

Evaluation of the Therapeutic Effects of *Telfairia Occidentalis* as an Adjunct in the Treatment of Malaria with Artemether/ Lumefantrine Regimen in Children with Acute Uncomplicated Malaria

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Abstract

Malaria, one of the deadliest diseases in Africa, continues to be a public health problem in Cameroon. *Anopheles gambiae* has been reported to be the very efficient vector of *Plasmodium falciparum* which is the species of parasite responsible for 98% of deaths among children in endemic areas. In the course of host invasion, malaria parasite attacks the red blood cells, leading to a decrease in hemoglobin levels and the development of anemia commonly reported in children and pregnant women. Malaria management involves the use of conventional drugs such as Artemether/Lumefantrine, (AL) which is the first drug of choice in the treatment of uncomplicated malaria. *Telfairia occidentalis* (Pumpkin Leaves), a vegetable plant consumed in most parts of the world and with potential antimalarial properties, could serve as an adjunct therapy in malaria management. Due to this effect, this study aimed, to evaluate the therapeutic effect of *Telfairia occidentalis* on the hemoglobin level, parasitaemia clearance rate and the liver activity in Cameroonian children below 16 years. In an open labeled randomized clinical trial, enrolled participants who had Hemoglobin level within normal ranges, and individuals who had uncomplicated *P. falciparum* malaria were randomly assigned to receive: Artefan (AL) + placebo only; AL and capsulated *Telfairia occidentalis* (2 x 1gram) dose for 3 days. On days 0, 1, 2 and 7. Hemoglobin levels were measured using a Mission Hb meter, gametocytes were also assessed and quantified by microscopy and quantification of liver activity enzymes using a

Humalyzer. Data analysis were carried out using Graph pad prism version 8.0.1 The overall findings showed that, parasite density affects the hemoglobin level and hematocrit with no significant average drop (Hb: 10.19g/dL to 10.19g/dL with p-value of ≥ 0.05 , Hct: 30.49% to 30.49% p-value: ≥ 0.05). The values of the Hemoglobin level and hematocrit were seen to increase after treatment with AL but a significant increase was seen with those treated with AL + Raw Pumpkin (RP) with mean \pm SE of Hb (11.08g/dL): 11.08 \pm 0.02950; $p \leq 0.05$, Hct (33.21%): 33.21 \pm 0.09449; $p \leq 0.0001$). Parasite density was seen to decrease in both groups from D0 to D7 but decreased more rapidly and significantly in the group receiving AL+RP compared to AL with mean difference of 337.5; p-value ≥ 0.05 , 937.5; p-value ≥ 0.05 , 300.0; p-value 0.01 and 0.000; p-value ≤ 0.05 on D0, D1, D2 and D7. Liver activity enzymes were as well seen to decrease in both groups from D0 to D7 but decreased more rapidly and significantly in the AL + RP group with mean \pm SE of AST: (40.53 IU/L) 40.53 \pm 3.739 p-value 0.0153, (29.73IU/L) 29.73 \pm 3.085 p-value 0.0012, (17.23 IU/L) 17.23 \pm 2.693 p-value < 0.0001 , (9.075IU/L) 9.075 \pm 2.131 p-value < 0.0001 , ALT:(48.71IU/L) 48.71 \pm 5.385 p-value 0.0332, (38.46IU/L) 38.46 \pm 4.884 p-value 0.0223, (15.29IU/L) 15.29 \pm 2.068 p-value 0.8397, (8.925IU/L) 8.925 \pm 2.105 p-value 0.9997. Also, signs and symptoms of malaria were seen to clear off more rapidly in the groups receiving *T. occidentalis* than in those receiving just AL. Conclusively, *Telfairia occidentalis* has an influence on the hemoglobin level and liver enzyme modulatory effects and works in synergy with AL for the treatment of uncomplicated malaria due to its antimalarial properties.

Keywords: *Telfairia occidentalis*; Hemoglobin level; Liver activity; Dietary Supplement Malaria; Artemether-Lumefantrine

Abbreviations: ACT: Artemisinin-Combined Therapy AL: Artemether-Lumefantrine CQ: Chloroquine Hb: Hemoglobin INTs: Insecticide-Treated Nets IPTP: Intermittent Preventive Treatment in Pregnancy IRS: Indoor Residual Sprays ITN: Insecticide Treated nets PB: Boiled Pumpkin PCR: Polymerase Chain Reaction RP: Raw Pumpkin SP: Sulfadoxine-Pyrimethamine SP: Sulfadoxine-Pyrimethamine WHO: World Health Organization D0: Day Zero

Background

In the world at large, over 200 million malaria cases occur yearly, out of which two million progresses to severe disease. According to WHO's latest world malaria report, there were an estimated 247 million malaria cases and 619 000 malaria deaths worldwide in 2021 [1], with the major causative agent being *Plasmodium falciparum* [1]. According to WHO latest World Malaria report released on the 6th of April 2022 there were 241 million cases of malaria in the year 2020 compared to 229 million cases in 2019. The estimated number of malaria deaths stood at 409,000 in 2020 compared to 411,000 deaths in 2018. In 2019 according to WHO statistics, nearly half of the world's population was at risk with most cases and deaths occurring in sub-Saharan Africa [2].

Despite all management measures put in place, malaria still tops the list of Cameroon's Public health concerns and other parts of Sub-Saharan Africa [3]. Also, children under 5 years of age were the most vulnerable group for malaria and accounted for 97% of all malaria deaths

worldwide in the year 2019 [1]. The management of malaria involves a coherent productive approach which includes preventive measures that aim at controlling the vectors such as use of insecticide-treated nets (ITNs), indoor residual sprays (IRS) and parasite elimination by use of conventional drugs including artemisinin monotherapies and artemisinin-based combination therapies (ACTs) [5]. Due to increase resistance of parasite to ancient drugs such as Chloroquine and mefloquine, ACT have been recommended as the first line treatment for patients infected with uncomplicated *Plasmodium falciparum* [2]. Artemether/Lumefantrine (AL) is one of the approved and most successful fixed dose ACT used in the treatment of uncomplicated malaria by *P. falciparum*. Unfortunately, recent partial artemisinin resistance has been reported, seen to be characterized by slow parasitological response defined as delayed parasite clearance to treatment during the 3 days' treatment with AL as recommended by the WHO as the stipulated time for complete parasite clearance from infected blood [6]. Early diagnosis and prompt treatment with an effective drug regimen remain important components of

malaria control and elimination strategies [7]. In Cameroon, one of the recommended drugs for the treatment of uncomplicated malaria caused by *Plasmodium falciparum*, is AL which is commonly known as Coartem. In a previous study designed to evaluate the efficacy of Amodiaquine-Artesunate versus Artemether Lumefantrine against uncomplicated malaria in children less than 14 years in Ngaoundere, Cameroon [8], it was reported that, both drugs were effective in parasite clearance and PCR-corrected cure. The rates were 100% and 96.4%. However, several laboratorial abnormalities occur in the course of the infection such as anemia, thrombocytopenia, and methemoglobinemia, [9]. Different substances or extracts have been used as adjuncts alongside ACTs. Many antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or are derivatives of plant components [10]. As such, plants have always been considered to be a possible alternative and rich source of new drugs. *T. occidentalis* for example (commonly known as pumpkin leaves); has been reported to exhibit antiplasmodial activity both in the 4-day early infection test and in established infection with a marked increase of the mean survival time [11]. The leaves of *Telfairia occidentalis* showed the presence of phenolic compounds, flavonoids, phytosterols, tannins, saponins, chlorophyll and glycosides which have been reported to exhibit chemo suppressive activity [12]. *Telfairia occidentalis* produced a dose-dependent improvement in the seminal fluid analysis and the histology of the testes, showing a near complete morphological regeneration and increased spermatogenesis [13]. The leaf extract of *T. occidentalis* increased sperm motility, sperm viability and sperm count in rat [14]. Other components of the plant such as arginine, vitamin C and zinc may also play important roles since studies have shown that nutritional therapies with zinc, vitamin C, vitamin E and arginine proved to be of benefit in the treatment of male infertility [12]. Both aqueous and ethanolic extracts of *T. occidentalis* leaf have hepatoprotective properties, although the aqueous extract was more effective than the ethanolic extract, which could be attributed to the higher antioxidant activity of the aqueous extract than the ethanolic extracts of *T. occidentalis* leaves [15].

The leaves are good sources of K, Cu, Fe and Mn, moderate sources of Mg and Zn which are essential in hu-

man and animal nutrition [16]. The high content of Fe in the young tender fluted pumpkin leaves serves as the basis for which the leaf extract is administered traditionally as blood tonic in the treatment of anemia and to convalescing patients. With these numerous therapeutic potentials, pumpkin leaves can therefore be used not only as a food supplement but also an adjunct therapy in the management of malaria alongside ACTs which is the first work carried out so far. This study aimed to investigate the effect of *Telfairia occidentalis* on the hemoglobin level of malaria patients, the clearance rate of parasite in the blood and its effects on liver activity of children less than 16 years of age.

Methods

Study Area

The study was carried out in the Bamenda Regional Hospital, Gilead Health Center and Solaman Multi-Care Foundation Laboratory which are all based in Bamenda situated in the Northwest Region of Cameroon, where it funds as the regional administrative headquarter. Bamenda is found in Mezam division and located between latitudes 5056 to 5058 North of Equator and between longitude 10008 and 10010 East of Greenwich Meridian [18]; and occupies a total surface area of 3,125 hectares [19]. It is bounded by Bafut subdivision to the North, flanked to the West by Mbengwi, to the South by Santa sub-division, to the Southwest by Bali sub-division and bordered by Tubah sub-division in the East.

Study Design

This study was a randomized open label clinical trial carried out from March 2022 to May 2022 to assess the effect of *Telfairia occidentalis* on the Hemoglobin level, the liver enzyme activity and parasite clearance rate when used in combination with artemether-lumefantrine (AL) treatment regimen in children. After a proxy-informed consent was gotten from parents/guardians, children aged 3 to 16 years who presented clinical signs and were diagnosed with uncomplicated malaria and parasitaemia count above 2000 parasites/ul blood, were recruited for this study through random sampling as case group. Subjects without malaria were recruited as control group. Participants screened for the presence of co-infections were excluded from the study.

The study was designed to have one control group and three treatment groups (Groups 1, 2, 3 and 4). Upon meeting all enrolment criteria, participants were randomized to receive either of the two treatment regimens; (i) AL (Coartem; Novartis Pharma AG, Basel Switzerland- 20 mg artemether and 120 mg lumefantrine) as a standard or (ii) therapeutic dose of AL and therapeutic dose of *Telfairia occidentalis*

Sample Population and Randomization

Overall, of 180 participants included in this study, 75 were females and 69 were males. Seventy participants were malaria positives giving a prevalence of 38.88% (70/180). Of the overall, 120 participants of all age groups were divided into 4 groups, 70 were study patients positive for malaria and 50 were the control group negative for malaria. Also, of the overall participants, a subset of 74 children between the ages of 3 to 15 belonging to the control and malaria infected group were selected for treatment. The children were enrolled and assigned to Four groups (control (RP, BP), AL, AL + RP and AL+BP groups) randomly including 70 malaria positives to the study groups 2, 3 and 4 (AL, AL + RP and AL+BP groups respectively) and 50 malaria negatives in the control group (Group 1). Follow-up was done for seven days for the assessment of Hemoglobin Level and Liver Enzyme Activity.

Drug Administration, Treatment and Grouping

The 70 patients recruited as malaria positive cases were placed into three groups (groups 2, 3 and 4). Group 2 received artemether lumefantrine only while group 3 received artemether-lumefantrine together with capsulated Raw *Telfairia occidentalis* and group 4 received artemether lumefantrine together with Boiled *Telfairia occidentalis*. Group one was made up of non-positive cases, that is children whose microscopic examination did not show the presence of *P. falciparum* or any other malaria species and were called normal individuals.

For group 2, artemether–lumefantrine was administered according to the manufacturer’s prescription which is based on the body weight of the child. The treatment lasted for a period of 3 days (72 hours) with a treatment interval of 8 hours as recommended by WHO. That is, drugs tak-

en twice a day; morning and evening [21]. For 5 kg to less than 15 kg body weight, patients took one tablet as an initial dose, 1 tablet again after 8 hours and then 1 tablet twice daily (morning and evening) for the following 2 days (total course of 6 tablets). For 15 kg to less than 25 kg bodyweight, two tablets as an initial dose, 2 tablets again after 8 hours and then 2 tablets twice-daily (morning and evening) for the following 2 days (total course of 12 tablets). For 25 kg to less than 35 kg bodyweight, three tablets as an initial dose, 3 tablets again after 8 hours and then 3 tablets twice-daily (morning and evening) for the following 2 days (total course of 18 tablets). For 35 kg bodyweight and above, four tablets as a single initial dose, 4 tablets again after 8 hours and then 4 tablets twice-daily (morning and evening) for the following 2 days (total course of 24 tablets). Each tablet of AL contains 20 mg of artemether and 120 mg of lumefantrine. For group 3 and 4, artemether lumefantrine was administered the same way as in group 2 *Telfairia occidentalis* was administered based on age ranges.

For follow-up, the treatment was initiated on D0 (enrollment Day) and participants were asked to return to study clinic on days 1, 2 and 7. On each follow-up day, study participants were assessed for any symptoms, possible adverse effects, concomitant medication, measuring axillary temperature and collecting blood samples. In case a participant did not show up at the clinic, a field worker was sent to the house to find out the reason and possibly to bring the participant at the clinic for clinical examination and sampling. At all visits, blood was collected by finger-prick for blood smears and 2mls of blood collected in red tubes for lipid parameter quantifications. A patients’ case report form was filled each day of follow up. This was to check for decrease of clinical signs of malaria (fever, temperature, vomiting, loss of appetite, abdominal pain, headache, dizziness and body weakness) and if there was any adverse effect of the drug administered.

Hemoglobin Measurement

Hb measurements were done using the mission plus Hb Hemoglobin Testing System VR (AconBiotech San Diego USA) intended for the quantitative determination of hemoglobin (Hb) and calculated hematocrit (Hct) in capillary and venous human whole blood. Before collecting the

sample, the individual's finger was cleaned with a cotton containing a prepared 75 % alcohol, after which a lancet was then used to prick the finger. The first blood was wiped off using a cotton and a light pressure applied to obtain a second drop of blood. About 10µL of capillary blood was then collected using a capillary transfer tube. The tube was held slightly downwards and its tip touched the blood drop. Capillary action automatically draws the sample to the fill line and stop. The Hb meter turned on after it was correctly set with the correct time and the appropriate test strip was inserted and its code shown on the screen of meter after insertion for confirmation. After some few seconds after inserting the test strip a signal is made for specimen to be applied in the specimen application area. The tip of the capillary transfer tube was then aligned with the specimen application area of the strip and the second drop of blood (approximately 10µL) applied. After which the Hb and Hct were read after a few seconds and the respective readings were noted at every occasion. This procedure was repeated for D1, D2 and D7 follow up of patients.

Malaria Parasite Count Estimation

The method used for quantification or estimation of parasite density in enrolled patients was microscopy which was performed by counting parasites within a given number of microscopic fields against counted white blood cells (WBCs) within the same fields [21] with an assumed WBC count of 8.0×10^9 cells/l. Blood samples were collected from patients on D0 upon recruitment by finger pricking using lancets and the slides labelled with patient ID number and day of collection. The blood was then made to drop on a clean sterilized microscopic slide (approximately 10µl) and a thick smear made in a circular motion. The slides were allowed to air dry before staining with giemsa. Air dried slides were fixed with methanol by dipping into the container of methanol for 2–3 minutes. The slides were then placed back-to-back in a staining trough containing giemsa solution making sure all fixed sample faced to one direction and allowed for 20 minutes. After which the slides were rinsed with clean water, allowed to air dry for 5 minutes then viewed under the microscope. The slides were examined under the x100 oil immersion objective lens of a

light microscope. The asexual parasites density was counted against WBCs counted in microscopic field examination. A patient was considered positive if *P. falciparum* was seen during the microscopic examination. This procedure was repeated for D1, D2 and D7 follow up of patients.

Liver Enzyme Activity Measurements

The method used for the measurement of ALT, AST and GGT was spectrophotometry using the Humalyzer 3500 machine which is automated with a built-in inventory management system. On D0, D3, D7 of follow-up, 2ml of blood was collected from patients via venipuncture and placed in to a 4ml EDTA test tube. Blood samples were centrifuged using a centrifuge at 3000 rpm for 15 minutes as per standard clinical laboratory procedures. The plasma was then separated and transferred into dry tubes and stored in a refrigerator set at 0 to 4°C. The samples were then analyzed using Humalyzer 3500 for AST, ALT and GGT which uses enzymatic colorimetric and spectrophotometric test. Blood analyzed were collected on D0 before treatment and D2 and D7 after treatment.

Data Analysis

Data were entered and analyzed using Microsoft excel 2013 and Graph pad prism version 8.0.1. Descriptive statistics were done. All values were expressed as Mean \pm S.E (Mean standard error of Difference). The analysis of variance (ANOVA) was employed to know mean differences of liver enzymes and parasite density. P-values <0.05 were considered as statistically significant and Confidence limit was set at 95%.

Results

Socio-Demographic Data

The baseline characteristics of participants (180) who met the inclusion criteria are displayed in Table 1. Of the overall, children enrolled had a mean weight of 36.4 kg. There was a significant difference in hemoglobin (Hb g/dL) study cases and control group ($p = 0.02$) and a significant difference in temperature.

Table 1: Socio-Demographic Data

Characteristics	Control (N=90)	Cases(N=90)	p-value (<0.05)
Sex-ratio (Male/female)	0.9 (35/37)	0.8 (34/38)	
Mean Hemoglobin(g/dL) (SD)	11.8 (0.58)	8.17(0.56)	0.02
Mean temperature(oC) (SD)	36.44 (0.25)	38.11(0.17)	0.04

Note*: SD: Standard Deviation, N: Number of participants, Kg: Kilogram, g/Dl: Gram per deciliter; : Descriptive characteristics of the study population

The combined effect of *Telfairia occidentalis* and AL on the parasite clearance rate in children infected with *Plasmodium falciparum*

We observed a positive response in individuals suffering from *P. falciparum* who received AL + Placebo as their only treatment. Their parasitemia dropped from higher to lower values of trophozoites/ul of blood with a significant change even though during the third day (D2) of treatment some trophozoites were still found in the blood samples of some patients as shown in the figure below (1a). It was observed that there was a significant difference in mean between D0 and D1 at 4000ul of blood and 1500ul of blood (3778 ± 378.3), 95% CI(1044 to 3467) with p-value 0.0015, as

well as between D0 and D7 at 4000ul of blood and The effect of AL+BP on the parasite clearance rate of *P. falciparum* infected children among the different treatment days showed a significant decrease in parasitemia between D0 and D7 from 3500 trophozoites/ul of blood (3450 ± 861.3) with a p-value of 0.02 and 0 trophozoites/ul of blood. Meanwhile the analysis also showed a decrease in parasitemia between D1 vs D2, D1 vs D7 and D2 vs D7 though not statistically significant (Figure 1b). The combined effect of AL + RP on the parasite clearance rate between the different days of treatment showed a significant decrease (p-value ≤ 0.05) in the number of parasites (Figure 1c). No trophozoites were observed on D2 and D7 showing a significant decrease with p-value 0.0037.

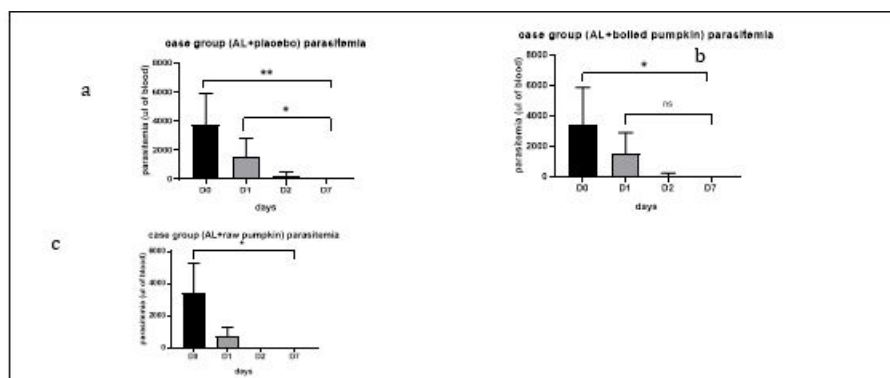


Figure 1: The effect of AL + RP, Artefan AL + Bp and Artefan + placebo treatment groups on parasite clearance. Effect of AL + placebo on parasitemia (a), effect of AL +BP (b) and the effect of AL +RP (c) on parasitemia of children infected with *Plasmodium falciparum*, asterisks represent statistical difference between the different treatment days (D0, D1, D2 and D7)

Comparison between BP+ AL and Placebo+ AL, RP + AL and Placebo + AL effects on parasite clearance

As shown in fig 2, parasite clearance differs, though not statistically significant, except for D2 in figure 2a which showed a significant decrease in mean from 3000 trophozoites/ul of blood (3000.0 ± 0.000), 95% CI 95.22 to

(504.8) with p-value ≤ 0.05 . The parasite clearance rate was higher in the BP+AL group from 3500 trophozoites/ul of blood (3756 ± 1170 , $p > 0.05$) to 100 trophozoites/ul of blood (112.5 ± 106.7 , $p > 0.05$) compared to the placebo + AL group from 3000 trophozoites/ul of blood (3475 ± 1170 , $p > 0.05$) to 800 trophozoites/ul of blood (300 ± 106.7 , $p > 0.05$). In the RP + AL group a small decrease in para-

sitemia was observed though not statistically significant as no parasites were seen after the third day of treatment (D2)

and D7 of follow up from 4000 trophozoites/ul of blood (3412.5 ± 1044 , $p > 0.05$) in D0 to 0 trophozoites/ul of blood.

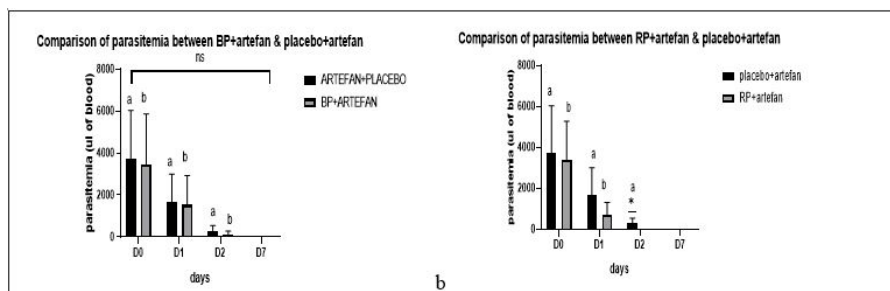


Figure 2: Effects of RP +AL, BP +AL and Placebo + AL on the parasite clearance rate and a comparison between BP+AL vs Placebo +AL (a), and RP + AL vs Placebo +AL (b) on the parasite clearance rate of plasmodium falciparum infected children. Asterisks represents statistical difference between treatment days (D0, D1, D2 and D7) as well as between the different treatment groups (BP+AL, RP+AL and AL+ Placebo) and also comparison between BP +AL vs placebo + AL and RP +AL vs Placebo + AL treatment groups while ns means not statistically significant

Hemoglobin levels in normal individuals and malaria patients

An increase in the means of Hb in children not affected by the parasite from that of individuals affected by the parasite was observed as follows. According to the different treatment days it was observed that, on D0 an increment was recorded from 10.30g/dL to 12.19 g/dL with a p-value of 0.02, D1 from 10.30g/dL to 12.19g/dL with a p-value of 0.02, D2 from a mean of 10.79g/dL to a mean of 12.31g/dL and a p-value of 0.11 and D7 from a mean of 11.08g/dL to 12.43 and with a p-value of 0.13, even though days 2 and 7 were not statistically significant. In the Sadik's multiple comparison of the means of Hb in normal, there was a general increase in the Hb level in the blood sample of normal children even though not statistically significant from days 2 and 7. There was a decrease in the means of Hb in children not affected by the parasite (RP vs BP)

from that of individuals affected by the parasite. D0 from 12.26g/dL to 12.19 g/dL with a p-value of 0.98, D1 from 12.26g/dL to 12.19g/dL with a p-value of 0.98, D2 from a mean of 12.49g/dL to a mean of 10.03g/dL and a p-value of <0.0001 and D7 from a mean of 13.00g/dL to 12.42g/dL and with a p-value of 0.43, even though days 0, 1 and 7 were not statistically significant, while a decrease in mean was as well observed between RP vs PA (Placebo + AL) though statistically significant. D0 from 12.26g/dL to 10.63g/dL p-value <0.0001 , D1 was from 12.26g/dL to 10.63g/dL with a p-value of <0.0001 , D2 from 12.49g/dL to 10.03g/dL with a p-value of <0.0001 and D7 was from 13.00g/dL to 10.21g/dL with p-value <0.0001 and Bp vs PA showed a decrease in mean as well though statistically significant D0 12.19g/dL to 10.03g/dL with p-value 0.003, D1 was from 12.19g/dL to 10.03g/dL with a p-value of 0.003, D2 from 12.31g/dL to 10.03g/dL with p-value 0.003 and D7 from 12.43g/dL to 10.21g/dL with a p-value of 0.002

Table 2: Comparing means of Hb levels in normal individuals

Days	Hb (g/dL) MEAN \pm SE (95%CI) Normal individuals	Hb (g/dL) MEAN \pm SE (95%CI) RP +BP	P-value
D0	10.30 \pm 0.5749 (-3.532 to -0.2430)	12.19 \pm 0.5749 (-3.532 to -0.2430)	0.02
D1	10.30 \pm 0.5749 (-3.532 to -0.2430)	12.19 \pm 0.5749 (-3.532 to -0.2430)	0.02
D2	10.79 \pm 0.6193 (-3.296 to 0.2456)	12.31 \pm 0.6193 (-3.296 to 0.2456)	0.11
D7	11.08 \pm 0.5796 (-3.004 to 0.3043)	12.43 \pm 0.5796 (-3.004 to 0.3043)	0.13

Note HB: Hemoglobin, CI: Confidence Interval, BP: Boiled pumpkin, RP: Raw pumpkin

Effects of Artefan +Placebo, RP and BP on the Hb level of Test and control groups

In the bar chart Tukey's multiple comparison, it was observed that there was an increase in Hb between RP vs BP (figure 3 b1) though not statistically significant, while a significant increase in Hb was observed between RP vs

PAL (b2) with a very tiny p-value of <0.0001 , as well as between BP vs PAL with a small p-value of 0.003. This observed increments in the hemoglobin levels as displayed by the graphs below can be attributed to the fact that the active ingredients iron specifically are still very much intact there by boosting the hemoglobin levels most especially in the RP group.

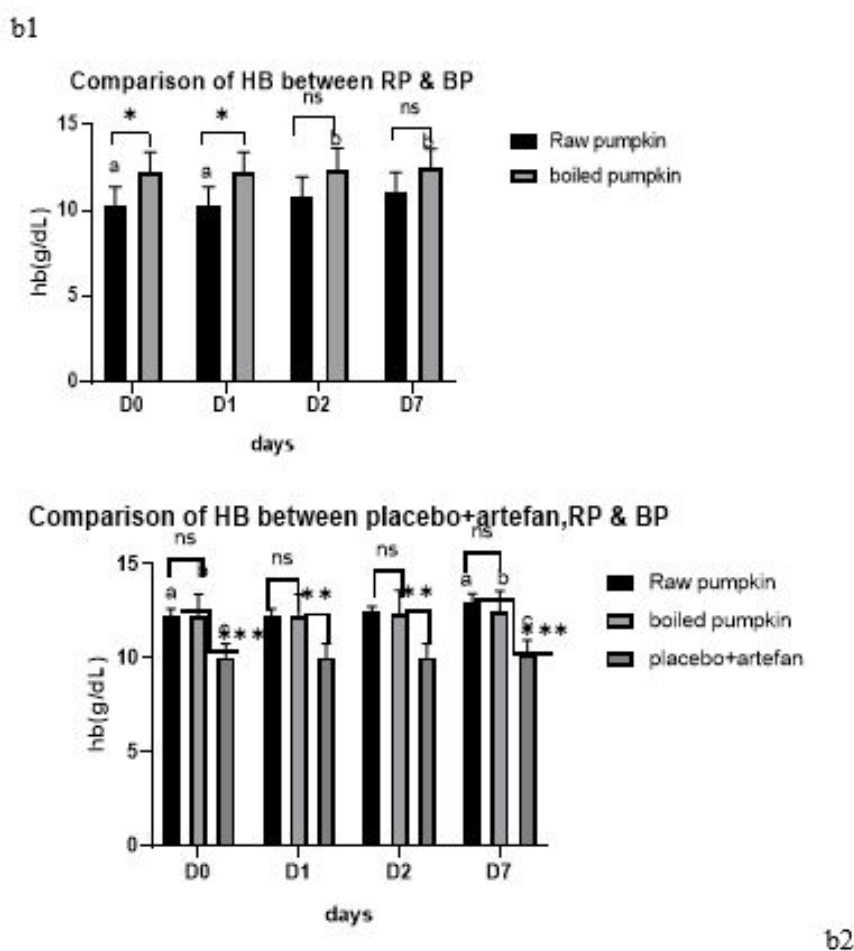


Figure 3: comparison of the effects of RP and BP (b1) on the Hb level of the control group and also comparison of the effects of AL + Placebo, RP and BP on the Hb level of Test and Control groups. Asterisks represents statistical difference between control groups (RP and BP) as well as between the test group (Placebo + AL), while the letters represent differences between control groups and test group

Therapeutic effect of *Telfairia occidentalis* on liver activity of *Plasmodium falciparum* infected children

Therapeutic effect of RP, BP and AL+ Placebo on liver activity

It was observed that, the AST, ALT and GGT levels in the RP group showed a great significant decrease ($p < 0.0001$) between the different days, compared to the BP

group which as well showed a significant decrease in these liver enzymes ($p < 0.001$). The level of these enzymes decreased significantly as well in the treatment group (AL + Placebo) ($p \leq 0.01$). An observed decrease in the three Liver enzymes in the RP + AL can be explained by the fact that the phytochemical contents of this functional food was still intact, unlike in the BP + RP group wherein we observed a small significant decrease can be due to thermal denaturation of most of these phytochemicals thereby decreasing its effect

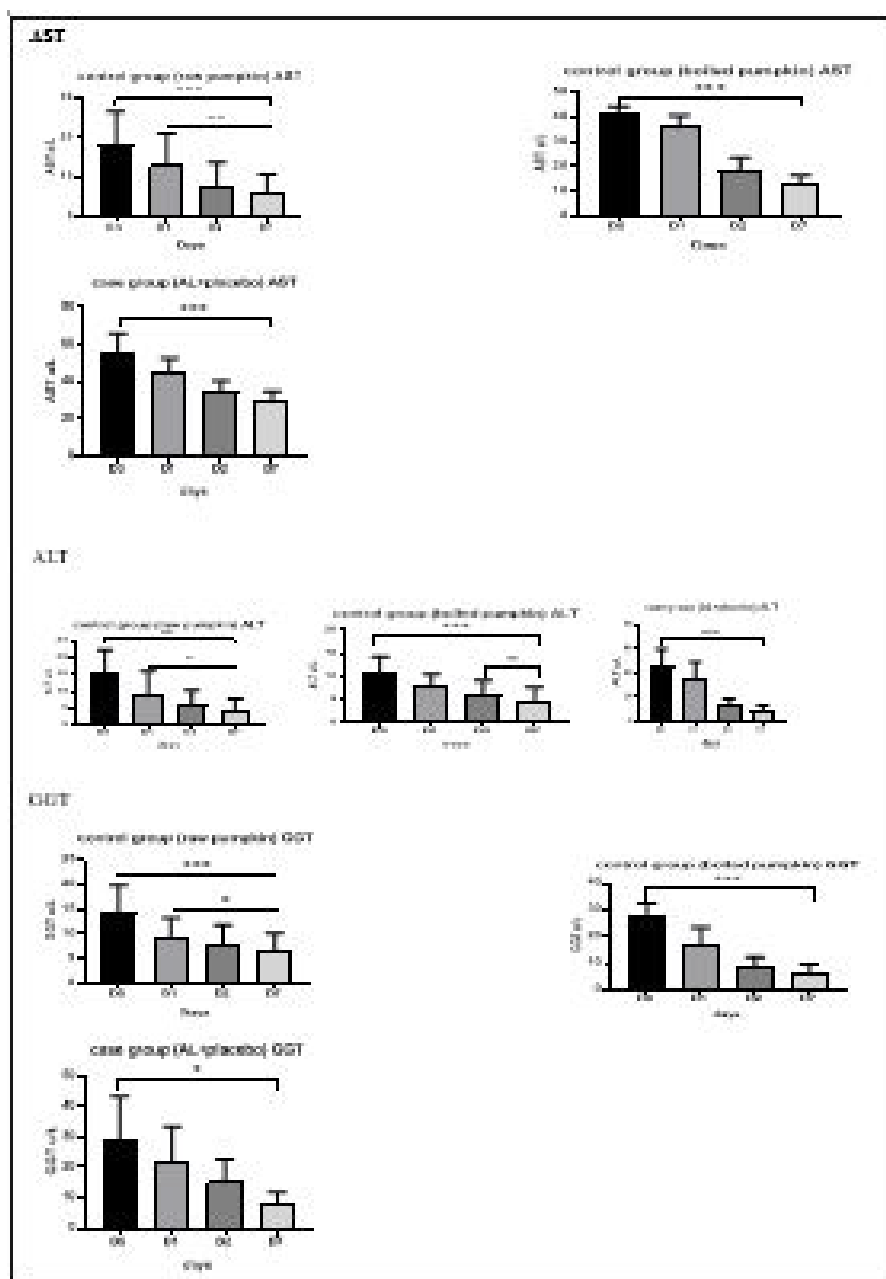


Figure 4: Effect of *T. falciparum* on the liver activity of non-infected children (Fig 4a and 4b) and children infected with *Plasmodium falciparum* (figure 4c). Asterisks represents statistical difference between the different treatment days (D0, D1, D2 and D7) while ns represents not statistically significant

Effect of BP +AL and RP + AL on liver activity of *Plasmodium falciparum* infected children

The figure 5 shows comparison of the effect of BP+ AL and RP + AL on the liver activity of the patients (treatment groups) which showed no significant difference

between the different liver enzymes (ALT, AST and GGT) during the different treatment days though it was observed that there was a decrease in mean during the different treatment days. Figure a showed a significant decrease in ALT for both treatment groups (BP +AL and RP +AL) on D0(28.74 ± 2.715 , p 0.003) and (40.53 ± 2.715 , p 0.003).

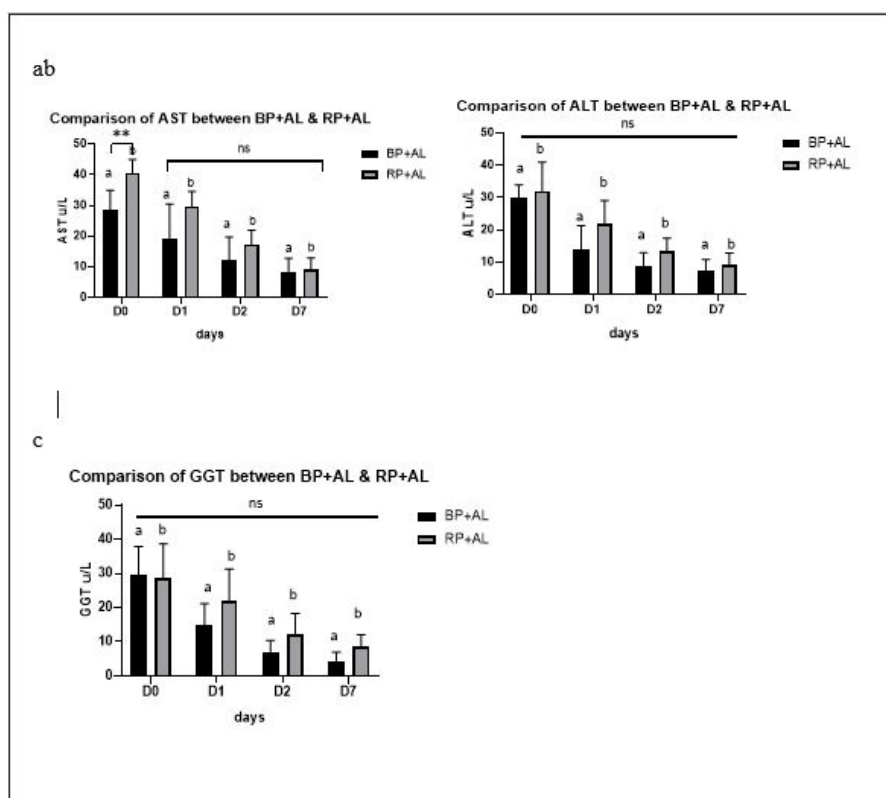


Figure 5: comparison of BP +AL and RP + AL effect on AST (fig 5a), ALT (fig 5b) and GGT (fig 5c) of *Plasmodium falciparum* infected children. Asterisks represents comparison between the two treatment groups (BP + AL and RP + AL) on the different days of treatment, ns represents statistically not significant while the letters represent the different treatment groups

Effect of BP +AL and Placebo+ AL on liver activity of *Plasmodium falciparum* infected children

The below figure 6 shows comparison of the effect of BP + AL and Placebo + AL on liver activity of the treatment group. It was observed in Figure 6(a) that there was a significant decrease in mean of AST between the two treatment groups with mean difference (21.87 ± 2.244 , $p < 0.0001$), as well as in means of ALT figure 6(b) with mean difference (6.390 ± 2.053 , $p = 0.0302$) though no significant difference was observed on D7 of follow up, but in there was no significant decrease in mean of GGT was observed between the two treatment groups.

Comparison between RP +AL and Placebo+ AL on

the liver enzyme activity of *Plasmodium falciparum* infected children

A comparison of the effect between RP + AL and AL + Placebo on the liver activity enzymes of *P. falciparum* infected children is shown in figure 7. It was observed that there was a significant increase in means of AST and ALT between the two treatment groups, AST (-13.99 ± 3.739 , $p = 0.0153$) on D0, (-15.39 ± 3.085 , $p = 0.0012$) on D1, (-17.87 ± 2.693 , $p < 0.0001$) and (-21.24 ± 21.24 , $p < 0.0001$) on D7, ALT (16.75 ± 5.385 , $p = 0.0332$) on D0, (16.56 ± 4.884 , $p = 0.0223$) on D1 and no significant difference was observed on D2 and D7 of treatment. There was no observed significant difference in GGT between the two treatment groups.

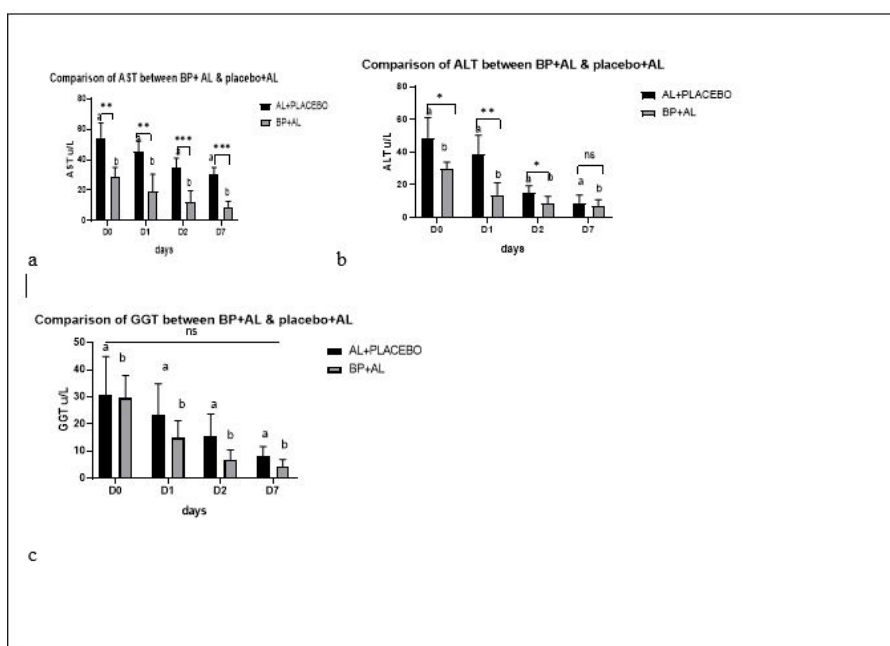


Figure 6: comparison of the effect between BP + AL and AL + Placebo on the AST (6a), ALT (6b) and GGT(6c) enzymes of Plasmodium falciparum infected children. Asterisks represents difference between AST, ALT and GGT levels during the different treatment days in BP + AL compared AL +Placebo treatment groups. Whereas a and b represent the difference in the serum levels of ALT, AST and GGTbetween BP+ALtreatment group compared to AL + Placebo treatment group

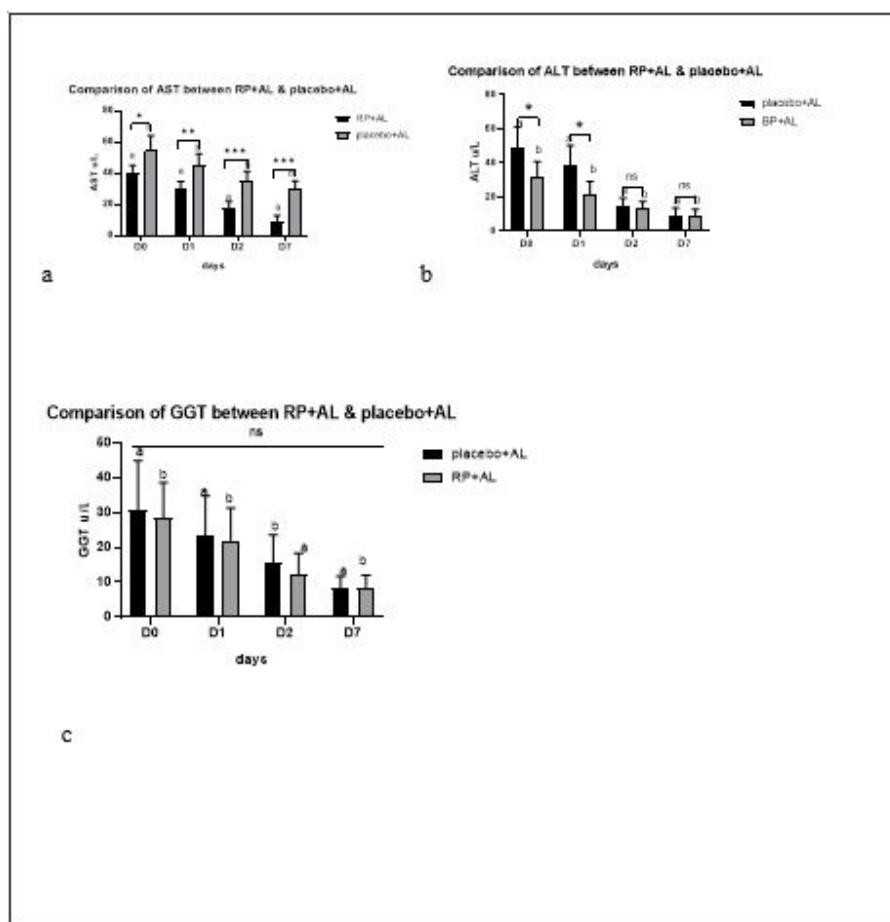


Figure 7: comparison of the effect between RP + AL and AL + Placebo on the liver enzymes activity, AST (a), ALT(b) and GGT(c) serum level of malaria positive children. Asterisks represents statistical difference between treatment groups (RP + AL and AL + Placebo) and also com-

parison between RP + AL treatment and AL + Placebo treatment groups. The letters represent the difference in serum levels between the RP + AL treatment group and AL + Placebo treatment group

Comparison of the different treatment groups

Parasitemia levels between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RPVs. AL + BP

Figure 8 below brings out comparison of parasite clearance rate among the different treatment groups, and it was observed that there was a significant parasite clearance rate in the AL + RP group as compared to the other two treatment groups that is AL +placebo and AL + BP groups.

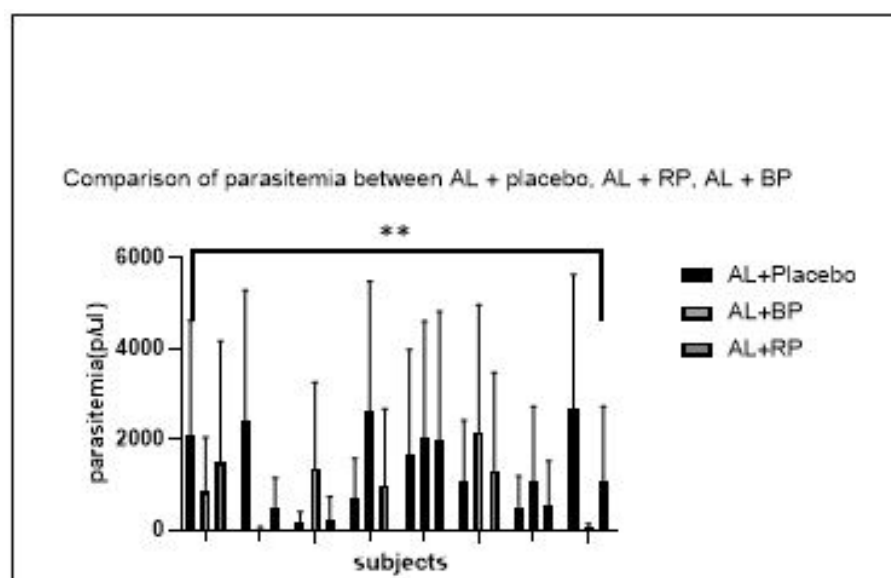


Figure 8: comparison of Parasitemia between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RP Vs. AL + BP treatment groups. Asterisks represents comparison between the three treatment groups (AL + placebo, AL + RP, AL + BP) among subjects of the different groups, ns represents statistically not significant while the letters represent the different treatment groups

HB level between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RPVs. AL + BP

Figure 9below brings out comparison of HB levels among the three different treatment groups, and the results obtained shows that there was a significant difference in

means of HB level of the AL + RP treatment group from (10.93 ± 0.1225) , (12.43 ± 0.1225) with p-value 0.0024 when compared with the AL + placebo group and from (9.37 ± 0.1500) , (12.23 ± 0.1500) with p-value 0.0007 when compared with AL + BP group.

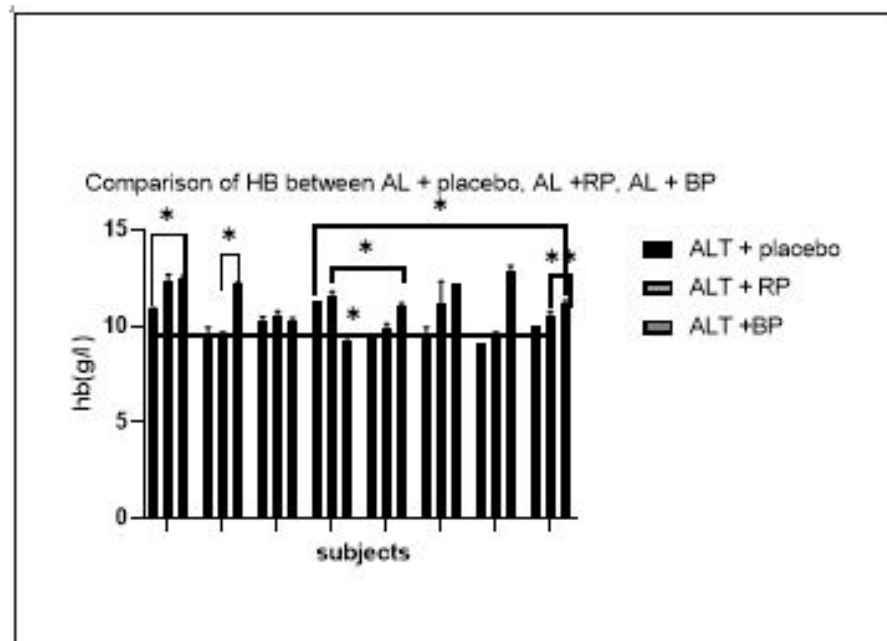


Figure 9: comparison of HB levels between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RP vs. AL + BP treatment groups. Asterisks represents comparison between the three treatment groups (AL + placebo, AL + RP, AL + BP) among subjects of the different groups, ns represents statistically not significant

Liver enzyme activity between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RP Vs. AL + BP

It was observed in figure 10 below when a comparison of the three liver enzymes activity (ALT, AST, GGT) were compared among the three treatment groups that, there was a significant decrease in AST in the AL + RP

group from (49.94 ± 1.532) , (22.61 ± 1.532) with p-value 0.0008 when compared with the AL + placebo group, main while no significant difference in the decreased liver enzyme (ALT) was observed when the AL + RP group was compared with the AL + BP group from (22.61 ± 3.466) , (9.175 ± 3.466) . For ALT and GGT no significant decrease was observed in these two liver enzymes among the different treatment groups when compared.

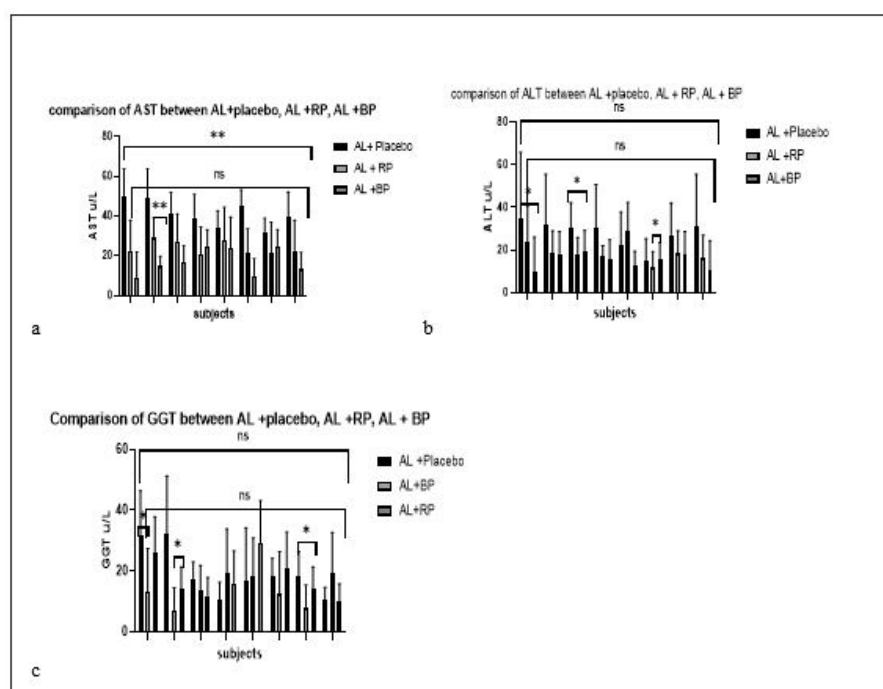


Figure 10: comparison of liver enzyme activity levels between AL + placebo vs. AL + RP, AL + placebo Vs. AL + BP and AL + RP vs. AL + BP treatment groups. Asterisks represents comparison between the three treatment groups (AL + placebo, AL + RP, AL + BP) among subjects of the different groups, ns represents statistically not significant

Discussion

Anemia is a common blood disorder that affects people of all age groups [21]. It is marked by a reduced red blood cell (RBC) count and hemoglobin (Hb) levels [22]. Iron deficiency induced anemia negatively affects motor and cognitive development, resulting in fatigue and low productivity. During parturition, it may be linked with reduced birth weight and an increased risk of maternal and perinatal death [23] [24]. In 2013, iron deficiency anemia accounted for approximately 90,000 deaths in both sexes across all age groups in developing nations [7]. Anemia is one of the several diseases asserted to have been successfully treated with plant principles by herb doctors [21]. In the recent past, an assortment of natural compounds from plant extracts has been studied by different researchers [25]. This study was aimed at evaluating the effect of *Telfairia occidentalis* on the hemoglobin level, parasite clearance rate, and liver enzyme activity in children in the age of range 2 to 15 years.

This study investigated the parasite clearance rate in three treatment groups. *T. occidentalis* in combination with AL recorded a high rate of parasite clearance, in the case group 3 (RP + AL) when compared with the case groups 2 (AL + Placebo) and 4 (BP + AL) where there was no recorded significant difference in parasite clearance rate between the two groups. In case group 3 it was recorded that there was a significant total parasite clearance on D2 as compared to case groups 2 and 4 where there was no significant difference in parasite clearance, but there was a complete parasite clearance on D7 in all three case groups. No significant difference was recorded for D0 and D1 for all the three case groups. Furthermore, signs such as high temperature, fever and vomiting were not the case for those who took AL+ RP, AL + BP during the treatment days but these were found to be the case for participants in the AL + Placebo group, whereas patients in the AL+ RP and AL + BP groups were not indifferent in the case of appetite from those in the AL+ Placebo group. It was also observed that the AL + RP group exhibited the highest parasite clearance rate when compared with the other treatment groups (AL + placebo, AL +

BP). The possible explanation for this could be that *T. occidentalis* played a role in the eradication of parasitemia in the host's blood, which had a combined effect with that already done by AL and subsequently increased the parasite clearance rate. This could be because of the rich content of flavonoids, tannins, alkaloids, saponins, and alkaloids. Previous research has shown that, *T. occidentalis* has a potent antioxidant capacity through its ability to prevent lipid peroxidation and DNA damage and to increase the production of antioxidant enzymes [26]. The antioxidant and anti-inflammatory effects of this plant result from the carotenoids, vitamins C, flavonoids, and other substances it contains. As the body cannot manufacture these nutrients, they must be supplied in the diet. Therefore, the synergistic antimalarial effect of *T. occidentalis* when used in combination with ACT likely resulted from the antioxidant activities that are associated with the beta carotene (carotenoids) and chlorophyll it contains [27]. The results obtained from this study are therefore in line with those of Okokon et al, [28], who reported significant antiparasmodial activity with the seed and leaf extracts of *T. occidentalis* during early and established infections. The seed extract exhibited schizontocidal activity. Antiplasmodic activity of fluted pumpkin was also validated by Adegbolagun et al, [29], who documented a reduction in parasitemia within 48 hours, when *Plasmodium berghei*-infected albino mice were administered an aqueous extract of *T. occidentalis* alone or in combination with artesunate.

Being one of the largest organs in the human body [30], the liver plays a key role in the regulation of varied processes, which include metabolism, storage, secretion, and detoxification of endogenous and exogenous compounds [31]. Hepatocytes are the functional cells of the liver [32]. Globally, liver disorders are a major problem. Orthodox drugs employed in the treatment of liver diseases are sometimes ineffective and can have grave untoward effects [33]. In addition, the treatment interventions are usually too expensive for resource-poor countries. Herbal medicines constitute a group of therapeutic agents with a low side effect profile.

In this study, all participants in groups 1A and B

registered a normal Hb level compared to the other groups. However, such levels were lowered, though not statistically significant in children with malaria compared to the healthy control. There was no significant difference in Hb levels between the two control groups (RP and BP), but a significant difference in Hb levels was observed between RP vs. placebo + AL and between BP vs. placebo + AL. There was a significant difference in Hb (increased in AL + RP) when the three treatment groups were well compared. This increase in hematological parameters (Hb and Hct) investigated could be as a result of some constituents such as iron and some B complexes vitamins that it possesses as these serve as hematopoietic factors that influence directly on blood production in the bone marrow [34]. This study agreed with the findings of [35], who reported that, consequently upon administration, the methanolic leaf extract of *T. occidentalis* stimulated significant erythropoietin and leukopoietin in mice. Atabo et al, [36], demonstrated that leaf, seed, and stem extracts of fluted pumpkin have the capacity to reduce endogenous methemoglobin (metHb) concentrations by converting metHb to Hb at a high rate. Ogbе., et al in 2010, [21] found that an oral daily dose of *T. occidentalis* showed significant antianemic activity in phenylhydrazine-induced anemia in rabbits. A significant increase in Hb was also reported by Iweala E., [37], who documented the hematinic activity of the aqueous crude extract. According to the findings of [38], preparations of fluted pumpkin significantly increased PCV, Hb concentrations, RBCs, and white blood cell (WBC) counts. This study also corroborates with the work of Salman et al. [39], who reported that there was a significant increase in the hematological parameters of rats that were treated for two weeks with the aqueous leaf extract of fluted pumpkin. Some scientists have proposed the use of fluted pumpkin in the treatment of anemia, following studies that reported that extracts of fluted pumpkin helped to maintain blood levels in subjects given their extracts [40]. This study further explored the combined effect of *T. occidentalis* and AL on the liver activity enzymes. The group control group 1A administered with RP showed a significant decrease in all three liver enzymes analyzed (AST, ALT and GGT) when the different treatment days were compared, as compared to the BP control group which showed a significant decrease in AST and GGT but no significant difference was observed for ALT when D2 was com-

pared with D7. While for case group 2 (AL + Placebo) a significant decrease in AST and ALT was recorded when the different treatment days were compared except for GGT where there was no significant difference when D1 was compared with D2. When a comparison between case groups 2 and 4 was made, it was observed that there was a significant decrease in AST, while a significant decrease in ALT was recorded just for D0 and D1 but no significant decrease on D2 and D7, comparison between case groups 3 and 4 was made, it was also observed that there was a significant decrease in AST and ALT except for D7 where no significant decrease was recorded, meanwhile no significant decrease was observed with GGT. When group 3 was compared with group 4, it was noticed that there was a significant decrease in AST on D0 and there was no observed significant decrease on days 1, 2 and 7, while no significant decrease was recorded for ALT and GGT from which we can draw a conclusion that RP group 1A and case group 3 recorded the greatest effect on decreasing the three analyzed liver enzymes. This can be attributed to the rich phenolic content of *T. occidentalis* as well as its antioxidant potentials. A significant difference in AST was also observed in AL + RP group when compared with the other treatment groups (AL + placebo and AL + BP), while there was no significant difference in ALT and GGT when the treatment groups were as well compared. The results of this study were in agreement with those of Oboh G., et al, [41] who reported that an aqueous extract of fluted pumpkin was more effective than the ethanolic extract in protecting hepatocytes against garlic-induced oxidative stress. Nwanna EE., et al [42] in their work found that both soluble free and bound phenolic extracts of the leaves protected hepatocytes from oxidative stress. However, soluble free phenolic extract showed significantly higher hepatoprotective activity as compared to bound phenolic extract. The hepatoprotective property of fluted pumpkin may be attributed to its rich phenolic content as reported by Oboh et al [21].

Conclusion

This study investigated the effectiveness of artemether/lumefantrine and its combination with raw or boiled leaves of *T. occidentalis* in the treatment of uncomplicated malaria. The degree of anemia was lowered as evidenced by a restored hemoglobin level and there was a

rapid parasite clearance upon administration of the combination of *T. occidentalis* and artemether/lumefantrine. The young leaves of *T. occidentalis* significantly normalized the level of hepatic enzyme activity in the course of the treatment of *P. falciparum* infected children. Globally, it can be concluded that, the combination artemether/ lumefantrine with the leaves of *T. occidentalis* can be effective in the treatment of malaria and that the effects are more pronounced in combination with the raw leaves of *T. occidentalis*. Thus, this study displayed *T. occidentalis* as an enriched functional food with outmost benefits to human health when consumed naturally.

Ethical Consideration

Ethical approval was sought and obtained from the Regional of public health Bamenda, Northwest Region

Cameroon through N/Ref: 028/ATT/NWR/RDPH/BRI-GAD of 8th FEBRUARY 2022; and the Ethical and Research committee of the University of Bamenda, Northwest Region Cameroon. A Proxy-informed consent was gotten from all individuals enrolled in the study or from parents and guardians of the children for their willingness to participate in the study after explaining the rational of the study and ensuring their confidentiality. Participation was voluntary.

Disclosure statement

The authors. declare there is no conflict of interest

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