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RESEARCH ARTICLE

COMBINATION THERAPY OF CARICA PAPAYA AND VERNONIA AMYGDALINA LEAF EXTRACTS ARE AS EFFICACIOUS AS ACTs

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ABSTRACT

The study focused on the comparative determination of the effects of the combination of leaf extracts of Carica papaya (CP) and Vernonia amygdalina (VA) and Artemether-based combination therapy (ACTs) on Plasmodium berghei infected male wistar rats. Fifty (50) male wistar rats, weighing 100 to 150g, were allotted into 5 groups (n=7). Group 2 (negative control) was infected but not treated, groups 3-5 which were infected received 250mg/kg b.w each of CP and VA in combination and 4mg/kg b.w of the two reference ACTs. Treatment was done orally once daily for 3 consecutive days, after which the animals were sacrificed and haematological analysis carried out. Afterwards, an observatory period of 7 days commenced after which another round of sacrifice was done. Before treatment, parasitemia count of animals in groups 3-5 was substantially ($p<0.05$) higher when juxtaposed with group 2. AST and ALT activities were significantly ($p<0.05$) elevated in group 3-5 when matched with group 2. *Plasmodium berghei* induction meaningfully ($p<0.05$) lowered white blood cell (WBC) at all groups. After treatment, the extracts and drug which notably ($p<0.05$) lowered plasmodium count, RBC, WBC, PCV, Hb and Platelet levels, did not meaningfully($p>0.05$) affect the activities of ALP, AST and ALT. After 7 days of observation, the extracts and drug efficaciously ($p<0.05$) reduced plasmodium count, WBC and ALP activity further at all groups. These results indicate that the leaf extracts in combination are as efficacious as the ACTs.

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INTRODUCTION

Malaria has been named one of the deadliest and foremost killer disease in tropical and developing countries of sub-Saharan Africa, including Nigeria (Owusu-Agyei, Asante and Adjuik, 2009). Malaria is a disease caused by the eukaryotic parasite of the genus plasmodium. Plasmodium is a type of parasite that destroys red blood cells (RBC) in humans, leading to the emergence of shock and fever characterized with high temperatures. Malaria is basically caused by five plasmodium species namely; malariae, ovale, vivax, knowlesi and falciparum (Singh, 2011). *Plasmodium falciparum* is responsible for most malaria deaths, especially in Africa and it is the deadliest and most common. The infection develops and produces several life-threatening complications, however with the right treatment, it is usually curable. Malaria parasite typically is transmitted to humans when bitten by mosquitoes belonging to the genus Anopheles. In few cases, one may contract malaria through contaminated blood or a fetus may become infected by its mother during pregnancy (Sarr *et al*, 2011). Nigeria accounts for a quarter of all malaria cases in Africa (World Health Organization, 2008a). In the southern part of the country, transmission occurs all year round while in the north it is more seasonal. Almost all malaria cases in the country are caused by *Plasmodium falciparum*, which is seen as the number one cause of death worldwide in 2004, from a single infectious agent (World Health Organization, 2008b). Malaria is the most common disease in Nigeria; according to the Federal Ministry of Health (2004), half of its population will have one or more malaria attacks annually. Treatment strategies of malaria focus on stopping the acute blood infection, to ameliorate and cure the symptoms, to eliminate the hypnozoites (the dormant liver form of the parasite) from the liver to prevent future relapses and to prevent the spread of infection. Various pharmacological treatment options are available, which include: chloroquine, mefloquine, quinine, primaquine, artemisinin derivatives like artesunate, artemether and amino alcohols like lumefantrine and halofantrine along

with tetracycline, doxycyclines and sulfadoxime etc. The greatest problem associated with this treatment is emergence of drug resistance (Tripathi, 2006). For malaria to be effectively managed and treated, standard procedures must be followed, which includes laboratory assessment before diagnosis and treatment. The first antimalarial therapy belongs to the class of amino quinines. Another class of antimalarials is those of the antifolates such as sulfonamide and pyrimethamine. Lumefantrine and halofantrine antimalarials belong to the amino alcohol class. Recently, artemisinin derivatives of the endoperoxidase class in the forms of arthemeter, artesunate, dihydroartemisinin are shown to be the most effective group of antimalarials which can exist as singles treatment or combined with other antimalarials as combination therapies (Krishnaa, Uhlemanna and Haynesb, 2004). Although the use of orthodox medicine has dominated malaria treatment, challenges such as drug resistance by the parasites have emerged. The World Health Organization (WHO) now recommends Artemisinin-based Combination Therapy (ACTs) for treating uncomplicated malaria. The ACTs combine an artemisinin-derivative with another longer lasting drug to try and reduce the risk of further resistance developing. Artemisinin based combinations are known to improve cure rates, reduce the development of resistance and they might decrease transmission of drug-resistant parasites. However, ACT use is limited due to its high costs, limited production of artemisinin derivatives to Good Manufacturing Practices (GMP) standards and toxicity (Haynes, 2001).

Theree are many medicinal plants indigenous to Nigeria have been implicated for malaria treatment across all ethnic and cultural groups in the country, for example, *Citrus aurantium*, *Vernonia amygdalina and Carica papaya.* Some other medicinal plants are the sources of classic antimalarials, for example, the two major antimalarial classes used to treat severe malaria; the cinchona alkaloids and artemisinins (Engwa, 2015). Most of these antimalarial plants are used in form of monotherapy, while quite a number are used in combination therapies of two, three, four and more. An example is the multi-herbal extract referred to as 'Agbo-Iba' which contains *Cajanus cajan* (pigeon pea) leaf, *Euphorbia lateriflora* leaf, *Mangifera indica* leaf and bark, *Cassa alata* leaf, *Cymbopogon giganteus* leaf, *Nauclea latifolia* leaf, and *Uvaria chamae* bark (Nwabuisi, 2002). However, it is the purpose of this study to determine the antimalarial effects of the combination therapy of the leaf extracts of *Carica papaya* and *Vernonia amygdalina* in comparison to two standard ACTs, viz; Arthemether and Lumefantrine (80mg/480mg) (ACT 1) and Dihydroartemisinin and Piperaquine (30mg/225mg) (ACT 2)

MATERIALS AND METHODS

MATERIALS

Collection and Identification of Plant Materials: Fresh leaves of *Vernonia amygdalina* and *Carica papaya* were harvested from a local farm in Otolo Nnewi, Nnewi North L.G.A of Anambra State, Nigeria. The plants were identified and authenticated in the Botany Department of Nnamdi Azikiwe University, Awka, Anambra State.

Animal models: Adult male Wistar rats of weights ranging between 100 to 150g were obtained from the animal breeding house at the College of Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The animals were acclimatized for 2 weeks under standard environmental conditions in the animal house of the Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus. Dark and light cycles were maintained at 12 hrs each. They were fed standard commercial rat pellets and water *ad libitum*. The animals were handled in adherence to the guidelines and recommendation of the ethics committee on the use of animals for research of Nnamdi Azikiwe University, Awka, Anambra, Nigeria.

Ethical approval: Studies were conducted in line with the protocol given by Nnamdi Azikiwe University, Faculty of Basic Medical Sciences, Nnewi Campus experimental model Ethics Review Committee on the humane handling of experimental animals. This is in agreement with Local and International Laws as well as Procedures for Standard Handling of Animal Models in Medical and Life Sciences Research.

Standard Drugs: NAFDAC registered and approved ACTs were obtained from Juhel Pharmacy, Awka, Anambra State. They are: Artesunate and Lumefantrine (80/480mg) and Dihydroartemisinin and piperaquine (30mg/225mg)

Malaria Parasite: The malaria parasite (*Plasmodium berghei* (Nk65)) was obtained from the department of Parasitology, University of Nigeria, Nsukka, Enugu, Nigeria.

METHODS

Body weight measurements: Each of the rats was weighed before the commencement of the experiment and then weighed again on day 7 after treatment was discontinued. Each cage was supplied with adequate amounts of food and water.

Plant Sample preparation: The harvested fresh leaves of Bitter leaf and Paw-Paw were washed and air-dried under ambient temperature for a period of 14 days and the dried specimens were grinded via an electric blender to a fine powder. This fine powder was used to prepare the extracts.

Ethanolic Extraction: A quantity of exactly 250 g of the powdered bitter leaves and paw-paw leaves each were weighed out and soaked in 1000ml of 98% absolute ethanol with a solute-solvent proportion of 1: 4 and allowed to stand for 48 hours at room temperature in a beaker. Afterwards, sieved using porcelain clothe and further filtered using a No. 1 filter paper into a clean glass beaker. The filterate was concentrated using a digital rotatory evaporator (TT-52 Technel and Technel USA) and dried using a thermostat oven (DHG- 9023A PEC medicals USA) into a paste/gel like substance and stored in a refrigerator (Nexus). The extracts were carefully kept in a dark or amber bottle to protect from heat and light which may cause evaporation and reduce quality and longevity.

Phytochemical Screening: The Phytochemical screening was carried out in the Pharmacy Laboratory, Faculty of Pharmaceutical Sciences Agulu, Nnamdi Azikiwe University, Awka. The presence or absence of flavonoids, saponins, glycosides, tannins, anthraquinones, alkaloids, terpenoids, phenols and phlobatannins were tested for by using the standard qualitative methods described by Trease and Evans (1996).

Lethality (LD_{50}) Test: The mean lethal dose LD50 of the extracts was determined in rats weighing between 100g to 150g in the laboratory of the Department of Human Physiology, Nnamdi Azikiwe University, Nnewi campus. The method according to Lorke (1983) and modified by Imafidon *et al*, (2015) was adopted.

Preparation of Extract Dose: The therapeutic doses that was adopted for this study was guided by the already determined oral LD50 of the plant extracts. These doses were taken to be less than 10% of the oral LD50. Hence, doses of 500 mg/kg of plant extracts were prepared as follows;

$$\frac{\text{Average weight of animals (Kg) x Dose (mg/ml)}}{\text{Stock solution (mg/ml)}}$$

1 g of plant extract was dissolved in 10 ml of distilled water to make a stock solution of 100 mg/ml of the extracts. The rats, therefore, 0.5ml/100g of two extracts each orally throughout the study period. Fresh samples were prepared every 48 h while left-overs were stored in a deep-freezer after use.

The ACTs were administered to the rats at 4mg/kg for three consecutive days orally. 560mg of artemether/ lumefantrine was dissolved in 20mls of distilled water to prepare a stock solution of 28mg/ml, and 255mg of dihydroartemisinine / piparaquine in 20mls of distilled water to prepare a stock solution of 12.75mg/mls.

Hence doses of 560mg/kg and 255mg/kg standard drugs were prepared as follows;

$$\frac{\textbf{Average weight of animals (Kg) x Dose (mg/ml)}}{\textbf{Stock solution (mg/ml)}}$$

Infection with Malaria Parasites: *Erythrocytes parasitized by Plasmodium berghei* were*obtained from donor mice (after confirmation of parasitemia) via ocular puncture. Collected blood* was *diluted in 0.9% normal saline in a blood-saline ratio of 1:20, such that*1 mL of blood which contains 5 × 10^7 infected RBCs was diluted in 20ml normal saline. *The rats*were infected via the intraperitoneal route with 0.2mL blood suspension, containing 1 × 10^7 P. *berghei* infected RBCs as described by (Singh *et al*, 2007) from donor mice. The newly parasitized erythrocytes on day 1 were closely monitored for manifestation of parasitemia for 3 days without treatment, after which parasitemia level was estimated as described byFidock, Rosenthal, Croft, Brun and Nwaka, (2004).

Experimental Design and Drug Administration

Experimental period: The experiment will last for 36 days (15 days acclimatization inclusive) and will be divided into various phases viz:

- **Phase 1 (P1):** Period of infection with *P.berghei* (**Day 1** (day of infection) to **Day 4** (day of confirmation of parasitemia)).
- **Phase 2 (P2):** Period of treatment and first set of sacrifice (**Day 5** to **Day 7:** 3 days of continuous treatment; **Day 8:** Day of sacrifice).
- **Phase 3 (P3):** Period of further observation and second set of sacrifice **(Day 9** to **Day 15**)

Experimental grouping: The rats were randomly picked and placed in 5 main groups of ten (10) male rats per group (totaling 50) and treated for 7 consecutive days according to Rane's test (a seven-day treatment method) as described by Iwalokun (2008). They received daily doses of normal saline, the extracts and two standard antimalarial drugs (Artemeter and Lumenfantrine 80/480mg and Dihydroartemisinin and piperaquine 30/225mg) by oral route.

Assessment of Treatment Progress: At the end of treatment, five (5) rats from each group were randomly selected and sacrificed (Day 8) and blood and liver (organ) samples collected. Parasitemia measurement and liver function tests, as well as haematology and liver histology studies were carried out. A week after, the remaining set of rats were sacrificed (second set of sacrifice) and observations, comparisons and analysis of parasitemia levels and liver function tests were carried out.

Sacrifice and preparation of samples: After treatment, two sets of sacrifice were carried out. The animals were fasted for 12 hours and thereafter anaesthesized with Diethyl ether. Blood samples (5 ml) were drawn via ocular puncture and stored. Blood samples were collected in Ethylene Diamine Tetra-Acetate Acid (EDTA) bottles and mixed properly for the analysis of haematological parameters. Another set of blood samples (5 ml) were collected in serum separating tube (SST) (plain bottles) and allowed to clot. The sera were separated by centrifugation using a Centrifuge at 2000 rpm for 10 minutes, decanted and separated from the clotted samples into serum tubes and then stored frozen in the refrigerator until needed for biochemical analysis.

Staining and Confirmation of Parasitemia: Peters' test as described by Peters (1967) was employed to estimate average parasitemia. Blood collected via tail puncture from each rat was applied on slides to make thin smears applied on microscope slides, which were dried and fixed with absolute methanol, stained with geimsa (10%) and allowed for about 10 minutes before being gently rinsed off using distilled water and left to air dry. To increase refractive index, glycerol was slightly applied to the stained slides and viewed under the microscope with a magnification of × 100. Ten different fields on each slide were examined to calculate the average parasitemia, such that:

Percentage (%) parasitemia= Number of parasitized RBC/ Total RBC number ×100.

Haematological Analysis: The anticoagulated blood samples (EDTA) were analyzed for haematology parameters using the modern Auto Haematology Analyzer (BC-20 Mindray) found in the laboratory. These parameters include white blood cell count (WBC), erythrocyte count (RBC), hemoglobin concentration (HGB), packed cell volume (HCT) and platelet counts (PLT).

Liver Function Tests: The following liver enzymes were estimated using the standard methods as recommended by WHO (2004), to evaluate the activities of the liver tissues:aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Statistical Analysis: The analysis will be carried out using the statistical package for the social sciences (SPSS version 25)

and the results will be expressed as mean ± SEM. Data for parasitemia count, liver function tests and haematological parameters were analyzed using analysis of variance (ANOVA) and figures were assumed significant at $p<0.05$.

RESULTS AND DISCUSSION

RESULTS

Survival and General Observations: There was no death recorded during and after treatment. However, a total of 10 rats died after infection with *Plasmodium berghei.* The animals improved physically as soon as treatment commenced and by the end of the experiment, they were fully back to normalcy as seen with agile movements around the cage and improved feeding habits.

Mean animal weights: There were significant changes in mean animal weights recorded before infection and after treatment with the leaf extracts.

Acute Lethality Dose (LD50)
LD50 =$\sqrt{A \times B}$
A = Maximum dose with 0% mortality
B = Minimum dose with 100% mortality.

The lethal dose of ethanolic extract of *V.amygdalina* (bitter leaf) was estimated to be over 5000mg/kg bodyweight in accordance with the work of Yusmazura *et al* (2016). The lethal dose of ethanolic extract of *Carica papaya* (paw paw) was still undetermined at 5000mg/kg body weight, as also reported by Oyegoke (2019).

Percentage Parasitemia: The groups infected developed parasitemia.The percentage parasitemia for day 14 was significantly lower in all the experimental groups when compared to the negative control group.There was a significant reduction of the percentage parasitemia in all test groups except group 2 on day 7 and a further reduction was seen after treatments on day 14 compared to days 1 and 7.

Haematological Analysis

AST: In comparison to the positive control (group 1):

- Day 4 (post-infection), there are significant higher values in all experimental groups.
- Day 7 and Day 14 recorded significant increase in AST levels in all experimental groups.

AST: Comparing between experimental periods

- Day 7: the negative control and all test groups showed significant decrease in AST levels compared to day 4.
- Day 14: further significant decrease occurred in comparison to day 4 and day 7.

ALT: In comparison to the positive control

- Day 4: Significantly higher values in all test groups except group 3.
- Day 7 recorded significant increase in groups 2.
- Day 14 showed no significant difference.

ALT: Comparing between experimental periods

- Day 7:group 2 showed significant increases compared to pre-infection day. groups 3, 4 and 5 showed no significant difference.

ALP: In comparison to the positive control

- Day 4, significant higher values were seen in groups 2 and 4, while significant lower values were seen in groups 3 and 5.
- Day 7 showed significant increase in group 2 and significant decrease in groups 3 and 5.

ALP: Comparing between experimental periods:

- Day 7: significant increase was seen in group 2 while all the test groups showed a significant decrease when compared to day 4.
- Day 14: all the groups showed no significant difference in comparison to day 7.

AST: In comparison to the negative control (group 1)

- Day 4: there were significant lower values in group 4.
- Day 7: significant decrease in all groups.
- Day 14: significant decrease in all groups.

ALT: In comparison to group 1

- Day 4: significant lower values were seen in groups 2 and 4.
- Day 7: significant decrease in all groups.
- Day 14: no significant difference.

ALP: In comparison to group 1

- Day 4: significant lower values in all groups.
- Day 7: significant decrease in all groups except group 3.
- Day 14: significant decrease in all groups.

AST: In comparison to the Artemether + Lumenfantrinegroup (group 1)

- Day 7: significant increase in group 2.

ALT: In comparison to group 1

- Day 4: significant lower values were seen in all groups.
- Day 7: significant decrease in all groups.
- Day 14: no significant difference.

ALP: In comparison to group 1

- Day 4: significant lower values in all groups.
- Day 7: significant increase in groups 2.
- Day 14: no significant difference in all groups.

AST: In comparison to the Dihydroartemisinin + Piperaquine group (group 1)

- **Day 4:** there were significant lower values in groups 2, 4 and 5.
- **Day 7:** significant increase in groups 2 and 4.

Table 1.0. Experimental Animal Grouping

S/N	Group	Number of rats	Treatment	Dosage
1	Positive control	10	Normal saline + feed.	Nil
2	Negative control	10	Malaria parasite (M.P) + water + feed.	0.2mL *P. berghei* infected diluted blood given intraperitoneally.
3	Bitter leaf + paw-paw leaves	10	M.P + bitter leaf extract + paw-paw leaf extract.	250mg/kg bitter leaf extract + 250mg/kg paw-paw leaf extracts.
4	Artemether and lumenfantrine	10	M.P + artemether and lumenfantrine.	4mg/kg.`
5	Dihydroartemisinin and piperaquine	10	M.P + dihydroartemisinin and piperaquine.	4mg/kg.

Table 2.0. Comparison of the Initial and Final Mean Animal Weights of the Different Experimental Wistar Rats

Experimental Group	Initial (pre-infection)	Final weight (day 7)	t-Value	P-value
1) Normal saline + feed	140.40±3.3	129.40±5.3	-1.405	0.233
2) Malaria parasite (M.P) + water + feed	141.00±6.74	122.80±6.74	-1.629	0.015*
3) M.P + bitter leaf extract + paw-paw leaf extract	121.20±9.13	113.00±8.33	-2.220	0.091
4) M.P + Artemether and lumenfantrine	126.00±5.33	116.20±6.81	-3.334	0.029*
5) M.P + Dihydroartemisinin and piperaquine	134.40±6.53	129.00±5.32	-2.903	0.044*

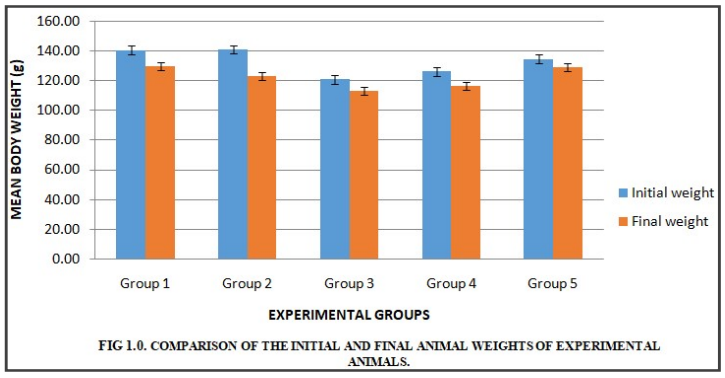

FIG 1.0. COMPARISON OF THE INITIAL AND FINAL ANIMAL WEIGHTS OF EXPERIMENTAL ANIMALS.

Table 3.0. Qualitative Phytochemical Screening for *V,amygdalina* leaf extract

S/N	Phytochemical	Qualitative Analysis
1	Alkaloids	+
2	Saponins	+
3	Tannins	+
4	Glycosides	+
5	Flavonoids	+
6	Terpenoids	+
7	Anthraquinones	-
8.	Phlobatanins	-

DOSE	*V.amygdalina*	*C.papaya*	Observation
10mg/kg	0/3	0/3	No death
100mg/kg	0/3	0/3	No death
1000mg/kg	0/3	0/3	No death
750mg/kg	0/2	0/2	No death
1500mg/kg	0/2	0/2	No death
3000mg/kg	0/2	0/2	No death
6000mg/kg	1/2	1/2	Two deaths

Table 4.0. Qualitative Phytochemical Screening for *C.papaya* leaf extract

S/N	Phytochemical	Qualitative Analysis
1	Alkaloids	+
2	Saponins	+
3	Tannins	+
4	Glycosides	-
5	Flavonoids	+
6	Terpenoids	+
7	Anthraquinones	-
8.	Phlobatanins	+

Key: + = Presence of phytochemical, - = Absence of phytochemical.

Table 6.0. The Percentage Parasitemia In Male Wistar Rats Infected With *P.Berghei*And Treated With Experimental Extracts And Standard Drugs

Experimental Group	Day 1	P-value	Day 7	P-value	Day 14	P-value
1) Normal saline +feed	0	0	0	0	0	0
2)Malaria parasite (M.P) + water + feed	25.40±0.68		37.80±0.66		12.80±0.37	
3) M.P + bitter leaf extract + paw-paw leaf extract	30.40±2.73	0.059	6.00±0.55	0.000*	4.20±0.49	0.000*
4) M.P + Artemether and lumenfantrine	25.40±0.68	0.100	3.40±0.51	0.000*	4.80±0.66	0.000*
5) M.P + Dihydroartemisinin and piperaquine	28.80±1.98	0.191	2.00±0.54	0.000*	2.00±0.32	0.000*

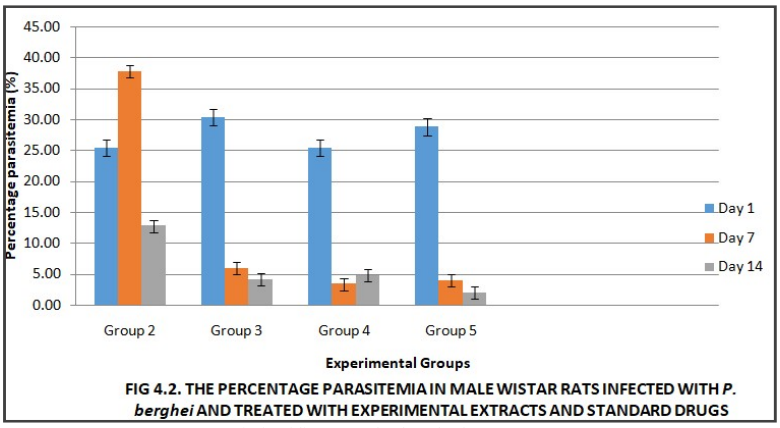

FIG 4.2. THE PERCENTAGE PARASITEMIA IN MALE WISTAR RATS INFECTED WITH *P. berghei* AND TREATED WITH EXPERIMENTAL EXTRACTS AND STANDARD DRUGS

*Significant at the level of P ≤0.05

Table 7.0. Comparison of the various Blood Parameters between the Positive Control and the Experimental Groups

Experimental Group	Wbc(*10^9/)	P-Value	Rbc	P-value	H/b	P-value	Pcv	P-Value	Platelets	P-Value
1) Normal saline + feed	11.64±0.308		7.28±0.17		13.20±0.27		47.3±0.320		808.00±8.0	
2) M.P + bitter leaf extract + paw-paw leaf extract	19.09±0.34	0.000*	8.90±0.33	0.000*	14.92±0.20	0.00*	46.54±0.37	0.005*	1221.20±3.64	0.00*
3) M.P + Artemether and Lumenfantrine	15.45±0.12	0.000*	9.12±0.12	0.000*	14.3±0.19	0.01*	45.28±1.54	0.10	495.40±82.60	0.00*
4) M.P + Dihydroartemisinin and Piperaquine	11.16±0.24	0.270	7.92±0.16	0.043*	12.74±0.11	0.11	41.22±0.71	0.025*	355.00±3.54	0.00*

*Significant at the level of P ≤0.05

Table 8.0. Comparison of the Various Blood Parameters between the Negative Control and the Experimental Groups

Experimental Group	Wbc(*10^9/L)	P-Value	Rbc	P-Value	H/b	P-Value	Pcv	P-Value	Platelets	P-Value
1) Malaria parasite (M.P) + water + feed	16.82±0.36		6.42±0.23		11.62±0.20		43.52±0.26		706.00±7.6	
2) M.P + bitter leaf extract + paw-paw leaf extract	19.09±0.34	0.00*	8.90±0.33	0.00*	14.92±0.20	0.00*	46.54±0.37	0.02*	1221.20±3.64	0.00*
3) M.P + Artemether and lumenfantrine	15.45±0.12	0.09	9.12±0.12	0.00*	14.3±0.19	0.00*	45.28±1.54	0.00*	495.40±82.60	0.01*
4) M.P + Dihydroartemisnin and piperaquine	11.16±0.24	0.00*	7.92±0.16	0.00*	12.74±0.11	0.00*	41.22±0.71	0.10	355.00±3.54	0.00*

*Significant at the level of P ≤0.05

Table 11.0. Comparison of the Liver function test result between the Positive Control and the Experimental groups

Experimental Group	Day 4			Day 7			Day 14		
	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
1) Normal saline + feed	31.60±4.75	22.60±1.29	295.03±16.85	35.40±3.944	21.00±1.82	299.40±2.80	32.60±2.66	19.80±0.80	392.73±38.97
2) Malaria parasite (M.P) + water + feed	173.40±1.60*	43.00±1.38*	451.60±4.39*	102.00±2.54*	67.80±1.80*	471.80±2.63*	63.80±2.65*	22.20±1.28	454.64±1.53
3) M.P + bitter leaf extract + paw-paw leaf extract	171.80±1.11*	24.00±0.95	206.00±3.33*	69.20±1.85*	21.00±1.00	145.20±1.32*	52.00±2.21*	22.00±0.95	146.01±2.16*
4) M.P + Artemether and Lumenfantrine	168.20±2.71*	38.80±1.39*	433.42±2.36*	55.00±1.6*	19.60±0.81	129.20±22.4	56.00±1.18*	22.80±0.91	144.36±0.72*
5) M.P + Dihydroartemisinin and Piperaquine	137.40±0.51*	29.00±1.50*	168.60±1.83*	59.20±3.02*	20.40±0.51	145.80±3.85*	55.40±4.06*	23.20±1.36	146.73±2.58*

*Significant at p ≤0.05

- **Day 14:** significant decrease in group 3 only.

ALT: In comparison to group 1

- Day 4: significant higher values were seen in group 3.
- Day 14: no significant difference.

ALP: In comparison to group 1:

- Day 4: significant higher values in groups 2 and 3.
- Day 7: significant decrease in groups 3.
- Day 14: no significant difference in all groups.

DISCUSSION

From the array of medicinal plants used for malaria therapy in Nigeria, this study has shown that indigenous medicinal plants exist and that they can be exploited in the development of new

Table 12.0. Comparison of the Liver function test result between the Negative Control and the Experimental groups

Experimental group	Day 4			Day 7			Day 14		
	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
1) Malaria parasite (M.P) + water + feed	173.40±1.60	43.00±1.38	451.60±4.39	102.00±2.54	67.80±1.80	471.80±2.63	63.80±2.65	22.20±1.28	454.64±1.53
2) M.P + bitter leaf extract + paw-paw leaf extract	171.80±1.11	24.00±0.95*	206.00±3.33*	69.20±1.85*	21.00±1.00*	145.20±1.32*	52.00±2.21*	22.00±0.95	146.01±2.16*
3) M.P + Artemether and Lumenfantrine	168.20±2.71	38.80±1.39	433.42±2.36	55.00±1.6*	19.60±0.81*	129.20±22.4	56.00±1.18*	22.80±0.91	144.36±0.72*
4) M.P + Dihydroartemisinin and Piperaquine (m.)	137.40±0.51*	29.00±1.50*	168.60±1.83*	59.20±3.02*	20.40±0.51*	145.80±3.85*	55.40±4.06*	23.20±1.36	146.73±2.58*

***Significant at p ≤0.05**

Table 13.0. Comparison of the Liver function test result between the Artemether + Lumenfantrine group and the Experimental Groups

Experimental groups	Day 4			Day 7			Day 14		
	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
1) M.P + Artemether and Lumenfantrine	168.20±2.71	38.80±1.39	433.42±2.36	55.00±1.6	19.60±0.81	129.20±22.4	56.00±1.18	22.80±0.91	144.36±0.72
2) M.P + bitter leaf extract + paw-paw leaf extract	171.80±1.11	24.00±0.95*	206.00±3.33*	69.20±1.85*	21.00±1.00	145.20±1.32*	52.00±2.21	22.00±0.95	146.01±2.16

*Significant at p ≤0.05

Table 14.0. Comparison of the Liver function test result between the Dihydroartemisinin + Piperaquine group and the Experimental Groups

Experimental group	Day 4			Day 7			Day 14		
	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
1) M.P + Dihydroartemisinin and Piperaquine	137.40±0.51	29.00±1.50	168.60±1.83	59.20±3.02	20.40±0.51	145.80±3.85	55.40±4.06	23.20±1.36	146.73±2.58
2) M.P + bitter leaf extract + paw-paw leaf extract	171.80±1.11*	24.00±0.95	206.00±3.33*	69.20±1.85*	21.00±1.00	145.20±1.32	52.00±2.21	22.00±0.95	146.01±2.16
3) M.P + Artemether and Lumenfantrine	168.2±2.71*	38.8±1.39*	433.42±2.36*	55.0±1.6	19.60±0.81	129.20±22.4*	56±1.18	22.80±0.91	144.36±0.72

*Significant at p ≤0.05

antimalarial drugs. The frequent and long term consumption of medicinal plants has necessitated a work load of toxicity studies to ensure their safety at certain doses. In order to elucidate such information, a proper toxicological evaluation was carried out in the experimental animals to predict toxicity and to select a safe dose for them. It was discovered that for both extracts, even at a dose of 5000mg/kg BW, no adverse effect was observed and no death recorded. This proved that the medicinal extracts do not pose any danger at such doses of consumption, however, the safety data on human subjects should be conducted through the various phases of clinical trial. The phytochemical screening of the ethanolic leaf extracts of *V.amygdalina* and *C.papaya* showed they both contain Terpenoids, Flavonoids, Saponins (which gives a bitter taste), Tannins and Alkaloids, however, Glycosides are present only in bitter leaf while Phlobatanins were seen only in paw-paw leaves. These results are in line with the findings of Muhammad, Sani, Sumayya and Muhammad (2019) and Arvind *et al*, (2013), where they asserted that the presence of these phytochemicals are responsible for the large spectrum of therapeutic potentials of these plants. Alkaloids, Flavonoids and Terpenoids in particular account for the anti-malarial**,** anti-microbial, antifungal, anti-parasitic, antiviral, anti-allergenic, anti-spasmodic, anti-inflammatory and immunomudulatory properties of these plants under study. The significant reduction in mean animal weight is suggestive that malaria can lead to weight loss, however, this improved with treatment with extracts and ACTs.

The elevated parasitemia count in all groups, before treatment may be adduced to significant chemo-activation of the parasite. However, the reduction in parasitemia count by the extract after the seven day treatment may be due to its percentage chemo-suppressive, prophylactic ability against P. berghei parasite as well as resultant reduction in the general clinical results of the disease-causing agent in rats (Bihonegn *et al*, 2019). Furthermore, the result showed that there was no significant difference in the parasitemia level of the groups treated with ACTs when compared with the group that took the combination leaf extract. This strongly suggests that the potency of the combination found in the two standard malaria drugs (Artemether and lumenfantrine and Dihydroartemisinin and Piperaquine) might be the same with bitter leaf extract + paw-paw leaf extract (combination therapy). This also suggests that bitter leaf extract only (monotherapy) might not be as effective as the ACTs. In the same vein, a significantly lower parasitemia level was recorded in the group treated with Dihydroartemisinin and Piperaquine when compared to the Artemether and Lumenfantrine group. This suggests that a combination of Dihydroartemisinin and Piperaquine could be more effective than the combination of Artemether and lumenfantrine in the treatment of malaria. Haematological parameters were studied to assess the effects of the extracts on blood components. White blood cells are known to play active roles in the Immune system.There was a significant increase in the WBC levels of the groups treated with a combination of bitter leaf and paw-paw leaf extracts when compared with the positive control. This indicates that a combination of bitter leaf + paw-paw leaf extracts could improve the immune system after the illness. The increase in RBC in the groups that received Dihydroartemisinni and Piperaquine and bitter leaf extract + paw-paw leaf extract (combination therapy), suggest the capacity of the extracts and ACT to resume and maintain the creation of new RBCs and its indices in the bone marrow. From the haematology viewpoint, the antimalarial activity of the combined doses of the extract have been suggested to occur by attenuation of the cell membrane of non-parasitized red blood corpuscles, thus restraining parasites' intrusion into wholesome red blood cells (Sairafianpour *et al*., 2003; Simelane *et al* 2013). This gives credence to the combination therapy of bitter leaf extract + paw-paw leaf extract as an efficacious antimalarial herbal recipe that boosts RBC count.
In comparison with the positive control, there was a significant increase in PCV in the group treated with a combination of bitter leaf extract + paw-paw leaf extract. This is an indicator of malaria's reduction effect on PCV and that a combination of bitter leaf extract + paw-paw leaf extract is effective improving the PCV levels in the blood.

The increase in platelet counts accounted for by the combination of bitter leaf and paw-paw leaf extracts may be a compensatory attempt to boost or modulate the immune suppression due to WBC depression since antigen A and B are known to be present on thrombocytes (Reid and Lomas-Francis, 2004). The measurement of the activities of various enzymes in tissues and body fluids play a significant and well known role in disease investigation and diagnosis and tissues cellular damage (Malomo, 2000). In the present study, there was a significant increase in ALP levels in the negative control and significant decrease in all the other test groups on Days 4, 7 and 14, when compared to the positive control. This indicates that pawpaw leaf + bitter leaf and Dihydroartemisinin and piperaquine reduces the ALP levels that was elevated as a result of the malaria parasite. The reduction in activity of ALP may be attributed to inhibition of the enzyme molecule, such reduction in ALP activity following administration of these extracts will limit or hinder adequate transportation of required ions or molecules across the plasma membrane. It may also lead to less availability of phosphate groups needed for the synthesis of some phospholipids (Akanji *et al*, 1993). AST and ALT are useful marker enzymes in assessing damage to the liver. Their presence in the serum may give information on tissue injury and organ dysfunction (Wells *et al*, 1986). The significant increase in the AST level of all the test groups during and after treatment when compared to the positive control may be attributed to increase in functional capacity of the liver as a result of the activities of the extracts and the ACTs. The significantly higher levels of ALT in the following test groups: i) the negative controlii) the group that treated with Dihydroartemisinin and piperaquine, when compared to the positive control, could be an indication that the presence of the parasite may cause injury to the liver. Histological examination of the liver cells in the treated groups showed the inability of the extract to cause any noticeable change in the liver structure of the test groups despite the fact that some of the test groups showed significant increase in the liver enzymes, this is suggestive that the used plant extracts and the ACTs might not be harmful to the liver.

CONCLUSION

In conclusion, the finding revealed that the reduction in parasite load were induced by both the plant extracts and ACTs. The study showed that combination therapy of bitter leaf and paw-paw leaf extracts were as efficacious as the two standard ACTs used. This repair and healing were further observed after 7 days post-treatment, indicating that the extracts confer prolonged healing and tissue repair even after

treatment. It also indicated that the combined extracts were of better effect for instance in the replenishment of various blood parameters. Therefore, in the treatment of malaria, combination therapy could prove to be more beneficial due to the synergistic effects of the phytochemicals present.

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