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Haematological evaluation and antiplasmodial activity of fractions and ethanolic extract of *Gongronema latifolium* in *Plasmodium berghei* infected albino mice

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Abstract

Gongronema latifolium (GL) is used by folkloric medicine practitioners especially in Africa, most specifically in Nigeria as multipurpose medicinal plant abundant medicinal potential with ameliorative, curative and preventive effect in diseases control and therapy. The plant, especially the leaves have been proven to possess medicinal effect on experimental animals. This study is set to investigate the antiplasmodial activity; suppressive, prophylactic and curative effects of GL leaves extract and fractions namely n-HEX (n-hexane fraction), ETOAC (Ethyl acetate fraction), AQF (Aqueous fraction), ACET (Acetone fraction), ETOH (Ethanol extract) on haematological parameters of *P. berghei* infected mice. Mice (157) were grouped into 3 assemblages for prophylactic, suppressive and curative antiplasmodial study. They were divided into 7 groups for prophylactic and suppressive treatments and 8 groups for the curative treatment. The groups were named as follows: MC (Malaria control), ACT (Positive control), NC (Normal control) and fractions namely ETOH (Ethanol fraction) n-HEX (n-Hexane fraction), Acetone fraction (ACET) ethyl acetate fraction (ETOAC), and Aqueous fraction (AQF). All the treatment batches obtained 500 mg/kg body weight (bw) of the respective extract or fractions. MC got coartem (10 mg/kg/day bw) and NC for curative treatment orally administered distilled HO (10 ml/kg bw). Treatment with GL in their different vehicles started after the parasites were induced, which involved another 4 days. This is the curative model. The suppressive aspect involved the administration of the different vehicles of the GL thirty minutes after which the induction had taken place. This process went on for four days. The various GL vehicle forms were given to the mice for consecutively three days before induction of the malarial parasites; thus, this constituted the prophylactic method. Later, Mean Survival Time, i.e MST and density of blood parasite were determined for prophylactic, curative and suppressive groups. The curative group animals got considered for haematological analysis. The result shows that ethanol extract and fractions significantly reduced the parasitaemia level in all the three models. The degree of anti-plasmodial activity was highest in prophylactic group while ACET group showed the highest chemo-suppressive potency of 93.48 % among the other treatment groups. In the suppressive model, the ethyl acetate fraction exerted the highest chemo-suppressive effect of 80.09 %. The findings further reveal that haematological indices were altered by the high parasitaemia level which also significantly ($P<0.05$) reduced, compared to MC. Active ingredients in the ethyl acetate fraction proved to be most effective in boosting erythropoietic status of the experimental mice.

Keywords: *Gongronema latifolium*; *Plasmodium berghei*; Haematological; Diseases; Antiplasmodial

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1. Introduction

Malaria has consistently become a public health problem with a very high mortality rate, especially the black world. This is partly as a result of some environmental and lifestyle issues. A disease that is life-threatening and parasitic, malaria is caused by Plasmodium parasites are spread by female Anopheles mosquitoes (WHO, 2010). The estimated number of people affected by this parasite is in 2017 is about 219 million worldwide, with Africa recording the highest with about 200 million or 92% of the global diseases burden (WHO, 2018). Children under age 5 years and below are the most vulnerable group accounting for 61 % death caused by malaria worldwide (WHO, 2018).

The malaria parasite (*P. falciparium*) is considered a haematological problem since most of it impacts is on basic blood components (Akinosoglou *et al.,* 20 I 3). The parasite depends solely on haemoglobin for growth and multiplication thus attacks and colonise the healthy red blood cells to produce new parasites that will further attack the healthy red blood cells (Kapishnikov, 2017). Certain changes in haematological parameters may be eminent upon the infestation by malaria parasite. This alteration in haematological parameters serves as the major complications in malaria and its pathogenesis (Manas *et al.,* 2014). Research has shown great reduction in white blood cells (WBCs), red blood cells (RBCs), lymphocytes, eosinophils and haemoglobin (Hb) levels in malaria afflicted individuals in relation to their uninfected counterparts (Bakhubaira, 2013; Erhart *et al.,*2004; Mohamed *et al.,* 2016). Manas *et al.,* (2014) also posited that Hb, RBC, MCH, MCHC, MCV, WBC, platelet count, neutrophil, and lymphocytes count are the most commonly changed haematological parameters in malaria- infected patients. Hence, understanding the haematological derangement in the incidence of malaria invasion is core in both treatment and prevention of anaemia (Innocent *et al.,* 2014). The changes in haematological parameters could lead to malaria-related anaemia (Mohamed *et al.,* 2016) as a consequence of the parasitic invasion on haemoglobin and afore listed which when left without prompt intervention can cause death mostly occurring among the pregnant women and children within 5 years (WHO, 2018). Of grave consequence is the increased mortality and morbidity due to malaria infection, especially among the endangered population (pregnant women and children) and symptomatic of anaemia, rigors, etc (Nussenblatt and Semba, 2002). Anaemia presents a potent key factor in accessing progress in the control of malaria; hence it can be used to ascertain the viability and effectiveness of treatments and possibly control measures. Thrombocytopenia during severe malaria is observed with known causes ranging from damage of the endothelium, abnormal platelets adhesion and enhanced platelets activation (Butthep and Bunyaratvej, I 992; Osim *et al.,* I 991).

Gongronema latifolium (GL) is a medicinal, non-deciduous, climbing, rain forest belt and edible plant indigenous of the southern part of Nigeria. This plant is described by some Nigerian local population as 'utazi' by the Ibo people, 'arokeke' in Yoruba dialect; 'utashi' by the Bishiris in Obanliku of Cross River State. It is called 'Efo' in Yakurr ethnic group, 'utasi' for the Efiks and lbibios of the Southern region of Nigeria. This herbaceous shrub taxonomically is a plant whose family is known as Asclepiadaceae (Morebise *et al.,* 2002: Okafor, 1987). Leaves of GL possesses great bioactivity that qualifies it to playing critical roles in management of a number of diseases including diabetes, malaria, cardiovascular complications etc. Ebong *et al.,* 2008; Atangwho *et al.,* 2009 and other researchers have reported the mineral elements, phytochemical, proximate and the vitamins compositions of this plant while investigating the antidiabetic mechanisms of GL.

Other researchers have Investigated the hepatoprotective, hypotensive, hypolipidemic, haematological, antiatherosclerotic and antiplasmodial effect of GL (Ahalike and Ahaneku, 2015; Chinedu *et al.,* 2013; Imo *et al.,* 2014 & Nwaka *et al.,* 2015). Traditionally, the leaves of GL are employed in malaria disease management mostly in Southern eastern region of Nigeria, with convincing evidence of laboratory successes. Thus, this study is also to investigate the modulative impact of GL leaves extract and fractions on the haematological parameters of *P. berghei* parasitized mice.

2. Materials and Methods

2.1. Plant Material: Collection and Identification

Fresh and mature GL leaves were harvested in February, 2016, from an Umuebechi Umunachi village botanical vegetable garden in Obowo LGA of Imo state, Nigeria. The GL leaves were then taken to a plant taxonomist called Dr Ebigwai in Biological Science Faculty, University of Calabar. Voucher number of ERU/2011/718 was assigned the specimen and thereafter subsequently deposited at the herbarium in order to reference it.

2.2. Crude Extract Preparation

The GL leaves were washed, rinsed several times using distilled H2O to take away particles of dust and other debris and the water was allowed to percolate. The next action involved shade drying of a sizeable amount the GL leaves at 25°C

for a fortnight. The resultant dried pulverized powdered leaves got weighed after blending with electric blender. Maceration of 2 Kg of the GL leaves in 1400 ml of 80% (v/v) absolute ethanol (BDH) was done (Ugochukwu and Babady, 2003). With the agitation of the suspension using electric blender to make for complete active ingredients extraction the arrangement was cooled for 48 hours at 4°C. Cheese cloth was used to filter first the suspension, later with Whatman No 2 filter paper to get homogenous blend of filtrate which was thereafter concentrated in vacuo within temperature of (37-40°C) to 1/10"of the initial volume by making use of rotary evaporator. The concentrated volume was left in the open in a water-bath (40°C) so as to allow it to dry completely. The final dried concentrate now called ethanol extract weighing 200 g. This ethanol extract was finally put in the refrigerator at temperature of between 2-8^{0}C, till fractionation and later animal studies.

2.3. Crude Extract Liquid-Liquid Fractionation

The principle of sequential fractionation of the GL crude extract by using differences of polarities of the organic solvent as adopted Joy and Neal (2012). Labelled amount (20 g) of crude extract was serially fractionated using separating funnel into 3 different solvents in their increasing magnitude of polarity in this wise ethanol: n-hexane, ethanol: ethyl acetate and ethanol: acetone. Supposed ten grams extract crude were dissolved in ten ml of alcohol (absolute) the solvent extraction medium. This is accompanied with step-by-step inclusion of part of the first solvent fractionated. Say, ninety ml n-hexane stirred intensely, later permitted to be still for between 5-10 minutes. This causes two visible layers to separate. The denser lower layer would then be gently poured into labelled beakers, different from the less dense part. This process would be replicated a number of times till a time when all the soluble constituents the crude extract in the first solvent fractionated would be obtained. This complete protocol would be done again but this time the second fractionation solvent would be used instead taking advantage of residue from the first separation. Finally, the resultant fractions would be available: (I) Residue fraction, (II) Ethyl acetate fraction, (III) Acetone fraction (IV) N-hexane fraction. Every fraction would be concentrated to get fractions with their various percentage yield determined. The fractionated and residue samples would then be refrigerated at -5°C till when required for phytochemical, anti-plasmodial and biochemical studies.

2.4. Crude Extract PhytochemicaJ Screening

Presence of photochemical in the leaves extract needed to be ascertained using the method described by Harbone (1973).

2.5. Animals and Experimental Protocol

One hundred and fifty-seven (157) Swiss male and female albino wistar mice of weight between 16-30g which were gotten from Pharmacology/Toxicology Department Animal House, Pharmacy Faculty, University of Uyo, Nigeria. The mice had unrestricted food and water access. They also enjoyed a 12: 12 hour dark/light cycle ambient temperature and later grouped.

2.6. Experimental Protocol/Design

A group of 157 mice was broken down into 3 batches as depicted by the table below:

Table 1 Experimental protocol

Curative	Suppressive	Prophylactic	Group	Group Acronym	Dose
10	5	6	Malarial control	MC	Distilled H_2O
10	-	-	Normal control	NC	Distilled H_2O
10	5	6	Positive control	ACT	10 mg/kg
10	5	6	n-Hexane fraction	n-HEX	500 mg/kg
10	5	6	Ethyl acetate fraction	ETOAC	500 mg/kg
10	5	6	Acetone fraction	ACET	500 mg/kg
10	5	6	Aqueous fraction	AQF	500 mg/kg
10	5	6	Ethanol extract	ETOH	500 mg/kg

Mice in the MC group for prophylactic batch were orally given distilled water (10 ml/kg bw). Conventional drug (ACT) got coartem (10 mg/kg/day bw). The rest of the treatment groups got five hundred mg/kg each of their various treatment substances.

For suppressive batch, treatment groups of GL were administered treatments half an hour after animals were passaged. This lapsed till day four during which MC mice were orally given 10 ml/kg distilled water. ACT group received 10mg/kg coartem per bwt. per day. Treatment groups got five hundred mg/kg b.wt each of the ethanol. extract, then respectively n-hexane and ethyl acetate, acetone and aqueous fractions.

For curative design, interventions using GL extract and fractions started four days after parasite induction, this continued consistently for another four days. MC group mice got ten (10) ml/kg distilled water. ACT got ten (10) mg/kg/day bw of the conventional drug; the test groups got five hundred mg/kg for their various fractions. group Ethanolic extract also got five hundred mg/kg bw. Substance administrations done through oral gavage. For Curative design NC group. MST or Mean Survival. Time and parasite density got determined for three designs, while mice for curative design got sacrificed; their blood tissue, liver harvested for haematological evaluation.

2.7. Experimental Malaria Parasite Induction.

Mice with plasmodium parasites were got from NIMR an acronym for National Institute for Medical Research located in Lagos. The mice were also maintained through sub-passage in mice which were normally used for research. Cardiac puncture method was used in parasitizing erythrocytes which were gotten from a donor-infected mouse (euthanized) by sterile syringes and needles.

This was dropped in anticoagulant-coated tubes. The mice were intraperitoneally inoculated and thereafter confirmed. The stock solution contains certain concentration of cells measured after dilution with physiological saline. Resultant inoculum, (0.2 ml) houses I x 10^7 erythrocytes affected by parasites. These inoculums are standard ones infecting a solitary mouse (Okokon *et al.,* 2017).

2.8. Infection Monitoring

The tail blood from each mouse was stained on slides to form thin and thick films later covered with Giemsa stain. The level of parasitaemia was measured by counting the number of parasitized white blood cells (WBC) out of 500 WBC or 200 erythrocytes in the microscope's random field. Automation Hematology Analyser Sysmex KX2l, SYSMEX Corporation Japan, (non-cyanide haemoglobin analysis Protocol) was employed in the measurement Full Blood Counts: Hematocrit (HCT), Red blood cell (RBC), platelet count (PLT), Haemoglobin (HGB), White blood cell (WBC) percentage count, lymphocyte count and red cell indices: Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH).

2.9. Data Analysis

Statistical analysis of various samples in this investigation was done via One-way Analysis of Variance and the Duncan's Multiple Range Test. These analyses were accompanied by SPSS software (version 20). Statistical differences at the level of $p<0.05$ were assumed to be significant. Data were expressed thus: mean ± SEM.

3. Results

Table 2 Curative Schizonticides Activity and Mean Survival time of Parasitized Mice of Test Groups

Test Group	%Growth Inhibition /Day6	%Growth Inhibition /Day8	Parasite Density			Mean Survival Time/ Day
			Day 4	Day 6	Day 8	
MC	-80.74	-122.08	102.73±26.00^{c}	185.68±16.78^{x}	228.13±7.56 ^{x}	9.50±0.73
ACT	76.20	100	81.02±5.10^{c}	19.29±12.43 ^{afx}	0±0.00 $^{a\,x}$	30.00±0.70
n-HEX	88.13	86.28	281.05±59.66	33.39±4.17 $^{a\,x}$	38.52±7.52^{abx}	20.00±1.07
ETOAC	76.54	85.25	120.03±62.28^{c}	27.69±4.5^{af}	17.72±1.68 ^{a}	18.00±1.48

ACET	51.90	45.29	77.04±27.16[c]	37.06±4.70[a]	42.16±19.98[ab]	12.50±1.21
AQF	46.64	63.76	109.09±34.39[c]	58.22±2.08[a]	39.55±11.21[ab]	13.00±1.04
ETOH	68.65	71.53	102.09±45.25[c]	31.99±5.42[af]	29.08±9.10[a]	16.00±1.82

Data are expressed: Mean ± SEM, n = 5. a = p<0.05 vs MC, b = p<0.05 vs ACT, c = p<0.05 vs n-HEX, f = p< 0.05 vs AQF, x = p<0.05 vs DAY 4.

Table 3 Suppressive, Schizonticides Activity and Mean Survival Time of Test Groups

Group	%Growth Inhibition	Parasite Density x 103	Mean Survival Time/ Days
MC	-	263.87 ± 63.98	9.75 ±1.27
ACT	99.90	0.23 ± 0.12[a]	29.00 ± 0.01
n-HEX	71.13	75.09 ± 11.53[a,b]	14.50 ± 1.78
ETOAC	80.08	50.16 ± 5.57[a,,f]	20.75 ± 3.97
ACET	73.38	68.67 ± 15.98[a,b]	15.50 ± 1.11
AQF	53.67	121.75 ± 24.04[a,b]	11.75 ± 1.84
ETOH	65.94	89.49 ± 24.33[a,b]	13.25 ± 5.17

Data are expressed: mean ±SEM, n = 6. a =p<0.05 vs NC, b=p<0.05 vs ACT, f = p<0.0 vs AQF.

Table 4 Repository Schizonticides Activity and Mean Survival Time (MST) of Test Groups

Group	%Growth Inhibition	Parasite Density x 103	Mean Survival Time/ Day
MC	-	163.93±57.74	9.10 ± 0.46
ACT	99.52	0.78 ± 0.04[a]	29.25±0.68
n-HEX	67.16	53.84 ±25.98[a]	19.00±0.52
ETOAC	62.24	61.90 ± 18.97[a,b]	16.00±0.58
ACET	93.48	10.68 ± 2.68[a]	25.67±1.47
AQF	54.83	74.05 ± 2.04[a,b,c]	13.50 ± 0.76
ETOH	42.64	94.03 ± 35.53[a,b,c]	12.50 ± 0.71

Data are expressed: mean ± SEM, n = 6.a =p<0.05 vs MC,b = p<0.05 vs ACT,e = p<0.05 vs ACET.

Table 5 Qualitative Screening of Phytochemicals Present in fractions and extract of GL

Fraction	Saponin	Alkaloid	Flavonoid	Steroid	Phenol	Tannin	Anthocyanin
n-HEX	+	+++	+	++	+++	+	+
ETOAC	+	+++	++	++	+++	+++	+
ACET	+	+++	++	++	+++	++	+
AQF	++++	+++	++	+	+	+++	+
ETOH	+	++++	+++	++	++++	+++	+

+ = Very low level present, ++ =Low level present, +++ = High level present, ++++ = Very high level present

Table 6 Curative Intervention with GL extract and fractions On Indices of Haematology of *P. berghei* infected mice after 4 days treatment

GROUPS	MC	NC	ACT	n-HEX	ETOAC	ACET	AQF	ETOH
RBCs×10^6µL	2.65±0.41	8.21±00.53^{a,g}	6.72±0.63^{a,f}	4.25± 0.29^{b,h}	6.48± 0.46^{a,c}	4.45± 0.22^{b,h}	4.34± 1.49^{d,h}	4.69± 0.54^{a,b}
WBC×10^3 µL	22.33±4.34	9.97±1.63^{a,c}	10.20±1.05^{a}	14.40± 1.87 ^{b}	10.80± 0.55^{a,c}	11.38± 2.34^{a,c}	12.13± 1.69^{a,c}	14.18± 1.25^{a,c}
HGB g/dl	3.95±0.84	11.33±0.93^{a,e}	10.18±0.69^{a,g}	6.78± 0.35^{a,b,h}	8.63± 0.69^{a}	6.30± 0.43^{b,h}	7.63± 1.93^{a,h}	7.18± 0.61^{a,h}
PLT×10^3µL	116.30±16.25	1127±94.05^{a}	877.8±129.5^{a}	866.3± 148.5^{a}	804.3± 263.2^{a}	585.3± 157.8^{a,h}	619.3± 94.33^{a,h}	312.5± 137.6^{h}
LYM%	108.50±1.55	65.8±5.48^{a}	66.05±11.49^{a}	83.00± 5.51^{a}	72.83± 4.77^{a}	71.70± 10.26^{a}	78.28± 6.42 ^{a}	83.50± 3.87 ^{a}
HCT%	13.50±1.54	39.83±3.43^{a}	35.85±2.48^{a}	22.75± 1.23^{b,h}	32.30± 2.31a	21.83± 1.74^{b,h}	23.20± 7.87^{b,h}	25.03± 1.91^{a,b,h}
MCV(fL)	41.73±3.35	51.33±1.90^{a}	53.83±1.68^{a}	53.75± 1.96^{a}	51.85± 1.65^{a}	49.25± 1.66^{a}	53.95± 0.72^{a}	51.60± 1.31^{a}
MCH(pg)	9.57±0.53	14.50±0.26^{a,f}	15.28±1.31^{a,f}	16.03± 1.17a	15.45± 0.72^{a}	14.23± 0.29^{a,f}	18.13± 2.29^{a}	14.70± 0.25^{a,f}
MCHC(g/dL)	22.45±2.38	28.50±0.32^{a}	28.43±0.39^{a}	29.80± 0.35^{a}	29.73± 0.75^{a}	29.00± 0.54^{a}	31.65± 2.12^{a}	28.50± 0.28^{a}

Data are expressed: Mean ± SEMn = 5. a =p<0.05 vs MC,b =p<0.05 vs ACT,c =p<0.05 vs n-HEX.d =p<0.05 vs ETOAC,e =p<0.05 vs ACET, f =p<0.05 vs AQF, g =p<0.05 vs ETOH, h = p<0.05 vs NC.

4. Discussion

The haematological and antiplasmodial activity of fractions and extract of GL leaves was investigated via conventional *P. berghei* designs of mice. There are a plethora of reports validated by scientific claims that medicinal plants exert biological activity against malaria parasites. Corroborating this, most medicinal plant interventions from folklore medicine in treatment of malaria have said a lot, but ascertaining the residual potency of the active ingredients of GL leaves in different solvent media and exploiting such information in treatment of malaria is yet undone. Consequently, this study aimed at scientifically validating these claims while exploiting GL leaves in management of malaria taking advantage offered by such solvent extraction media.

In general, the test extraction groups observed positive effect in significantly reducing ($p<0.05$) density of parasites in the 3 models, thus revealing that antiplasmodial activity of the test groups each had some varying degree of antiplasmodial potency against *P. berghei* parasitized mice. The curative batch, n-hexane fraction showed the greatest antiplasmodial activity (86.29 %) with respect to the rest of the treatment groups. The ethyl acetate fraction of the suppressive group showed the greatest chemo-suppressive effect (80.09 %). This confirmed the earlier findings of Ettebong *et al.,* 2012). Conversely, the Acetone fraction of prophylactic batch exhibited the greatest chemo-suppressive effect (93.48 %) when compared with the other test groups. The observation of acetone and ethyl acetate fractions respectively showing the greatest prophylactic and suppressive effects compared with other test groups presupposes that active ingredients repository in this plant possessing antimalarial property may be localized there. Certainly, this strongly positive antiplasmodial activity demonstrated by these test groups must have been associated with activity of bioactive substance such as revealed by a plethora of flavonoids, alkaloids, etc in GL phytochemistry. Some known alkaloids are potent antibiotics exhibiting antiplasmodial activity in their mechanism by inhibiting or interrupting the synthesis of protein of *P. falciparum* parasite (Ettebong *et al.,* 2012). Perhaps too, an element of synergistic action of multiple compounds locked up in GL might have played a bioactive role (Esume *et al.,* 2011 and Okwu, 2004). Besides, flavonoids, saponin and tannins are known to play antioxidant and scavenging roles of free radicals and therefore capable of counteracting the oxidative lesion induced by *P. berghei* (David *et al.,* 2004). Flavonoids are known to induce

chelation by utilizing base-pairing of nucleic acid of the Plasmodium. Such scenario might have been implicated in the reversal of parasitic activities against the different plasmodial parasite species. Malarial control mice group showed significant decrease ($p<0.05$) in the level of RBC, HCT, MCV, HGB, PLT, MCH and MCHC with respect to NC. Etkin (2003) reported haemolysis of the erythrocytes which caused haemolytic anaemia during malaria pathogenesis. This observation so corroborated the claims of Khursheed, (2010) and Kotepui et al. (2017) as they noted that red cell indices RBCs, HGB, MCH, HCT, MCV and MCHC were significantly reduced in malarial patients. Almost all test groups in this investigation significantly elevated ($p<0.05$) the values of RBC count, HGB, PLT, MCH, HCT, MCV and MCHC close to the values of the Malarial control. group. But most strikingly, ETOAC appeared most effective in boosting the erythropoietic status of the test mice. Conversely, WBC and the percentage lymphocyte counts recorded significant increase ($p<0.05$) with respect to NC group. Components of leucocyte serve to engulf plasmodium or even pathogens originating from bacteria and possess the ability to elicit change in WBC counts (Baker *et al.,* 2007). However, this is not in line with accounts of Akinnuga et al., (2011) and McKenzie *et al.,* (2005) who posited leukopenia and lymphocytopenia in sufferers afflicted with *P. falciparum* and *P.vivax* . Following infection of plasmodium parasite in the blood, localization of white blood cells occur outside peripheral circulation and the spleen and other marginal pools instead of the actual stasis or depletion (McKenzie *et al.,* 2005: *Taha et al.,* 2007 and Sumbele *et al.,* 2010).

Malaria-associated decrease in lymphocyte count observed in the MC group is notable since lymphocytes, particularly T-cells, greatly function in immunity to falciparum malaria by unleashing pro-inflammatory cytokines (TNF-α, interferon-y) and other cytokines, thus exciting other inflammatory cells. However, excess production of pro-inflammatory cytokines add to the intensity of the disease (Biemba *et al.,* 2000). In addition, lymphocytes are sources of serum immunoglobulin antibodies and function in immunologic reactions (Asiwe *et al.,* 2023). Dacie and Lewis, 1991 reported that lymphocytosis in humans during lymphatic leukemia and viral infections. Decreased lymphocyte count (lyphopenia) took place in chemotherapy. Treatment with the test groups significantly lowererd WBC counts and percentage lymphocyte, causing great improvement of the immune system and may imply anti-inflammatory action of the test groups (Charles *et al.,* 2004).

5. Conclusion

In summary, this research has investigated the haematological and antiplasmodial evaluation of fractions and ethanol extract of GL leaves in *P. berghei* infected mice. The anti-plasmodial activity of the extract and fractions of GL has exhibited significant chemo-suppression and chemotherapeutic effects against *P. berghei* in mice. Acetone, ethyl acetate and n-hexane fractions respectively recorded percentage parasite growth inhibition of 93.48 %, 80.09 % and 86.29 % in the prophylactic, suppressive and curative groups. The phytochemistry of GL leaves has shown high amounts of alkaloids, flavonoids, phenols and saponins which are responsible for antiplasmodial activity. Extract and it various fractions possess both haematopoietic and antiplamodial activity against *P. berghei* infected mice, suggestive of complementary role in the management of malaria.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present work was conducted in strict accordance with the Biological Science Faculty, University of Calabar ethics committee rules for the care and use of laboratory animals.

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