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Antiplasmodial effects of *Carica papaya* extract on haematological markers of Albino Wistar rats infected with *Plasmodium berghei*

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Abstract

In view of resistance of the malaria parasite to antimalarial drug therapy, which leads to drug failure, new drugs or drug combinations are urgently required for the treatment of malaria infections from traditional medicinal plants. The antiplasmodial effects of *Carica papaya* extract on haematological markers of albino rats infected with *Plasmodium berghei* was determined using three control groups (Normal, negative, and positive control) and five experimental groups with each representing the extract concentrations (100, 300, 500, 800 and 1000mg/kg). After initiation of malaria infection through intraperitoneal inoculation, measurement of the level of parasitaemia over a five-day period revealed a progressively decrease in the treated group. On the other hand, the untreated group showed a progressive increase in parasitemia level with average percentage parasitized red cells as 11.33±1.97 on the first day post inoculation and 18.15±1.49 on the fifth day. While the haematological result showed a significant ($p<0.05$) decrease in values of Red blood cell, Packed cell volume, Haemoglobin and neutrophils in the inoculated groups, there was an increase in the treated groups, all of which were concentration-dependent. Conclusively, it can be inferred that the leaves of *Carica papaya* have antiplasmodial potentials and can therefore be purified employed in the development of antimalarial drugs.

Keywords: Antibiotic; Resistance; Medicinal plants; Malaria; *Carica papaya*; *Plasmodium*

1. Introduction

The Nigeria tropical forests are biologically loaded with diverse ecosystem of plant whose potential value as natural pharmacy is yet to be fully discovered. In developing countries, remedies from plants are readily used in the treatment of various kinds of diseases. This usually involves the use of medicinal plants and related products in health management. This is despite the recent revolution in medical practice as a result of technology [1]. Aside the prominent use of herbal plants in traditional medicine, other natural products like animal parts is frequently used. WHO [2] puts the estimate at about 80% of the population of the world relying on drugs from plant origin for their health needs. Every culture of the world has plants and plant products used in folkloric medicine. Despite the availability of modern medicine, a great majority of people still depend on this ethno pharmacological product for health care management due to obvious reasons such as availability and affordability. In Nigeria, people of all classes depend so much on plant based medications for the management of different illnesses although modern medication are available [3]. Different medicinal plants possess diverse therapeutic potential as no single plant has all the medicinal properties [4]. Ethno medicinal plants have been believed to have fewer side effects, this is however an erroneous impression [5, 6]. Many of the medicinal potentials of plants used in folkloric medicine have been subjected to scientific investigation and this has warranted their wide spread use as an alternative or complement to orthodox medicines.

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Malaria is one of the most prevalent, devastating parasitic infectious diseases in the world. Each year, 300- 500 million clinical cases and 1.5 – 2.7 million deaths associated with malaria are reported globally [7]. The burden of malaria is the greatest in Africa, representing 90% of the estimated malaria-associated death [8]. It is the greatest cause of mortality and hospitalization among children <5 years of age in Sub-sahara Africa [9].

Available therapeutic agents are already limited in their efficacy, while drug resistance threatens the ability to prevent and treat the infection [10]. Malaria is the leading cause of illness and death in sub-Saharan Africa with an annual mortality of approximately one million children under five [11]. In Nigeria and the rest of the world, malaria infection continues to pose a major health challenge. In view of resistance of the parasite to antimalarial drug therapy, which leads to drug failure, new drugs or drug combinations are urgently required for the treatment of malaria infections from traditional medicinal plants [12]. Traditional medicinal plants play an important role in the medical system in Nigeria; however, plant materials remain an important resource to combat serious diseases in the world. Currently used plants have always been considered to be a possible alternative and rich source of new drugs, and some of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates [13]. Hence, this study was aimed at determining the antiplosmodial and toxicological effects of *Carica papaya* extract on biochemical and haematological markers of albino rats infected with *Plasmodium berghei.*

2. Material and methods

2.1. Collection of Plant Sample and Extraction

The leaf parts of *Carica papaya* were used. The plants were gotten from fruit garden D-Line in Port Harcourt and were identified by the herbarium unit of the department of Plant Science and Biotechnology University of Port Harcourt. The dried leaves were extracted using absolute ethanol as a solvent in a Soxhlet extractor. The solvent was completely removed with the aid of a rotary evaporator. The crude extracts were stored at −20°C until used. Before experiments, the extracts were then dissolved in appropriate volume of water to make the respective stock solutions.

2.2. Phytochemical Screening of *Carica papaya Leaves*

The qualitative and quantitative phytochemistry of the *leaves of Carica papaya* were carried out.

2.3. Experimental Animals

A total of fifty-two (52) adult rats of both sexes weighing between 116 to 130g were used. They were acquired from Department of Physiology, University of Port Harcourt. The animals were housed in a specially designed plastic/wire gauze animal cage and were placed on standard feed and given access to water *ad libitum*. They were kept under observation (Acclamatization) for about 10 days before the onset of the experiment to exclude any form of infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25 ± 5 °C) and normal 12-hour light/dark cycle.

2.4. Malaria Infection of Experimental Rats

The malaria infection was carried out according to the method described by Okolie and Obiajunwa [14]. *Plasmodium berghei* ANKA (65) strain was originally obtained from the Nigerian Institute of Medical Research in Yaba, Lagos. The parasite was then maintained at the Malaria Research Laboratory of the University of Port Harcourt. Malaria infection in rat was initiated by intraperitoneal (IP) inoculation of 0.5 ml blood which contains about 2 x 10^7/ml Parasitized Red Blood Cells (PRBC) from a donor mouse infected with *P. berghei*. Controls to malaria-infected rat were given an equivalent volume and dilution of normal uninfected red blood cells.

2.5. Estimation of Percentage Parasitemia

Thin and thick blood films were prepared with blood collected from the tail of each mouse on the fourth day of inoculation. The thin films were fixed with methanol, stained with Giemsa stain and the percentage parasitemia was determined by microscopic examination using the formula:

$$\% \text{ Parasitemia } = \frac{\text{No of parasitized RBC}}{\text{No of total RBC}} \times 100$$

2.6. Experimental Design

Rats were infected with *P. berghei* as described above and were divided into thirteen groups in a plastic wire cages. combisunate@ was used as a standard and was dissolved in distilled water and a dose of 10mg/kg (oral administration) were given in a single daily dose at midday starting from day 1 until day 4 following four days of inoculation. Normal Control group was introduced which were exposed to the same experimental conditions as others and were allowed access to food and water only, the group is also known as the uninfected rat group. An untreated group was also created. That is, a group inoculated with *P. berghei* for four days like every other inoculated groups but was not treated when others are receiving treatment for four days post inoculation process. Five groups were created with each category representing the ethanolic extracts of *Carica papaya*. The five groups represent the five concentrations of 100, 300, 500, 800 and 1000mg/kg ethanolic extracts administerterd for four days for the treatment of the parasite.

Table 1 Experimental design for control groups

S/N	Group	No. of rat	Food +water Only	Parasite induction	Treatment	100 mg/ kg	300 mg/ kg	500 mg/ kg	800 mg/ kg	1000 mg/k g	10mg/kg combisunate@
1	normal control	4	yes	No	no						
2	negative control	4	yes	yes	no						
3	positive control	4	yes	yes	yes						yes

Table 2 Experimental Design for *C. papaya*

S/N	Group	No. of rat	Food+ water Only	Parasite induction	treatment	100 mg/kg	300 mg/kg	500 mg/kg	800 mg/kg	1000 mg/kg	10mg/kg combisunate@
4	GA1	4	yes	yes	yes	yes					
5	GA2	4	yes	yes	yes		yes				
6	GA3	4	yes	yes	yes			yes			
7	GA4	4	yes	yes	yes				yes		
8	GA5	4	yes	yes	yes					yes	

Where: GC1-GC5 represent 100mg/kg, 300mg/kg, 500mg/kg, 800mg/kg and 1000mg/kg ethanolic extract of C*. papaya* respectively.

2.7. Estimation of Curative Test against *P. berghei* Infection in Rat

Ethanol extracts of *Carica papaya* were assessed for *in vivo* activity in a four- day curative test against *P. berghei* infection in rat. Rats were inoculated with 0.5ml of 2 x 10^7 PRBC intravenously as described above. All extracts were dissolved in 2.5% tween 80 and diluted with water (for injection) to provide doses of 100, 300, 500 and 1000 mg/kg body weight. The extracts were administered in a single daily dose orally according to their body weight from day 1 until day 4 post infection. Parasitaemia development in the infected rat was monitored on the first, third and fifth day of the treatment.

2.8. Blood Collection

After four days of treatment, the rats were sacrificed on the fifth day using chloroform. Blood was collected directly from the heart of the animals through 2 ml syringe into EDTA (ethylene diamine tetra acetic acid) bottles for white blood cell count and packed cell volume and another hematological test. Heparinized bottles were also used to collected blood in order to obtain plasma for biochemical analysis.

2.9. Haematological Estimations

The blood samples were collected into tubes containing EDTA and were immediately used for determination of haematological parameters. Total red blood cell (RBC) and white blood cell (WBC) counts were estimated according to

the visual method of Dacie and Lewis [15]. The percentage Packed Cell Volume (PCV) was determined according to the hematocrit method while the blood haemoglobin (Hb) concentration in all samples was estimated according to the cyanomethaemoglobin method using Drabkin's reagent [16]. Differential white blood cell counts were estimated using the method of Osim *et al.,* [17].

2.10. Statistical Analysis

Data from this study were statistically analyzed using SPSS software version 20. Descriptive statistics were done. An independent t-test was used to show mean differences. Analysis of variance was employed to know mean differences among groups. P-values <0.05 were considered as statistically significant. Statistical analysis of the data obtained in this study was performed by one-way ANOVA followed by a single post hoc test. P<0.05 was taken as statistically significant.

3. Results

3.1. Qualitative phytochemical composition of *Carica papaya*

The qualitative evaluation of phytochemical composition of *Carica papaya* showed that Alkaloids, Flavonoids, Tannins, Anthraquinones, Triterpenoid, Glycosides, Saponins, Ssteroids and Phytate were all present in the plants (Table 3).

Table 3 Qualitative phytochemical composition of *Carica papaya*

S/N	Phytochemical composition of Carica papaya	Remark
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Anthraquinones	-
5	Triterpenoid	+
6	Glycosides	+
7	Saponins	+
8	Steroids	+
9	Phytate	+

Key: + = Present; - = Absent

3.2. Quantitative phytochemical composition

Table 4 Quantitative phytochemical composition of *Carica papaya*

S/N	Sample	phytochemical composition of *Carica papaya* [Mg/100gm]
1	alkaloid	0.76
2	flavonoid	1.67
3	tannins	0.951
4	phenols	2.478
5	saponin	0.79
6	glycosides	0.223
7	terpenoid	0.76
8	steroid	0.014
9	papain	0.276
10	oxalate	1.02

The quantitative phytochemistry of *Carica papaya* showed that alkaloid, flavonoid, tannins, Phenols, oxalate, Saponin, terpenoids, steroid, papain, glycosides, in *Carica papaya* have values of 0.76mg/100g, 1.67mg/100g, 0.95mg/100g, 2.48mg/100g, 1.02mg/100g, 0.79mg/100g, 0.76mg/100g, 0.014mg/100g), 0.28mg/100g, 0.22mg/100g, respectively.

3.3. Curative ability of ethanolic extracts of *Carica papaya* against *P. berghei*

The parasitemia level for the treated groups decreased progressively for the five days period. This is indicative in the mean number of the percentage parasitized red cells of 1000mg/kg doses as 9.79±0.73 on the first day post inoculation and 0.21±0.03 by the fifth day. The decrease is also observed in the 100, 300, 500 and 800mg/kg groups of each sample. Except for the untreated group which showed a progressive increase in parasitemia level showing the mean number of the percentage parasitized red cells as 11.33±1.97 on the first day post inoculation and 18.15±1.49 by the fifth day. The result of plant extracts on parasitemia density in rat is presented in Table 5 and 6.

Table 5 Curative activity of control groups of ethanolic extracts of *Carica papaya* against *P. berghei*

S/N	Group	Day 1	Day 3	Day 5
1	normal control	0.00±0.00 ^{bc}	0.00±0.00	0.00±0.00 ^{c}
2	negative control	11.33±1.97 ^{ac}	14.95±2.65 ^{ac}	18.15±1.49 ^{ac}
3	positive control	9.17±1.39 ^{ab}	0.35±0.12 ^{b}	0.06±0.06 ^{a}

KEY = Data are expressed as Mean ± SEM. n=4; a= Values found in a column with common superscript letter a are significantly different ($p<0.05$) when compared to the normal control; b= Values with superscript b, are significantly different ($p<0.05$) relative to the negative control; c= values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

Table 6 Curative activity of ethanolic extracts of *Carica papaya* (leaf) against *P. berghei*

S/N	group	Administration of ethanolic extracts in mg/kg of *C. papaya*	day 0	day 3	day 5
1	GA1	100	8.23±1.11 ^{ac}	3.35±1.36 ^{b}	1.35±0.29 ^{ac}
2	GA2	300	12.61±5.96 ^{ac}	1.87±0.32 ^{b}	1.18±0.09 ^{ac}
3	GA3	500	9.71±0.99 ^{ac}	1.73±0.28 ^{b}	0.72±0.15 ^{ac}
4	GA4	800	9.92±1.20 ^{ac}	1.98±0.37 ^{b}	0.34±0.08 ^{abc}
5	GA5	1000	9.79±0.73 ^{ac}	2.99±1.45 ^{b}	0.21±0.03 ^{ab}

KEY = Data are expressed as Mean ± SEM. n=4; a= Values found in a column with common superscript letter a are significantly different ($p<0.05$) when compared to the normal control; b= Values with superscript b, are significantly different ($p<0.05$) relative to the negative control; c= values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

3.4. Effects of Ethanolic Extracts of *Carica papaya* on Some Hematological Indices of Rats

Table 5 shows the hematological parameters in normal control, untreated and treated groups of wistar rats. The result indicated a significant ($P < 0.05$) difference in the hematological parameters of normal and the untreated group. Hematological parameters as an investigating tool for cases of early malaria infections and mostly help to detect early complications associated with serious malaria infection. Reduction in red blood cells-RBC could lead to anemia which could result to death. To prevent death that may result from such complications, the need to carry out proper blood count. The report from this study shows that the untreated group tends to have significantly lower red blood cell count (RBC) 3.55±0.13 lower than that of non-infected subjects 4.82±0.11. The treated groups showed a linear and a progressive increases in RBC relative with increase in dose. The PCV and Hb levels were also noted to be significantly lower in the untreated control ($P<0.05$) as compared with the normal control and those groups receiving treatment.

Table 7 Effects of ethanolic extracts of *Carica papaya* (leaf) on some hematological indices of rats.

S/N	Group	Administration of ethanolic extracts in mg/kg of *C. papaya*	PCV	Hb	RBC	WBC	NEU	LYM	MON
1	GA1	100	32.50±1.04 abc	11.32±0.24 abc	3.77±0.04 abc	10.92±0.22 abc	64.75±0.85 abc	28.50±1.19 abc	1.25±0.25 abc
2	GA2	300	38.25±0.85 abc	12.75±0.21 abc	4.37±0.11 abc	9.97±0.20 abc	65.75±1.10 abc	24.00±1.08 abc	0.50±0.28 abc
3	GA3	500	40.25±0.62 abc	13.87±0.14 abc	4.57±0.04 abc	9.67±0.08 abc	68.50±0.64 abc	23.00±0.40 abc	0.25±0.25 abc
4	GA4	800	42.00±0.40 abc	14.05±0.09 abc	4.72±0.06 abc	9.32±0.04 abc	69.50±0.64 abc	21.75±0.62 abc	0.25±0.25 abc
5	GA5	1000	44.50±0.64 abc	14.82±0.18 abc	4.97±0.04 abc	7.50±0.14 abc	71.50±0.64 abc	20.00±0.40 abc	0.00±0.00 abc

KEY= Data are expressed as Mean ± SEM. n=4; a= Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control; b= Values with superscript b, are significantly different ($p<0.05$) relative to the negative control; c= values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

There was an increase in WBC in groups receiving treatment and the inoculated but untreated group of rats compared to the normal control group. In general, the total WBC, the absolute lymphocytes and monocytes were significantly higher in the malaria infected patients than in the normal control ($p<0.05$). However, the PCV, Hb RBC and absolute neutrophils count were lower significantly in the malaria infected rats than in the normal control group ($p<0.05$).

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

4. Discussion

The results of the standard 4-day curative test against *P. berghei* infected rats were summarized in Table 5 and 6. Several studies have attributed the antiplasmodial properties of plants to their alkaloids, flavonoids, terpenoids, anthraquinones, and glycosides contents [7]. The phytochemical analysis reviewed in this study confirmed the existence of alkaloids, flavonoids, coumarins, glycosides, and terpenoids in these plants. *C. papaya*, documented to possess several medicinal properties, has been extensively researched for various pharmacological properties. The laboratory studies and clinical trials provide a strong scientific base supporting the various ethnobotanical/ethno pharmacological reports from across the world. In addition, as *C. papaya* propagates easily and thrives in almost all the climatic conditions, it is widely available for medicinal use as well as commercial applications. Thus, *C. papaya* extract with its multiple medicinal properties needs to be further developed for wider use for the treatment of malaria. Identification and isolation of promising compounds for the development of products are also needed.

This study showed a reduction in the average percentage parasitemia in a correlated pattern with the various doses administered for the plant extract. An average percentage parasitemia of 8.23±1.11, 12.61±5.96, 9.71±0.99, 9.92±1.20, 9.79±0.73 and on the first day and 1.35±0.29, 1.18±0.09, 0.72±0.15, 0.34±0.08, 0.21±0.03 on the fifth day post inoculation, corresponding with the ethanolic extract doses for *C. papaya* ranging from 100, 300, 500, 800 and 1000mg/kg body weight. Unlike other groups, the untreated (negative) control group had 11.33±1.97 average percentage parasitemia value on day 1 post inoculation and 18.15±1.49 on the fifth day indicating an increase in the parasitemia level compared with the group treated with combisunate as showed in table 3. Judging from the results displayed in tables 3 and 4, no single group in all the groups inoculated with *P. berghei* had total clearance.

This study showed a lower RBC count in *P. berghei* infections compared to the normal non-inoculated control. The cause and effect of malaria and anemia is complex and not fully understood. Infected RBCs display a reduced deformability and altered surface characteristics, which usually would lead to them being filtered and cleared by the spleen. However, the malaria parasite *P. berghei* has found a way to counter this protective measure. They modify their host cell membrane, which ultimately results to the cyto adherence of RBCs onto the endothelium. Anaemia in acute malaria is due to increase in haemolysis and decrease in the rate of production of red blood cells, increased destruction of parasitized red blood cells and accelerated removal of both parasitized and non-parasitized red blood cells. Other factors contributing to anaemia in malaria include increased red blood cell deformability, splenic phagocytosis and/or pooling [18]. In addition to anemia, a reduction in the number of PCV is another one of the more well-known haematologic changes observed in rats with malaria. This study supported that lower PCV among rats infected with *P. berghei* in comparison to the control group were notably important. Trends between increasing parasite density and an increase in the level of haematologic parameters were observed in this study. Leukocyte counts, especially lymphocytes and monocytes, were significantly higher in rats with high parasitemias compared to those with low and moderate parasitemias. However, lymphocyte and monocyte numbers were significantly higher in patients with low parasitemias compared to those with moderate and high parasitemias. The trend of decreasing Hb concentration with increasing levels of parasitemia was observed in this study. Low Hb concentrations were associated with increased parasite density.

5. Conclusion

The investigation of the antiplasmodial property of the ethanolic leaf extract of *Carica papaya* showed that the extract at all levels of dose do not have curative effects after four day administrations, but possessed antimalarial activity as revealed by the significant percentage parasitemia reduction. The extract also showed haematopoietic potential, increasing the levels of haemoglobin, packed cell volume and red blood cells. These further established the antimalarial potential of the plant extracts considering that antiplasmodial activity is closely related to haematopoietic activity and the lowering of liver enzymes concentrations. The result of the finding did not demonstrate a curative effect against

plasmodial infection in the treated groups. The antiplasmodial, haematopoietic, hepatic-enhancing and renalgesic effects of *Carica papaya* might therefore be as a result of any one or combination of the phytochemicals present in the plant. The use of the plant material in folkloric medicine is thus verified by this study.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

Ethical Approval for the use of adult wistar rats in this research work was obtained from Imo State Ministry of Agriculture and Natural Resources.

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