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Original paper

Carica papaya augments anti-malarial efficacy of artesunate in *Plasmodium berghei* parasitized mice

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ABSTRACT. Drug-herb interaction may lead to therapeutic failure or toxicities. This study investigates the effect of methanol extract of *Carica papaya* (papaya) on anti-malarial efficacy of artesunate and on hepato-renal toxicities in *Plasmodium berghei* infected mice. Five groups comprising of twenty-five mice were used for the study. Group 1 mice were non-infected and served as normal control while groups 2–5 were all parasitized. Group 2 mice were without treatment and served as parasitemia control. Group 3 mice were treated with 400 mg/kg of the extract alone while group 4 mice received 5 mg/kg of artesunate. The last group received a combination of 400 mg/kg of the extract and 5 mg/kg of artesunate. The treatment lasted five consecutive days during which daily packed cell volume and parasitemia levels were evaluated. At the end of the treatment period, mice were euthanized and blood samples were collected to determine some haematological parameters, liver and kidney function parameters and levels of oxidative stress. Co-administration of *Carica papaya* and artesunate significantly (P<0.01) reduced daily parasitemia load and significantly (P<0.01) mitigated drastic reduction in packed cell volume, red blood cells and haemoglobin levels. The combination significantly (P<0.01) attenuated oxidative stress and does not adversely affect white blood cells and differential white blood cells count as well as hepato-renal markers. Short-term co-administration of *Carica papaya* and artesunate in *Plasmodium berghei* infected mice is a positive drug-herb combination. This should be clinically explored for the purpose of malaria treatment in humans.

Keywords: artesunate, *Carica papaya*, malaria, drug interaction, *Plasmodium berghei*, efficacy

Introduction

Malaria has over the years remained a serious public health concern. It is caused by parasites of *Plasmodium* species and affects over 40% of the world's population [1]. Malaria is a major cause of morbidity and mortality especially in sub-Saharan Africa where pregnant women and children are at higher risks [2]. Globally, there were 219 million cases of malaria in 2017 and 435,000 related mortality. Africa recorded 92% and 93% of the prevalence and deaths respectively [3]. Adequate treatments aimed at reducing morbidity and related deaths are therefore required.

Artesunate has proven very effective against multi-drug resistant *Plasmodium falciparum* and hence a mainstay in malaria therapy. It has found use in the treatment of severe malaria with faster relieve of fever than seen with quinine [4,5]. Though a derivative of artemisinin, artesunate has a better absorption profile than the artemisinin combination, hence provides an advantage [6].

Medicinal plants used traditionally as anti-malarial agents from Africa's rich flora are part of newer areas where malaria therapy is sought [7,8]. One of such rich reservoirs is the plant *Carica papaya*. *Carica papaya* Linn (papaya) is the most common species of the Caricaceae family being the most cultivated. It is a tree of large berry fruits which reaches 3–10 centimeters in height. The fruits are consumed as food and provide a rich source of fibre, calcium, potassium, vitamin C, thiamine, foliate niacin, vitamin A, riboflavin, and iron [9]. The traditional uses of different parts of *Carica*

papaya for medicinal benefits have long been reported [10,11]. Some of the investigated biological activities include antimicrobial potentials [12], wound healing [13], anti-diabetic [14], antibacterial [15], antioxidant [16], anti-asthma, treatment of diarrhoea [17] and anti-malarial [18].

The concurrent use of orthodox medications alongside herbal remedies is common in West Africa. This could be before, during or after conventional anti-malarial therapy [19]. This practice which is usually without the consent of the healthcare expert may predispose to deleterious drug-herb interactions [20]. In such cases, the pharmacokinetic profile and hence pharmaco-dynamic outcomes of medications may be affected, to bring about either therapeutic failure or toxicity [21]. With the vast medicinal benefits of *Carica papaya* and generally reported safety [22] there may be possibility of concurrent use with artesunate for the purpose of treating malaria among rural dwellers.

This study investigated the effect of methanol leaf extract of *Carica papaya* on the anti-malarial efficacy of artesunate and on hepato-renal toxicities in parasitized mice.

Materials and Methods

Plant collection and preparation

Fresh leaves of *Carica papaya* were collected on a cold morning of February 2018 in Samaru-Zaria, Kaduna State, Nigeria. They were thereafter identified and authenticated by a taxonomist in the Biological Sciences Department of Ahmadu Bello University Zaria. The leaves were detached from the stem and washed under running water. The freshly collected leaves were allowed to dry at room temperature for about 14 days and afterwards pounded into coarse powder with a mortar and pestle. 100 g of the powdered plant material was extracted by cold maceration with 70% methanol. The weighed powder was soaked in 500 mL of the extraction solvent in an Erlenmeyer flask and left to stand for 48 hours within which intermittent shaking was done, and then filtered. The filtrate was concentrated by evaporation in a water bath at 40°C. The extract was weighed and stored in an air tight container. The percentage yield of the plant was calculated as shown below:

$$\text{percentage yield} = \frac{\text{weight of dried extract x100}}{\text{weight powdered plant}}$$

Animals

Male albino mice with an average weight of 21 grams were obtained from the Pharmacology Department, University of Nigeria, Nsukka and kept in clean cages in the animal house unit, College of Medicine, Gregory University, Uturu, Abia State. They were allowed to acclimatize for two weeks prior to the study and allowed access to food and water *ad libitum*. The Gregory University ethical considerations for animal use and care which complies with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were observed.

Drugs and chemicals

Artesunate (Mekophar Pharmaceuticals VD-10618-10) Methanol (Sigma Chemical Co. USA), Chloroform (Sigma Chemical Co. USA), Methanol extract of *Carica papaya* (MECP).

Phytochemical screening

This was carried out to determine the presence or absence of secondary metabolites according to the method of Trease and Evans [23].

Parasite inoculate

Donor mouse infected with *Plasmodium berghei* NK65 was obtained from National institute of Pharmaceutical Research and Development, Abuja, Nigeria. Parasitized erythrocytes were obtained via cardiac puncture from the donor mouse. Mice for the study were inoculated on day zero by administering 0.2 ml blood suspension intra-peritoneally which contains 10^6–10^7 of parasitized erythrocytes [24]. Four days after inoculation, mice with parasitemia level of 9–12% were selected and divided into four groups of 5 mice per group.

Experimental design

Group 1: Non-infected mice, served as normal control without treatment

Group 2: Infected mice, served as parasitemia control without treatment

Group 3: Infected mice, treated with 400 mg/kg of MECP

Group 4: Infected mice, treated with 5 mg/kg artesunate

Group 5: Infected mice, treated with 400 mg/kg of MECP and 5 mg/kg artesunate

All treatments were done orally for five continuous days within which parasitemia and packed cell volume were determined. After all drug

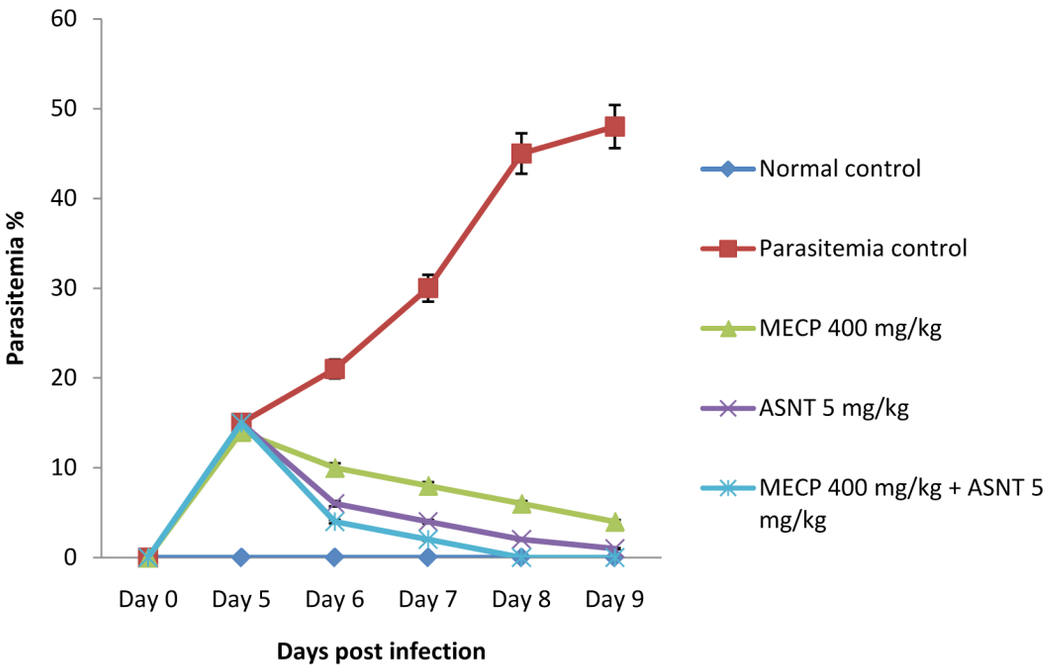

Figure 1. Effect of MECP and artesunate co-administration on daily parasitemia count in *P. berghei* parasitized mice
Values are Mean ± SEM, n = 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 5 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, Data analysed by split plot ANOVA

treatments, mice were euthanized and blood samples were collected into plain and EDTA sample containers for determination of biochemical and haematological parameters respectively.

Parasitemia determination

Blood samples were collected from the tail of each mouse with which thin blood film smears were made on microscopic slides [25]. The slides were fixed in methanol and thereafter stained using Giemsa stain with pH of 7.2. Three counts were made for erythrocytes in each 10–50 fields. The mean count was used as the parasitemia load of each animal.

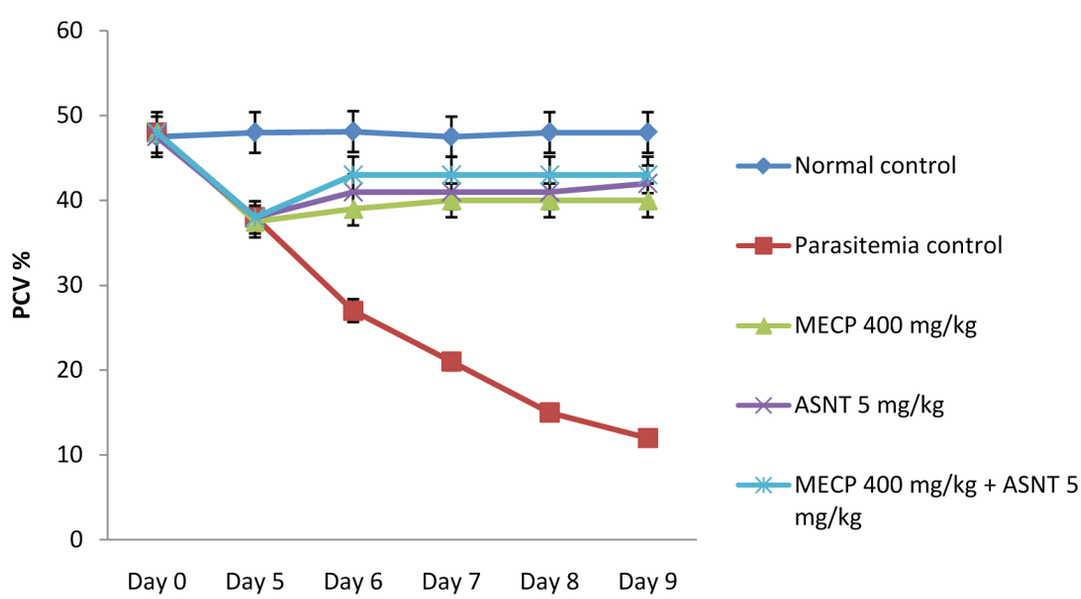

Figure 2. Effect of MECP and artesunate co-administration on daily PCV in *P. berghei* parasitized mice
Values are Mean ± SEM, n = 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 5 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, Data analysed by split plot ANOVA

Table 1. Effect of MECP and artesunate co-administration on renal function markers in *P. berghei* parasitized mice

Groups	Urea (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Normal control	33.31±0.84	4.14±0.90	4.31±0.37	1.13±0.43	1.60±0.71
Parasitemia control	67.21±0.44**	1.04±0.07*	1.76±0.65*	21.08±0.22**	19.02±.11**
MECP 400 mg/kg	34.73±0.11a	3.60±0.22 a	3.92±0.80a	2.18±0.21a	2.04±0.33a
ASNT 5 mg/kg	35.15±0.21 a	4.82±0.41a	4.82±0.31a	1.83±0.25a	1.99±0.31a
MECP 400 mg/kg + ASNT 5 mg/kg	36.16±0.31 a	3.17±0.17a	3.99±0.14a	1.03±0.61a	1.71±0.21a

Values are Mean ± SEM, n= 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 5 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, a = Significant different (p<0.05) compared to parasitemia control, * = Significant different (p<0.05) compared to normal control, ** = Significant different (p<0.01) compared to normal control, after analysis with one way ANOVA

Packed Cell Volume (PCV) determination

Blood samples were collected from tail of the mice with which PCV determinations were done. The formula below was used to determine the relative volume blood that is occupied by erythrocytes in percentage [1]:

Packed Cell Volume =

$$\frac{\text{Volume of erythrocytes in given volume of blood}}{\text{Total blood volume}}$$

Biochemical parameters

Levels of renal (urea, creatinine, uric acid, albumin and total protein) and hepatic markers [alkaline phosphatase (ALP), alanine amino-tranferase (ALT) and aspartate aminotransferase (AST)] were determined using the protocol described in Randox® diagnostic kits.

Haematological parameters

Some haematological parameters such as red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), neutrophils (N), lymphocytes (L), and eosinophils (E) were assessed by means of automated hematology analyser sysmex KX21, (SYSMEX, Corporation, Japan) [26].

Antioxidant assay

Malondialdehyde (MDA) was determined as described by Buege and Aust [27], while superoxide dismutase (SOD) was assayed using the method of Sun and Zigman [28]. The assay of catalase (CAT) was according to Aebi [29] and glutathione (GSH) as described by Sedlak and Lindsay [30].

Data analysis

Data analysis was done using SPSS version 22.

Table 2. Effect of MECP and artesunate co-administration on liver function markers in *P. berghei* parasitized mice

Groups	Alanine Transferase (U/L)	Aspartate Transferase (U/L)	Alkaline Phosphatase (U/L)
Normal control	31.26±1.40	39.90±0.60	40.11±1.01
Parasitemia control	91.12±1.17**	94.23±1.23**	96.23±1.89**
MECP 400 mg/kg	30.41±2.11a	41.11±1.90a	42.37±1.90a
ASNT 5 mg/kg	31.91±2.11a	40.22±1.68a	43.32±2.20a
MECP 400 mg/kg + ASNT 5 mg/kg	31.86±1.20a	40.35±1.75a	41.24±1.75a

Values are Mean ± SEM, n= 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 5 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, a = Significant different (p<0.05) compared to parasitemia control ** = Significant different (p<0.01) compared to normal control, after analysis with one way ANOVA

Haematological and biochemical data were analysed using One Way Analysis of Variance followed by Bonferroni post hoc test. Data for daily PCV and parasitemia load were analysed using split plot ANOVA followed by Bonferroni post hoc test. Data were considered significant at (P<0.05).

Results

Extract yield and phytochemistry

The percentage yield of the methanol leaf extract of *Carica papaya* was 17.2%. Phytochemistry revealed the presence of alkaloids, cardiac glycosides, anthraquinones, flavonoids, tannins, reducing sugars, saponins and steroids.

Effect of MECP and artesunate co-administration on daily parasitemia count in P. berghei parasitized mice

On the first day of treatment (day 5), all parasitized mice had significantly (P<0.05) higher parasitemia load compared to pre-infection (day 0). The mean parasitemia level of mice treated with MECP and artesunate either as single agents or in combination showed a significant (P<0.01) reduction in daily parasitemia load when compared to pre-treatment values. Interestingly, the parasitemia load for the group of mice treated with a combination of MECP and artesunate showed lower levels of parasitemia when compared to the mice that received both agents singly (Fig. 1).

Effect of MECP and artesunate co-administration on daily PCV in P. berghei parasitized mice

At the start of treatment (day 5), all parasitized rats had PCV levels significantly (P<0.05) lower than the pre-infection values (day 0). Untreated infected mice (parasitemia control) showed a drastic reduction in PCV values in the successive days which was significantly (P<0.01) lower when compared to normal values. This reduction was significantly (P<0.01) prevented following treatment with MECP and artesunate singly and in combination. In addition, mice that received a combination of MECP and artesunate had higher PCV values than when they were administered singly (Fig. 2).

Effect of MECP and artesunate co-administration on renal markers in P. berghei parasitized mice

All markers for renal function accessed were significantly different (P<0.01) in the non-treated infected mice when compared to normal control. Mice treated with MECP and artesunate either singly or in combination did not show any significant change in any of the markers when compared to the normal control (Table 1).

Effect of MECP and artesunate co-administration on liver function markers in P. berghei parasitized mice

Liver function markers (AST, ALT and ALP) were significantly (P<0.01) higher in the parasitemia control mice when compared to the normal control. These markers were not significantly altered after treatment with either MECP, artesunate or both when compared to the values of the normal control mice (Table 2).

Table 3. Effect of MECP and artesunate co-administration on some haematological markers in *P. berghei* parasitized mice

Groups	RBC $\times 10^6$/µl	Hb g/dl	WBC $\times 10^6$/µl	N (%)	M (%)	L (%)	E (%)
Normal control	56.31±0.14	16.12±1.10	15.31±0.37	18.33±1.43	81.60±0.11	0.39±0.01	0.41±0.01
Parasitemia control	17.31±0.64**	4.14±0.17*	41.16±0.65*	32.11±0.12**	119.02±.21*	11.01±0.09	10.10±0.11
MECP 400 mg/kg	51.13±0.21[a]	14.10±0.22 [a]	13.94±0.21[a]	16.28±0.21[a]	82.14±0.13[a]	0.90±0.19	0.81±0.12
ASNT 5 mg/kg	50.10±0.81[a]	14.88±0.51[a]	13.12±1.31[a]	16.81±0.23[a]	80.19±0.36[a]	1.01±0.01	0.99±0.90
MECP 400 mg/kg + ASNT 10 mg/kg	53.12±0.31[a]	14.17±0.97[a]	13.12±1.14[a]	17.14±0.61[a]	80.71±0.71[a]	1.02±0.90	0.70±0.01

Values are Mean ± SEM, n = 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 10 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, [a] = Significant different (p<0.05) compared to parasitemia control ** = Significant different (p<0.01) compared to normal control, after analysis with one way ANOVA

Table 4. Effect of MECP and artesunate co-administration on oxidative stress markers in *P. berghei* parasitized mice

Groups	MDA (µg/ml)	CAT (IU/l)	SOD (IU/l)	GSH (IU/l)
Normal control	3.61±0.74	44.56±0.89	48.11±0.37	21.11±0.67
Parasitemia control	77.21±0.84**	8.84±1.02*	8.17±1.75*	4.98±1.12**
MECP 400 mg/kg	4.63±0.91	40.69±0.88	43.12±0.10	20.11±0.71
ASNT 5 mg/kg	5.05±0.71	39.99±0.71	41.82±0.71	19.63±0.98
MECP 400 mg/kg + ASNT 5 mg/kg	4.19±0.81	44.01±0.19	46.12±0.65	21.03±0.41

Values are Mean ± SEM, n = 5, oral route, ASNT 5 mg/kg = treated with artesunate at dose of 5 mg/kg, MECP 400 mg/kg = treated with methanol extract of *Carica papaya* at a dose of 400 mg/kg, ** = Significant different (p<0.01) compared to normal control, after analysis with one way ANOVA

Effect of MECP and artesunate co-administration on some haematological markers markers in P. berghei parasitized mice

The parasitemia control group showed significantly lower RBCs, Hb and significantly ($P \leq 0.05$) with higher WBC and differential white blood count when compared to the normal control. Values for these markers after treatment with MECP and artesunate either singly or in combination did not differ from that of the normal control (Table 3).

Effect of MECP and artesunate co-administration on oxidative stress markers in P. berghei parasitized mice

Concurrent administration of MECP and artesunate significantly (P<0.01) reduced levels of lipid peroxidation in comparison to the parasitemia control. In addition, this combination significantly (P<0.01) increased the levels of endogenous antioxidants when compared to the parasitemia control. Also, mice treated with MECP in combination with artesunate showed better prognosis for oxidative stress than with either agent (Table 4).

Discussion

Plant based medicines are known to contain some active ingredients which are responsible for their characteristic smell, colour or biological activities [31]. Phytochemical constituent present in the methanol extract of *Carica papaya* such as alkaloids, cardiac glycosides, anthraquinones, flavonoids, tannins, reducing sugars, saponins and steroids in this study corroborates earlier reports [18]. Some of these constituents like flavonoids are reported to possess anti-malarial activity [32] and hence may be responsible for the observed anti-malarial activity shown by the extract. Active ingredients such as terpenoids, flavonoids, tannins and polyphenols have been documented to have enzyme-inhibitory potentials as well as protein binding ability [33,34]. This may suggest that the extract acts by possibly inhibiting some key enzymes within the parasite.

Drug interaction may ensue between two or more drugs (drug-drug interaction) or with plant based medicines (drug-herb interaction). The resulting effects may be additive or synergistic where the drug's efficacy is enhanced or antagonistic where drug's efficacy is reduced. Also, a new response which neither of both agents may possess may be produced leading to toxicities. Prevention of the drastic reduction in Hb, RBCs and PCV levels, an index for anaemia [35] in mice treated with a combination of the extract and artesunate suggests an additive or synergistic effect in ameliorating anaemia, which is a characteristic of malaria infected mice [36]. This synergy is consistent with the rapid eradication of parasitemia load observed when both agents were concurrently administered. This result is comparable to findings from similar reports where the anti-malarial efficacy of halofantrine [19], chloroquine [37] and artesunate [38] were significantly enhanced by *Khaya grandifolia, Vernonia amygdalina* and *Telfaria occidentalis* respectively.

Several reports have shown impairment of renal function as a clinical feature in malaria patients [39–41]. Increase in blood levels of urea, uric acid

and creatinine with reduced albumin and total protein levels have been used as markers for kidney toxicity [42]. Changes in these markers in parasitized control mice in this study are indicative of renal dysfunction. Treatment with methanol extract of *Carica papaya* alone did not affect renal indices and hence suggests its relative safety on the kidneys. This agrees with previous findings where renal markers were unchanged after oral chronic dosing with *Carica papaya* at similar and higher doses [22]. Similarly, renal markers after concurrent administration of *Carica papaya* and artesunate did not differ from that of the normal mice. This may indicate renal safety of this drug-herb combination in *P. berghei* parasitized mice.

The role of the liver in metabolism and detoxification of harmful substances makes it a potential target for toxicity. All markers for liver function were significantly elevated in the parasitized non-treated mice, indicating signs of hepato-toxicity [43]. The values for these markers were similar with that of the normal mice after treatment with the extract alone and in combination with artesunate. This may imply that co-administration of both agents does not adversely affect liver functions.

Pathophysiologic mechanisms involved in malaria due to *P. falciparum* originate from different sources of oxidative stress [44,45]. This was manifested in this study by significant elevation of MDA in non-treated parasitized mice and a decline in levels of CAT, SOD and GSH. Values for these markers obtained after treatment with methanol extract of *Carica papaya* alone revealed that the extract prevented oxidative stress. This is in agreement with earlier reports on the use of the plant as an antioxidant [16]. Also, end product of lipid peroxidation and the values of endogenous antioxidants did not differ from normal control when the extract was combined with artesunate. This suggests that the combination of the two agents may not mediate oxidative stress but may mitigate oxidative stress in *P. berghei* parasitized mice.

Carica papaya does not affect anti-malarial efficacy of artesunate after short term co-administration in *P. berghei* parasitized mice. In addition, liver and kidney functions as well as some haematological (WBCs, neutrophils, monocytes and eosinophils) and oxidative stress markers were not adversely affected by the combination. Hence, this combination may be further explored clinically for the treatment of malaria in humans.

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