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Original article

Effects of the methanolic seeds extract of Carica Papaya on plasmodium Berghei infected mice

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Abstract

Objective: The leaves extract of Carica Papaya(*C. Papaya*) papaya has been shown to possess antimalarial activity, thus this work aims at finding out if the plants antimalarial activity is present in or extended to the seeds. **Methods:** The seeds of C. papaya were collected from its fruit, air dried for 5 days and ground into fine powder. 80.65 g of the powder was then soaked for 48hours in 300 mL of methanol. Filtration was carried out using Whatman No.1 filter paper. The filtrate was evaporated to dryness by a three-day continuous heating on a hot plate of 30℃. The dry extract yield was scraped out of the Petri dish weighed and refrigerated until required. The percentage extract yield was calculated out from the initial powder weight. A preliminary phytochemical study was done by re-dissolving the appropriate amount of the dry extract in distilled water and appropriate test reagent added. The LD_{50} of the seeds of C. papaya was carried out using arithmetic method. Swiss albino Mice of both sexes and of average weight of 18-25 g were used as animals for antimalarial activity. They were housed in standard animal house, fed on Rats/Mice pellets and had non restricted excess to both feed and water throughout the 60day study period. While the non pregnant female Mice were used as test animals, the male animals were used as malaria parasite donors. Precautions were taken to ensure that all animals in the study groups were free from infection with Eperythrozoon coocoides. The female animals were then divided into three main groups (A-C) of 25 animals per group. Group A was used for malaria suppressive study (early infection-day 0-3) and was further subdivided to 5subgroups (a-e) of 5animals per group. Group B was used for malaria curative study (established malaria infection-day 3-7) and was further subdivided to 5subgroups (a-e) of 5animals per group. Group C was used for malaria prophylactic study (repository-4days treatment prior to malaria parasite infection) and was also further subdivided into 5subgroups (a-e) of 5animals per group. At the appropriate time, 50 mg/kg/day, 100 mg/kg/day and 200 mg/kg/day of crude extract of C. papaya were administered orally to the different subgroups(b-d) within the three main groups. One subgroup(a) in each main group also received orally, 5 mg/kg/day of chloroquine phosphate as positive control while one subgroup (e) in each main group also received orally, 0.2 mL/kg/day of distilled water as negative control. Malaria parasites infected red blood cells numbering 1×10^7 and suspended in 0.2 mL of physiological saline was inoculated intraperitoneally, to each animal of the subgroups (a-d) in each of the three main groups at the appropriate time. Blood smears were made from animals´tail, stained with Lieshman and examined microscopically

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at 100 × for the presence of malaria parasite. Percentage malaria parastaemia was calculated as well as average percentage malaria parasitaemia suppression. **Results**: Extraction yield of 25.29% was obtained while the LD_{50} was 620 mg/kg. The phytochemistry showed the richly presence of alkaloids, as well as glycosides, carbohydrates, resins, fats and fixed oils. The suppressive study at doses of 200,100 and 50mg/kg/day showed 53.02%, 43.43% and 19.83 % suppressive activity against Plasmodium berghei respectively. This activity compared to that of chloroquine, a standard antimalaria drug that gave 95.95% suppressive anti-parasitaemia. The prophylactic study at doses of 200, 100 and 50 mg/kg/day showed 63.85%, 61.12% and 48.08% prevention to malaria parasitaemia respectively as against 94.78% showed by chloroquine. The curative study however, at doses of 200, 100 and 50 mg/kg/day failed to suppress malaria parasitaemia with a mean survival range of 6-8days as against 27.2 days showed by chloroquine. The seeds extract of C. papaya showed a significant malaria parasitaemia suppressive activity ($P \leqslant 0.05$). These activities are dose dependent and comparable to those of Chloroquine phosphate. **Conclusion**: The results above suggest that the seeds extracts of C. papaya possess antimalarial activity like the leaf extracts. The antimalarial activity may be attributable to the richly presence of alkaloids and or the presence of its proteolytic enzyme (Papain). The present finding justifies the inclusion of the seeds of C. papaya in the treatment of malaria by local herbalists. The seeds extracts therefore, if well purified and characterized may be used in treatment of very early plasmodiasis as well as a good prophylactic drug in human. This work at the moment is limited to animals, thus clinical trials in humans is be recommended particularly, when C. papaya seeds are non harmful/non toxic.

Keywords: Plasmodium berghei; Plasmodiasis; Seeds of Carica papaya; Malaria; Medicinal plants

INTRODUCTION

Malaria is one of the world highest killer diseases affecting most tropical countries especially Africa. Mostly, children below 5, pregnant women and non-immuned individuals are predisposed with over one million children dying annually from malaria and malaria related complications[1].

In most regions of the world, malaria parasites have become unresponsive to conventional antimalarial drugs and most antimalarial drugs have been associated with unsatisfactory efficacy, tolerability, safety profiles, as well as complicated and expensive dosage regimens[2]. Consequently, there is an urgent need for new antimalarial drugs that are effective, safe and affordable.

Plants form good sources of cheap and affordable drugs and medicinal plants possess therapeutic efficacy like their orthodox drugs counterpart yet, they exhibit less or no side/adverse unwanted effects[3-5].

Carica papaya L(C. papaya) (*caricaceae*) commonly known as pawpaw is easily recognized by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal of cluster of large and long-stalked leaves. It is rapidly growing and on a good soil, it can grow up to 20 meters tall. It is widely distributed throughout West Africa and may be cultivated or it can come up spontaneously on a fertile soil and occurs as monoccious or dioccious plant[6]. Earlier works done on C. papaya have shown that traditional medicine healers use every part of the plant for treatment of a wide range of ailments. Its leaves are boiled together with those of Lemon grass and guava for treatment of malaria. Its leaves also possess sedative, muscle relaxant, antioxidant and anticonvulsant effects. While the roots extracts are purgatives, the seeds and pulp possess antibacterial activity[6]. C. papaya has its purified active constituents as proteolytic enzymes (Papain that is found in most part of the plant and Chymopapain that is found in the latex). Earlier works on C. papaya have shown the clinical and pharmacological importance of these proteolytic enzymes[6-8]. Higher levels of these enzymes are found more in younger plants than the older ones[9]. Anti inflammatory, antipyretic and analgesic activities of the leaves extracts have also been demonstrated[6].

Animals are not left out in the dividends from C. papaya. In Indonesia and Philippines, C. papaya is used as de-wormers, reduction in body-weight and as "after-feed" in post parturition animals perhaps to encourage lactation[10, 11].

MATERIALS AND METHODS

The matured but, unripe fruits of C. papaya were harvested within the campus of the University of Port Harcourt, Nigeria. The taxonomy of the plant has been done earlier[6]. The fruits were cut open and the black seeds were collected into a clean dry metal

plate.

Plasmodium berghei prep was obtained from National Institute for Product Research and Development (NIPRD) Abuja. Nigeria. While chloroquine phosphate tablets were purchased from University of Port Harcourt's Hospital Pharmacy.

All animals were bought from University of Port Harcourt's Animal House.

Seeds extraction

The seeds were air dried for 5 days and ground into a fine powder using a Coronal manual grinder. 80.65 g of the fine powder was immersed into (Macerated in) 300 mL of Methanol and allowed to stand for 48 hours at room temperature. Filtration was then carried out using a Whatman No. 1 filter paper. The filtrate was poured into a clean-dry Conical flask and evaporated to semi-solid on a hot plate of 30℃. The semi-solid filtrate was poured into an evaporating dish and further evaporated to dryness at 30℃ for 2 days. The dry solid was scraped out, weighed and refrigerated until required. The percentage extract yield was calculated out from the initial fine powder weight.

Phytochemistry

A preliminary phytochemical study was done by redissolving the appropriate amount of the dry extract in distilled water and appropriate test reagent added[12]. Phytochemical screening was carried out to test for the presence of alkaloids, flavonoids, proteins, sugars/reducing substances, glycosides, saponins, resins, fats/oils and starch.

Animal experiment Ⅰ (LD_{50} study)

With the approval of the University's ethical committee on the use and handling of animals, 30Swiss albino male Mice of 18-25 g weight, divided into 5groups of 6 per group were selected for the LD_{50} study. The animals were allowed to be acclimatized to their new mates and cages overnight. They were fed on Rats/Mice pellets (Pfizer Feeds PLC) and had no restricted access to both feeds and good drinking water throughout the experiment. An initial pilot study was carried out to determine the minimum dosage that killed all animals in the study group and the maximum dosage that killed no animal.

The animals in their cages of 6/cage(A-E) received crude drug extract administered. Intraperitoneally (ip) in the ranges of 50, 100, 200, 400 and 800 mg/kg respectively. The negative control group (F) received 0.25 mL of Normal saline ip. The symptoms of toxicity and, or death were observed within 24 hours and recorded. Any dead animal was removed from cage as soon as possible. The LD_{50} was calculated as the probit of the minimum dose of the extract that killed half the number of the animals in the study group or the mean of two doses where applicable[13].

Animal experiment Ⅱ (antimalarial activity of C. papaya)

This aspect of the work was divided into three testing subgroup to make room for a clearer justification of our goals. Thus namely, (a): Suppressive Test, (early infection. Antimalaria started at day 0-3 post MP inoculation) to demonstrate the ability of C. papaya to inhibit plasmodiasis in an infected animal (Inhibition of Virulence). (b): Curative Test (established malaria infection. Antimalaria started at day 3-7post MP inoculation), to demonstrate the ability of C. papaya to kill malaria parasites, thus reverse an already established plasmodiasis. (c): Preventive or Prophylactic Test (4days treatment prior to malaria parasite inoculation) to demonstrate the ability of C. papaya to inhibit infectivity.

Swiss albino Mice of both sexes and of average weight of 18-25 g were used as animals for antimalarial activity. They were housed in standard animal house, fed on Rats/Mice pellets and had non restricted excess to both feed and water throughout the 60day study period. While the non pregnant female Mice were used as test animals, the male animals were used as malaria parasite donors. Precautions were taken to ensure that all animals in the study groups were free from infection with Eperythrozoon coocoides. The female animals were then divided into three main groups (A-C) of 25 animals per group. Group A was used for malaria suppressive study (early infection-day 0-3) and was further subdivided to 5 subgroups (a-e) of 5 animals per group. Group B was used for malaria curative study (established malaria infection-day 3-7) and was further subdivided to 5 subgroups (a-e) of 5animals per group. Group C was used for malaria prophylactic study (repository-4days treatment prior to malaria parasite infection) and was also further subdivided into 5 subgroups (a-e) of 5 animals per group. At the appropriate time, 50 mg/kg/day, 100 mg/kg/day and 200 mg/kg/day of crude extract of C. papaya were administered orally to the different subgroups (b-d) within the three main groups. One subgroup

(a) in each main group also received orally, 5 mg/kg/day of chloroquine phosphate as positive control while one subgroup (e) in each main group also received orally, 0.2 mL/kg/day of distilled water as negative control. Malaria parasites infected red blood cells numbering 1×10^7 and suspended in 0.2 mL of physiological saline was inoculated intraperitoneally, to each animal of the subgroups (a-d) in each of the three main groups at the appropriate time.

Blood smears were made from animals´ tail, stained by Lieshman´s stain method and examined microscopically at 100 × for the presence of malaria parasite.

Percentage malaria parastaemia was calculated as well as average percentage malaria parasitaemia suppression. These calculations were done using the below formula, Where WBC = White blood cells, No = number and MP = malaria parasites[6].

$$\text{thus:} \frac{\text{- No of MP} \times 100}{\text{Total No WBC} \times 1.}$$

Twenty microscopic fields with an average of 50WBC per field were counted to give a total of 1 000WBC as counted No of WBC. Average percentage suppression was also calculated thus:

$$\frac{\text{Average \% parasitaemia in Control} - \text{Average \% parasitaemia in Treated} \times 100}{\text{Average \% parasitaemia in Control}}$$

All results and data were subjected to statistical analysis and expressed as mean ± Standard deviation. Rane graphical plot was used to show results differences and percentage calculations were used to score results. Thus Percentage malaria parastaemia was calculated as well as average percentage malaria parasitaemia suppression. Student *T*-test was used were possible to show differences in observations.

RESULTS

Extraction

From the starting ground seeds weight of 80.65g, 2.40g was obtained as extraction yield. Thus, giving a 2.53% yield.

Phytochemistry

While glycosides was averagely present in the seeds of C. papaya, alkaloids was abundantly present. Resin, carbohydrates, fats and fixed oils were detected in low values.

LD_{50}

Lethal dose was calculated as the mean of the Probit of the highest dose (400 mg/kg) that killed less than half of the number of animals in its group and the lowest dose (800 mg/kg) that killed half of the number of animals in its group dosage[13]. Our LD_{50} was thus estimated to be 620 mg/kg. Estimation of LD_{50} of the leaves has been gotten to be 560 mg/kg[6]. Our result being higher may indeed be justifiable since the seeds are easily swallowed or eaten during eating of the pulp and has never reported to have harmed any known being. Man does not eat the leaves unless for medicinal purposes.

Antimalarial activity of C. papaya

Suppressive study

The methanolic extract of the seeds of C. papaya, at the various doses employed produced a dose dependent Chemosuppressive effect on plasmodiasis ($P \leqslant 0.05$) by reducing virulence of Malaria parasites (% suppression) or outright killing of the parasites (% free of MP) stood at 19.83%, 43.43% and 53.02% respectively. These were comparable to that of Chloroquine, a standard Schizonticidal drug, that stood at 95.95%. See Table 1.

Preventive (Repository or Prophylactic) study

The methanolic extract of the seeds of C. papaya also produced a dose dependent repository activity ($P \leqslant 0.02$). % of RBC free of MP for 50, 100 and 200 mg/kg/day stood at 48.08%, 61.12% and 63.85% respectively. These were equally comparable to that of Chloroquine, a standard Schizonticidal drug, that stood at 94.78%. See Table 2.

Curative study

This was assessed through the rate of survival of the Mice post crude drug extract administration in established plasmodiasis. The mean survival of the Mice receiving the various doses 50, 100 and 200 mg/kg/day of the methanolic extract of the seeds of C. papaya were 6.60 ± 0.45, 7.20 ± 0.39 and 7.20 ± 0.39 respectively. Unlike those of repository and suppressive studies, the results of the Curative were far away from that of Chloroquine, a standard Schizonticidal drug, that stood at 27.20 ± 0.23. It can be seen from the results that the results of curative study were not really different from that of control 6.00 ± 0.42. ($P \geqslant 0.05$). See Table 3 and Figure1 (Rane Comparative Graphic analysis).

Table 1 Blood schizonticidal activity of methanolic extract of the seeds of C. papaya. (suppressive study results)

Crude Drug Extract	Dose(mg/kg/day)	Average % parasitaemia	Average % Suppression
C. papaya seeds	50	32.87 ± 0.62	19.83
C. papaya seeds	100	23.18 ± 0.49	43.43
C. papaya seeds	200	19.25 ± 0.56	53.02
Chloroquine. positive	5	1.66 ± 0.11	95.95
Comparative control			
Distilled water	0.2mL	40.98 ± 0.15	—
Negative control			
N = 5	*N* = 5	*N* = 5	*N* = 5

Table 2 Blood schizonticidal activity of methanolic extract of the seeds of C. papaya. [Repository (prophylactic or preventive) study]

Crude Drug Extract	Dose(mg/kg/day)	Average % parasitaemia	Average % Suppression
C. papaya seeds	50	20.12 ±0.27	48.08
C. papaya seeds	100	15.12 ±0.15	61.12
C. papaya seeds	200	14.06 ±0.15	63.85
Chloroquine positive	5	2.03 ±0.16	94.78
Comparative control			
Distilled water	0.2mL	38.89 ±0.17	—
Negative control			
N = 5	*N* = 5	*N* = 5	*N* = 5

Table 3 Mean survival period of Mice receiving the various doses of C. papaya. (Curative study)

Crude Drug Extract	Dose(mg/kg/day)	Mean Survival Time in Days
C. papaya seeds	50	6.60 ± 0.45
C. papaya seeds	100	7.20 ± 0.39
C. papaya seeds	200	7.20 ± 0.39
Chloroquine positive	5	27.20 ± 0.23
Comparative control		
Distilled water	0.2mL	6.00 ± 0.42
Negative control		
N=5	*N*=5	*N*=5

DISCUSSION

The primary aim of our present study was to find out if the already demonstrated antimalarial activity of the leaves of C. papaya is extended or present in the seeds. Malaria remains a major tropical health problem and the resurgence of malaria, especially drug resistant cases[14], has renewed the urgent need for Newer, Cheap and Effective Drugs.

Our results showed the ability of the methanolic extract of C. papaya to prevent malaria parasites infectivity and reduce the virulence caused by malaria parasite infestation.

In most regions of the world, malaria parasites have become unresponsive to conventional antimalarial drugs and most antimalarial drugs have been associated with unsatisfactory efficacy, tolerability, safety profiles, as well as complicated and expensive dosage regimens[2]. Consequently, there is an urgent need for new antimalarial drugs that are effective, safe and Cheap. Plants form good sources of cheap and affordable drugs and medicinal plants possess therapeutic efficacy like their orthodox drugs counterpart yet, they exhibit less or no side/adverse unwanted effects[3-5].

In our present study, Chloroquine Phosphate, a standard Schizonticidal drug showed antimalarial activity in all the three categories of the study. These activities of Chloroquine demonstrated that the malaria parasites species were sensitive to the drug, thus a well positive controlled study, ruling out the possibility of influence of drug resistance and false antimalarial activity of the crude extract.

The seeds extract demonstrated a dose-dependent suppressive and repository($P \leqslant 0.02$ and $P \leqslant 0.05$) activity. Antimalarial activity may stem from the richly presence of alkaloids[15,16] and the proteolytic enzymes which may not only act as catalyst in certain biological reactions; but also break malaria parasite vacuole thus killing and reducing the number of malaria parasites. Mice and Rats are easily susceptible to Plasmodium berghei and not Plasmodium falcipa-

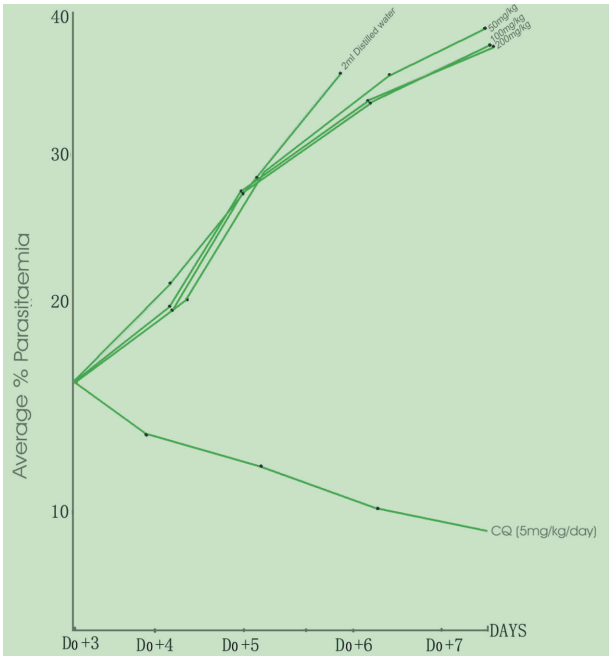

Figure 1 Mean survival period of Mice receiving the various doses of C. papaya. (curative study). Methanolic extract of the seeds of C. papaya on rane test. each point is a mean of five observations.

rum that is actually the major threat to humans, particularly in Africa. But, having demonstrable activity against Plasmodium species that are susceptible to animals is usually extrapolated to man. Such is the case with the antimalarial activity of the leaves of C. papaya extract that was carried on Mice infected with Plasmodium yoelli[17]. Malaria kills ten times the rate of HIV/AIDS, hence an intensified search towards new drugs development to fight this malaria ugly menace must have reduced or less bureaucratic/ethical rules before being tried on humans especially, where the medicinal plant is edible.

The present finding justifies the inclusion of the seeds of C. papaya in the treatment of malaria by local herbalists. The seeds extracts therefore, if well purified and characterized may be used in treatment of very early plasmodiasis as well as a good prophylactic drug in human. This work at the moment is limited to animals, thus clinical trials in humans may be recommended particularly, when C. papaya seeds are non harmful/non toxic.

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