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ANTI-MALARIAL EFFECTS OF FIVE TRADITIONAL NIGERIAN MEDICINAL PLANT EXTRACTS ON *PLASMODIUM BERGHEI*-INFECTED RATS

[1]Ugochukwu Vincent Igbokwe, [2*]Ejike Daniel Eze, [3]Moses Dele Adams, [4]Karimah Mohammed Rabiu, [5]Iliya Ezekiel, [6]Prisca Ojochogu Ajeka, [1]Peace Ogechukwu Okpara

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[2]Department of Physiology, School of Medicine, Kabale University, Uganda.
[3]Department of Biochemistry, Faculty of Computing and Applied Sciences, Baze University, Abuja, Nigeria.
[4]Department of Biological Sciences, Faculty of Science, Yobe State University, Damaturu, Yobe, Nigeria.
[5]Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University, Wukari, Nigeria.
[6]Department of Human Kinetics and Sport Science, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.

[*]**Corresponding Authors Email: edeze@kab.ac.ug**

ABSTRACT
This work focusses on comparative determination of the effects of plant extracts: bitter leaf (BL), sour lime (SL), grape (G), pawpaw (PP) and unripe pineapple (UPA) in female rats induced into malaria with *Plasmodiumberghei*. Thirty female rats weighing 120-160 g were allotted into five groups (n=6). Group A (negative control) were infected but not treated. Animals in Groups B–E which were infected were given 500 mg/kg body weight (BW) of malanter DS (reference antimalarial drug), 500 mg/kg BW of BL, 250 mg/kg BW each of SL and BL as well as 250 mg/kg BW each of G, PP and UPA. Treatment was done orally once daily for 14 days after which a few related analyses were carried out. Before treatment, parasitemia count of animals in groups B-E was substantially ($p<0.05$) higher when juxtaposed with group A. AST and ALT activities was substantively ($p<0.05$) elevated in group B-E when matched with group A. *Plasmodium berghei* induction notably ($p<0.05$) lowered white blood cell (WBC) and monocyte (Mono) levels at all groups. After 7 days of treatment, the extracts and drug which appreciably ($p<0.05$) lowered plasmodium count, RBC, WBC, PCV, Hb, Plat, Lymph, Mono, Granul levels did not meaningfully($p>0.05$) affect the activities of ALP, AST and ALT. After 14 days of treatment, the extracts and drug exceptionally ($p<0.05$) reduced plasmodium count, WBC and ALP activity at all groups. These results give suggestive evidence that the plant extracts either singly or combined, could be a promising anti-plasmodial candidate.

Keywords: Malaria, *Plasmodium berghei*, *Vernonia amygdalina*, Parasitemia.

INTRODUCTION

Malaria is taken as one of the lethal clinical conditions brought about by protozoan parasite that belongs to the plasmodial genus (Junaid *et al*., 2017). *Plasmodium berghei* is the disease-causing organism for most of the deadliest reported cases of malaria; however, other forms like *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium vinckei* among others have been identified (Ezenyi and Salawu, 2016). Although *P. berghei*is the parasitic organism that leads to malaria in some rodents, studies have shown it is the major animal model for human malaria research (Junaid *et al*., 2017). Malaria remains one of the global diseases (WHO and UNICEF, 2005) with the prevailing anti-malarial drug resistance which portends a huge setback to malaria treatment (Clarkson *et al*., 2004; Kato *et al*., 2016). According to a 2019 Malaria Report by World Health Organization (WHO), about 228 million malaria cases and 405,000 deaths were estimated in year 2018 worldwide. Africa had above 90% of these cases/deaths and about 70% of the global malaria casualties were in children below age five (WHO, 2019). Thirteen African countries accounted for about 80% of such cases, and more than half in nations including Nigeria, Congo, Ethiopia, Tanzania and Kenya (WHO, 2008a). For Nigeria, a quarter of all malaria incidence in Africa affect her (WHO, 2008b), chiefly brought about by *Plasmodium falciparum*(Adebayo and Krettli, 2011) with about 100 million malaria incidence and more than 300,000 yearly deaths (WHO, 2010).

This burden has resulted in more scientific studies with a view to finding plant-based substitute for malaria treatment

(Rappuoli and Aderem, 2011). The malaria parasite is able to resist drugs used in the treatment of malaria but for artemisinins. Following this resistance, they are used with other antimalarials, a synergy called Artemisinin-Combination Therapies (ACTs) (Onaku *et al*., 2011). However, there are emerging evidences that disease-causing models have developed resistance to most chemical agents thereby making anti-malarial drugs less active (Arese, 2001). This greatly limits the treatment of malaria. Furthermore, most antimalarial drugs are not easily accessed by sufferers and this prompted the option to settle for self-medication using natural products (Muregi *et al*., 2003; Obisesan and Owoseni, 2017).

Traditional medicinal plants play key role in the health care delivery system of Nigeria; however, plant components remain a veritable tool to tackle harmful diseases in the world. (Tapsell *et al*., 2006; Oche, 2016). Plants-in-use are known to be possible substitute and potent means of drug synthesis and some of the antimalarials presently used like quinine and artemisinin were either solely obtained from plants or produced via chemical architecture of plant–based components as templates due to the drug resistance to malaria (Akerele, 1993; Basco *et al*., 1994).

Among others, plants which have been found by traditional medical practionist are *V. amygdalina, C. papaya, C. aurantium, C. paradisi*, and *A. comosus*, and can be used in the treatment of malaria. *V. amygdalina*mostly called bitter leaf is the most largely grown species of the genus Vernonia which has about 1,000 brand of shrubs (Muanya, 2013). It pertains to the family of *Asteraceae*. The leaves have been used as a quinine substitute in treating ailments (Masaba, 2000; Farombi and Owoeye, 2011). Medically, the plant has been successfully applied for healing of different metabolic and clinical conditions like diabetes, malaria, infertility, nausea, liver diseases, kidney diseases, bacterial infections, parasitic infestations as well as diseases of the gastrointestinal tracts. It has anti-parasitic, anti-malaria, anti-tumor, anti-bacterial and anti-inflammatory properties (Ilondu *et al*., 2009).

Carica papaya commonly called pawpaw, belongs to the family *Caricaceae*. It is a widely cultivated papaya, a tropical fruit plant. It is being used to treat malaria (Titanji *et al*., 2008).*Citrus paradisi* (*Rutaceae*) is a subtropical perennial tree (grapefruit) known for its likely large sour to semi-sweet, a bit bitter fruit. Raw grapefruit or grapefruit juice is most useful in regulating the extent of malarial attack. It comprises a natural quinine-like constituent that can remove malarial signs (Bruce, 2015).*Citrus aurantium* L. (*Rutaceae*), commonly known as bitter orange or sour lime is normally used as a flavoring and acidifying agent for groceries (Khan *et al*., 2013; Karabıyıklı, *et al*., 2014). *Ananas comosus* is a tropical plant with an edible fruit, also called pineapple, and a major economically important crop in the family *Bromeliaceae* (Coppens and Leal, 2003).

It is therefore important to investigate some of the plants used in Nigeria either alone or combined with other plants to contain malaria. This study might also assist in providing an alternative drug source from the plants studied in the control of malaria resistance.

MATERIALS AND METHODS

Plant materials

The new specimens of *V. amygdalina* leaf, *C. papaya* fruit, *C. aurantium*, *C. paradisi* and *A. comosus* were obtained from Nkwo Market in Nnewi, Anambra State, Nigeria.

Experimental animals

Thirty (30) female Wistar rats weighing 120g-160g were used for this research work.They were housed at a room temperature of 29 ± 2 ^{0}C and a relative humidity of 40-55%. They were freely allowed to feed on water and standard rat food and allowed to get acquainted with the new environment for twelve days prior to start of the experimental study.

Drugs and chemicals

The reference anti-malaria drug (malanter DS) which was procured from Christ de King Pharmacy, Opposite Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria was a product of Panzonar Laboratory Supplies, Button Road, Canada. *Plasmodium berghei* was procured from Parasitology and Entomology Department, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Ethical approval

Studies involving all animal models 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 the template in line with Local and International Laws as well as Procedures for Standard Handling of Animal Models in Medical and Life Sciences Research.

Production of ethanol extract of *V. amygdalina* leaf and juice extracts of *C. papaya, C. aurantium, C. paradisi* and *A. comosus* fruits

Reasonable amount of newly collected *V. amygdalina* (bitter leaf) were rinsed and devoid of impurities. The leaves were dried under room temperature for three weeks and the dried specimens were grinded via an electric blender. 250 g of the grinded leaves was soaked in 100 ml of 98% absolute ethanol (BDH England) and placed in a mechanical checker (UNISCOPE SM 101 England) for 24 hours. After the mixture was separated using cloth sieve and it was thereafter further separated using Number One Whatman Filter Paper into a clean glass beaker. The filtrate was concentrated using digital rotary evaporator (π/52 techmel and techmel USA) and was further

dried using thermostat oven (DHG-9023 A PEC medicals USA) into a jelly-like substance and stored in a cooling system (NEXUS) for usage in the experimental study process. Also, fruits of *C. papaya* (pawpaw), *C. aurantium* (sour lime), *C. paradisi* (grape) and *A. comosus* (unripe pineapple) were washed, seed removed and cut into smaller pieces with the back inclusive. The juice extracts were prepared by blending their fruits using electric blender (Binatone, Model: X38P, Japan) with 100 ml of water. Their liquid content was sieved using a clean handkerchief into a clean glass beaker. One ml of each juice was placed in a petri dish which had earlier been oven dry and weighed. The concentration of the juice was determined by calculating the weight difference between the empty petri dish and the dried one with sample, which gave 0.25 g 0.09 g, 0.11 g, 0.09 g respectively.

Inoculation of experimental animals and assessment of parasitemia

Blood collected from the malarial infected mice was dissolved in normal saline (1 in 20 ml) and 0.2 ml of the parasitized red blood cell was used to induce malaria into the rats via intra-peritoneal route (Anowai *et al*., 2015). The induced animals were allowed to stay for 3 days after which parasitemia was assessed by taking blood from the veins at the tail region of the animals prior to administration for treatment.

Experimental design and animal grouping

Thirty female Wistar rats weighing (120-160 g) were grouped into 5, each containing six rats as shown below. The animals were kept for two weeks, to adapt to the environment, before grouping.

GROUP A- Inoculated with Plasmodium but received no treatment (Negative Control)

GROUP B- Infected and treated with known antimalarial drug [malanter DS] (Positive Control)

GROUP C- Infected and treated with only bitter leaf extract

GROUP D- Infected and administered sour-lime + bitter leaf extract as a combined dose

GROUP E- Infected but treated with grape + pawpaw + unripe pineapple extracts as a combined dose

Extract administration

The extracts in single and combined dose were administered to the rats through careful insertion of a cannula connected to a syringe into the mouth except for the negative control group. This was done daily over a14-day period. Blood cells were taken on weekly basis during which parasitemia counting, hematological and biochemical assays were done.

Sacrifice of the animals

Each animal was sacrificed by putting it into a container containing cotton wool soaked in diethyl ether till it lost consciousness. Blood sample was collected through ocular puncture via a capillary tube inserted into the ocular sinus. Blood flows through the tube into EDTA and plain sample tubes. Blood cells in the EDTA tubes was used for hematological analysis while the one in plain bottle was centrifuged to give a supernatant which was the serum and the sediments. The blood cells contained in sterile tubes was transferred into a test tube and centrifuged after which the serum was decanted into another plain bottle, stored at 20 ^{0}C till it was used for biochemical analysis.

Hematological analysis

Blood haematology was analysed as handed down by Dacie and Lewis (1995).

Determination of serum enzyme activities

The estimation of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the serum of the animals was effected by a protocol explained by WHO (2004).

Staining and counting of parasitemia

Parasitemia staining and counting was done as put forward by Anowi *et al* (2015) with some modifications. Briefly, the blood taken from the tail of the infected rats was placed on a sterile glass slide placed horizontally on the work stand. The slide and the spreader were positioned facing a preferred angle, drawn backward touching the dripping blood cell on the slide and spread along it. The film was attached to methanol and subjected into the already synthesized Giemsa stains (1ml of Giemsa + 19 ml of buffer) with staining allowed for 35 minutes. The slide was raised off the staining mixture using a forceps, residue stain was cleaned away and allowed to dry under room temperature. Then, parasitemia was viewed using a microscope layered with oil-coated lens and the parasitemia count was obtained by counting red blood cells out of 200 red blood cells in a random environment of microscope.

Protocol for data analysis

Figures were statistically evaluated using statistical package for social sciences (SPSS version 23). Figures were denoted as average ± S.E.M of six determinations. Data for parasitemia count, liver function test and hematological parameters were analyzed using analysis of variance (ANOVA) and figures were assumed significant at $p<0.05$.

RESULTS AND DISCUSSION

Results

Plasmodium berghei meaningfully ($p<0.05$) increased plasmodium count in groups B, D, and E but decreased it in group Cwhen matched with to group A. After 7 days of treatment, *P. berghei* infection appreciably ($p<0.05$) lowered plasmodium count in groups C-E when set side by side with group A. After 14 days of treatment, *P. berghei* induction substantially ($p<0.05$) decreased plasmodium count in groups B-E when placed side by side with group A (Table 1).

Table 1: Effect of BL, SL, G, PP, UPA and malanter DS on plasmodium count on malaria-induced rats before treatment, after 7 and after 14 days of treatment

GROUP	MEAN ± SEM		
	BEFORE TREATMENT	AFTER 7 DAYS TREATMENT	AFTER 14 DAYS TREATMENT
Group A (NC)	12.67±0.33	15.66±1.20	14.33±1.20
Group B (AD)	14.67±0.00	14.00±2.08	3.00±0.57
Group C (BL)	11.00±0.57	6.66±0.33	2.33±0.33
Group D (SL+BL)	13.33±0.33	5.66±0.33	3.43±0.56
Group E (G+PP+UPA)	18.33±0.33	5.33±0.33	4.33±0.33

NC = Normal Control; **AD =** Antimalarial Drug; **BL =** Bitter Leaf; **SL+BL =** Sour Lime + Bitter Leaf; **G+PP+UPA =** Grape + PawPaw + Unripe Pineapple

While *P. berghei* induction substantively ($p<0.05$) decreased RBC level in group B, it raised the level in groups D and E, but group C gave a non-comparable ($p>0.05$) value when liken to group A. Hemoglobin concentration was sufficiently($p<0.05$) increased in groups C, D and E while its level was decreased in group B when weighed up with group A (Table 2).
P. berghei administration, before treatment, remarkably ($p<0.05$) decreased the level of packed cell volume in groups B-D, while group E had a non-statistical ($p>0$.05) effect when juxtaposed with group A (Table 2). *P. berghei* substantially($p<0.05$) decreased WBC level in groups B-E when measured with group A. Platelet count result showed a non-appreciable ($p>0.05$) reduction in groups C and D while group B and E had a notable ($p<0$.05) decrease in comparison with group A (Table 2). Infection of rats with *P. berghei* showed a glaring ($p<0.05$) drop in lymphocytes level in groups B-D while group E had a non-substantive ($p>0.05$) effect when related with group A. Monocytes result showed an appreciable ($p<0.05$) fall at all groups when matched with group A. *P. berghei* induction gave a notable ($p<0.05$) reduction in granulocytes at groups B and E while a non-appreciable ($p>0.05$) value was obtained in groups C and D when compared with group A (Table 2).

After seven days of treatment, extract and drug administration appreciably ($p<0.05$) decreased RBC level in all groups when liken with group A (Table 3). Hemoglobin and packed cell volume concentration were exceptionally ($p<0.05$) decreased in all groups in comparison with group A (Table 3).

Treatment with extract and drug after seven days meaningfully ($p<0.05$) lowered WBC level in groups B, D, and E while its level did not substantively ($p>0.05$) affect animals in group C when compared to group A. Platelet count result showed a non-substantive ($p>0.05$) effect in all groups when matched up with group A (Table 3).
Lymphocyte level decreased in group D and E while group B and C had no comparable ($p>0.05$) effect when measured with group A (Table 3). Extract and drug administration showed a sufficient ($p<0.05$) drop in group B and a reasonable ($p<0.05$) increase in group D, while group C and E had a non-appreciable ($p>0.05$) effect, after seven days treatment, when weighed up with group A (Table 3). Granulocyte result showed a notable decrease ($p<0.05$) in group B and C, while group D and E had a non-appreciable ($p>0.05$) effect, when set by side by side with group A (Table 3).

Table 2: Red blood cell, white blood cell and their selected differentials before treatment

INDICATOR	GROUP	MEAN ± SEM
Red Blood Cell (x10^12/l)	Group A (NC)	6.75±0.01
	Group B (AD)	5.15±0.00
	Group C (BL)	7.05±0.01
	Group D (SL+BL)	7.57±0.08
	Group E (G+PP+UPA)	7.49±0.06
Hemoglobin (g/dl)	Group A (NC)	12.35±0.28
	Group B (AD)	9.55±0.05
	Group C (BL)	13.30±0.05
	Group D (SL+BL)	13.55±0.50
	Group E (G+PP+UPA)	13.85±0.05
Packed Cell Volume (%)	Group A (NC)	42.55±0.43
	Group B (AD)	29.75±3.50
	Group C (BL)	39.70±2.40
	Group D (SL+BL)	41.35±3.06
	Group E (G+PP+UPA)	42.70±2.73
WBC (x10^9/l)	Group A (NC)	18.10±0.10
	Group B (AD)	12.40±0.10
	Group C (BL)	17.25±0.05
	Group D (SL+BL)	16.70±0.05
	Group E (G+PP+UPA)	15.00±0.00
Platelet Count (x10^9/l)	Group A (NC)	527.50±0.50
	Group B (AD)	648.50±0.50
	Group C (BL)	526.50±0.50
	Group D (SL+BL)	524.50±0.50
	Group E (G+PP+UPA)	325.00±5.00
Lymphocyte (%)	Group A (NC)	11.00±0.00
	Group B (AD)	8.35±0.15
	Group C (BL)	9.85±0.05
	Group D (SL+BL)	9.85±0.05
	Group E (G+PP+UPA)	10.65±0.15
Monocyte (%)	Group A (NC)	2.35±0.05
	Group B (AD)	0.95±0.05
	Group C (BL)	2.05±0.05
	Group D (SL+BL)	1.95±0.05
	Group E (G+PP+UPA)	1.45±0.05
Granulocyte (%)	Group A (NC)	4.65±0.05
	Group B (AD)	3.15±0.05
	Group C (BL)	5.45±0.05
	Group D (SL+BL)	4.75±0.05
	Group E (G+PP+UPA)	2.85±0.05

NC = Normal Control; **AD =** Antimalarial Drug; **BL =** Bitter Leaf; **SL+BL =** Sour Lime + Bitter Leaf; **G+PP+UPA =** Grape + PawPaw + Unripe Pineapple; **SEM =** Standard Error of Mean

Table 3: Red blood cell, white blood cell and their selected differentials after 7 days of treatment

INDICES	GROUP	MEAN ± SEM
Red Blood Cell (x10^12/l)	Group A (NC)	8.38±0.01
	Group B (AD)	6.44±0.52
	Group C (BL)	6.41±0.13
	Group D (SL+BL)	6.74±0.41
	Group E (G+PP+UPA)	6.69±0.07
Hemoglobin (g/dl)	Group A (NC)	15.10±0.22
	Group B (AD)	11.23±0.20
	Group C (BL)	11.67±0.26
	Group D (SL+BL)	12.30±0.90
	Group E (G+PP+UPA)	12.10±0.40
Packed Cell Volume (%)	Group A (NC)	53.73±0.43
	Group B (AD)	41.10±3.50
	Group C (BL)	42.50±2.40
	Group D (SL+BL)	46.36±3.06
	Group E (G+PP+UPA)	44.33±2.73
White Blood Cell (x10^9/l)	Group A (NC)	9.43±0.96
	Group B (AD)	5.96±0.06
	Group C (BL)	9.30±0.56
	Group D (SL+BL)	7.70±0.00
	Group E (G+PP+UPA)	5.10±0.60
Platelet Count (x10^9/l)	Group A (NC)	672.00±94.00
	Group B (AD)	450.66±59.66
	Group C (BL)	442.33±34.33
	Group D (SL+BL)	639.33±34.33
	Group E (G+PP+UPA)	639.33±81.33
Lymphocyte (%)	Group A (NC)	6.26±1.03
	Group B (AD)	4.70±0.10
	Group C (BL)	7.86±0.33
	Group D (SL+BL)	9.40±1.40
	Group E (G+PP+UPA)	3.70±0.20
Monocyte (%)	Group A (NC)	0.90±0.10
	Group B (AD)	0.33±0.03
	Group C (BL)	0.77±0.20
	Group D (SL+BL)	1.56±0.20
	Group E (G+PP+UPA)	0.47±0.26
Granulocyte (%)	Group A (NC)	2.37±0.03
	Group B (AD)	1.70±0.00
	Group C (BL)	0.73±0.63
	Group D (SL+BL)	2.56±0.44
	Group E (G+PP+UPA)	1.27±0.33

NC = Normal Control; **AD =** Antimalarial Drug; **BL =** Bitter Leaf; **SL+BL =** Sour Lime + Bitter Leaf; **G+PP+UPA =** Grape + PawPaw + Unripe Pineapple

After fourteen days of treatment with all extracts and drug, there was a notable ($p<0.05$) rise in RBC level in group C, while its level was non-comparable with groups B, D and E when liken to group A. Hemoglobin result showed an appreciable ($p<0.05$) reduction in group B while groups C-E had a non-comparable

(*p>0.05*) effect when placed side by side with group A (Table 4).

The extracts and drug sufficiently (*p<0.05*) decreased the level of packed cell volume in group C, while groups B, D and E showed a non-appreciable (*p>0.05*) effect when measured with group A (Table 4).

After fourteen days of treatment, all the extracts and drug substantively (*p<0.05*) reduced the WBC level in groups B, C, and E while group D animals showed a non-appreciable (*p>0.05*) effect when matched with group A. Platelet count result showed a meaningful (*p<0.05*) reduction in group D, while groups B, C, and E gave a non-substantial (*p>0.05*) value when placed side by side with group A (Table 4). The level of lymphocytes and monocytes were not sufficiently (*p>0.05*) altered in all groups by the extracts and drug when juxtaposed with group A. At the end of fourteen days treatment with all extracts and drug, the level of granulocytes remarkably (*p<0.05*) grew in all groups when measured with group A, but the increase was exceptional (*p<0.05*) at group D (Table 4).

Table 4: Red blood cell, white blood cell and their selected differentials after 14 days of treatment

VARIABLE	GROUP	MEAN ± SEM
Red Blood Cell	Group A (NC)	7.81±0.94
(x10^12/l)	Group B (AD)	7.21±0.08
	Group C (BL)	9.95±0.94
	Group D (SL+BL)	6.87±0.00
	Group E (G+PP+UPA)	7.68±0.05
Hemoglobin	Group A (NC)	14.10±0.22
(g/dl)	Group B (AD)	12.10±0.20
	Group C (BL)	12.85±0.26
	Group D (SL+BL)	13.00±0.90
	Group E (G+PP+UPA)	13.10±0.40
Packed Cell Volume	Group A (NC)	55.35±1.93
(%)	Group B (AD)	49.55±5.48
	Group C (BL)	37.05±10.90
	Group D (SL+BL)	51.85±1.47
	Group E (G+PP+UPA)	50.40±2.65
White Blood Cell	Group A (NC)	14.25±1.18
(x10^9/l)	Group B (AD)	10.35±0.08
	Group C (BL)	8.45±0.77
	Group D (SL+BL)	14.50±1.09
	Group E (G+PP+UPA)	8.40±1.03
Platelet Count	Group A (NC)	549.50±14.90
(x10^9/l)	Group B (AD)	496.50±13.39
	Group C (BL)	580.00±12.70
	Group D (SL+BL)	185.89±15.14
	Group E (G+PP+UPA)	584.00±16.39
Lymphocyte	Group A (NC)	0.94±0.00
(%)	Group B (AD)	0.64±0.27
	Group C (BL)	0.91±0.00
	Group D (SL+BL)	0.85±0.02
	Group E (G+PP+UPA)	0.91±0.01
Monocyte	Group A (NC)	0.17±0.07
(%)	Group B (AD)	0.04±0.00
	Group C (BL)	0.05±0.00
	Group D (SL+BL)	0.08±0.01
	Group E (G+PP+UPA)	0.20±0.14
Granulocyte	Group A (NC)	0.02±0.00
(%)	Group B (AD)	0.03±0.00
	Group C (BL)	0.03±0.00
	Group D (SL+BL)	0.06±0.01
	Group E (G+PP+UPA)	0.03±0.00

NC = Normal Control; **AD =** Antimalarial Drug; **BL =** Bitter Leaf; **SL+BL =** Sour Lime + Bitter Leaf; **G+PP+UPA =** Grape + PawPaw + Unripe Pineapple

Before treatment, the extracts and drug notably ($p<0.05$) increased AST activity in groups B, D, and E while group C had a non-comparable ($p>0.05$) effect when set side by side with group A. ALT activity was remarkably ($p<0.05$) increased in all groups in comparison with group A. ALP activity showed a non-appreciable ($p>0.05$) rise in all groups, by the extracts and drug, when liken to group A (Table 5).

After seven days of treatment with all extracts and drug, a non-comparable ($p>0.05$) effect in AST activity at all groups was observed when juxtaposed with group A. ALT activity was non-appreciable ($p>0.05$) in all groups when matched with group A. ALP result showed a non-appreciable ($p>0.05$) decreased activity in all groups when related with group A(Table 5).

After fourteen days of treatment, the extracts and drug showed a non-comparable ($p>0.05$) effect in AST activity at all groups when weighed up with group A (Table 5). ALT result showed a non-substantive ($p>0.05$) effect in group B while group C and D animals showed an appreciable ($p<0.05$) increase, but those in group E had a reasonable ($p<0.05$) reduction matched with group A. ALP activity was greatly ($p<0.05$) increased in all groups by all extracts and drug, after fourteen days of treatment, when measured with group A (Table 5).

Table 5: Effect of BL, SL, G, PP, UPA extract and malanter DS on AST, ALT and ALP activities in malaria-induced rats before treatment, after 7 days and 14 days of treatment

MARKER	GROUP	MEAN ± SEM		
		BEFORE TREATMENT	AFTER 7 DAYS TREATMENT	AFTER 14 DAYS TREATMENT
AST (IU/L)	Group A (NC)	130.50±0.50	94.66±2.60	116.33±1.85
	Group B (AD)	133.50±0.50	89.33±0.3	107.67±5.04
	Group C (BL)	132.50±0.50	90.00±2.60	118.00±10.53
	Group D (SL+BL)	134.00±1.00	89.66±0.63	94.00±21.03
	Group E (G+PP+UPA)	135.50±0.50	96.33±2.67	124.33±12.67
ALT (IU/L)	Group A (NC)	18.50±0.50	31.33±0.33	20.00±0.57
	Group B (AD)	20.00±0.00	31.00±1.00	21.00±0.00
	Group C (BL)	21.50±0.50	32.66±0.33	27.33±0.33
	Group D (SL+BL)	23.00±0.00	29.33±0.33	2.33±0.33
	Group E (G+PP+UPA)	24.50±0.50	32.66±1.67	18.00±1.00
ALP (IU/L)	Group A (NC)	298.21±0.21	313.74±12.38	250.21±0.85
	Group B (AD)	300.70±0.70	299.92±1.06	278.59±0.36
	Group C (BL)	296.72±0.72	307.91±0.72	304.16±6.14
	Group D (SL+BL)	298.72±0.71	312.43±13.12	269.46±0.64
	Group E (G+PP+UPA)	297.75±2.25	321.59±18.50	273.64±1.39

NC = Normal Control; **AD =** Antimalarial Drug; **BL =** Bitter Leaf; **SL+BL =** Sour Lime + Bitter Leaf; **G+PP+UPA =** Grape + PawPaw + Unripe Pineapple; **SEM =** Standard Error of Mean; **AST =** Aspartate Aminotransferase; **ALT =** Alanine Aminotransferase; **ALP =** Alkaline Phosphatase

DISCUSSION

Malaria is among the main global diseases (WHO, 2012) with large spectrum anti-malarial medication resistance which portendenormous concern to contain malaria (Clarkson *et al*., 2004). This consequently result in scientific studies aimed at finding fresh substitute for management and treatment of malaria, not without the involvement of natural products (Yerbanga *et al*., 2012).

The elevated parasitemia count in all groups, before treatment, may be adduced to significant chemo-activation of parasite which might have favoured a rise in body weight. However, the reduction in parasitemia count in all groups by the extract after seven- and fourteen-days 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).The antimalarial capacity shown in this work by the

extract might be claimed to single or synergistic effect of the extracts which could be by creation of a carbon-centralized radical, suppression of aggregation of heme (Haynes *et al*., 2013), manufacture of free radicals and modification of membrane transport features of the malaria parasite, which slows down the nutrient movement in the parasite.

The relationship between blood-diseased anaemia and malaria have been ascribed to the phagocytosis of parasitized and non-parasitized red blood cells, erythrocytic repression as well as bone marrow dyserythropoiesis resulting in acute ill health development (Gboeloh *et al*., 2014; White, 2018). The reduction in RBC level of group B when liken with group A after malaria induction by *Plasmodium berghei* may indicate anemia and inflammation which are common pathophysiology of malaria. It could also be an indication of abnormal synthesis of RBC and hemoglobin (Zeleke *et al*., 2017; Laryea and Borquaye, 2019). The occurrence of anemia in malaria has been linked to increased non-enzymatic rupture of RBC membrane proteins thereby lowering RBC level. The increase in RBC at group C, platelet at groups C and D as well as granulocytes at group D by the extract and drug after fourteen days of treatment suggest the capacity of the extricate to resume and maintain the creation of new RBCs and its indices in the bone marrow. From the hematology viewpoint, the antimalarial activity of the single and 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).

The assessment of enzymaticaction in organs and blood cells of the biological system is of prime importance and play a vital role in disease inspection and testing (Coodley, 1970). Enzymes including phosphatases and transferases enter the blood via openings from deranged cell membranes in impaired tissues (Adaramoye *et al*., 2008).

Alkaline phosphatase (ALP) is an enzyme that indicates alteration level in the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). The non-comparable action of *Plasmodium berghei* on ALP activity of the serum before treatment when liken with group A is an indication that there is no intrusion of the enzyme on plasma membrane wholeness as well as other metabolic processes carried out by ALP in the hepatic system (Panteghini and Bais, 2008). The absence of notable action in serum ALP activity also implies that induction of malaria may not predispose to hepatobiliary obstruction (Wright and Plummer, 1974).

Aminotransferase activity is a definite indicator of hepatocyte alteration for evaluating organ status (Panteghini and Bais, 2008). The elevation of serum AST and ALT activities before treatment, in this study, may imply activation of enzyme activity *in vivo*. The non-significant effect of serum ALP, AST and ALT activities by the extract after seven days of treatment suggest maintenance of liver architecture. The increase in serum ALP activity after fourteen days of treatment indicates enhanced enzyme production.

CONCLUSION

The study indicated that the extract facilitated decease in malaria parasitemia count and repair of damage in liver/blood-related markers. This action may be due to sole or joined activity of the studied plants.

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CONFLICT OF CONCERNS

Authors did not have any issue of concern as per this study.

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