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Evaluation of Antimalarial Activity of Methanolic Extract of *Carica Papaya* (*Carecaccaea)* Yellow Leaf in Mice

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Abstract

Malaria still remains a public health problem in developing countries. Malaria is one of the most prevalent diseases as people still rely on traditional medicine as source of treatment for this disease. Malaria causes mortality and morbidity with social economic impact in developing countries where the burden is high. Reports showed that the high global health challenges is partly due to multidrug resistance *P. falciparum* develop on existing and available antimalarial drugs and that has spur the search of alternate treatment with low or less side effect. The purpose of this study is to evaluate the antimalarial potential of methanolic extract of *Carica papaya* yellow leaf on animal model. In vivo screening for antimalarial drug discovery is of the recommended stream line process for new compound in path from drug discovery to developing. The Rane's curative method of established infection was employed in vivo for assessing antimalarial activity. Swiss albino mice of both sexes weighing between 23-27 g and aged 6 weeks were infected with $1x10^{-7}$ *P. berghei* (NK-65) RBC/ml intraperitoneally and were treated with various dose (100, 200 and 400mg/lg bt.wt) of *C. papaya* yellow leaf methanolic extract. Acute oral toxicity test was employed using OECD method. The mice treated with 400 mg/kg b.wt of *C. papaya* yellow leaf extract showed significant ($P < 0.05$) antimalarial activity. In acute oral toxicity result showed that the maximum tolerated dose was found to be 5000 mg/kg body weight. *C. papaya* leaf extract showed antimalarial activity and study has validated its use by locals in the treatment of malaria in most developing countries. Recommendations are made for the isolation and identification of bioactive substance for possible drug development.

Keywords: Antimalarial activity, *P. berghei* (NK-65), infected mice, *C. papaya* yellow leaf.

1. Introduction

Malaria is a serious pathogenic disorder, which causes mortality and morbidity with social and economic impact most especially in developing countries where the burden of malaria is very high. According to WHO 2021, there were an estimated 241 million malaria cases and 627,000 malaria related cases worldwide which represent 14 million more case in 2021 compound to 2019, and 69,000 more death. Which were linked to disruption to the provision of malaria prevention, diagnosis and treatment drug the pandemic. This disease has positioned about 3.3 billion people at risk. The recommended preventive drugs include a combination of sulfadoxine, pyrimethamine and amodiaquine, while the therapeutic strategy includes use of first lane drugs – artemisinin

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compound therapy in areas where *P. falciparum* is endemic and chloroquine (CQ), in the areas where it is still efficient.

The high global challenges developed by existing and partly available antimalarial drug due to multidrug resistance *P. falciparum* has led to the urgent need in search for newer treatments to eradicate malaria most especially in developing countries.

Although the available synthesized drugs may have side effects, indigeneous drugs are better alternate sources for the treatment of malaria due to low or less side effect. The resistance in malaria parasite is believed to have emerged through mutation in the active sites of drug targets or from biochemical changes in drug receptors.

The plant *C. papaya L* (English Paw-Paw; Hausa: Gwanda; Marghi; Ron: Mban) is a large tree-like plant belonging to the *Caricaceae* family, it is commonly called "Paw-Paw". *C. papaya* is widely grown in tropical and subtropical lowlands region. Among the developing countries, India, Indonesia, Mexico, Nigeria and Thailand are the largest papaya grown countries (Wimalawansa, 1981).

In contains pawpaw, chymoparinad vegetable pepsin (Wimalawansa, 1981). The presence of papaya endopeptidase II and *Carica papaya* endopeptidase IV were also reported. Protein, carpaine were also found to be present (Gills, 1992), leaves contain Carposide and seed contain Myrosinase carian and Sinigrin glycoside (Otskia et al., 2010). Phytochemicals present includes tanins, flavonoid, saponin, anthroquinones, steroids reducing sugars, cardinolides and phenolics (Owoyele et al., 2008).

2. Material and methods

Collection and authentication of plant materials

The yellow leaf of C. papaya were collected from university permanent site Naraguta Campus in April 2021 and was authenticated by a taxonomist at the Department of Plant Science and Biotechnology, Faculty of Natural Science University of Jos, Nigeria and specimen with voucher number JUHN21000418 was deposited in the departmental herbarium.

Preparation of the yellow leaf extract

After the collection of the yellow leaf water to remove any dirt and foreign particles and then shade dried. The dried yellow leaves were then crushed and grounded to get a powder form and weighed the powdered material (50 g) was weighed and extracted with 300 ml of methanolic for 72 hrs as described by Mankilik et al., 2021a, Mankilik et al., 2021b the solution obtained was sieved and extract kept in a desiccator to evaporate. It was then kept in a clean sterile bottle for further use.

Chemicals

All chemicals and reagents used were of analytical grade procured from various certified local and international suppliers.

Acute oral toxicity studies

A safe oral dose of the extract was determined by accurate oral toxicity test of Organization of Economic Cooperation and Development (OECD, 2008) as per 425 guidelines as described by Mankilik et al., 2021a. The animals were observed for any sign of toxicity OECD, 2008.

Experimental animals

Swiss albino mice weighing 18-30 g of either sex were used in the study. All the animals were housed for a week in a ventilated standard environment condition. The animals were used in this study were obtained from animal house unit of Pharmacology Department, Faculty of Pharmaceutical Sciences, University of Jos. The experiment was approved by the ethical committee Animal experimental unit, Department of Pharmacology and experiment were conducted as per guidelines of Institutional Animal Case and Use Committee (IACU) in collaboration with Office of Laboratory Animal Welfare office of laboratory animal (OLAW) with approval number F17-00379.

Maintenance of *P. berghei*

The chloroquine (CQ) sensitive rodent malarial parasite *P. berghei* (NK-65) strain was purchased from the National Institute of Medical Research, Ibadan, Oyo State, Nigeria. The blood stage parasite was maintained in an adult donor mice y serial blood passage and mice with 20-30 % rising parasiteamia was aneasthesized using chloroform dapped in a cotton wool. The blood

collected in heparinized tubes contain 0.5 % trisodium citrate buffer and adjusted with 0.9 % physiological saline so that each 0.2 ml aliquate contain $1x10^{-7}$ infected red blood cell/ml of blood.

Antimalarial test – Rane's curative assay

In the curative assay on Day 0 (D_0) mice were intraperitoneally infected with $1x10^{-7}$ *P. berghei* infected erythrocytes followed by random division of into four groups of 3 mice, each group 72 hrs after observation, the groups 1-3 orally received the plant extract of 200, 300 and 400 mg/kg bwt/day respectively. The group 4 (negative control) and group 5 (positive control) received 0.2 ml of vehicle and CQ phosphate 25 ml/kg b.wt/day respectively. The animals were dosed accordingly once daily for 5 consecutive days (D_2-D_7). On D_8, the blood parasiteamia in the all the groups were determined by using Giemsa stain. The mean survival of the treatment group were. Arithmetically determined by calculating the average survival time starting from day of infection to 30 days (D_0-D_{29}), in the Rane's test, the body weight was measured 3 hrs between infection on day 0 and consecutively from D_3 – D_8 during treatment period to establish the effect of plant drug on malarial mice (Ryley, Peters, 1970).

Determination of mean survival time

Mortality in each group was monitored daily from the time of parasite inoculation to death in the treatment and control groups throughout the study period. In the curative assay (Rane's test) the survival was observed for 30 days (D0 – D29). The Mean Survival Time (MST) of each group was calculated as:

$$\text{MST} \frac{\textit{Sum of survival time of all mice on a group}}{\textit{total number of mice in a group}}$$

Statistical data analysis

Each experiment was done in triplicate and data's were expressed as mean ± standard error on mean. Statistical significance was determined by one way analysis variance (ANOVA). The $P \leq 0.05$ from percentage parasiteamia were considered statistically significant when compared to control group.

3. Results

Phytochemical screening

The phytochemical screening of *C. papaya* yellow leaf methanolic extract reveals the presence of flavonoid, tannins, balsam, phenols and resins (Table 1).

Acute toxicity

The result of the toxicity study was depicted in Table 2. The extract was found to be safer up to 5000mg/kg b.wt.

Body weight

Result of Figure 1 showed that body weight of mice in the control group (CQ = 25 mg/kg) significantly increased, compared to negative control and other treatment groups. Dose dependent decrease was seen in treatment group (400 > 300 > 200).

PCV

Extracts did not ameliorate reduction in PCV level (Figure 2) reduction in PCV treatment group is dose is dependent as standard drug treatment group prevented reduction in PCV.

Antiplasmodial activity of plant extract and chloroquine

The result showed that the percentage increase in mice treated with extract at different doses on day 2 (D_2) while the parasiteamia of the mice treated with CQ 10 mg/kg did not increase compound compared to to those treated with *C. papaya* extract on day 2. In the chloroquine treated group, there was significant reduction in *P. berghei* to non-detectable level (D_8 – D_{10}). (Figure 3) there was significant ($P < 0.05$) decrease in parasiteamia level of mice infected with *P. berghei* and treated with 400mg/kg of *C. papaya* yellow leaf extract on Day 10 (D_{10}).

Mean Survival Time

All extract prolonged survival time, with 400 mg/kg b.wt of extract surviving being standard drug experimental period compared to the infected untreated (Table 3).

Table 1. Phytochemical profile of the Yellow Leaves Methanolic Extract On *Carica papaya*

PHYTOCHEMICAL	RESULT
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	-
Balsams	+
Carbohydrates	-
Phenols	+
Resins	+

KEY
- = absent
+ = present

Table 2. Acute toxicity of plant extract in mice with behavioral changes

Plant	Behavioral changes
C. papaya Yellow leaf methanolic extract	2000 mg/kg b.wt 5000 mg/kg b.wt No behavioral changes or mortality, except hair erection, no other symptoms were observed, No signs of toxicity and mortality was observed.

Table 3. Mode of Survival Time (MST) of Swiss albino mice

GROUPS	SURVIVAL RATE
200 mg/kg b. wt	18 days
300 mg/kg b. wt	16 days
400 mg/kgb.wt	Lived beyond experimental period
Infected/Untreated	13 days
25 mg/kgb. wt of chloroquine	Lived beyond experimental period

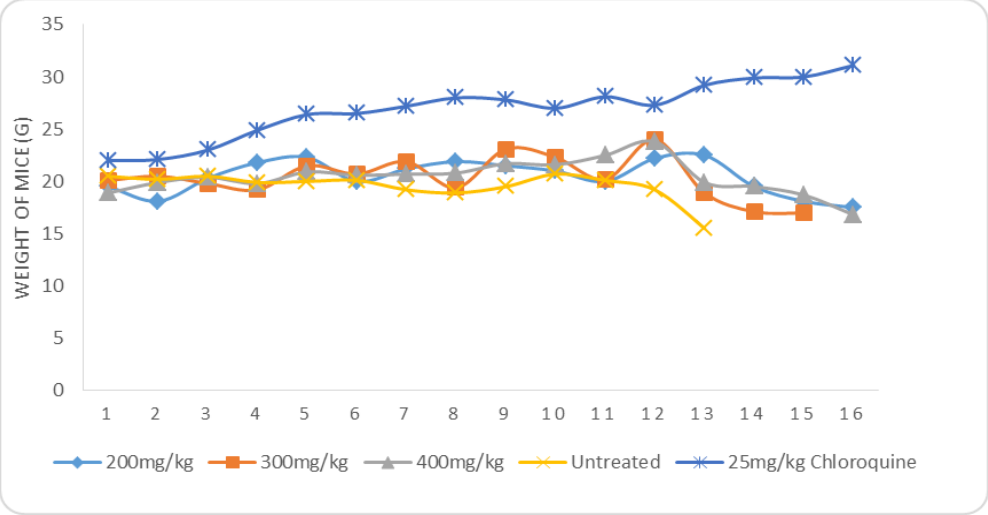

Fig. 1. Effect of yellow leaves methanolic extract of *Carica papaya* on body weight of *Plasmodium berghei* mice

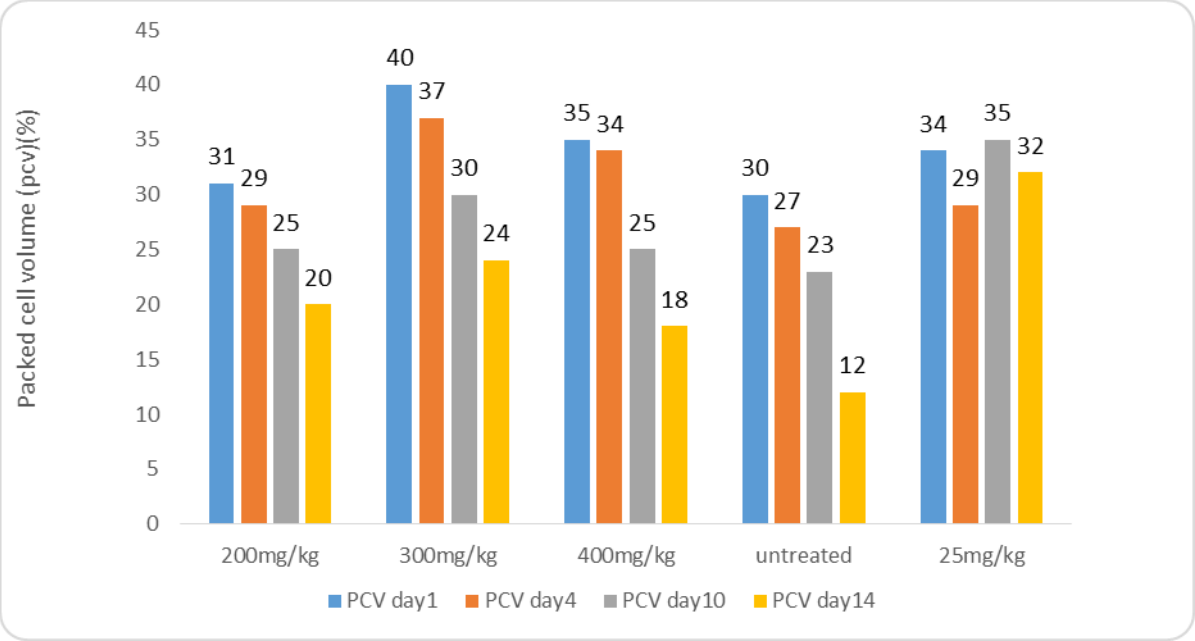

Fig. 2. Effect of yellow leaves methanolic extract of *Carica papaya* on PCV of *Plasmodium berghei* mice

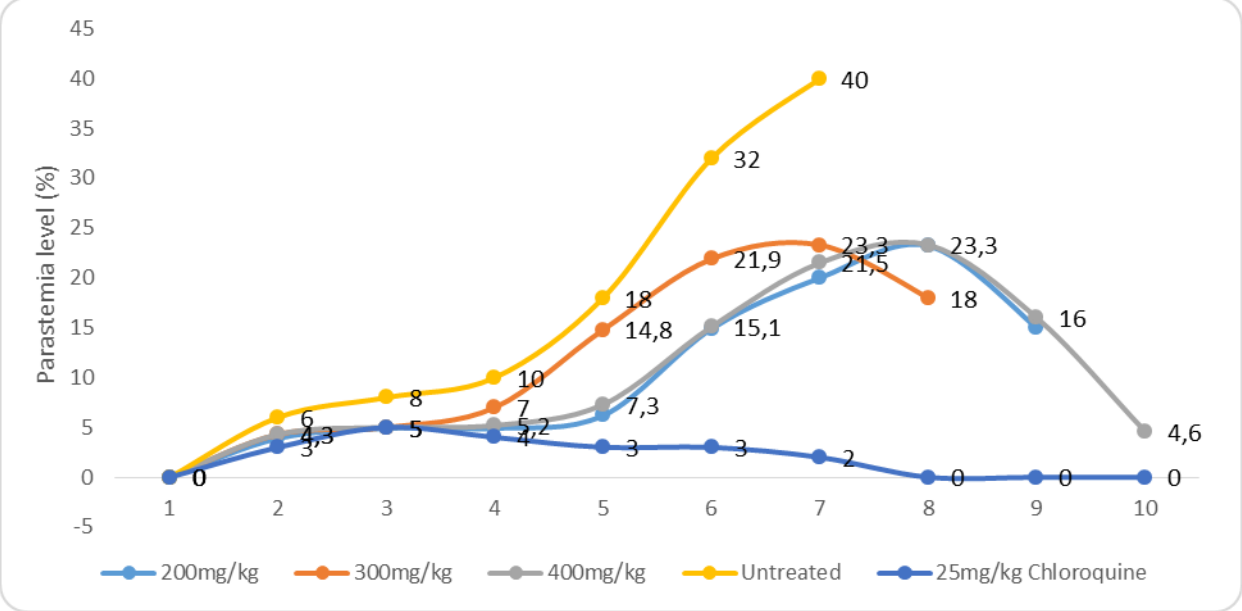

Fig 3. In vivo antiplasmodial activity of yellow leaves extract of *Carica papaya* against *Plasmodium berghei* infected mice

4. Discussion

Malaria remain the most vital pathogen disease. The rodent malaria parasite *P. berghei* is reported to be analogous to human malaria parasites in most essential aspect of structures, physiology life-cycle and model for the investigation of biology of malaria parasites, parasite – host interaction, vaccine development and drug testing (Fidock et al., 2004).

In toxicity test of yellow leaf methanol extract of *C. papaya* showed no visible toxic effects up to 5000 mg/kg b.wt. This study is similar to our previous study on the aqueous and methanolic extract of *A. aspera* shoot (Mankilik et al., 2021a; Mankilik et al., 2021b).

In the antiplasmodial study, the chloroquine sensitive *P. berghei* (NK-65) strain was used to check the efficacy of *C. papaya* yellow leaf methanolic extract along with conventional antimalarial

agent chloroquine. Hence in the antimalarial screening the drug is expected to prevent body weight loss under parasiteamia. The crude extract of *C. papaya* at various doses prevented weight loss induced with increased in parasiteamia level. In our study there was steady decrease in weight by extract treated group, except for dose 400 mg/kg b.wt where steady increase was recorded. Decrease in weight has been associated with decreased food intake, disturbed metabolic function and likely hypoglycaemia (Atkinson et al., 2000; Bashar et al., 2012).

The plant extract was effective in curative assay exhibiting above 50 % antiplasmodial activity. In our studies there was a remarkable increase of parasiteamia level by infected untreated groups of mice with remarkable decrease in chloroquine treated group from day 8 to a non-detectable level. The extract treated groups also reduced *P. berghei* levels compared with infected untreated. The present study was also comparable to our previous antiplasmodial study reported (Mankilik et al., 2021a; Mankilik et al., 2021b) on *A. aspera L.; Vernoma amgadalina* (Longdet et al., 2021); *Eucalyptus cameldulensis* ethylacetate extract (Longdet et al., 2020) with array of positive approaches for medicinal plants in malaria. The methanolic extract revealed the presence of bioactive component contained in the plant such as, terpenoids, alkaloids, flavonoids, tannins and phenol. Proving that the may be the major constituent for antimalarial activity. This activity could be attributed to the presence of good concentration of active compounds in higher doses (Zaruwa et al., 2018).

The observation of mean survival time in days revealed that the drug treated group prolonged the survival time of mice compared to the untreated group. The chloroquine group animals' survival beyond the experimental period (D_{29}) compared to negative control which displayed 13 days of survival. At higher doses of 400 mg/kg b.wt of *C. papaya,* survival days were beyond the experimental period, while at dose 200 mg/kg and 300 mg/kg b.wt prolonged survival of mice for 18 and 16 days respectively. These could be due to the reduced parasiteamia could and overall reduction in pathologic effect of the parasite strain on the study mice and thus agreed with the report that bioactive compounds of medicinal plants that prolonged survival time greater than twelve days in considered as active (Ural et al., 2014). This study is consistent with previous study (Mankilik et al., 2021a; Mankilik et al., 2021b).

From our study, there was remarkable decline in PCV levels of mice in all treatment group from D_2-D_{10} compared to the standard control. The low PCV observed in the infected and untreated groups may be as a result of heamolysis due to growing infection. This is in tandem with report that induced reduction in PCV levels during malaria in rodent occurred approximately 48 hrs post infection (Mace et al., 2015). The destruction of RBC either by parasite multiplication or spleen reticuloendothelial cell action in the pressure of many abnormal RBC stimulate the spleen to produce many phagocytes which could lead to aneamia *P. berghei* infected mice (Chinchilla et al., 1998). *Carica papaya* displayed antimalarial effect this has ability to reduce the erythrocytes stages (merozoites, schizonts and gametocytes) of *P. berghei* and hence justify its traditional usage of malaria remedy. This is in tandem with previous research that *C. papaya L.* extracted from leaf was reported having antidengue, anti-inflammatory and antimalarial (Charan et al., 2016; Pandey et al., 2016; Ansari, 2016).

5. Conclusion

The study showed that yellow leaf methanolic extract of *Carica papaya* leaf could possess useful phytocompounds and may be a potential candidate for isolation of antimalarial compound and may provide scientific support for its use in traditional medicine. Extract is safe as no sign of mortality or morbidity was seen.

6. Conflict of interest

The authors declare that they have no competing interest.

7. Acknowledgements

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Ethics and consent approval to participate:

The study was approved by the institute of Animal Ethical Committee, University of Jos, Ethical clearance obtained with approval number UJ/FPS/F17-00379.

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