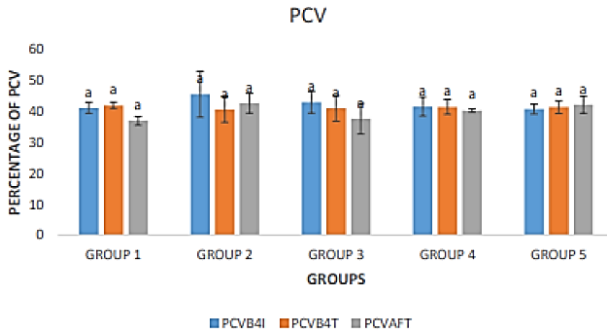


Figure 2 – Packed Cell Volume (PCV) values before, during, and after treatment

Data are expressed as mean ± standard deviation (SD) for the experimental animals in each group key.

Group 1 – Negative control (infected but untreated)

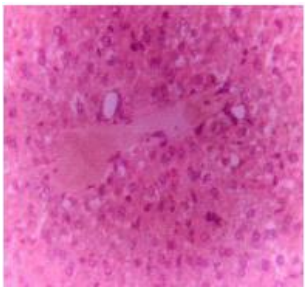
Group 2 – Infected and treated with *Carica papaya* decoction

Group 3 – Infected and treated with *Citrus aurantifolia* decoction

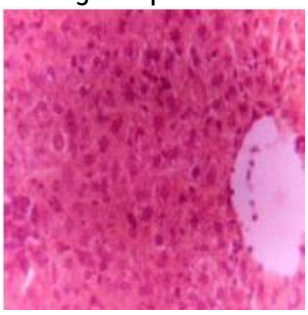
Group 4 – Infected and treated with a combined *Citrus aurantifolia* and *Carica papaya* decoction plus Artemether

Group 5 – Infected and treated with a combined *Carica papaya* and *Citrus aurantifolia* decoction

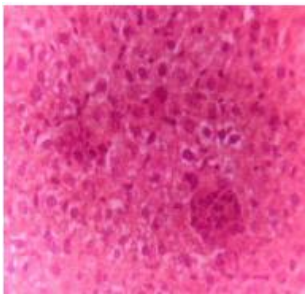
Effect of *C. aurantifolia* and *C. papaya* on hepatic tissues of *P. berghei*-infected mice: infected, untreated mice (negative control) exhibited vascular congestion with slight hepatic necrosis. In contrast, all mice in group 2 that received 0.2 ml of *Carica papaya* decoction showed regular features after treatment. In group 3 (treated with 0.2 ml of *Citrus aurantifolia* decoction), the mice showed slight hepatic necrosis. Mice in Group 4 (treated with 0.2 ml *Citrus aurantifolia* and *Carica papaya* + Artemether) showed hyperplasia of inflammatory cells. In contrast, mice in Group 5 that received 0.2 ml of *Carica papaya* and *Citrus autantifolia* decoction showed vascular congestion with mild hepatic necrosis.



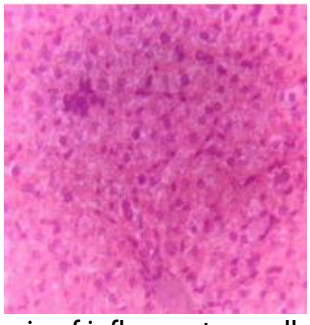
(a) Liver section of infected-untreated mice (negative control) showing vascular congestion with slight hepatic necrosis



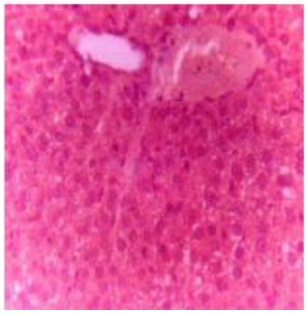
(b) Liver section of mice treated with *Carica papaya* decoction (0.2 ml) showing regular features



(c) Mice treated with *Citrus aurantifolia* decoction (0.2 ml) presented with slight hepatic necrosis



(d) Hyperplasia of inflammatory cells seen in the liver tissue of mice treated with *Citrus aurantifolia* and *Carica papaya* + Artemether (0.2 ml)



(e) Vascular congestion with mild hepatic necrosis seen in mice treated with *Carica papaya* and *Citrus autantifolia* (0.2 ml)

Figure 3 – Plates a-e: sections of hepatic tissues of *P. berghei*-infected mice

Malaria remains a significant public health burden, especially in endemic regions like Nigeria. Natural remedies and botanical extracts offer potential advancements in malaria management. The researchers evaluated the effects of single and combined treatments with decoctions of *Citrus aurantifolia* and *Carica papaya*. The chemosuppression of parasitemia observed in infected mice following single or combined therapy with *Citrus aurantifolia* and *Carica papaya* was consistent with the authors' report [9], who showed that the combination of *Citrus aurantifolia* and honey extract demonstrated considerable antimalarial potency by significantly reducing parasitemia and enhancing the survival rate of infected mice. However, the results showed that *C. papaya* treatment resulted in the highest level of chemosuppression. These findings underscore variations in treatment responses across groups and suggest that *C. papaya* warrants further investigation for its potential therapeutic value in managing *Plasmodium berghei* infection. The variations in mean parasitemia recorded in the treatment groups can be attributed to several factors, including the effectiveness of the treatments, dosage, duration, and specific components of each treatment.

The results of the haematological indices showed no noticeable/measurable changes in packed cell volume across the entire groups of mice used in the investigation. These results indicate that the combined treatment did not result in statistically significant changes in PCV compared with the control or other treatment groups; this is consistent with the findings of authors [10], who reported no significant difference in the assessment of the extract's effect on haematological parameters compared with the control group.

The histopathological results provided significant insights into the health and tissue conditions of mice used in the study. The changes in liver tissues observed in mice that received either the decoction of *C. aurantifolia* or *C. papaya* + *C. aurantifolia* indicated that the decoction was not protective against *P. berghei*-induced liver alterations. That was in contrast to the findings of authors [11], who reported that the decoction of *Citrus aurantifolia* and *Camellia sinensis* (Lipton tea) did not impair the architecture of the liver and kidneys in Wistar rats. However, the tissue-protective effects of *C. papaya* decoction observed in this study agree closely with the reports of authors [12], who showed that the seeds of *Carica papaya Linn* exhibited hepatoprotective properties against carbon tetrachloride-induced liver damage.

CONCLUSIONS

Treatment with *C. papaya* decoction was more efficacious than either *C. aurantifolia* or the combination of *C. papaya* and *C. aurantifolia* in ameliorating *P. berghei*-associated parasitemia and hepatological changes.

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