



## Article Info

Received: 21<sup>st</sup> August 2023Revised: 18<sup>th</sup> December 2023Accepted: 20<sup>th</sup> December 2023

<sup>1</sup>Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa state, Nigeria.

<sup>2</sup>Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa state, Nigeria.

<sup>3</sup>Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa state, Nigeria.

<sup>4</sup>Department of Biochemistry, Faculty of Life Sciences Modibbo Adama University, Yola, Adamawa State, Nigeria.

\*Corresponding author's email:

[jadasm84@gmail.com](mailto:jadasm84@gmail.com)

Cite this: *CaJoST*, 2024, 1, 45-53

## Effect of Methanolic Extract of *Ficus trigonata* Leaves on Lipid Profile and Liver Function Indices in *Plasmodium berghei* Infected Mice

Mahmoud S. Jada<sup>1\*</sup>, Hyelda Stephen<sup>2</sup>, Hauwa A. Zailani<sup>3</sup>, and Abdullahi U. Wurochekke<sup>4</sup>

The research was designed to evaluate the effect of methanolic extract of *Ficus trigonata* leaves on lipid profile and liver function indices in *Plasmodium berghei* infected mice. The extract was prepared by maceration and tested for antioxidant activity using Diphenyl-picryl hydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. Thirty-six (36) mice were used for the study. The extract was administered orally at doses of 100, 200 and 300 mg/kg to infected mice for four days and compared to standard drug chloroquine and normal saline controls. Lipid profile and liver function indices were determined using standard methods. The results showed that the extract exhibited concentration-dependent antioxidant activity in both DPPH and FRAP assays, with an IC<sub>50</sub> values of 0.49 and 0.37 mg/mL, respectively. The elevated levels of Triglycerides and Total cholesterol were significantly (P<0.05) reduced by the extract compared to negative control. The extract significantly (P<0.05) reduced Low Density Lipoprotein cholesterol levels from 93.00 ± 2.00 mg/dL in negative controls to 81.33 ± 4.67 mg/dL, 90.33 ± 2.91 mg/dL, and 89.00 ± 3.06 mg/dL in the 100, 200 and 300 mg/kg extract treatment groups respectively. The elevated level of High Density Lipoprotein from 60.33 ± 2.96 mg/dL in naïve mice was also significantly (P<0.05) reduced in the treatment groups. The increased levels of Aspartate Transaminase, Alanine Transaminase, and Alkaline Phosphatase observed in negative control were also significantly (P<0.05) reduced by the extract. The research clearly established the potential of methanolic extract of *Ficus trigonata* leaves to ameliorate malaria-induced dyslipidaemia and hepatotoxicity.

**Keywords:** *Ficus trigonata*, Leaves, *Plasmodium berghei*, Dyslipidaemia and Hepatotoxicity

## 1. Introduction

Malaria is a devastating global health problem known as the world's most important tropical parasitic infectious disease to humans (Greenwood *et al.*, 2022). According to the World Health Organization (2022), there were an estimated 247 million cases of malaria worldwide in 2021, taking the estimated number of malaria deaths to about 619,000.

Malaria is caused by Plasmodium parasites and it is transmitted through the bites of Anopheles mosquitoes that are infected. Five Plasmodium species cause malaria in humans, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, with *Plasmodium falciparum* accounting for the majority of malaria morbidity and mortality (WHO, 2022). Rodent malaria species like *Plasmodium berghei* have proven useful experimental models

for evaluating potential new antimalarial agents (Mekonnen, 2015).

Lipid profile and liver function indices are important biomarkers that reflect the metabolic and hepatic status of an organism. Malaria infection can alter the lipid profile and liver function indices, leading to dyslipidaemia and hepatotoxicity. These conditions can affect the immune response and the outcome of malaria infection (Okagu *et al.*, 2022).

*Ficus trigonata* Linn., commonly known as 'chalate', is a tree species in the Moraceae family that is native to North and South America (Berg, 1989). It belongs to the subgenus *Urostigma*, which comprises about 150 species of figs that have aerial roots and milky latex. *Ficus trigonata* is distributed from Mexico to Brazil, and also occurs in the Caribbean islands. It grows in wet

tropical forests, savannas, and disturbed areas (Berg and Villavicencio, 2004; Grandtner, 2005). *Ficus trigonata* is a multipurpose tree that is used for food and medicine by local people. The syconia are edible and have a sweet taste. They are eaten raw or cooked. The leaves are also edible and are used as fodder for livestock. The latex has antiseptic and anti-inflammatory properties and is applied to wounds, ulcers, skin infections, and insect bites (Stevens *et al.*, 2001). Additionally, despite widespread traditional use, potential toxicity has not been extensively evaluated. The study therefore investigated the effects of methanolic leaves extract of *F. trigonata* on lipid profile and liver function indices in mice infected with *Plasmodium berghei*.

## 2. Materials and Methods

### 2.1 Plant Sample Collection and Identification

The leaves of *Ficus trigonata* were collected from Hong Local Government Area of Adamawa State, Nigeria. The plant was identified by a plant taxonomist from the department of Plant Science, Modibbo Adama University, Yola. The fresh leaves of the plant were air-dried under shade and grounded into homogenous matter with an electric mill and stored in airtight containers.

### 2.2 Extraction

Methanol extracts were obtained through maceration method as described by Amah *et al.* (2021). Using the cold maceration technique, 300g of powdered material was suspended in 1L of methanol and allowed to stand for 72 hours while being stirred occasionally. The suspension was then put through a mesh filter. Whatman No. 1 filter paper was used for further filtration to get rid of tiny residues. To get the methanol extract, the filtrate was concentrated using a rotary evaporator (Heidolph Laborata 4001, Germany) at 45 °C. Prior to usage, the extract was kept in an airtight screw bottle at 5 °C.

### 2.3 Experimental Animals

Thirty-six (36) mice weighing about 20g to 24g were used for the research. The mice were procured from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The mice were kept in cages barred with steel nets and allowed to acclimatize and were constantly been supplied with standard feed and clean water *Ad libitum* for a period of 7 days prior to the commencement of the experiment.

### 2.4 Malaria Parasites

*Plasmodium berghei* (NK65) was obtained from National Institute for Medical Research (NIMR),

Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal transmission every five (5) days in mice. The re-infected mice were moved to the Animal House of the Department of Biochemistry, Faculty of Life Science, Modibbo Adama University, Yola, where the research was carried out. Prior to commencement of the experiment, one of the infected mice was kept and observed to reproduce signs of disease similar to human malarial infection.

## 2.5 Antioxidant Assay

### (a). Diphenyl-picryl hydrazyl (DPPH) assay

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay was carried out to evaluate the free radical scavenging activity of the plant extract as described by Mishra *et al.* (2012). This is based on the reduction of the stable DPPH free radical, which is deep violet in colour, to the yellow-coloured diphenyl-picryl hydrazine by antioxidants. Briefly, 1 ml the extract (0.2 – 1mg/ml) in methanol was added to 4ml (0.004% w/v) methanol solution of DPPH. After 30mins the absorbance of the solution was measured at 517nm using a UV spectrophotometer which was compared with the corresponding percentage inhibition of standard ascorbic acid. The free radical scavenging activity (FRSA) was calculated using the formular:

$$\% \text{ scavenging} = \frac{[\text{Abs (control)} - \text{Abs (sample)}]}{\text{Abs control}} \times 100.$$

### (b). Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was carried out to determine the antioxidant activity of the plant extract according to method described by Benzie and Strain (1996). The principle is based on the reduction of ferric ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ) ions by antioxidants which causes formation of a coloured ferrous-probe complex that can then be measured spectrophotometrically. The experimentation was initiated by mixing 1ml of the test sample extract (at varied concentration from 0.2 – 1 mg/ml), mixed with 1ml of 2M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide separately. The reaction mixture was incubated in a temperature-controlled water bath at 50°C temperature for 20mins, followed by addition of 1ml of 10% trichloroacetic acid. The mixture was centrifuged for 10mins at room temperature. The supernatant obtained (1mL) was added to 1mL of deionized water and 200ml of 0.1%  $\text{FeCl}_3$  and absorbance was measured at 700nm. The FRAP radical scavenging ability was calculated using the formula:

$$\% \text{ scavenging} = \frac{[\text{Abs}(\text{control}) - \text{Abs}(\text{sample})/\text{Abs}(\text{control})]}{\text{Abs}(\text{control})} \times 100.$$

## 2.6 Parasite Inoculation

Parasitized erythrocytes were obtained from donor mice by cardiac plexus puncture and was diluted with trisodium citrate. Mice were inoculated intraperitoneally with 0.2ml blood suspension containing  $10^6$ - $10^7$  parasitized erythrocytes on day 0.

## 2.7 Experimental Design

Thirty-six (36) mice were divided into six (6) groups of six (6) mice each. Group 1 received normal saline as a normal control. Group 2 was infected with  $1.0 \times 10^7$  *P. berghei* parasites intraperitoneally and given 0.2 ml normal saline as a negative control. Group 3 was infected and then treated with the antimalarial drug chloroquine diphosphate (CDP) at 10 mg/kg bw/day for 4 days, as a positive control. The remaining 3 groups were also infected and then orally treated with different doses (100, 200, 300 mg/kg bw/day) of a *Ficus trigonata* leaves extract for 4 days. After the final treatment, the mice were fasted overnight, then sacrificed by cervical dislocation under mild anaesthesia. Blood samples were collected via cardiac puncture into lithium heparin bottles for analysis.

## 2.8 Lipid Profile

### (a). Determination of Total Cholesterol

The cholesterol concentration was determined enzymatically using method described by Roeschlau *et al.* (1974). The assay was performed by first pipetting 10  $\mu$ L of sample or 200 mg/dL cholesterol standard into labelled test tubes. Then, 1000  $\mu$ L of the enzymatic reagent containing cholesterol esterase, cholesterol oxidase, peroxidase and the chromogens phenol and 4-aminoantipyrine were added to the sample, standard and blank tubes. The contents were thoroughly mixed by vortexing and incubated at 37°C for 5 minutes. Absorbance was measured against the blank and cholesterol concentration was calculated using the formula:

$$\text{Cholesterol (mg/dL)} = (\text{Abs. Sample}) / (\text{Abs. Std}) \times \text{Conc. Std.}$$

### (b). Determination of Triacylglyceride

The triglyceride concentration was determined enzymatically based on the method described by Bucolo and David (1973). 10  $\mu$ L of sample and 200 mg/dL triglyceride standard was pipetted into sample and standard labelled test tubes followed by 1000  $\mu$ L of the enzymatic reagent containing lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase and chromogens 4-aminophenazone and then 4-chlorophenol was added to the sample, standard and blank tubes. The tubes were mixed and incubated at 37°C for 5 minutes to allow the enzymatic reactions to

occur. Absorbance was then read against the reagent blank and triglyceride concentration was calculated using the formula:

$$\text{Triglycerides (mg/dL)} = (\text{Abs. Sample}) / (\text{Abs. Std}) \times \text{Conc. Std.}$$

### (c). Determination of HDL Cholesterol

HDL cholesterol was quantified enzymatically based on the method described by Demacker *et al.* (1997). 2 mL of the precipitation reagent containing magnesium chloride was added to 5 mL of serum sample. This was mixed and allowed to stand for 10 minutes at room temperature before centrifuging at 1500 x g for 30 minutes. The clear supernatant containing HDL was then assayed for cholesterol: 10 $\mu$ L supernatant or 25mg/dL HDL standard along with 1000 $\mu$ L cholesterol reagent were added to the blank, standard and sample tubes. The reaction mixture was incubated kinetically at 37°C with a 10mm light path and absorbance read at 505nm against the reagent blank. HDL cholesterol concentration was calculated using the formula:

$$\text{HDL Cholesterol (mg/dL)} = (\text{Abs. Sample} / \text{Abs. Std}) \times \text{Conc. Std} \times 2.$$

Where, 2 is the dilution factor from the deproteinization step.

### (d). Determination of LDL Cholesterol

LDL cholesterol concentration was calculated using the Friedewald formula as described by Friedewald *et al.* (1972). This is calculated using the formula:

$$\text{LDL Cholesterol (mg/dL)} = \text{Total Cholesterol} - (\text{HDL Cholesterol} + (\text{Triglycerides}/5))$$

## 2.9 Liver Function Indices

### (a). Determination of Aspartate Transaminase (AST) Activity

The activity of aspartate aminotransferase (AST) was assayed using the method of Reitman and Frankel (1957) as modified by Schmidt (1961). 0.1 mL serum sample was pipetted into a test tube followed by addition of 0.5 mL buffer and mixing. This was incubated for 30 minutes at 37°C. After that, 0.5 mL of 2,4-dinitrophenylhydrazine was added, mixed and incubated for 20 minutes at 25°C. Then, 5 mL of 0.4N NaOH was added, mixed and the absorbance was read after 5 minutes against a reagent blank at 546 nm. AST activity was calculated using the formula:

$$\text{AST } (\mu\text{L}) = 1768 \times \text{Absorbance.}$$

### **(b). Determination of Alanine Transaminase (ALT) Activity**

The activity of alanine aminotransferase (ALT) was determined using the method of Reitman and Frankel (1957) as modified by Schmidt (1961). Exactly, 0.1 mL of serum sample into a test tube to which 0.5 mL of buffer substrate was added. The mixture was incubated for 30 minutes at 37°C, after which 0.5 mL of 2,4-dinitrophenylhydrazine was added and incubated for 20 minutes at 25°C. Then, 5 mL of 0.4N NaOH was added and the absorbance was read after 5 minutes against a reagent blank at 340 nm. The ALT activity in U/L was calculated using the formula:

$$\text{ALT } (\mu\text{L}) = 1746 \times \text{Absorbance.}$$

### **(c). Determination of Alkaline Phosphatase (ALP) Activity**

The activity of ALP was evaluated using the method of Wright *et al.* (1972) and a Randox kit. Exactly 0.01 mL of serum sample was pipetted into a test-tube followed by addition of 0.5 mL of buffer substrate and mixing. The initial absorbance was read and timed simultaneously, after which the absorbance was read again after 2-3 minutes. ALP activity was dependent on the rate of increase in absorbance resulting from the formation of p-nitrophenol.

### **(d). Determination of Total Protein**

The total protein was estimated using the biuret method as described by Weichselbaum (1946). One mL of serum was added to the standard and sample tubes while 0.2 mL distilled water and 0.2 mL standard were added to the blank and standard tubes, respectively. The absorbance of the standard and samples, was measured against the blank at 546 nm and protein concentration was calculated using the formula:

$$\text{Total protein concentration} = \left( \frac{\text{sample/standard}}{\text{Standard concentration}} \right) \times$$

### **(e). Determination of Bilirubin Level**

Total and Direct Bilirubin were assayed using the method described by Jendrassik and Grof (1938). The assay utilized four reagents: a 0.5% sulphanilic acid solution in 1N HCl, a 0.1% sodium nitrite solution, a 1% caffeine in 0.1M sodium benzoate accelerator solution, and a bilirubin sample containing the conjugated bilirubin for measurement. To determine the Direct bilirubin level, 10  $\mu\text{L}$  of the sample was added to a test tube and 10  $\mu\text{L}$  of distilled water was added to another test tube as blank. After that, 200  $\mu\text{L}$  of the sulphanilic acid solution was pipetted into two test tubes (one for the blank and one for the sample). Then, 50  $\mu\text{L}$  of the sodium nitrite solution was added to the sample

tube, followed by 1000  $\mu\text{L}$  of 0.1M sodium benzoate accelerator solution to both tubes. The reaction mixture was thoroughly mixed, then 200  $\mu\text{L}$  of the bilirubin sample was added to each tube. The reaction mixture was mixed once again, then the test tubes were left to stand for 10 minutes in darkness. The absorbance was measured at 550 nm using a spectrophotometer, with the blank serving as a reference. The measured absorbance indicated the formation of the blue azo-bilirubin complex, proportionate to the original conjugated bilirubin concentration in the sample. Total bilirubin was determined repeating the above procedure but 1% caffeine in 0.1M sodium benzoate was used instead. The presence of the caffeine releases the albumin-bound bilirubin. The absorbance was also measured at 550 nm using a spectrophotometer, with the blank serving as a reference

### **(f). Determination of Albumin Level**

The serum albumin level was estimated based on its quantitative binding to the indicator bromocresol green (BCG) as described by Doumas *et al.* (1997). The albumin-BCG complex absorbs maximally at 578nm, with the absorbance proportional to the serum albumin concentration. The procedure involved preparing test tubes for the sample, standard, and blank. 10 $\mu\text{L}$  of sample or standard along with 1000 $\mu\text{L}$  of the working reagent were added to the respective tubes, while 1000 $\mu\text{L}$  of the working reagent and 10 $\mu\text{L}$  distilled water were added to the blank. The tubes were mixed, incubated for 20 mins at 25°C, and the optical density was read at 620nm. The albumin concentration was calculated using the formula: (Optical density of test)/(Optical density of standard)  $\times$  Concentration of standard.

## **2.10 Statistical Analysis**

The data collected were evaluated using SPSS version 26 software using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to separate the means that are statistically different. Data were expressed as mean  $\pm$  SEM and a P-value  $<$  0.05 was considered to be statistically significant. Charts were designed using Origin 2022.

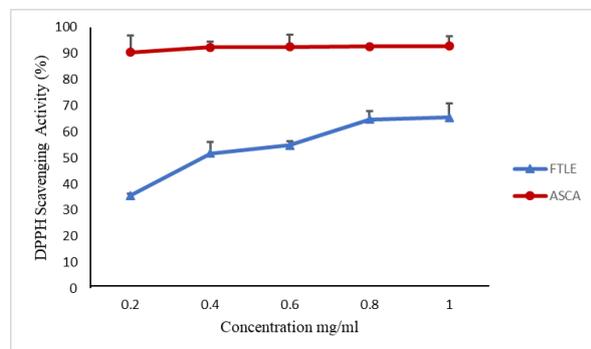
## **3. Results and Discussion**

### **3.1 Results**

#### **(a). The Antioxidant Activity**

The free radical scavenging activity of *Ficus trigonata* leaves extract (FTLE) and the standard antioxidant ascorbic acid (ASCA) based on the DPPH assay revealed that across the concentration range of 0.2 to 1 mg/mL tested,

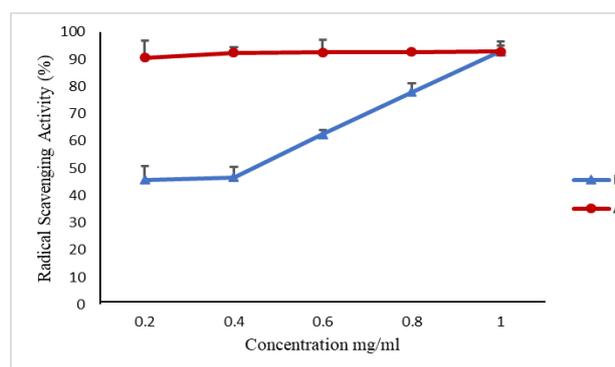
*Ficus trigonata* leaves extract showed a concentration-dependent increase in DPPH radical scavenging activity from  $35.71 \pm 0.12\%$  to  $65.46 \pm 1.1\%$ . In comparison, ASCA demonstrated over 90% radical scavenging activity at all concentrations. The  $IC_{50}$  value of FTLE was 0.49 mg/mL as shown in Figure 1.



**Figure 1:** DPPH free radical scavenging activity against concentration.

**FTLE:** *Ficus trigonata* leave extract; **ASCA:** Ascorbic acid.  $IC_{50}$ : 0.49.

The antioxidant capacity of FTLE in comparison to the standard antioxidant ascorbic acid (ASCA) based on FRAP assay showed that the reducing power increased with increasing concentrations of FTLE from 0.2 to 1 mg/mL. At the highest concentration of 1 mg/mL, the FRAP value for FTLE was  $92.9 \pm 2\%$ , comparable to that of ASCA ( $92.8 \pm 3.75\%$ ). The extract exhibited concentration-dependent reducing power, indicative of its antioxidant potential. The  $IC_{50}$  value determined from the dose-response curve was 0.37 mg/mL as shown in Figure 2.

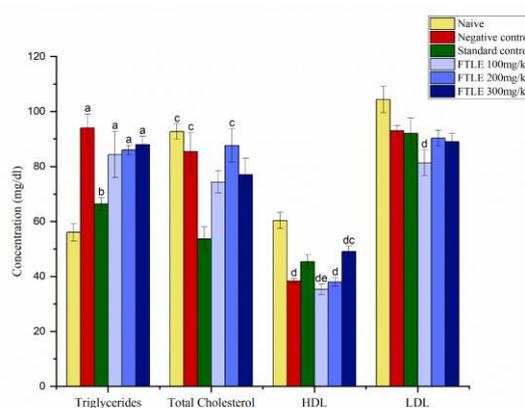


**Figure 2:** Radical scavenging activity using FRAP method. FTLE: *Ficus trigonata* leaves extract; ASCA: Ascorbic acid.  $IC_{50}$ : 0.37

### (b). The Lipid Profile

As shown in Figure 3, compared to naïve controls ( $104.33 \pm 4.81$  mg/dL), LDL cholesterol was significantly ( $P < 0.05$ ) decreased in all treatment groups: 100 mg/kg ( $81.33 \pm 4.67$

mg/dL), 200 mg/kg ( $90.33 \pm 2.91$  mg/dL), and 300 mg/kg ( $89.00 \pm 3.06$  mg/dL). A marked reduction in HDL cholesterol was observed in extract-treated groups compared to naïve mice ( $60.33 \pm 2.96$  mg/dL), with the lowest level seen at 100 mg/kg ( $35.33 \pm 1.86$  mg/dL). In contrast, total cholesterol was significantly elevated in the extract groups relative to standard controls ( $53.67 \pm 4.33$  mg/dL), but lower than naïve values ( $92.67 \pm 2.73$  mg/dL). No significant difference in total cholesterol was found between 200 mg/kg extract ( $87.67 \pm 6.06$  mg/dL) and negative controls ( $85.33 \pm 6.98$  mg/dL). Triglyceride levels were increased in all extract doses compared to naïve mice ( $56.00 \pm 3.21$  mg/dL), but decreased compared to negative controls ( $94.00 \pm 5.13$  mg/dL) and standard treatment ( $66.33 \pm 2.33$  mg/dL).

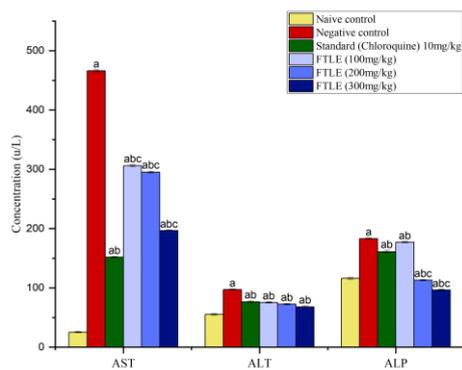


**Figure 3:** Effect of methanol leaves extract of *Ficus trigonata* on lipid profile in mice infected with *Plasmodium berghei*. FTLE: *Ficus trigonata* leaves extract. Data were expressed as mean  $\pm$  SEM;  $n=5$ . <sup>a</sup> $P < 0.05$  higher than naïve, <sup>b</sup> $P < 0.05$  lower than the negative control, <sup>c</sup> $P < 0.05$  higher than the standard control, <sup>d</sup> $P < 0.05$  lower than the normal control, and <sup>e</sup> $P < 0.05$  lower than the standard control.

### (c). The Liver Function Indices

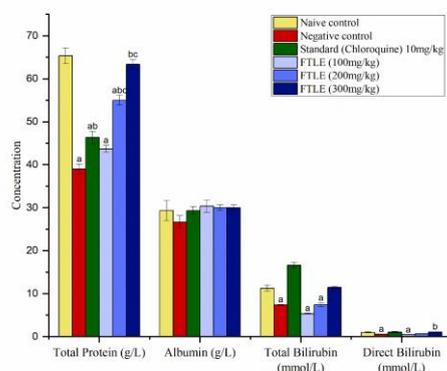
Aspartate aminotransferase (AST) levels were markedly increased from  $25.33 \pm 1.45$  U/L in naïve mice to  $466.00 \pm 1.73$  U/L in negative controls, indicating liver injury induced by malaria infection. Treatment with 100, 200 and 300 mg/kg *F. trigonata* leaf extract significantly ( $P < 0.05$ ) decreased AST to  $306.00 \pm 1.73$ ,  $295.00 \pm 2.30$  and  $196.67 \pm 0.88$  U/L respectively compared to negative controls. However, AST remained higher than the standard drug ( $152.00 \pm 1.16$  U/L) and normal mice. A similar pattern was observed for alanine aminotransferase (ALT), with elevated levels of  $97.00 \pm 1.16$  U/L in infected untreated mice reduced to  $75.33 \pm 1.20$ ,  $72.66 \pm 1.20$  and  $68.00 \pm 1.52$  U/L in the extract groups. ALP was raised from  $116.33 \pm 1.76$  U/L in naïve controls to  $183.33 \pm 1.20$  U/L in negative controls, and treatment lowered ALP to  $177.00 \pm$

1.16,  $113.00 \pm 1.15$  and  $96.67 \pm 0.88$  U/L as shown in Figure 4.



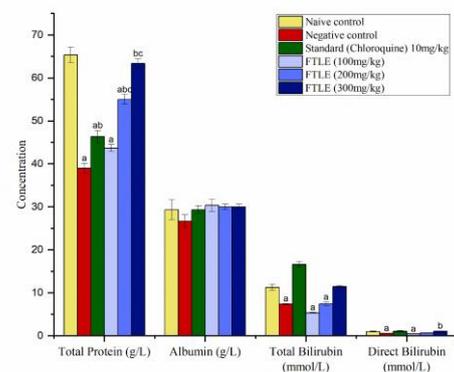
**Figure 5:** Effect of methanol extract of *Ficus trigonata* leaves on Liver enzyme markers in mice infected with *Plasmodium berghei*. FTLE: *Ficus trigonata* leaves extract. Data were expressed as mean  $\pm$  SEM; n=5. <sup>a</sup> $P < 0.05$  higher than normal, <sup>b</sup> $P < 0.05$  lower than negative control, <sup>c</sup> $P < 0.05$  higher than standard control.

The total protein levels observed were significantly decreased ( $P < 0.05$ ) from  $65.33 \pm 1.86$  g/L in naïve controls to  $39.00 \pm 1.16$  g/L in negative controls due to malaria infection. Treatment with 100, 200 and 300 mg/kg extract increased total protein to  $43.67 \pm 0.87$ ,  $55.00 \pm 1.12$  and  $63.33 \pm 1.20$  g/L respectively, though the 100 mg/kg group remained lower than normal. Total bilirubin was reduced in untreated infected mice ( $7.37 \pm 0.15$  mmol/L) compared to naïve animals ( $11.27 \pm 0.75$  mmol/L), and extract doses of 100 and 200 mg/kg further lowered total bilirubin to  $5.33 \pm 0.12$  and  $7.44 \pm 0.40$  mmol/L. A similar pattern was seen for Direct Bilirubin. Albumin levels were unaffected by infection or extract treatment. These observations are presented in Figure 6



**Figure 6:** Effect of methanol extract of *Ficus trigonata* leaves on serum protein markers in mice infected with *Plasmodium berghei*. FTLE: *Ficus trigonata* leaves extract. Data were expressed as mean  $\pm$  SEM; n=5.

<sup>a</sup> $P < 0.05$  lower than normal, <sup>b</sup> $P < 0.05$  higher than negative control, <sup>c</sup> $P < 0.05$  higher than standard control.



**Figure 6:** Effect of methanol extract of *Ficus trigonata* leaves on serum protein markers in mice infected with *Plasmodium berghei*. FTLE: *Ficus trigonata* leaves extract. Data were expressed as mean  $\pm$  SEM; n=5. <sup>a</sup> $P < 0.05$  lower than normal, <sup>b</sup> $P < 0.05$  higher than negative control, <sup>c</sup> $P < 0.05$  higher than standard control.

### 3.2 Discussions

#### (a). The Antioxidant Activity

The antioxidant potential of *Ficus trigonata* leaf extract was evaluated in this study using two standard assays - DPPH and FRAP. These assays provide reliable preliminary evidence on the free radical scavenging and reducing power of plant extracts, which enables inference on their antioxidant capacity (Huang *et al.*, 2005). The extract exhibited concentration-dependent activity in both models, though lower than the standard antioxidant ascorbic acid. The  $IC_{50}$  values signified potent antioxidant properties, likely attributable to the presence of phenolic compounds (Vuolo *et al.*, 2019) like flavonoids in *F. trigonata* leaves as reported previously (Pandey and Rizvi, 2009). Phytochemicals can act as antioxidants through multiple mechanisms including electron donation to stabilize radicals, metal ion chelation, inhibition of oxidizing enzymes, and regeneration of membrane-bound antioxidants (Erb and Kliebenstein, 2020). The extract may exert its effects via these pathways to mitigate oxidative stress.

#### (b). The Lipid Profile

According to reports, malaria causes mild alterations in a person's lipid profile, with a usual increase in blood triglyceride levels and a decrease in HDL levels (Faucher *et al.*, 2002). The study investigated the impact of *Ficus trigonata* leaves extract on lipid profiles in *Plasmodium berghei* infected mice. The results demonstrated a significant reduction in LDL cholesterol across all treatment groups

compared to naïve controls, indicating the potential of the extract in lowering LDL cholesterol levels which resulted from malaria complication which also agrees with another study (Laufs *et al.*, 2019). Interestingly, HDL cholesterol, often referred to as the 'good' cholesterol due to its role in removing excess cholesterol from the bloodstream (Jomard and Osto, 2020), was found to be markedly reduced in the extract-treated groups. This could suggest a potential adverse effect of the extract on HDL levels, which could have implications for cardiovascular health, given that higher HDL levels are generally associated with lower risk of heart disease (Jomard and Osto, 2020). Total cholesterol was observed to be significantly elevated in the extract groups compared to standard controls but remained lower than naïve values. This could indicate a modulatory effect of the extract on total cholesterol levels (Calling *et al.*, 2019). Triglyceride levels were found to be increased in all extract doses compared to naïve mice but decreased compared to negative controls, although not to the level standard treatment. High triglyceride levels are often associated with increased risk of heart disease (Reiner, 2017), suggesting that while the extract may increase triglyceride levels relative to naïve mice, it may still offer some curative effects compared to negative controls and standard treatment.

#### (b). The Liver Function Indices

The extract demonstrated hepatocurative effects in malaria-infected mice as evidenced by dose-dependent reductions in elevated serum AST, ALT and ALP. These aminotransferases are released into circulation following liver injury, making them sensitive indicators of hepatocellular damage (Giannini *et al.*, 2005). Elevations induced by malaria infection are attributable to inflammatory cytokine mediated oxidative stress and hepatic dysfunction (Sobolewski *et al.*, 2005). By lowering AST, ALT and ALP, *F. trigonata* leaves extract may mitigate these processes and stabilize hepatocyte membranes to prevent enzyme leakage.

The reductions in total protein levels due to malaria infection agree with previous reports, attributable to impaired liver function and nutrient malabsorption (Ojo *et al.*, 2022; Okagu *et al.*, 2022). By restoring protein levels, the extract likely supports hepatic protein synthesis and intestinal absorption. Of note, extract treatment had negligible effects on albumin despite raising total protein. The maintained albumin levels could signify enhanced liver function to sustain albumin production coupled with elevated non-albumin proteins (Ajayi *et al.*, 2017). Raised total bilirubin level is associated with severe malaria

infection (Mathews *et al.*, 2019), total and direct bilirubin were decreased in infected untreated mice and further lowered with extract doses of 100 and 200 mg/kg in this study. This reduction could be attributable to impaired hepatic uptake and conjugation of unconjugated bilirubin (Ruiz *et al.*, 2021). The extract may dose-dependently stabilize these processes to regulate bilirubin metabolism. At 300 mg/kg, bilirubin was restored to near normal levels, further confirming the potential of *F. trigonata* to mitigate malaria-induced effects on bilirubin regulation.

## 4. Conclusion

This study provides evidence that *Ficus trigonata* leaves extract can mitigate some of the alterations in lipid profile and liver function indices induced by *Plasmodium berghei* infection in mice. The extract exhibited antioxidant properties and demonstrated potential to ameliorate malaria-associated dyslipidaemia and hepatocellular injury.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

The authors sincerely appreciate Biochemistry department of Modibbo Adama University, Yola, Adamawa State, Nigeria, for allowing them to use the departmental laboratory and other necessary facilities for the success of this study.

## References

- Ajayi, E. I. O., Adeleke, M. A., Adewumi, T. Y., Adeyemi, A. A. (2017). Antiplasmodial activities of ethanol extracts of *Euphorbia hirta* whole plant and *Vernonia amygdalina* leaves in *Plasmodium berghei*-infected mice. *Journal of Taibah University for Science*, 11(6), 831-835. <https://doi.org/https://doi.org/10.1016/j.jtusci.2017.01.008>
- Amah, C. C., Enechi, O. C., Ekpo, D. E., Okagu, I. U., Ononiwu, C. P., Joshua, P. E. (2021). Safety assessment and antimalarial property of methanol extract of *Fagara zanthoxyloides* root-bark on *Plasmodium berghei*-infected mice. *Comparative Clinical Pathology*, 30(2), 217-228. <https://doi.org/10.1007/s00580-021-03202-7>
- Benzie, I. F. F., and Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP

- Assay. *Analytical biochemistry*, 239(1), 70-76.  
<https://doi.org/https://doi.org/10.1006/abio.1996.0292>
- Berg, C. C. (1989). Classification and distribution of *Ficus*. *Experientia*, 45(7), 605-611.  
<https://doi.org/10.1007/BF01975677>
- Berg, C. C., and Villavicencio, X. (2004). *Taxonomic studies on Ficus (Moraceae) in the West Indies, extra-amazonian Brazil, and Bolivia*. Universitetet i Bergen.
- Bucolo, G., and David, H. (1973). Quantitative Determination of Serum Triglycerides by the Use of Enzymes. *Clinical Chemistry*, 19(5), 476-482.  
<https://doi.org/10.1093/clinchem/19.5.476>
- Calling, S., Johansson, S.-E., Wolff, M., Sundquist, J., Sundquist, K. (2019). The ratio of total cholesterol to high density lipoprotein cholesterol and myocardial infarction in Women's health in the Lund area (WHILA): a 17-year follow-up cohort study. *BMC cardiovascular disorders*, 19(1), 239.  
<https://doi.org/10.1186/s12872-019-1228-7>
- Demacker, P. N. M., Hessels, M., Toenhake-Dijkstra, H., Baadenhuijsen, H. (1997). Precipitation methods for high-density lipoprotein cholesterol measurement compared, and final evaluation under routine operating conditions of a method with a low sample-to-reagent ratio. *Clinical Chemistry*, 43(4), 663-668.  
<https://doi.org/10.1093/clinchem/43.4.663>
- Doumas, B. T., Ard Watson, W., Biggs, H. G. (1997). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica chimica acta*, 258(1), 21-30.  
[https://doi.org/https://doi.org/10.1016/S0009-8981\(96\)06447-9](https://doi.org/https://doi.org/10.1016/S0009-8981(96)06447-9)
- Erb, M., and Kliebenstein, D. J. (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. *Plant physiology*, 184(1), 39-52.
- Faucher, J.-F., Ngou-Milama, E., Missinou, M., Ngomo, R., Kombila, M., Kremsner, P. G. (2002). The impact of malaria on common lipid parameters. *Parasitology research*, 88(12), 1040-1043.  
<https://doi.org/10.1007/s00436-002-0712-6>
- Friedewald, W. T., Levy, R. I., Fredrickson, D. S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, 18(6), 499-502.  
<https://doi.org/10.1093/clinchem/18.6.499>
- Giannini, E. G., Testa, R., Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*, 172(3), 367-379.  
<https://doi.org/10.1503/cmaj.1040752>
- Grandtner, M. M. (2005). *Elsevier's Dictionary of Trees: Volume 1: North America*. Elsevier.
- Greenwood, B., Zongo, I., Dicko, A., Chandramohan, D., Snow, R. W., Ockenhouse, C. (2022). Resurgent and delayed malaria. *Malaria Journal*, 21(1), 77.  
<https://doi.org/10.1186/s12936-022-04098-6>
- Huang, D., Ou, B., Prior, R. L. (2005). The Chemistry behind Antioxidant Capacity Assays. *Journal of agricultural and food chemistry*, 53(6), 1841-1856.  
<https://doi.org/10.1021/jf030723c>
- Jendrassik, L., and Grof, P. (1938). Quantitative determination of total and direct bilirubin in serum and plasma. *Biochem Z*, 297, 81-89.
- Jomard, A., and Osto, E. (2020). High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential [Mini Review]. *Frontiers in cardiovascular medicine*, 7.  
<https://doi.org/10.3389/fcvm.2020.00039>
- Laufs, U., Parhofer, K. G., Ginsberg, H. N., Hegele, R. A. (2019). Clinical review on triglycerides. *European heart journal*, 41(1), 99-109c.  
<https://doi.org/10.1093/eurheartj/ehz785>
- Mathews, S. E., Bhagwati, M. M., Agnihotri, V. (2019). Clinical spectrum of *Plasmodium vivax* infection, from benign to severe malaria: A tertiary care prospective study in adults from Delhi, India. *Trop Parasitol*, 9(2), 88-92.  
[https://doi.org/10.4103/tp.TP\\_2\\_19](https://doi.org/10.4103/tp.TP_2_19)
- Mekonnen, L. B. (2015). In vivo antimalarial activity of the crude root and fruit extracts of *Croton macrostachyus* (Euphorbiaceae) against *Plasmodium berghei* in mice. *Journal of Traditional and Complementary Medicine*, 5(3), 168-173.  
<https://doi.org/https://doi.org/10.1016/j.jtcm.2014.07.002>
- Mishra, K., Ojha, H., Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food chemistry*, 130(4), 1036-1043.

- <https://doi.org/https://doi.org/10.1016/j.foodchem.2011.07.127>
- Ojo, R. J., Jonathan, I. G., Adams, M. D., Gyebi, G., Longdet, I. Y. (2022). Renal and hepatic dysfunction parameters correlate positively with gender among patients with recurrent malaria cases in Birnin Kebbi, Northwest Nigeria. *The Egyptian Journal of Internal Medicine*, 34(1), 77. <https://doi.org/10.1186/s43162-022-00164-2>
- Okagu, I. U., Aguchem, R. N., Ezema, C. A., Ezeorba, T. P. C., Eje, O. E., Ndefo, J. C. (2022). Molecular mechanisms of hematological and biochemical alterations in malaria: A review. *Molecular and biochemical parasitology*, 247, 111446. <https://doi.org/https://doi.org/10.1016/j.molbio para.2021.111446>
- Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2, 270-278.
- Reiner, Ž. (2017). Hypertriglyceridaemia and risk of coronary artery disease. *Nature Reviews Cardiology*, 14(7), 401-411. <https://doi.org/10.1038/nrcardio.2017.31>
- Reitman, S., and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *American journal of clinical pathology*, 28(1), 56-63. <https://doi.org/10.1093/ajcp/28.1.56>
- Roeschlau, P., Bernt, E., Gruber, W. (1974). Enzymatic determination of total cholesterol in serum. *Zeitschrift fur klinische Chemie und klinische Biochemie*, 12(5), 226.
- Ruiz, A. R. G., Crespo, J., Martínez, R. M. L., Iruzubieta, P., Mercadal, G. C., Garcés, M. L., Lavin, B., Ruiz, M. M. (2021). Measurement and clinical usefulness of bilirubin in liver disease. *Advances in Laboratory Medicine / Avances en Medicina de Laboratorio*, 2(3), 352-361. <https://doi.org/doi:10.1515/alm-2021-0047>
- Schmidt, F. H. (1961). Die enzymatische Bestimmung von Glucose und Fructose nebeneinander. *Klinische Wochenschrift*, 39(23), 1244-1247. <https://doi.org/10.1007/BF01506150>
- Sobolewski, P., Gramaglia, I., Frangos, J. A., Intaglietta, M., Heyde, H. v. d. (2005). "Plasmodium berghei" Resists Killing by Reactive Oxygen Species. *Infection and immunity*, 73(10), 6704-6710. <https://doi.org/doi:10.1128/iai.73.10.6704-6710.2005>
- Stevens, W. D., Ulloa, C., Pool, A., Montiel, O. M. (2001). *Flora de Nicaragua* (Vol. 85). Missouri Botanical Garden Press St. Louis.
- Vuolo, M. M., Lima, V. S., Maróstica Junior, M. R. (2019). Chapter 2 - Phenolic Compounds: Structure, Classification, and Antioxidant Power. In M. R. S. Campos (Ed.), *Bioactive Compounds* (pp. 33-50). Woodhead Publishing. <https://doi.org/https://doi.org/10.1016/B978-0-12-814774-0.00002-5>
- W. H. O. (2022). *World malaria report 2022*. World Health Organization.
- Weichselbaum, C. T. E. (1946). An Accurate and Rapid Method for the Determination of Proteins in Small Amounts of Blood Serum and Plasma. *American journal of clinical pathology*, 16(3\_ts), 40-49. [https://doi.org/10.1093/ajcp/16.3\\_ts.40](https://doi.org/10.1093/ajcp/16.3_ts.40)
- Wright, P., Leathwood, P., Plummer, D. (1972). Enzymes in rat urine: alkaline phosphatase. *Enzymologia*, 42(4), 317-327.