Comparative Effect of Aqueous Extract of Psidium guajava Leaves and Arthemetar/Lumefantrine (coartem) on non-targeted parameters in Plasmodium berghei- Infected Mice

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Abstract

Objective

To study the effect of aqueous extract of *Psidium guajava* and arthemeter/lumefantrine (coartem) on non-targeted parameters in *Plasmodium berghei*-infected mice.

Methods

Twenty five mice (out of thirty mice weighing between 30 and 35 g) were intraperitoneally infected with 0.2 ml of 1×10^5 /ml parasitized erythrocytes of *Plasmodium berghei* (NK 65 strain) and randomly grouped into six (G2-G6) with five (5) mice each. G1 (normal control) received distilled water and animal feed. G2 (parasite control) received 1ml of distilled water daily. Groups (G3-G6) were treated with 28 mg/kg b.w of coartem, 200 mg/kg b.w, 400 mg/kg b.w and 600 mg/kg b.w of *Psidium guajava* extract respectively. Data were entered and analyzed using SPSS-21 statistical software. A $p < 0.05$ was taken as statistically significant.

Results

The *Psidium guajava* aqueous extract revealed presence of saponins, alkaloids, tannins, terpenoids and flavonoids. All the groups treated with *Psidium guajava* aqueous extract showed a dose-dependent significant ($p < 0.05$) increase in percentage clearance rate of the malaria parasite, PCV, HB, and RBC count, antioxidant status, antioxidant enzymes activities and a significant ($p < 0.05$) decrease in MDA level, ALP, AST and ALT activities as against the coartem treated and the parasite control groups.

Conclusion

Aqueous extract of *Psidium guajava* leaf could be useful in the treatment of diseases and symptoms caused by plasmodium parasites, especially berghei species with milder side effects than coartem.

Introduction

Unfortunately, most of the anti-malaria drugs like artemisinin combination therapy exert their antiparasmodial effect with a concomitant release of free radicals (2). These free radicals are both friends and foes to the host metabolic processes- Friend in the sense that the parasite is destroyed, but in return endangers host cells and tissues leading to necrosis and inflammation especially the red blood cells and hepatocytes (3).

In search of a better treatment option, phytochemicals from plants origin have been proven over the years to be highly potent as anti-malaria agents (due their antioxidant activities) together with their chemo-

protective effects against ailments (4). Guava is a plant in the myrtle family (*Myrtaceae*) genus *Psidium*. Extracts from guava leaves or bark have been implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain (4, 5, 6). Essential oils from guava leave have anti-cancer activity in vitro (7).

Guava leaves are used in folk medicine as a remedy for diarrhea and fever, malaria; the bark extracts acts as antimicrobial and as an astringent agent (8). Guava leaves and bark are used in traditional medicine for management of diabetes (9). Phytochemical analyses of guava leaf revealed the presence of alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, flavonoids (especially quercetin), lectins, phenols, saponins, tannins, triterpenes, and vitamin C (10).

Material And Methods

Collection of plant materials and authentication

The leaves of *Psidium guajava* were obtained from the Alumni Garden of Alex Ekwueme Federal University Ndufu Alike on 20th October, 2020. The leaves were identified and authenticated at the Department of Plant Science, Ebonyi State University, Abakaliki, Nigeria. The voucher specimen of the plant is available. A sample of the plant portion was deposited at the Departmental Herbarium for future purpose.

Preparation of aqueous extract of *Psidium guajava*

The leaves of *Psidium guajava* were air-dried at room temperature. The air-dried leaves were ground to powder using electric blender. Exactly 50 g of the sample was soaked in 1000 mL of de-ionised water for 24 hours at room temperature, under occasional shaking. It was then filtered using a Whatmann filter paper (No.1). The filtrate was concentrated to dryness using a rotary evaporator at 45°C to obtain the aqueous crude extract and the extract was stored in the fridge at 4 °C until needed.

Experimental Animals

A total of thirty mice of both sexes weighing between 30 to 35 g were acquired from Nigerian Institute of Medical Research, Lagos. They were acclimatized for seven days before use. The animals were housed in a plastic/wire gauze animal cage and were placed on standard feed and free access to water *ad libitum*.

Experimental Design

Thirty mice weighing between 30 and 35 g were used for this work. Twenty five mice were intraperitoneally infected with 0.2 ml of 1×10^5 /ml parasitized erythrocytes of *Plasmodium berghei* (NK 65 strain) and randomly grouped into six (G2-G6) with five (5) mice each while the remaining five mice served as the normal control and all the mice in the respective groups were treated as follows:

Group 1(Normal control) received distilled water and animal feed

Group 2 (Parasitic control) received 1mL of distilled water daily

Group 3: Treated with 28 mg/kg b.w of coartem orally

Group 4: Treated with 200 mg/kg b.w. of aqueous extract of *Psidium guajava* leaf (AEPGL) orally

Group 5: Treated with 400 mg/kg b.w. of aqueous extract of *Psidium guajava* leaf (AEPGL) orally

Group 6: Treated with 600 mg/kg b.w. of aqueous extract of *Psidium guajava* leaf (AEPGL) orally

The animals were maintained at a room temperature of $24 \pm 2^{\circ}\text{C}$ with 12 hours light/dark cycle, under the principles and guidelines of the Nigeria Council on Animal Care as outlined in Guide for the care and use of Laboratory Animals’.

Determination of biochemical parameters

Liver enzyme biomarkers

The activities of Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline phosphatase (ALP) were assayed according to the method of Reitman and Frankel (17).

Estimation of antioxidant parameters

Serum ascorbic acid, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT) activities and malondialdehyde (MDA) were assessed by the method of Arthur and Boyne (20), Paglia and Valentine (21), Aebi, *et al.* (22) and Walin *et al.* (23) respectively.

Determination of hematological parameters

Pack Cell Volume (PCV), Hemoglobin concentration (HB), and Red blood cell count (RBC) were determined following the procedure described by Ochei *et al.* (18). While the Total White blood Cell counts (WBC), lymphocyte count, and eosinophil count were determined by hemocytometry method as described by Cheesbough (19).

Statistical analysis

The result of the replicates were pooled and expressed as Mean $\pm$ Standard deviation and One-way analysis of variance (ANOVA) followed by Tukey’s test were used to check the level of significance at $p < 0.05$.

Results

Table 1: Effect of aqueous extract of *Psidium guajava* leaf on percentage clearance of *plasmodium berghei* in mice

The result (table 1) below showed a dose-dependent clearance percentage of the aqueous extract compared to the parasite group and the coartem treated group, though a greater percentage was recorded in coartem treated group.

S/N	Groups	Percentage (%) clearance
1	NC	0.00
2	PC	14.24
3	28 mg/kg coartem	82.10
4	200 mg/kg AEPGL	48.95
5	400 mg/kg AEPGL	53.95
6	600 mg/kg AEPGL	65.59

Key: NC = Normal control, PC = Parasitic control and AEPGL = Aqueous extract of *Psidium guajava* leaf.

Table 2: Antioxidants and antioxidant enzymes concentration of the various treatment groups

There was a significant ($p < 0.05$) decrease in serum vitamin C (ascorbic acid), GPx and CAT of the parasite control group and coartem treated group compared to the normal control and the extract treated groups. There was no statistically significant ($p > 0.05$) difference in the activities of SOD among all the groups except 600 mg/kg AEPGL which was significant ($p > 0.05$) compared to parasite control group. There was a dose-dependent increase in serum vitamin C (ascorbic acid), GPx, CAT and SOD among the extract treated groups though not statistically significant ($p > 0.05$)

Parameters / Group	NC	PC	28 mg/kg coartem	200 mg/kg AEPGL	400 mg/kg AEPGL	600 mg/kg AEPGL
VIT C (mg/dl)	1.178±0.03	0.74±0.08 ^a	0.73±0.10 ^a	1.13±0.05 ^b	1.12±0.02 ^b	2.15±0.02 ^{bc}
CAT (U/mg)	3.59±0.34	2.61±0.11 ^a	2.45±0.05 ^a	4.03±0.02 ^{ab}	4.54±0.01 ^{ab}	5.77±0.04 ^{bc}
SOD (U/mg)	11.66±0.02	11.00±0.07	11.41±0.03	11.27±0.09	11.60±0.05	12.99±0.19 ^b
GPX (U/mg)	31.12±0.07	28.63±0.04 ^a	29.97±0.43 ^a	31.75±0.29 ^b	32.70±0.05 ^{bc}	33.50±0.38 ^{bc}

Results are presented as Mean ±SD. Values with different superscripts indicate significant difference at P<0.05.

Key: NC = Normal control, PC = Parasitic control, AEPGL = Aqueous extract of *Psidium guajava* leaf, U/mg = units per mg of enzyme proteins.

Table 3: Effect of aqueous extract of *Psidium guajava* leaf on lipid peroxidation of *plasmodium berghei*-infected mice.

The result of table 3 indicated high level of lipid peroxidation in both parasite control and coartem treated groups unlike the extract treatment groups. Interestingly, the extract induced a dose-dependent protective effect against lipid peroxidation when compared to the parasite control and coartem treated groups

S/N	Groups	MDA (mg/dl)
1	NC	2.69±0.52
2	PC	4.70±0.35 ^a
3	28 mg/kg coartem	4.33±0.23 ^a
4	200 mg/kg AEPGL	2.94±0.24 ^{bc}
5	400 mg/kg AEPGL	2.86±0.23 ^{bc}
6	600 mg/kg AEPGL	2.75±0.23 ^{bc}

Results are presented as Mean $\pm$ SD. Values with different superscripts indicate significant difference at $P < 0.05$.

Key: NC = Normal control, PC = Parasitic control, AEPGL = Aqueous extract of *Psidium guajava* leaf,

Table 4: Effect of aqueous extract of *Psidium guajava* leaf on hematological parameters of *plasmodium berghei* infected mice.

The result of table 4 reveals significant ($p < 0.05$) reduction in PCV, HB and RBC levels in parasite control and coartem treated groups when compared to the extract treated groups and normal control. Increased level of WBC, eosin, and lymph was recorded in parasite control group when compared to the other treatment groups and the normal control. However, the aqueous extract caused a dose-dependent ameliorative effect on most of the hematological parameters except on eosin when compared to the parasite control group.

Parameters \ Groups	NC	PC	28 mg/kg coartem	200mg/kg AEPGL	400mg/kg AEPGL	600mg/kg AEPGL
PCV (%)	56 $\pm$ 1.23	38 $\pm$ 2.48 ^a	35.5 $\pm$ 2.63 ^a	47.35 $\pm$ 1.32 ^{bc}	47.75 $\pm$ 1.33 ^{bc}	47.85 $\pm$ 2.93 ^{bc}
HB (mg/dl)	18.65 $\pm$ 0.39	12.65 $\pm$ 0.80 ^a	11.83 $\pm$ 0.86 ^a	15.93 $\pm$ 0.45 ^{bc}	15.70 $\pm$ 1.50 ^{bc}	15.78 $\pm$ 0.98 ^{bc}
RBC (X10 ¹² /L)	7.94 $\pm$ 0.35	5.54 $\pm$ 0.21 ^a	5.8 $\pm$ 0.38 ^a	7.98 $\pm$ 0.18 ^{bc}	8.00 $\pm$ 0.02 ^{bc}	7.95 $\pm$ 0.46 ^{bc}
WBC (X10 ⁹ /L)	9.45 $\pm$ 0.40	10.80 $\pm$ 0.25 ^b	9.60 $\pm$ 0.59 ^a	9.79 $\pm$ 0.16 ^a	9.68 $\pm$ 0.11 ^a	9.10 $\pm$ 0.30 ^a
Eosin (%)	1.25 $\pm$ 0.25	5.00 $\pm$ 0.40 ^a	1.75 $\pm$ 0.47 ^b	2.50 $\pm$ 0.29 ^b	2.35 $\pm$ 1.11 ^b	2.00 $\pm$ 0.41 ^b
Lymph (%)	67.25 $\pm$ 1.38	68.75 $\pm$ 0.63 ^a	57.5 $\pm$ 2.63 ^b	61.5 $\pm$ 4.64 ^b	59.50 $\pm$ 1.12 ^b	58.75 $\pm$ 0.75 ^b

Results are presented as Mean $\pm$ SD. Values with different superscripts indicate significant difference at $P < 0.05$.

Key: NC = Normal control, PC = Parasitic control, AEPGL = Aqueous extract of *Psidium guajava* leaf.

Table 5: Effect of aqueous extract of *Psidium guajava* leaf on liver enzymes of *plasmodium berghei* infected mice

The result of table 5 indicates increased activities of the liver enzymes (AST, ALT and ALP) on parasite control group and coartem treated group unlike the other treatment groups. The aqueous extract reversed

the increased activities of those liver enzymes in a non-dose-dependent order.

Parameters	Groups	NC	PC	28 mg/kg coartem	200mg/kgAEPGL	400mg/kgAEPGL	600mg/kgAEPGL
AST (U/L)		95.00±2.0	113.80±4.73 ^a	101.00±3.08 ^a	77.50±4.79 ^{bc}	88.89±4.55 ^{bc}	96.50±4.79 ^{bc}
ALT (U/L)		80.00±5.78	126.30±2.39	106±5.72 ^a	90.75±4.15 ^b	90.55±6.33 ^b	80.00±9.13 ^b
ALP (U/L)		11.84±1.22	16.25±1.09 ^a	10.81±1.29 ^b	9.60±0.70 ^b	9.75±5.35 ^b	9.84±3.36 ^b

Results are presented as Mean ±SD. Values with different superscripts indicate significant difference at P<0.05.

Key: NC = Normal control, PC = Parasitic control, AEPGL = Aqueous extract of *Psidium guajava* leaf.

Discussion

Malaria is a major public health problem in Sub-Sahara region with wide-ranging hematological and biochemical alterations. This disease is caused by parasitic protozoan, the *Plasmodium* species and it poses a severe public health problem which is characterized by increased parasitemia, anemia, reduced availability of nitric oxide, and increased systemic expression of white blood cells etc. (46).

Phytochemicals are functional foods derived from plants which possess antioxidant, nutraceuticals, phytonutrients, anti-nutrients and phytotoxins properties etc. The presence of alkaloids, saponin flavonoids and tannins in AEPGL proves the medicinal value of the plant. Flavonoids have been documented to possess anti-oxidant activities, anti-inflammatory activities, anti-cancer activities and anti-microbial properties (47). Tannins are a group of polymeric phenolic compounds which have the ability to inactivate and kill microorganisms. Their pharmacological effects are used in the treatment of varicose ulcers, hemorrhoids, minor burns etc. Alkaloids serve as local anesthetic and stimulants with both anti-bacterial and antimalarial activities (e.g. quinine). Saponins are glycosides found widely in plants that have detergent, antioxidant, antiinflammatory, antiapoptosis and immunostimulant properties (48).

In this study, the percentage clearance of parasitemia was determined for all the groups, a significant ($p < 0.05$) increase in percentage clearance of malaria parasite after treatment compared with the baseline among all the treated groups was recorded. With comparison to the extract treated groups and the coartem treated group, the percentage malaria parasite clearance (82.10%) was significantly ($p < 0.05$) higher than that of the aqueous extract of *Psidium guajava* leaf (AEPGL) treated groups (table 1). However, the AEPGL treated groups showed a dose-dependent clearance rate with the highest concentration (1000 mg/kg b.w) having the highest percent of malaria parasite clearance (65.59%) compared to the parasite control group (14.28%). It is therefore evident that the chemical drug (coartem)

is more potent in clearing *plasmodium* parasite than the extract. This result is consonance with findings of other researchers (24, 25, 26).

As shown in table 2, there was a significant ($p < 0.05$) decrease in serum vitamin C (ascorbic acid) of the parasite control group and coartem treated group compared to the normal control and the extract treated groups. This decrease in serum vitamin C level among those groups is a symbol of oxidative stress due to action of the malaria parasite and effect of the drug metabolism on P450 enzymes (33). The AEPGL treated group showed a significant ($p < 0.05$) increase of serum ascorbic acid level when compared to the normal control and parasite control. This is possibly because of the potentiating ability of antioxidant property of vitamin C by the extract. Hence, scavenging and chelating reactive oxygen species produced by the body's defense mechanisms (parasite control) and during drug metabolism (coartem treated group) (34).

Oxidative stress biomarkers including SOD, GPx, GST, CAT, and GSH are accountable for guarding the living system against oxidative stress through the maintenance of physiological concentration of free radicals (49). Antioxidant enzymes specifically Glutathione peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD) activities in serum were assayed. Although there was no statistically significant ($p > 0.05$) difference in the activities of SOD among all the groups (table 3), but there was a significant ($p < 0.05$) decrease in GPx and CAT activities of the parasite control group and the standard drug (coartem) treated group compared to the normal control group (table 3). Superoxide dismutase transforms superoxide radicals via the dismutation process to hydrogen peroxide. CAT converts hydrogen peroxide into water whereas GPx detoxifies lipid peroxides (49). The observed decrease in SOD activity is a confirmation of oxidative stress in both the parasite control group and the standard drug treated group. In contrast, there was a significant ($p < 0.05$) increase in GPx and Catalase activities among the extract treated groups when compared to the parasite control group and the standard drug treated group. The increased antioxidant enzyme activities could be attributed to the high antioxidant contents of AEPGL and the synergetic action of the likely bioactive compounds in the extract which must have mitigated the generation of free radicals and supplied reducing equivalent to the enzymes (38, 49).

Systemic expression of serum malondialdehyde (MDA) has been categorized as a biomarker of lipid peroxidation due to free radicals attack on membrane lipids of macromolecules. The accumulation of free radical trigger lipid peroxidation (assayed as MDA), a practice that encourages lipids of the cell membrane experiences catabolism, resulting in organ impairment, probably due to breakdown of the polyunsaturated fatty acids in the liver and kidney. There was a significant ($p < 0.05$) increase in serum malondialdehyde (MDA) of the parasite group compared to the normal control and the extract treated groups as presented (in table 4). However, no statistically significant ($p > 0.05$) difference on the concentration of MDA between the standard drug (coartem) treated group and the parasite control group. This could be attributed to increased rate of lipid peroxidation occasioned by increased free radical generation incurred on the course of drug metabolism by the body system. Interestingly, a momentous reduction in the level of serum MDA was observed among the extract treated groups in a dose-dependent order compared to standard drug (coartem) treated group, parasite control and normal control groups.

This could be as a function of the phytochemicals present in AEPGL playing their antioxidant and pharmacological effects thus ameliorating lipid peroxidation among the extract treated groups. The observed results in this study corroborate the findings of other previous research (35, 36, 37).

Pack cell volume (PCV), hemoglobin concentration (HB) and red blood cell count (RBC) are hematological indices for the evaluation of anemia (39). The result of this study (table 4) showed a significant ($p < 0.05$) decrease in PCV, HB, and RBC of both the parasite control group and the standard drug (coartem) treated group compared to the normal control group. Of course, lipid peroxidation arising from free radicals and the metabolic effects of the drug are possible reasons for this decrease (36). In contrast, a significant ($p < 0.05$) increase in these parameters was observed among the extract treated group compared to the former groups. The combined effect of the various antioxidants such as flavonoids, tannins and alkaloids present in the extract could be responsible for mopping up the free radicals thereby guarding the cell membranes of the red cells against lipid peroxidation and hemolysis (40).

Studies have shown that white blood cell (WBC) total count and WBC differential counts are indices for evaluating the level of infection (41, 42). Although there was no statistically significant ($p < 0.05$) increase in total WBC and lymphocytes among the parasite control group compared to the normal control group but its eosinophil count demonstrated a significant ($p < 0.05$) increase when compared to the normal control group. This increased eosinophil in response to malaria infection is in line with the report of Kotepui, *et al* and Kurtzhals *et al* (43, 44). Interestingly, a significant ($p < 0.05$) decrease in the eosinophil count of all the treated groups (G3-G6) compared to parasite control group was recorded. This confirms the capability of the extract against infectious diseases, probably due to the existence of tannins, flavonoids and alkaloids (48).

Alkaline phosphatase (ALP) is a useful tool used in the detection of renal damage in a disease condition. Enzyme activity in the organs is employed as a means of confirming the toxic nature of consumed xenobiotics. ALP is a membrane-bound enzyme and has the highest concentration in the kidney, and is found in substantial amounts once the cell membrane ruptures or burst (49).

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) are two of the intracellular enzymes whose activities in serum increases when there is tissue or cellular necrosis and inflammation especially within the red blood cells and hepatocytes (27, 28). The result of this study showed a significant ($p < 0.05$) increase in serum ALP, AST and ALT activities of the parasite control group and coartem treated group compared to the normal control group (table 5). This increase could be linked to necrosis and hemolysis caused by parasite infection in the hepatocytes and the red blood cells (29, 30). Conversely, those negative effects were protected against by the AEPGL as recorded in the extracted treated groups.

Unlike AST and ALT, there was no significant ($p > 0.05$) difference in ALP between the coartem treated and extract treated groups compared to the normal control, possibly because there was no hepatobiliary obstruction or bone disease (27). But a statistically significant result was obtained in the parasite control group which is indication of renal damage probably due to oxidative stress accumulation in those

animals. The excellent performance of AEPGL in normalizing this adverse effect of parasite infection in those mice could be linked to the existence of antioxidants as well as phytochemicals in the extract. This observation is in consonance with the reports of other previous researchers (49, 32, 32).

Conclusion

From the results of this study, it was concluded that though both coartem and aqueous extract of *P. guajava* leaf (AEPGL) specifically destroyed the *Plasmodium berghei* parasites, the AEPGL demonstrated a more positive effect on non-target parameters than the coartem. Hence, AEPGL is apparently a safer option than arthemeter/lumefantrine as an antimalarial having milder side effects, easily accessible and affordable especially for the rural dwellers.

Declarations

Availability of Data

All data will be made available upon reasonable request

Author contribution

Concept development: Kanu Shedrach. **Experimental work:** Kanu Shedrach and Ejeje Jerius. **Data analysis:** Uket Obeten. **Data Interpretation:** Kanu Shedrach , Uket Obeten, Ejeje Jerius, Eleazu Obeten and Nwabueze Elom. **Writing:** Kanu Shedrach and Ejeje Jerius. All authors read and approved the final manuscript.

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Ethics approval

The study protocol was reviewed and ethical clearance was obtained from the Ethics Committee of Faculty of Biological sciences, Alex Ekwueme Federal University, Ndufu-Alike Ikwo Ebonyi State, Nigeria.

Conflict of interest

The authors declare no competing interest.

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