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Original paper

Beneficial interaction between *Ficus platyphylla* and artesunate on cytokines TNF-α and IL-10 and oxidative stress in *Plasmodium berghei*-infected mice

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ABSTRACT. This study investigates the effects of *Ficus platyphylla* and artesunate combination on the prognosis of malaria in parasitized mice. Five groups (n=6) of mice were used. Groups one and two were normal control (NC) and parasitemia control (PC) respectively. Groups 3–5 were all parasitized and administered 300 mg/kg of the extract (FPE_{300}), 5 mg/kg artesunate (ART_5), and a combination of both (ART_5+FPE_{300}) respectively. Within the five days of oral treatments, daily packed cell volume (PCV) and parasitemia load were measured. The experiment was terminated by cervical dislocation. Blood samples were immediately taken by cardiac puncture and separated into plasma and serum. Plasma samples were used to determine erythrocytes, haemoglobin and leukocytes while some cytokines (TNF-α, IL-10), antioxidant profile (malondialdehyde, reduced gluthathione, catalase, superoxide dismutase), renal (urea, creatinine, uric acid), and hepatic markers (alanine transferase, aspartate transferase, alkaline phosphatase) were assessed from serum. Administration of ART_5+FPE_{300} significantly (P<0.01) reduced daily parasitemia load and PCV compared to PC, with erythrocytes, haemoglobin and leukocytes values being comparable to NC. In addition, this drug-herb combination significantly (P<0.05) mitigated inflammatory response, oxidative stress and hepato-renal toxicities respectively compared to PC. Co-administration of *Ficus platyphylla* and artesunate improves the prognosis of malaria and the resulting pathological consequences by inhibiting inflammatory response and oxidative stress in parasitized mice.

Keywords: *Ficus platyphylla*, artesunate, malaria, oxidative stress, drug-interaction, *Plasmodium berghei*

Introduction

Malaria, a *Plasmodium* parasite disease has persisted globally as a public health menace affecting almost one third of human population across the world. This disease has led to numerous deaths especially in sub-Saharan Africa where it affects mainly children and women in gestation [1]. About 219 million malaria cases leading to 435,000 mortalities were recorded worldwide as at 2017 [2]. Hence, treatment strategies that will mitigate malaria related morbidity and deaths are of paramount interest. Artesunate is a drug of choice used in treatment of multi-drug resistant malaria. It is useful in severe malaria and produces faster relieve for fever than quinine [3]. Although artesunate is a derivative of artemisinin, it provides an advantage over the artemisinin combination because of its good pharmacokinetics profile [4]. In recent years, attention has been given to medicinal plants as possible sources for development of newer and more effective antimalarial agents [5] or for the purpose of combination therapy [6]. These plants are usually accepted in Tropical Africa because they are easily accessible at little or no cost and due to high costs of conventional orthodox medications [7]. One of such plants is *Ficus platyphylla*, a plant based medicine widely distributed across Africa. It is predominantly common in Ghana and Northern Nigeria where it is used locally by Hausa natives for the management of pain and inflammation, pyschosis, insomnia, depression, epilepsy and malaria [8]. Some of these claims have been scientifically validated in rodents. For instance, the anticonvulsant, psychopharmacology and neuro-behavioral studies have been documented [9,10].

Additionally, the high-performance liquid chromatography (HPLC) finger print as well as phytochemical constituents have been studied, and reported to contain tannins, saponins and flavonoids [11]. In the past decades, 1, 4-dimethylbenzenedicarboxylic acid ester, flavonoids and coumarins content of *Ficus platyphylla* were isolated and characterized [12,13]. Furthermore, the antimalarial efficacy and safety of the plant has been established scientifically in rat models [14,15]. A more recent study also reported hepato-renal safety after artesunate and *Ficus platyphylla* were co-administered in rats [16]. Thus, it is not uncommon to see concurrent use of this plant alongside artesunate for perceived antimalarial benefits among rural dwellers. This is because concurrent use of orthodox medications alongside medicinal plants is commonly reported in West Africa. However, there is no documented scientific validation supporting this drug-herb combination for the purpose of malaria treatment. Hence, this research investigates the effect of *Ficus platyphylla* and artesunate combination on the prognosis of malaria in *Plasmodium berghei*-parasitized mice.

Materials and Methods

Plant collection and extraction

Large stem of the plant were obtained from a local farm in May of 2018 in Tudun Wada, Zaria. It was thereafter authenticated by a botanist at the Department of Plant Sciences, A.B.U., Zaria where it was compared with a previously deposited voucher number (900106). The barks were detached from the stems and were allowed to dry at approximately 27ºC before they were pounded with a mortar and pestle. The coarsely powdered plant material was extracted by cold maceration by soaking about 250 grams of the powder in methanol as previously described in [6,18,22]. This was allowed to stand in a flask that was shaken at intervals for forty-eight hours. The mixture was afterwards filtered while the filtrate was collected in a conical flask and placed on a water bath maintained at 50ºC to obtain a dark-brownish extract. Fresh stock solutions were made daily from the extract and administered to the animals.

Animals

Swiss albino mice were purchased from Federal University of Agriculture Umudike, Abia State. The animals were transported in clean fabricated aluminium cages with saw dust beddings. Acclimatization period was 15 days before the research started. Access to animal feed and water were ensured. The study was conducted under natural 12 hours day/night cycle at approximately 26ºC in a relatively noise free environment.

Drug, chemicals and equipments

Artesunate (Kermel, 1690), methanol extract of *Ficus platyphylla*, chloroform (JHD 9.01863.205), rat's immunosorbent kits (ElabScience® USA) Randox kits®.

Microhaematocrit centrifuge (Hermle, Z207H), microhaematocrit reader (Hawsley, England), weighing scale (Ohaus NJ07054, USA), (spectrophotometer Jenway, 6850).

Parasite inoculate

A *Plasmodium berghei* NK65-infected mouse was purchased from NIPRD Abuja to serve as a donor. Parasites were collected from the donor by means of cardiac puncture to obtain parasitized red blood cells. The cells were introduced into the experimental animals by means of intraperitoneal administration of 0.2 ml of 10^6–10^7 parasitized cells in a blood suspension. The day of inoculation was recorded as day zero. Four days later, the mice were assessed for parasitemia levels. Those with parasitemia level of between 10 to 12 percent were grouped for the study.

Experimental design

Five groups (n=6) of mice were used. A group of non-treated normal mice and another of parasitized mice served as normal control (NC) and parasitemia control (PC), respectively. The third group composed of parasitized mice and received 300 mg/kg of *Ficus platyphylla* extract (FPE_{300}). This was the dose at which the antimalarial activities were previously reported [14]. The fourth group composed of parasitized mice and received treatment with 5 mg/kg artesunate (ART_5) while the fifth group composed of parasitized mice which received a combination of artesunate and *Ficus platyphylla* (ART_5+FPE_{300}). Within the five days of oral treatments, packed cell volume (PCV) and parasitemia load were measured. The experiment was terminated by cervical dislocation. Blood samples were immediately taken by cardiac puncture into heparinized and plain vacutainers and centrifuge 4000 g for 10 minutes to obtain serum and plasma samples respectively which were stored

at 4ºC until they were analyzed. Plasma samples were used to determine some haematological parameters [erythrocytes (RBCs), haemoglobin (Hb), and leukocytes (WBCs)]. Some cytokines (TNF-α, IL-10), antioxidant profile [malondialdehyde (MDA) reduced gluthathione (GSH), catalase (CAT), superoxide dismutase (SOD)], renal (urea, creatinine, uric acid), and hepatic markers [alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP)] were assessed from serum.

Parasitemia determination

Smear of thin blood films were made from samples collected from snapped tail tip of the mice using microscopic slides [17]. The slides were thereafter stained with Giemsa at pH of 7.2 after fixing in methanol. Triplicate erythrocyte counts were taken in each 10–50 fields while the mean count was recorded as parasitemia load for individual mouse. Percentage parasitemia and suppression levels were obtained using Equation 1:

$$\% \text{ parasitemia} = \frac{\text{No. of parasitised RBCs}}{\text{Total No. of RBCs}} \times 100$$

Packed Cell Volume (PCV) determination

By means of heparinized capillary tubes, blood samples were collected from the mice centrifuged using microhaematoctrit centrifuge (Hermle, Z207H). PCV for each animal was calculated was using Equation 2:

$$\text{PCV} = \frac{\text{Volume of RBCs in a given blood volume}}{\text{Total volume of blood}} \times 100$$

Haematological parameters

Blood indices such as RBCs, Hb, and WBCs were assayed using an automated hematology analyser sysmex KX21, (SYSMEX, Corporation, Japan).

Biochemical indices

Malondialdehyde (MDA), an index for oxidative stress and endogenous antioxidants reduced glutathione (GSH) catalase (CAT) and superoxide dismutase (SOD) was measured using spectophotometry method previously described by Oraebosi et al. [18]. Interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) were assayed using specific rat's immunosorbent kits (ElabScience® USA) according the protocol described in the manual. Renal (urea, creatinine, uric acid), and hepatic markers [alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP)] were determined using procedure described in the manual of Randox® diagnostic kits as previously adopted [16].

Data analysis

This was done using SPSS version 23. Single point data (haematology and biochemistry) were analyzed with one way ANOVA and Tukey post hoc test. Those collected over time (daily parasitemia and PCV) were analyzed using split plot ANOVA and Bonferroni post hoc test. *P*-value of less than or exactly 0.05 were taken as significant.

Ethical statement

The European Communities Ethical Council directives (86/609/EEC) which corroborates the regulations of Gregory University Committee on Animal Use and Care (GUCAUAC) and Standard Ethical Guidelines (NIH Publication No. 85-23, revised, 1996) were adhered.

Results

Effect of Ficus platyphylla and artesunate combination on daily parasitemia load in P. berghei parasitized mice

At the commencement of treatment (fifth day), the percentage parasitemia for all groups of infected mice was significantly ($P<0.05$) higher than values before inoculation. There was a progressive significant ($P<0.01$) increase in parasitemia load in the PC group throughout the study. All of the groups treated with either FPE_{300}, ART_5 or FPE_{300}+ART_5 showed significant ($P<0.01$) decrease in levels of daily parasitemia levels when compared to their respective initial values. It is however of interest to note that parasitemia level observed following treatment with FPE_{300}+ART_5 was progressively lower than with FPE_{300} or ART_5 singly. The result is shown in figure 1.

Effect of Ficus platyphylla and artesunate combination on PCV levels in P. berghei parasitized mice

The PCV values for all groups of infected mice were significantly ($P<0.05$) lower at the start of treatments (fifth day) than values before inoculation (day zero). There was however a significant ($P<0.01$) and progressive reduction in PCV levels for the PC group throughout the study. In contrast,

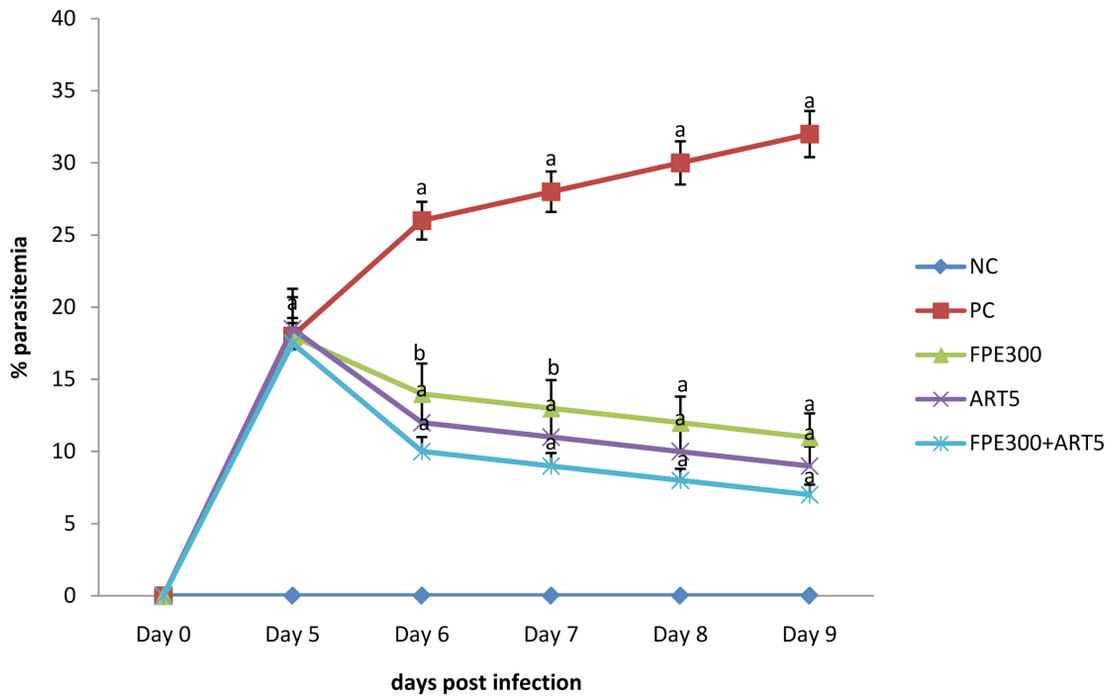

Figure 1. Effect of *Ficus platyphylla* and artesunate combination on parasitemia progression in *P. berghei* parasitized mice. Values are mean±SEM, n=6, NC=normal control, PC=parasitemia control, oral route, ART_5 =artesunate 5 mg/kg, FPE_{300}=*Ficus platyphylla* 300 mg/kg, [a]=(P<0.01) versus day 0, [b]=(P <0.05) versus day 0, Split plot ANOVA

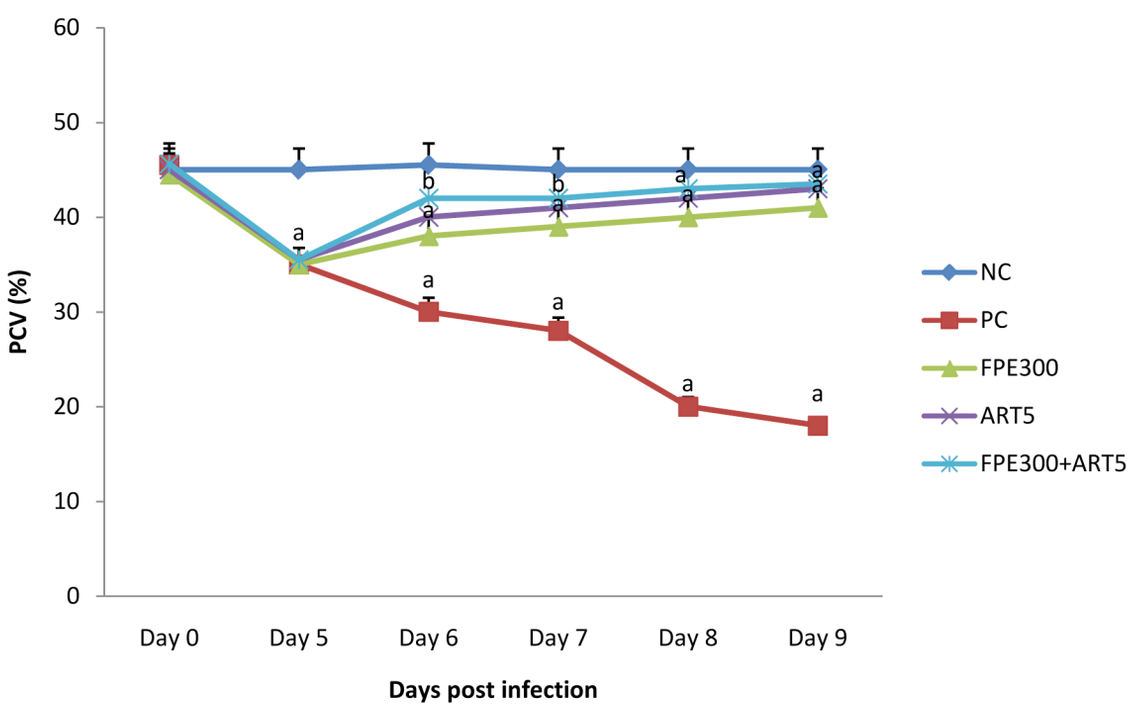

Figure 2. Effect of *Ficus platyphylla* and artesunate combination on PCV levels in *P. berghei* parasitized mice. Values are mean±SEM, n=6, NC=normal control, PC=parasitemia control, oral route, ART_5 =artesunate 5 mg/kg, FPE_{300}=*Ficus platyphylla* 300 mg/kg, [a]=(P<0.01) versus day 0, [b]=(P <0.05) versus day 0, Split plot ANOVA

treatment with either FPE_{300}, ART_5 or FPE_{300}+ART_5 produced significantly (P<0.01) higher PCV values than the PC group. Also, PCV value for FPE_{300}+ART_5 treated group was higher

Table 1. Effect of ALA and artesunate combination on RBCs, Hb and WBCs levels in *P. berghei* parasitized mice

Groups	RBC $\times 10^6/\mu l$	Hb g/dl	WBC $\times 10^6/\mu l$
NC	55.61±0.11	16.86±1.21	16.45±0.34
PC	15.21±0.14^c	3.19±0.76^b	44.76±0.22^b
FPE_{300}	45.18±0.89^a	10.18±0.74^a	21.11±0.81^a
ART_5	50.19±0.99^a	14.95±0.67^a	19.11±1.53^a
FPE_{300}+ART_5	52.19±0.61^a	14.99±0.32^a	18.82±1.89^a

Explanations: values are mean±SEM, n=6, NC=normal control, PC=parasitemia control, oral route, ART_5=artesunate 5 mg/kg, FPE_{300}=*Ficus platyphylla* 300 mg/kg, ^{a}=(P<0.05) versus PC ^{b}= (P<0.05) versus NC, ^{c}=(P<0.01) versus NC, one way ANOVA and Tukey post hoc

than the values when both agents were administered individually. The result is presented in figure 2.

Effect of Ficus platyphylla and artesunate combination on RBCs, Hb and WBCs levels in P. berghei parasitized mice

These values are shown in table 1. The values for RBCs and Hb were shown to be significantly (P≤0.01) lower with significantly (P≤0.05) higher WBCs in the PC when compared to NC. The values for these indices following treatment with either FPE_{300}, ART_5 or FPE_{100}+ART_5 is comparable to that of the NC group. Also, the group of mice treated with FPE_{100}+ART_5 showed improved prognosis for blood indices than with either agent.

Effect of Ficus platyphylla and artesunate combination on antioxidant levels in P. berghei parasitized mice

This is presented in table 2. Levels of lipid peroxidation end product was significantly (P<0.01) higher in the PC group with lower protective antioxidant enzymes (CAT, GSH, SOD) when compared to the NC. Co-administration of *Ficus platyphylla* and artesunate produced a significant (P<0.01) decrease in lipid peroxidation in comparison to PC. Additionally, concurrent administration of both agents significantly (P<0.01) elevated SOD, GSH and CAT in comparison to PC.

Effect of Ficus platyphylla and artesunate combination on TNF-α level in P. berghei parasitized mice

The PC group had significantly (P<0.01) higher TNF-α value in comparison to NC. Administration of the extract either alone or alongside artesunate produced significantly (P<0.05) lower TNF-α values in comparison to PC. However, the group treated with the drug-herb combination had apparently lower values than when the agents were administered singly. The result is shown in figure 3.

Effect of Ficus platyphylla and artesunate combination on 1L-10 levels in P. berghei parasitized mice

The PC group had significantly (P<0.01) lower

Table 2. Effect of *Ficus platyphylla* and artesunate combination on antioxidant levels in *P. berghei* parasitized mice

Groups	MDA (µg/ml)	CAT (IU/l)	SOD (IU/l)	GSH (IU/l)
NC	4.01±0.99	47.36±0.76	49.14±0.33	23.16±0.87
PC	69.52±0.81^b	9.94±1.32^b	9.23±1.35^b	3.18±1.32^b
FPE_{300}	5.11±0.29^c	43.25±0.98^c	43.12±0.10^c	19.13±0.74^c
ART_5	7.12±0.97^c	39.45±0.71^c	40.44±0.21^c	16.43±0.91^c
FPE_{300}+ART_5	4.19±0.12^c	44.01±0.19^c	46.12±0.65^c	21.03±0.41

Explanations: see table 1

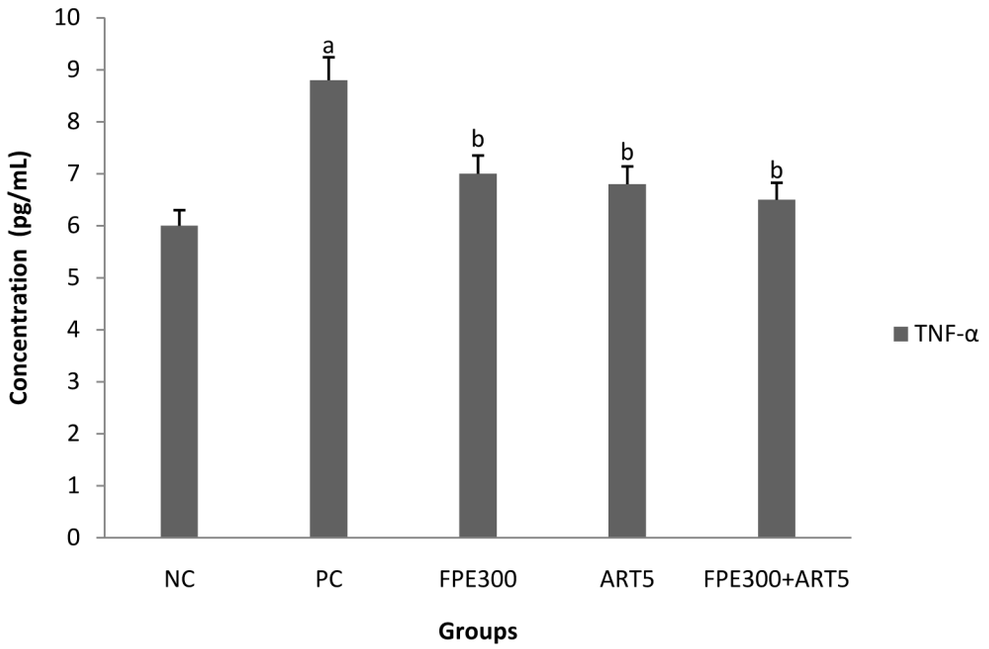

Figure 3. Effect of *Ficus platyphylla* and artesunate combination on TNF-α levels in *P. berghei* parasitized mice Values are mean±SEM, n=6, NC=normal control, PC=parasitemia control, oral route, ART_5 =artesunate 5 mg/kg, FPE_{300}=*Ficus platyphylla* 300 mg/kg, [a]=(P<0.01) versus NC [b]=(P<0.05) versus PC, one way ANOVA and Tukey post hoc

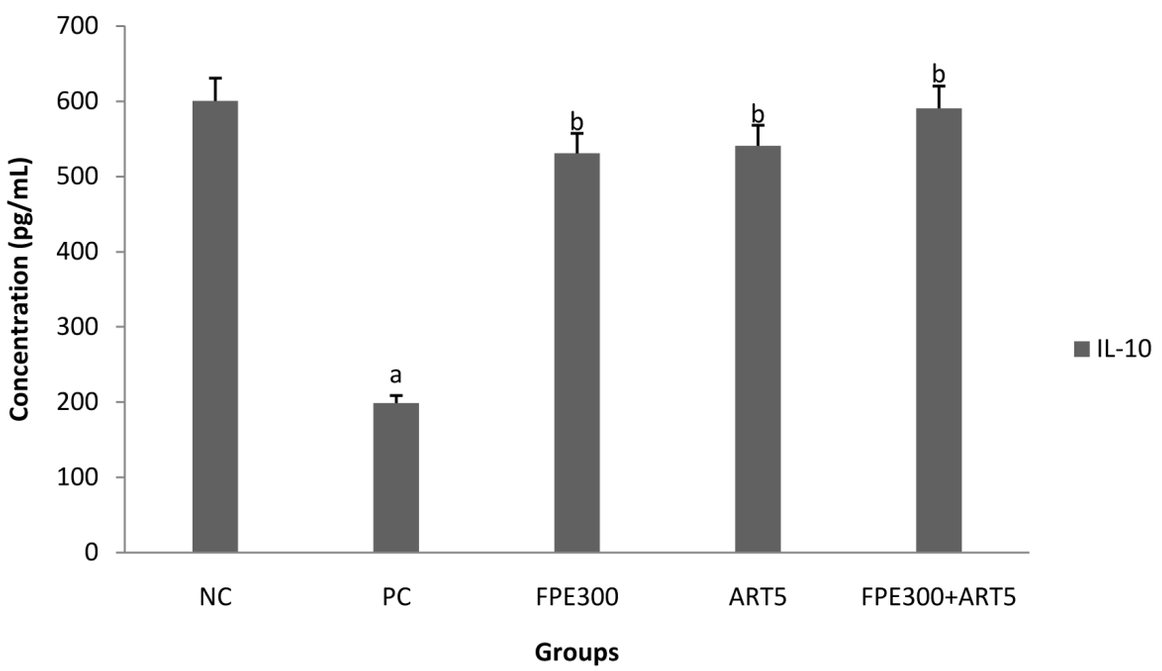

Figure 4. Effect of *Ficus platyphylla* and artesunate combination on 1L-10 levels in *P. berghei* parasitized mice Values are mean±SEM, n=6, NC=normal control, PC=parasitemia control, oral route, ART_5 =artesunate 5 mg/kg, FPE_{300}=*Ficus platyphylla* 300 mg/kg, [a]=(P<0.01) versus NC [b]=(P<0.05) versus PC, one way ANOVA and Tukey post hoc

IL-10 value in comparison to NC. Administration of FPE_{300} alone or when combined with ART_5 produced significantly (P<0.05) higher IL-10 values in comparison to PC. Also, the group that received FPE_{300}+ART_5 had apparently higher values of IL-10 than when the agents were administered singly.

Table 3. Effect of *Ficus platyphylla* and artesunate combination on hepato-renal indices in parasitized mice

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Alanine Transferase (U/l)	Aspartate Transferase (U/l)	Alkaline Phosphatase (U/l)
NC	34.11±0.24	1.18±0. 30	1.80±0.31	30.56±1.70	39.11±0.30	40.67±1.34
PC	68.41±0.74^{b}	20.10±0.11^{b}	20.12±.11^{b}	93.16±1.07^{b}	95.55±1.93^{b}	95.73±1.29^{b}
FPE_{300}	35.76±0.21^{a}	2.78±0.33^{a}	2.17±0.43^{a}	32.61±1.11^{a}	42.51±1.30^{a}	43.67±1.30^{a}
ART_5	36.15±0.54 ^{a}	1.92±0.45^{a}	2.01±0.31^{a}	32.98±1.71^{a}	41.13±1.45^{a}	43.99± 1.40^{a}
FPE_{300}+ART_5	35.23±0.71 ^{a}	1.08±0.32^{a}	1.83±0.41^{a}	30.89 ±1.56^{a}	40.95±1.23^{a}	41.02±1.65^{a}

Explanations: see table 1

The result is shown in figure 4.

Effect of Ficus platyphylla and artesunate combination on hepato-renal indices in parasitized mice

All renal (urea, creatinine, uric acid) and hepatic (AST, ALP, ALT) markers for the PC group were significantly (P<0.01) different from NC group. Values for these markers were significantly (P<0.01) restored after individual and combined treatment with FPE_{300} and ART_5. The group receiving the combination showed better prognosis with values similar to that of the NC group. This is shown in table 3.

Discussion

Malaria is a serious public health challenge especially in developing countries and throughout the sub-Saharan Africa. It is even more worrisome that various resistant strains of malaria parasites have emerged for currently used antimalarial agents such as artemisinin [19] and chloroquine [20]. Hence, there is need for search for newer remedies from plant based medicines. Medicinal plants are known to be rich reservoirs for biologically active substances which confer on them their characteristic smell, taste and pharmacological activities. Previous studies have shown that *Ficus platyphylla* possess flavonoids, tannins and saponins [11]. Flavonoids were previously shown to possess antimalarial potentials and could be the reason for the antimalarial properties of the extract as seen in this study. In addition, flavonoids have been previously reported to have good protein binding as well as enzyme inhibitory potentials [21]. Hence the antimalarial activity observed from the extract may be as a result of inhibitory effects of the extract on some important enzymes within the parasite as previously suggested [14].

A drug-herb interaction may occur when drugs and medicinal plants are concurrently administered. This may either be beneficial where medicinal plants abrogate known and suspected drug toxicities [18,22] and/or augments drug's efficacy [6,23] or harmful where drug toxicities are increased or efficacy reduced. In this study, the drug-herb combination was seen to rapidly eradicate daily parasitemia levels and improve prognosis of malaria markers in mice. Reduction in haemoglobin, RBCs and PCV are used as indicators for malaria in mice [24]. Hence prevention of severe decrease in these indices observed in this present study following the drug-herb combination is indicative of a beneficial drug-herb interaction. This may suggest an additive or synergistic interaction between artesunate and the extract against malaria in mice. These findings corroborate similar studies where *Telfaria occidentalis* and *Carica capaya* enhanced antimalarial efficacy of artesunate in mice [6,25]. In addition, the antimalarial efficacy of chloroquine and halofantrine were shown to be augmented by *Vernonia amagdalina* [26] and *Khaya grandifolia* [5] respectively in similar reports.

Malaria parasite infection in its host triggers series of events that lead to synthesis and release of reactive oxygen species (ROS). Infection with *Plasmodium falciparum* is reported to initiate hepatic release of free radicals from the mitochondria and may explain the manifestation of oxidative stress and apoptosis associated with the infection [27]. Furthermore, studies have shown that free radical production in *Plasmodium falciparum*-infected red blood cells is twice higher than in normal conditions [28]. This is similar to findings from this present study where oxidative stress was manifested with a dramatic increase in MDA levels followed by drastic reduction in protective endogenous antioxidant enzymes in the parasitemia control group. Oxidative

stress was however ameliorated after treatment with the extract with a decrease in MDA levels and increase in protective enzymes. This corroborates recent findings that reported the antioxidant potentials of the *Ficus platyphylla* [29]. Interestingly, the protective antioxidant enzymes and levels of oxidative stress marker were similar to that of the normal control following treatment with artesunate alongside the extract. This may imply that the extract combined with artesunate to mitigate plasmodium parasite-mediated oxidative stress in mice.

During the process of breakdown of haemoglobin, free radicals are produced in the food vacuole of the plasmodium and are known to trigger the release of TNF-α and IL-1 with other pro-inflammatory cytokines [30]. This is usually due to the release of haemozoin at the erythrocytic phase of malarial infection. This process releases free heme, a strong initiator of ROS. This may explain the elevated levels of TNF-α observed in the infected control group in this study and agrees with findings of Oluba et al. [31] who reported elevated TNF-α and decreased IL-10 in parasitized mice. IL-10 is cytokine with anti-inflammatory properties, thus, higher levels of IL-10 may inhibit levels of TNF-α and its pro-inflammatory activities. The lower levels of TNF-α and increase in IL-10 following treatment with *Ficus platyphylla* either singly or in combination with artesunate may suggest that this combination may be capable of improving the prognosis of malarial infections and the resulting pathological consequences by inhibiting haemazoin accumulation. These is because some conventional antimalarial drugs act through this pathway and have innate haemozoin inhibitory effects which helps promote parasite destruction by stimulating free heme buildup.

Over the years, elevated serum levels of uric acid, creatinine and urea and that of ALT, ALP and AST have served as indices for kidney and liver diseases respectively [16]. Values for these markers in the infected control group signify renal and liver dysfunction. Treatment with the extract either alone or combination with artesunate shows that levels of these markers did not differ from that of the normal control. This agrees with previous studies where administration *Ficus platyphylla* singly for 28 continuous days did not alter renal and hepatic functions in rodent models [15]. Interestingly also, co-administration of *Ficus platyphylla* and artesunate has been recently shown not to affect kidney and liver functions in rats [16]. Findings from the current study may further suggest the efficacy and hepato-renal safety of this drug-herb combination in parasitized mice.

In conclusion, co-administration of *Ficus platyphylla* and artesunate improves the prognosis of malaria with subsequent mitigation of inflammatory response and oxidative stress in *Plasmodium berghei* parasitized mice. The drug-herb combination also prevents hepato-renal dysfunction in *Plasmodium berghei* parasitized mice. This may give an insight for similar studies in human subjects for the purpose of exploring potential clinical benefits.

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