FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 2, April, 2024, pp 29 - 37
DOI: https://doi.org/10.33003/fjs-2024-0802-2160

MODULATION OF CYTOKINE EXPRESSION AND ANTIMALARIAL ACTIVITY OF RESIDUAL AQUEOUS FRACTION OF THE METHANOL LEAF EXTRACT OF *FICUS ASPERIFOLIA* IN *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT

Malaria is an inflammatory cytokine driven disease that sometimes results in death due to lyses of red blood cells. The disease presents a major health challenge to the world especially in tropical and subtropical areas. The aim of this research was to evaluate the modulation of cytokine expression and *in vivo* antimalarial activity of the residual aqueous fraction (RAF) of methanol leaf extract of *Ficus asperifolia* (FaMLE). The extract was dissolved in distilled water and serially partitioned with solvents of different polarity to obtain the residual aqueous fraction. Standard protocol was used to determine the preliminary phytochemical and OECD method was used to evaluate the acute toxicity of the extracts. The antimalarial activity was evaluated in mice infected with chloroquine sensitive *plasmodium berghei*. Oral LD_{50} of the extracts were estimated to be >5000 mg/kg. The RAF at all doses produced a significant ($p<0.05$) suppressive, prophylactic, and curative antimalarial activity. The extract also significantly prolonged the survival time of the treated mice compared to the distilled water group. There was a statistically significant ($p<0.05$) reduction in the mean values of pro inflammatory cytokines assayed (IL-1β, TNFα and IFN-γ) in the RAF treated groups compared with the distilled water treated group. In conclusion RAF possesses antimalarial activity which might have been achieved through modulation of cytokine expression among other possible mechanisms.

Keywords: Antimalarial, Cytokines, *Ficus asperifolia*, *Plasmodium berghei*, RAF

INTRODUCTION

Cytokines seem to be involved both in the protection and pathology of malaria infection. Early and effective inflammatory response, mediated by gamma interferon (IFN-γ) in the interleukin 12 (IL-12) and 18 (IL-18) dependent manner, seems to be crucial for the control of parasitaemia and resolution of malaria infection through the mechanisms of the tumor necrosis factor α (TNFα) (Day *et al*., 1999). Induction and enhanced release of the anti-parasitic reactive nitrogen and oxygen radicals also plays an important role (Malaguarnera and Musumeci, 2002; Artavanis-Tsakonas *et al.*, 2003). On the other hand, severe malaria has long been associated with high circulating levels of pro-inflammatory cytokines such as TNFα, IFN-γ, interleukin 1 (IL-1) and interleukin 6 (IL-6) (Malaguarnera and Musumeci, 2002). Their excessive production may affect the disease outcome through their direct systemic effect and by increasing adherence of parasitized erythrocytes to the endothelium via up-regulation of adhesion molecules in *Plasmodium falciparum* infections (Day *et al*., 1999). It has been observed that plant materials possessing both antiplasmodial and immunodulatory activities are better candidates for antimalarial drug development (Afolayan *et al*., 2020).

Medicinal plants have been used for a long time in history. They are regarded as the first source of biologically active compounds and many documented medicinal plants have been scientifically proven to have therapeutic applications (Faustino *et al*., 2010). Research on medicinal plants has led to the discovery of novel lead compounds for potential development as drugs (Adebayo *et al*., 2015; Seo *et al*., 2018). *Ficus asperifolia* can be an epiphyte, shrub or a tall tree plant, which is widely distributed across Africa. Its presence has been reported in Nigeria, Senegal, Cameron, Sudan, Central and East African countries (Burkill, 1997). Traditionally, the latex, leaves, bark and roots of the plant are generally used to treat pain, inflammation, malaria and diabetes mellitus among others (Burkill, 1997; Watcho *et al*., 2009). Research conducted on different parts of the plant has scientifically proven many of the claims that the plant is use for in traditional medicine. The water-based fruit extract from the plant has been found to possess uterotonic activity, while research by Nkafamiya *et al*. (2010) highlighted that the crude fiber from *Ficus asperifolia* leaves contains higher levels of protein and minerals compared to certain Nigerian vegetables. The plant has also been reported to possess gastroprotective and hypoglycemic properties (Raji *et al*., 2011; Omoniwa and Luka, 2012). Analysis of *Ficus asperifolia* leaves identified phytochemical constituents like saponins, tannins, cardiac glycosides, terpenes, steroids, and flavonoids (Omoniwa *et al*., 2013). Various studies have established the antibacterial activity of the aqueous bark extract of the plant (Nwanko and Ukaegbu-obi, 2014) and antioxidant potential of the aqueous leaf extract (Ojo and Akintayo, 2014), and antimicrobial properties of the essential oils extracted from the leaves of the plant (Lawal *et al*., 2016). Investigations carried out have demonstrated the significant anti-inflammatory and analgesic activities of the methanol leaf extract of *Ficus asperifolia* (Abdullahi *et al*., 2020). Most recently, the work of Abdullahi *et al*., (2023) revealed that the methanol leaf extract possesses significant suppressive, prophylactic and curative antiplasmodial properties but the active fraction and the possible mechanism of activity has not been suggested making it a justification for further research.

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice of both sexes weighing 100-150g and 20-25g respectively were obtained from the Department of Pharmacology and Therapeutics, Bayero University, Kano. The animals were allowed free access to standard feed and

water ad libitum. They were kept in clean cages filled with saw dust, which was replaced every three days.

Preparation of plant material

The fresh leaves of Ficus asperifolia Miq were collected from Toro district, Toro Local Government Area of Bauchi State, Nigeria. The plants were identified and authenticated at the herbarium unit of the Department of Biological Sciences, Bayero University, Kano, Nigeria. A voucher specimen number BUKHAN 0106 was collected for *Ficus asperifolia*.

Extraction

Fresh leaves of *Ficus asperifolia* were collected from the plant, rinsed with clean water and shade-dried. The plant materials were then pulverized into a fine powder using a porcelain mortar and pestle and sieved. Powdered plant material weighing 2kg was macerated with 7L of 70% v/v methanol at room temperature for 7 days with occasional agitation of the mixture. At the end of the extraction, the crude methanol extract was filtered using What man filter paper (1mm mesh size) and then concentrated in a water bath maintained at 45 ºC until greenish-black residues were obtained and stored in a desiccator.

Fractionation of crude extract

The methanol crude extract of *Ficus asperifolia* leaf was subjected to liquid-liquid partitioning to separate the extract into different fractions. The extract was reconstituted with 300 ml of distilled water. The reconstituted extract was placed in a separating funnel and 300 ml of chloroform was added sequentially as a 1:1 (v/v) solution and shaken (Abdullahi *et al*., 2023). The sample was left to stand for 30 minutes in the separating funnel until a fine separation line appeared indicating the supernatant from the sediment before desorption. The process was repeated at least thrice until chloroform fraction was exhaustively collected in a container and concentrated in a water bath maintained at 45 ºC. The same process was sequentially repeated using ethyl acetate (EAF), n-butanol (NBF) to obtain their fractions as well as the residual aqueous (RAF) fraction.

Phytochemical Screening

The preliminary phytochemical composition of the residual aqueous fraction (RAF) of the methanol leaf extract of *Ficus asperifolia* was determined using phytochemical screening (Abdullahi *et al*., 2023).

Acute toxicity study in mice

The study strictly adhered to ethical guidelines for the use and care of laboratory animals. The ethical committee at Bayero University approved the utilization of animals in the experimental procedures (BUK/CHS/REC/VII/54). Median Lethal Dose (LD_{50}) determination was conducted using Organization for Economic Co-operation and Development guide lines 420 (OECD, 2001) in rats and mice. In this method, a dose of 2000 mg/kg was administered to one mouse and observed for 48 hours. There was no death and the test proceeded to the second phase. A dose of 5000 mg/kg was administered to another mouse and observed for 24 hours. There was no death recorded except in the ethyl acetate fraction (EAF) treated groups and the observation continued daily for 14 days. Changes monitored include skin, eyes, mucous membranes, somatic activity and behavior pattern. Animals were also observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.

Inoculation of mice

A *Plasmodium berghei* infected mouse (parasitemia of 34 %) was used as a parasite donor and blood sample was collected retro-orbitally into an EDTA containing bottle. The inoculum was prepared by determining the percentage parasitemia and erythrocyte count of the donor mouse and further diluting the blood with isotonic saline (Okokon and Nwafor, 2009).

Parasitemia determination

Thin blood smears were applied on microscope slides, fixed with absolute methanol for 10 minutes and stained with 10% Geimsa stain for 30 minutes. The number of parasitized red blood cells was counted using light microscope with an oil immersion eye piece of 100x magnification power (Laychiluh, 2011). Parasitemia and percentage suppression were then determined.

Antiplasmodial activity against early infection (Suppressive test)

Swiss albino mice weighing 22 g to 24g were infected by intraperitoneal (i.p) injection with standard inoculums of *Plasmodium berghei* with 1 × 107 infected erythrocytes (Peters, 1967). The mice were randomly divided into 6 groups of 6 mice each. Group I was given RAF 1000 mg/kg, group II 500mg/kg, group III 250 mg/kg orally for 4 consecutive days. Group IV treated daily with 5 mg chloroquine kg-1 (positive control-I) and Group V received 2 mg kg artesunate (positive control-II). Group VI received distilled water 10 ml kg-1 (Negative control). On day 5, the blood was collected from the tail of each mouse and smear made (Khan *et al*., 2015). The blood films was fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia determined microscopically (Peter *et al*., 1975).

The average % suppression was calculated as follows:

$$Average\ \%\ suppression = \frac{A - B}{A} \times 100$$

Where A = Average percentage parasitaemia in negative control group, and B = Average percentage parasitaemia in test group

Determination of packed cell volume

The packed cell volume (PCV) of each mouse was measured before infection and on day 4 after infection. Blood was collected from the retro orbital sinus and centrifuged at 12,000 rpm for 5 minutes. The PCV measurement was taken using micro haematocrit reader using the modified Win Trobe's Method (Munzer *et al*., 1980). PCV volume is calculated as follows:

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

Prophylactic (repository) test

The prophylactic activity of the extract was assessed using the method described by Peters (1967). Adult mice were randomly divided into 5 groups of 6 mice each. Group I received 10 ml/kg of distilled water (negative control), Group II, III and IV received 250, 500 and 1000 mg/kg RAF respectively. Group V received 1.2 mg/kg of pyrimethamine orally (Positive control). Treatment was continued for five consecutive days (D0–D4). On the sixth day (D5), the mice were inoculated with *Plasmodium berghei*. After 72 hours later, the blood was collected by tail bleeding and parasitemia was determined.

Antiplasmodial activity against established infection (Rane or Curative test)

Evaluation of the schizontocidal activity of the extract against established infection was carried out as described by Ryley and Peters (1970). Adult mice were inoculated with *Plasmodium berghei berghei* on the first day (D0). 72 hours later (D3), the mice were divided randomly into 6 groups of 6 mice each. Group I received 10 ml/kg of distilled water (negative control), Group II, III and IV received 250, 500 and 1000 mg/kg of the leaf extract respectively. Group V received 5 mg/kg of chloroquine (positive control-I) and Group VI received 2 mg/kg of artesunate (positive control-II) for five consecutive days (D3–D7) orally. The blood sample was collected from each mouse by tail bleeding on day three (post parasite inoculation). On the day seven (post treatment), the parasitemia was determined microscopically

Determination of mean survival time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite to time of death was recorded (Mengistie *et al*. 2012). The mean survival time (MST) for each group was calculated as follows:

$$MST = \frac{Sum\ of\ survival\ time\ (days) of\ all\ mice\ in\ a\ group}{Total\ number\ of\ mice\ in\ that\ group}$$

Data analysis

Results were expressed as Mean ± Standard Error of Mean (S.E.M) and were analyzed using one way analysis of variance (ANOVA) followed by Dunnett ' s post hoc test. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Constituents of the Methanol Leaf Fractions of *Ficus asperifolia*

The results of the preliminary phytochemical screening of *F. asperifolia* methanol leaf fractions (CRF) Chloroform fraction, (EAF) Ethyl acetate fraction, (NBF) N-butanol fraction and (RAF) Residual aqueous fraction revealed the presence of various phytochemicals such as cardiac glycosides, tannins, flavonoids, alkaloids, saponins, steroids and terpenoids. However, some of the constituents are only present in some solvent fractions while others are absent (Table 1).

Table 1: Phytochemical Constituents of Methanol Leaf Fractions of *Ficus asperifolia*

Chemical constituents	CRF	EAF	NBF	RAF
Alkaloids	-	-	-	+
Anthraquinone	-	-	-	-
Steroid	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	-	+	+	+
Tannins	-	+	+	+
Flavonoids	-	+	+	+

+ =present, **- =** absent, CRF = chloroform fraction, EAF =ethyl acetate fraction, NBF = n-butanol fraction and RAF = residual aqueous fraction.

Median Lethal Dose (LD_{50}) Values

The oral median lethal dose (LD_{50}) of the crude extract in mice was above 5000 mg/kg. The oral LD_{50} of CRF, NBF and RAF in mice were also above 5000 mg/kg. However, oral LD_{50} of the EAF was above 2000 mg/kg in mice indicating higher toxicity of this fraction (Table 2).

Table 2: Median Lethal Dose (LD_{50}) of Fractions of *Ficus asperifolia* Leaf

Fraction	Animal Specie	Route	Value (mg/kg)
CRF	Mice	Oral	>5000
EAF	Mice	Oral	>2000
NBF	Mice	Oral	>5000
RAF	Mice	Oral	>5000

CRF = chloroform fraction, EAF = ethyl acetate fraction, NBF = n- butanol fraction, RAF = residual aqueous fraction.

In vivo* Antiplasmodial Screening of the Residual Aqueous Fraction of Methanol Leaf Extract of *Ficus asperifolia

Suppressive test

There was a statistically significant ($p<0.05$) reduction in the mean percentage parasitaemia values of the RAF treated groups compared to the distilled water control group. The RAF (1000, 500 and 250 mg/kg) revealed percentage suppression in a dose dependent manner with 70.5, 65.3 and 61.3% respectively. However, the standard drug chloroquine 5mg/kg and artesunate 2 mg/kg caused a suppression of 84.7 and 90.3 respectively (Table 3). Furthermore, result of PCV determination after 4days suppression test with regards to the effect of RAF showed no statistically significant ($p<0.05$) difference from that of the negative control (Table 4).

Table 3: Suppressive Effect of the Residual aqueous Fraction of Methanol Leaf Extract of *Ficus asperifolia* in Mice Infected with *Plasmodium berghei*

Treatment	Dose (mg/kg)	Average % Parasitemia (D4)	% Suppression
D/W	10 ml/kg	20.69±2.15	_
RAF	(1000)	6.10±1.04*	70.5
RAF	(500)	7.18±0.96*	65.3
RAF	(250)	8.01±1.12*	61.3
CQ	(5)	3.17±0.77*	84.7
ART	(2)	2.01±0.29*	90.3

Values are presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at p<0.05 using one-way ANOVA and Dunnett's Post Hoc tests. RAF = Residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia*, DW = Distilled water, D4 indicates day 4, ART = Artesunate, CQ = Chloroquine Phosphate.

Table 4: PCV Values in Mice Infected with *Plasmodim berghei* and Treated with Residual Aqueous Fraction of the Methanol Leaf Extract of *Ficus asperifolia*

Dose (mg/kg)	Average PCV (%)		% Change
	D0	D4	
D/W 10ml/kg	53.10±1.08	48.13±1.08	- 9.3
RAF (1000)	52.97±1.71	52.17±1.12	-1.5
RAF (500)	50.83±1.99	48.07±2.14	- 5.4
RAF (250)	53.66±1.07	50.58±1.67	- 5.7
CQ (5)	58.33±2.21	55.67±2.24	- 4. 6
ART (2)	59.12±1.06	57.20±2.06	- 3.2

Values presented as Mean ±SEM, and percentage, n=6, No significant difference from control at p<0.05 using one-way ANOVA and Dunnett's Post Hoc tests. RAF = Residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia*, D4 indicates day 4, ART = Artesunate, CQ = Chloroquine Phosphate.

Prophylactic test

There was a statistically significant (p<0.05) reduction in the mean percentage parasitaemia values of the RAF treated groups compared to the distilled water control group in the prophylactic activity screening test. The RAF at 1000, 500 and 250 mg/kg doses, exhibited prophylactic activity in a manner that is not dose dependent with 74.8, 56.0 and 39.7 percent respectively. However, the standard drug pyrimethamine 1.2 mg/kg caused prophylactic activity of 86.7% (Table 5).

Table 5: Prophylactic Effect of the Residual aqueous Fraction of Methanol Leaf Extract of *Ficus asperifolia* in Mice Infected with *Plasmodium berghei*

Treatment	Dose (mg/kg)	Average % Parasitemia (D7)	% Prophylaxis
D/W	10ml/kg	24.36±2.11	_
Pyrimethamine	(1.2)	3.24±0.81*	86.7
RAF	(1000)	6.14±1.02*	74.8
RAF	(500)	10.70±1.14*	56.0
RAF	(250)	14.68±1.56	39.7

Values presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at p<0.05 using one-way ANOVA and Dunnett's Post Hoc tests. RAF = Residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia*, D7 indicates day 7.

Curative test

There was a statistically significant (p<0.05) reduction in the mean percentage parasitaemia values of the RAF treated groups compared to the distilled water control group. The RAF at 1000, 500 and 250 mg/kg doses, showed percentage parasite clearance in a dose dependent manner with 81.6, 76.8 and 65.9% respectively. However, the standard drug chloroquine 5mg/kg and artesunate 2 mg/kg showed a clearance of 85.7 and 91.8% respectively (Table 6). Furthermore, the RAF at 1000 mg/kg, 500 mg/kg and 250 mg/kg significantly extended the mean survival of the animals to 24, 25 and 24 days respectively. However, the standard drugs (Artesunate 2mg/kg and Chloroquine 5mg/kg) treated groups showed average survival days of 26 and 24 respectively (Table 6).

Table 6: Curative Effect of the Residual aqueous Fraction of Methanol Leaf Extract of *Ficus asperifolia* in Mice Infected with *Plasmodium berghei berghei*

Treatment	Dose (mg/kg)	Average % Parasitemia		% Clearance	Mean Survival (Days)
		Pre (D4)	Post (D7)		
D/W	10ml/kg	21.00±2.13	26.11±1.15	_	12.83±3.61
ART	(2)	20.04±1.97	1.64±0.21*	91.8	26.15±1.28*
CQ	(5)	19.82±1.44	2.84±0.18*	85.7	24.31±0.33*
RAF	(1000)	21.62±2.12	3.97±0.21*	81.6	24.67±0.41*
RAF	(500)	21.17±2.01	4.91±0.44*	76.8	25.11±1.10*
RAF	(250)	19.87±1.21	6.78±0.82*	65.9	24.30±1. 02*

Values presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at p<0.05 using one-way ANOVA and Dunnett's Post Hoc tests. RAF = Residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia*, D7 indicates day 7, D/W = Distilled water, ART = Artesunate, CQ = Chloroquine Phosphate, % = Percentage.

Effect of Extract and RAF on Cytokine Expression in Mice Infected with *Plasmodium berghei* in a Four Day Suppressive Test

There was a statistically significant ($p<0.05$) reduction in the mean values of pro inflammatory cytokines assayed (IL-1β, TNFα and IFN-γ) in both the FaMLE and RAF treated groups compared with the distilled water treated group. However, there was no observed statistically significant ($p<0.05$) change in the anti-inflammatory cytokine (IL-10) assayed in both the FaMLE and RAF treated groups compared with the distilled water treated group. Similarly, the standard drug chloroquine 5mg/kg showed reduction in pro-inflammatory cytokines assayed while there was no significant change in the anti-inflammatory cytokine assayed compared with the distilled water treated group (Table 7 and Figure 1).

Table 7: Serum Cytokines values in Mice Infected with *Plasmodium berghei* and Treated with Crude and Residual aqueous Fraction of the Methanol Leaf Extract of *Ficus asperifolia* in a Four day Suppressive Test

Treatment (mg/kg)	Mean Cytokines (pg/ml)			
	IL-1β	**TNFα**	**IFN-γ**	**IL-10**
UNI	2.61±0.97	3.02±1.01	2.88±0.86	3.14±0.99
DW 1ml/kg	27.64±1.71	145.31±4.12	36.43±1.87	14.67±3.02
FaMLE (1000)	9.24±1.12*	46.19±2.01*	12.09±1.16*	18. 36±1.17
RAF (1000)	11.13±1.09*	55.68±2.14*	14.15±1.04*	17. 51±1.21
CQ (5)	8.93±1.03*	49.17±2.67*	5.28±0.47*	15.12±1.94

Values presented as Mean ±SEM, n=6, * significantly different from negative control analysed using one-way ANOVA at $p<0.05$ and dunnett's post hoc tests. UNI = Uninfected untreated mice, DW = Infected mice treated with distilled water, IL-6 = interleukin-6, TNF = tumor necrosis factor, IFN Y = interferon Y and IL-10. RAF = Residual aqueous fraction, CQ = chloroquine Phosphate.

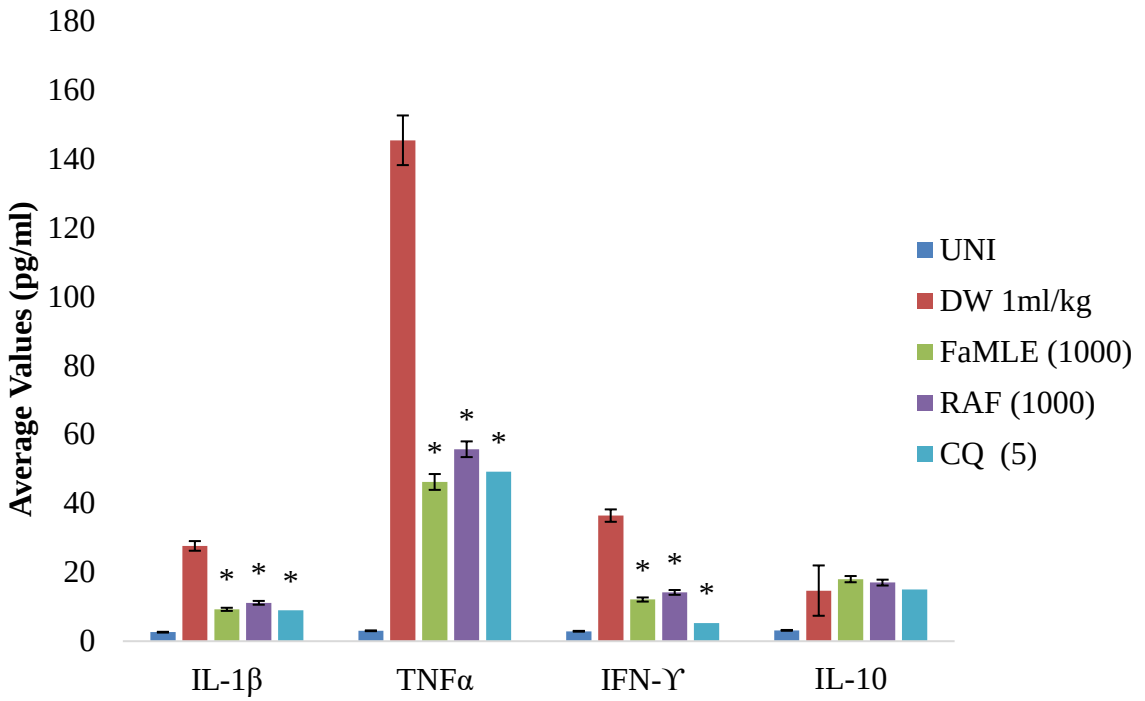

Figure 1: Effect of crude and residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia* on serum cytokines values in mice infected with *P. berghei* in a four day suppressive test. UNI = Uninfected untreated mice, DW = Infected mice treated with Distilled water, IL-6 = interleukin-6, TNF = tumor necrosis factor, IFN Y = interferon Y and IL-10. RAF = Residual aqeous fraction, CQ = Chloroquine Phosphate.

Discussion

Preliminary phytochemical screening is a valuable method for identifying various classes of secondary metabolites in plants and assessing their potential medicinal significance (Shabbir *et al*., 2013; Imafidon *et al*., 2018). Analysis of leaf extracts from *Ficus asperifolia* indicated the presence of saponins, flavonoids, tannins, alkaloids, terpenoids, steroids, and cardiac glycosides. This report agrees with the findings of Omaniwa and Luka (2012) and Abdullahi *et al*. (2023). Secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and steroids play crucial roles in the biological activities of plants (Edewor-Kuponiyi, 2013; Rungsung *et al*., 2015). Many plants contain diverse phytochemicals as their bioactive components, and some have been reported to exhibit antiplasmodial activity (Alshawsh *et al*., 2007; Matur *et al*., 2009). While certain studies attribute antiplasmodial activity to alkaloids, flavonoids, and terpenoids (Akuodor *et al*., 2010; Philip, 2020; Tajjuddeen *et al*., 2021), others suggest saponins also demonstrate such activity (Akanbi *et al*., 2018; Nafiu *et al*., 2021).

Acute toxicity studies are carried out to ascertain the dosage range that could be harmful to animals and the estimation of the therapeutic index for drugs and xenobiotics (Maikai *et al*., 2008). The oral LD_{50} values for methanol leaf extract of *Ficus asperifolia* and its fractions were found to be above 5000

mg/kg, indicating relative safety which agrees with the reported work of Abdullahi *et al*. (2020). However, the ethyl acetate fraction (EAF) was found to be less than 5000 mg/kg signifying relatively lower level of safety compared to the extract and other fractions.

In vivo screening offers a comprehensive method for assessing the effectiveness of drugs in a biological context (White, 2008). However, it is crucial to acknowledge that various host factors, including drug disposition and inherent drug antiparasitic activities, can influence the outcomes of in vivo studies, impacting the drug's efficacy. Therefore, achieving favorable disposition characteristics is not guaranteed even if a drug demonstrates efficacy *in vivo*. For instance, despite its suboptimal disposition in animals, artemisinin exhibits notable efficacy *in vivo* (White, 2008; Asanga *et al*., 2017), emphasizing that the success of an antimalarial drug goes beyond its disposition properties. *Plasmodium berghei* is commonly employed in in vivo screening for antimalarial drugs due to its accessibility and widespread use in drug discovery research (Fidock et al., 2004). However, it is essential to recognize that certain genes specific to *Plasmodium berghei*, adapted to rodents, may vary from those found in the human pathogen *Plasmodium falciparum* thereby presenting limitations in the direct interpretation of outcomes in rodents as compared to humans (Asanga *et al*., 2017). In this study, the antimalarial activity of RAF was evaluated using the *in vivo* suppressive, prophylactic and curative antiplasmodial screening tests in mice infected with *Plasmodium berghei* parasites.

The chemo suppressive test revealed a significant reduction in the mean percentage parasitaemia values of the RAF treated groups compared to the distilled water control group. It had been reported that some plants extracts exerted their antimalarial activity through the inhibition of protein synthesis in the parasites (Kirby, 1997). Also, since alkaloids have been implicated in blocking protein synthesis in *Plasmodium falciparum* (Nergiz, 1993) and alkaloids have been found to be one of the phytochemical constituents in RAF therefore, it presupposes that the relative availability of alkaloids in the fraction may have contributed to the chemo suppression of the *Plasmodium berghei* possibly by inhibiting the replication processes and blocking protein synthesis in the parasite thereby affecting growth and reproduction processes in the parasites.

The prophylactic activity showed that the parasite density in all the treatment groups were significantly decreased when compared with that of the negative control, pointing out that the RAF had varying degrees of repository activity hence, suggesting that the consumption of plants prior to malaria infection may help to reduce the upsurge of the signs and symptoms that result from malaria attack. The RAF demonstrated prophylactic activity comparable to that of Pyrimithamine. This result is consistent with the report by Nkafamiya *et al.* (2010) on the nutritious value of the leaves of *Ficus asperifolia* as it may serve as immune boaster in preventing malaria. Therefore, the prophylactic efficacy is indicative of non-selectivity of action on the different stages of malaria parasite, since prophylactic drugs work either by the disruption of the initial development of malaria parasite in the liver or by suppressing the emergent asexual blood stages or even by the prevention of the relapses induced by hypnozoites (Hill *et al*., 2006). Moreover, cardiac glycosides are one of the revealed preliminary phytochemicals in the RAF and have been implicated in prophylactic activity as their relative broad stage specificity of action had been reported to extend the ability to impede developments of gametocytes (Yoo *et al*., 2008).

The result of curative activity screening showed that the RAF had significantly reduced parasite densities when compared with that of the distilled water group. Malaria is known as a complicated syndrome involving many inflammatory responses which may enhance cell-to-cell interaction, cell stimulation involving malaria derived antigens/toxins and the host derived factors such as cytokines (Boampong *et al*., 2013). The inflammatory conditions of malaria are marked with free radical generation, activation of phospholipase activity resulting in the generation of prostaglandins or even tumour necrotic factors. Therefore, the established curative properties of the extracts/fraction may either be due to the inhibition of the production and/or the release of these inflammatory mediators associated with malaria or even by direct cytotoxic effect on the parasites. In addition, there were significant increases in the mean survival time (MST) of all the treatment groups when compared with that of the negative control group indicating that the treatment is efficacious, as mean survival time is used as an indicator for drug efficacy in antimalaria drug researches (Udobre *et al*., 2013). The result of mean survival time (MST) in the curative model showed that the groups of mice treated with chloroquine, artesunate, and RAF significantly increased in their MST when compared with the negative control group. The prolonged survival times of mice is evidenced by the high parasitemia clearance by the standard drugs and the extracts/fraction. Mean survival time (MST) has often been used as one of the indicators for evaluating drug efficacy mostly, as it provides an insight to the potency of a drug in clearing the host system of parasite infection. The longer the survival time, the higher the chance of the animal surviving and recovering from the oxidative changes induced by the parasite and hence, the better the activity of the extract or drug (Udobre *et al*., 2013). The MST is a very important parameter to evaluate the antimalarial activity of plant extracts (Fidock, 2004). The RAF prolonged the survival time of *Plasmodium berghei* infected mice which might be due to the presence of secondary metabolites that prevent the overall pathologic effect of the parasite in the infected mice, like antioxidant, anti-inflammatory effects in addition to good parasites suppression (Toma *et al*., 2015).

Malaria is an inflammatory cytokine driven disease that sometimes results in death due to lyses of red blood cells. It is marked with oxidative changes in animals due to the parasites' numerous metabolic activities (Asanga *et al*., 2017). Studies suggest that the successful resolution of malaria infection is dependent on the ability of the host to induce adequate levels of pro-inflammatory cytokines early at the acute phase of the attack (Afolayan *et al*., 2016; Gebrehiwot *et al*., 2019; Afolayan *et al*., 2020). When the pro-inflammatory cytokines are adequately regulated by anti-inflammatory cytokines disease progression is hampered (Chen *et al*., 2018). Thus, effective immune response to *P. falciparum* requires a balance of pro-inflammatory and anti-inflammatory cytokines (Crompton *et al*., 2014). The imbalance of these inflammatory responses accounts for the pathology observed in malaria. The increased levels of cytokines and chemokines during cerebral malaria cause the sequestration of leukocytes in cerebral malaria (Dunst *et al*., 2017). In this study, the significant increase in cytokines level in the infected control mice may be attributed to increased schizogony and fever paroxysms resulting in increased TNF-α, IFN-γ and nitric oxide (NO) levels. The cytokines may affect the receptors expression on vascular endothelium by either redistributing or up regulating the receptors; thus, increasing the cytokines level (Heddini, 2002). On the other hand, the treatment of the infected mice with FaMLE and RAF extracts might have alleviated the inflammatory effect

of the *Plasmodium berghei* parasite by direct inhibitory effect on cytokines production, including TNF-α, IFN-γ, NO and also might have generated many kinds of antioxidants in vivo with protective activities against protozoal infections (Awasthi *et al*., 2003). The presence of phytochemicals may be the basis of the immune modulatory activity exhibited by the extracts which also agrees with reported work of Ojo and Akintayo, (2014) on the antioxidant activity of FaMLE and its aqueous fraction. Flavonoids have been reported to induce TNF- α, and anti-inflammatory responses via their ability to inhibit reactive oxygen or nitrogen compounds and to modify intracellular signaling pathways in immune cells. Saponins are also good immune stimulants that enhance macrophages actions and stimulate IL-6 (Yahfoufi *et al*., 2018).

CONCLUSION

The residual aqueous fraction of the methanol leaf extract of *Ficus asperifolia* exhibited suppressive, prophylactic, and curative antiplasmodial activity. The extract also significantly prolonged the survival time of the treated mice compared to the distilled water group. There was a statistically significant reduction in the mean values of pro inflammatory cytokines assayed (IL-1β, TNFα and IFN-γ) in the RAF treated groups compared with the distilled water treated group. This therefore implies that RAF possesses antimalarial activity which might have been achieved through modulation of cytokine expression among other possible mechanisms.

ACKNOWLEDGEMENT

The authors acknowledged the technical support of Malam Aliyu Ahmad of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical sciences, Bayero University, Kano, Nigeria.

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