Nigerian Journal of Biochemistry and Molecular Biology 2015; 30(1): 7-24

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.org/

NJBMB/039/15

Antiplasmodial and Haematopoietic Activities of Ethanolic Extract of some Chinese Green Tea in *Plasmodium berghei*-infected Mice

***Akande, I. S., Oshilaja, R. T., Oderinde, A. O. and Adeshakin, A. O.**

Department of Biochemistry, College of Medicine, University of Lagos, P.M.B 12003, Idi Araba, Lagos, Nigeria.

Abstract: In the present study, we evaluated the antiplasmodial and haematopoietic activities of 200 mg/kg and 400 mg/kg body weight of crude ethanolic extracts of three commercial Chinese green tea (BIA 849, TD 570 and GB/T19598) using the 4-day suppressive and Rane's curative anti-malarial assays in mice infected with *Plasmodium berghei* (NK65 strain). Effect of the extracts on weight was also determined. Ethanolic extract of the green tea produced a significant ($p<0.05$) dose dependent decrease in the parasitemia level in the test groups comparable to the chloroquine treatment group. 400 mg/kg bw of TD570 and GB/T19598 green tea produced significant ($p<0.05$) increase in parasitemia suppression while BIA 849 suppressive activity did not change significantly with dose. The curative and chemo suppression activities of TD570 and GB/T19598 at 4 days post treatment were significantly ($p<0.05$) higher than 5 mg/kg bw of chloroquine having 100 % curative activities at 2 and 3 days post treatment, respectively and 100 % chemo suppression. Chemosuppression activity of BIA 849 did not change significantly ($p>0.05$) at 400 mg/kg bw. compared with same dose of TD570 and GB/T19598 green tea. Significant ($p<0.05$) increase in haematopoiesis during suppressive and curative treatment of malaria were respectively shown by 200 mg/kg and 400 mg/kg bw. of these tea. BIA 849 produced significant ($p<0.05$) increased haematopoiesis compared to the other two. There was significant ($p<0.05$) increase in weight in mice treated with 200 mg/kg TD 570 compared to the controls while the weight of mice treated with 400 mg/kg GB/T19598 decreased significantly ($p<0.05$) compared with positive control group. GB/T19598 green tea at the dose of 400 mg/kg bw. produced best effects and is thus recommended as the ideal antiplasmodial agent with possibly best therapeutic value.

KEYWORDS: Chemosuppression, Chinese tea, Haematopoiesis, Malaria, Parasitemia

1.0 Introduction

Malaria, a life threatening disease caused by a parasitic infection of the red blood cells, is undoubtedly the single most destructive and dangerous infectious agent in the developing world, predominantly tropical and subtropical regions, including parts of America, Asia and Africa (Winter *et al.*, 2006). Each year 300 to 500 million new cases are diagnosed and approximately 1.5 million people die of the disease (Greenwood *et al.*, 2005). Almost 90% of these deaths occur in sub-Saharan Africa, where it is the greatest cause of hospitalization and directly responsible for one in five childhood deaths among children age 6 months to 5 years (Quattara *et al.*, 2006). It indirectly contributes to illness and deaths from respiratory infections, diarrhoeal disease and malnutrition (WHO, 1999).

The re-emerging of malaria in many parts of the world is due to the rapid increase of resistance to most of the available anti-malarial drugs, as well as resistance of vectors to insecticides (Zirihi *et al.*, 2005). Drug resistant strains of *Plasmodium* have been found in many endemic areas of the world and many of conventional anti-malarial drugs have been associated with treatment failure. The evolution of drug-resistant *Plasmodium* strains, difficulty of creating efficient vaccines and also adverse side-effects of the existing anti-malarial drugs highlight the urgent need for novel, well-tolerated and effective anti-malarial drugs for

***Corresponding Author**
Tel.: +2348033187864
E-mail: iakande@unilag.edu.ng

both prophylaxis and treatment of malaria (Winstanley, 2000).

Plants have been a great source of medicine useful in the treatment of various diseases including malaria (Bako *et al.,* 2005). Both quinine and artemisinin, antimalaria drugs in use, have been derived from traditional medicine and plant extracts (Gessler *et al.,* 1994). The recommendation of artemisinin, a plant extract derivative by the World Health Organization as a component of the first-line treatment of malaria, ACT, has encouraged the continued search for new natural product-derived anti-malarial drugs (Mutabingwa, 2005). Furthermore, several studies have been undertaken to evaluate not only the inhibitory effects of various plant extracts on *P. falciparum* (Wanyoike, 2004) using *in vitro* culture, but also *in vivo* anti-malarial properties on *Plasmodium berghei*-infected mice (Sudhanshu *et al.,* 2003)**.** One of such plants which researchers have developed interest in of recent is the tea plant.

Tea (*Camellia sinensis*) was first discovered as a drink and medicine in China around 2737 B.C. Since then, tea has become so popular making it the second beverage to water in terms of worldwide consumption (Scharbert *et al.,* 2004). In recent years, the health benefits of consuming green tea, including the prevention of cancer and cardiovascular diseases, the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, antiplasmodium and cholesterol-lowering effects of green tea and isolated green tea constituents are under investigation. Tea constituents including flavonoids, caffeine and theanine have been investigated and linked to the health benefits such as prevention of cancers and cardiovascular diseases, reduction of the risks of obesity and diabetes, and improvement of immune system (Zhang *et al.,* 2007., Basu *et al;* 2010, Akande *et al.,* 2012). Despite the enormous research that has been conducted on the plant, there is still very limited literature on the antimalaria activity of tea plant. Interest in medicinal plants as possible sources of new anti malarial drugs has been stimulated since the isolation and clinical use of artemisinin from a Chinese plant, *Artemisia annua* L. (Asteraceae) (Castilho *et al*, 2008). This plant, employed for the treatment of febrile diseases and malaria has for centuries been used in traditional Chinese medicine and is included in the current pharmacopoeia of China (Hsu, 2006). *Artemisia annua* is usually used to prepare a tea and when found to containing effective amounts of artemisinin, it might be used as a self-reliant treatment of malaria (Blanke et al., 2008; Antonella *et al*, 2012).

The health-promoting effects of green tea are mainly attributed to its polyphenol content, particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight. Recently, many of the aforementioned beneficial effects of green tea were attributed to its most abundant catechin, (-)-epigallocatechin-3-gallate (EGCG). Green tea extracts are more stable than pure epigallocatechin gallate, one of the major constituents of green tea, because of the presence of other antioxidant constituents in the extract (Zhang *et al.,* 2007). The chemical composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose,fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and a-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds: aldehydes, alcohols, esters, lactones, hydrocarbons (Zhang *et al.,* 2007. Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves and their infusion. Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophylline. In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as theanine.

The major flavonoids of green tea are various catechins, which are found in greater amounts in green tea than in black or Oolong tea. There are

four kinds of catechins mainly find in green tea: epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG. The preparation methods influence the catechins both quantitatively and qualitatively; the amount of catechins also varies in the original tea leaves due to differences in variety, origin, and growing conditions. The preparation of fresh green tea cannot totally extract catechins from the leaves; therefore, the concentration found differs from the absolute values determined through the complete extraction of leaves; moreover, catechins are relative. Thus, comparison of ingested doses in animal studies is not possible because the catechin quantification before administration is often not known unstable and could be quantitatively and qualitatively modified during the time frame of an experiment

The re-emerging of malaria in many parts of the world is due to the rapid increase of resistance to most of the available anti-malarial drugs, as well as resistance of vectors to insecticides and high cost of treatment (Ridley *et al.,* 2002; Zirihi *et al.,* 2005). Hence there is a need to search for a potent and affordable alternative which might be conveniently exploited to design new and/or more effective combination therapies.

The overall objective of this study was to determine the suppressive, curative and heamatological effects of three Chinese green tea on *Plasmodium berghei*-infected albino mice.

2.0 Materials and Methods

2.1 Green Tea

Three brands of Chinese green tea, namely BIA 849, TD 570 and GB/T19598 produced by China Tea (Human), An Hua Tea Factory, were sourced from Guangzhuo, China.

2.2 Animal Handling

Healthy Swiss albino mice (18.5 ± 0.5 g) of either sex were used for the study. The animals were procured from the animal house of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria and were allowed to acclimatize to the new environment for a period of two weeks prior to the study. The mice, maintained on standard rodent feed (Ladokun Feeds Ltd., Ibadan, Nigeria) and water *ad libitum*, were housed in polypropylene cages at room temperature throughout the study and were maintained under standard conditions of humidity (40-45%), room temperature (25^0C) and 12 h light/12h darkness cycle.

2.3 Rodent Parasite Strain

The rodent parasite *Plasmodium berghei* NK 65 used in this study was obtained from National Institute for Medical Research (NIMR) Lagos, Nigeria. The strain of parasite was maintained by continuous intraperitoneal passaging of the parasite into uninfected mice for two weeks.

2.4 Preparation of Extract

A known weight (40 g) each of the three brands of Chinese green tea namely, BIA 849, TD 570 and GB/T19598 tea was micronized and extracted exhaustively in 800 ml 80 % ethanol at boiling point for 120 minutes (Vuong *et al.,* 2010). The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator (Stuart RE300, Bibby Scientific, UK) to give 9.45 g, 11.20 g and 9.76 g corresponding to 11.81%, 14.00% and 12.20% percentage yield of green coloured pastes respectively stored in an air-tight container and kept in the refrigerator maintained at 4^0C.

2.5 Animal Grouping

One hundred and eighty (180) mice (weighing 18-25 g) were divided randomly into nine groups of twenty mice each according to the treatment protocol in Table 1.

2.6 Determination of Mean Body Weight

To determine the effectiveness of the extract in preventing loss of body weight by the parasite, weights of the mice were measured before parasite inoculation and treatment in all the extract treated and control groups using digital sensitive weighing balance (Wigger, Hauser model: GR 200, Germany). The mean

body weight was calculated according to the following mathematical equation:

$$\text{Mean body weight} = \frac{\text{Total weight of mice}}{\text{Total number of mice}}$$

(Yeshanew and Mekonnen, 2013)

Table 1: Animal Grouping and Administration of Chinese Tea Extract Administration

Groups	Treatments
A	5 ml/kg body weight of distilled water (Uninfected negative control)
B	*P. berghei* + 5 ml/kg body weight of distilled water (Negative control)
C	*P. berghei* + 5 mg/kg body weight of chloroquine phosphate solution (Positive control)
D	*P. berghei* + 200 mg/kg body weight ethanolic extract of Chinese green tea BIA 849
E	*P. berghei* + 400 mg/kg body weight ethanolic extract of Chinese green tea BIA 849
F	*P. berghei* + 200 mg/kg body weight ethanolic extract of Chinese green tea TD 570
G	*P. berghei* + 400 mg/kg body weight ethanolic extract of Chinese green tea TD 570
H	*P. berghei* + 200 mg/kg body weight ethanolic extract of Chinese green tea GB/T1959
I	*P. berghei* + 400 mg/kg body weight ethanolic extract of Chinese green tea GB/T1959

2.7 Antiplasmodial Activity Screening

In vivo anti-plasmodial and haematological activities of the three green tea extracts were assayed in established malaria infection models in chloroquine-sensitive *Plasmodium berghei* NK65-infected mice (Awe and Opeke, 1990). Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harbouring rodent *Plasmodium species.*

2.7.1 Chemosuppression Test

Evaluation of suppressive potential of the extracts was done using Knight and Peters 4-day suppressive test against *P. berghei berghei* infection in mice (Knight and Peters, 1980; David *et al.,* 2004). Parasitized erythrocytes were obtained from a donor infected mouse by cardiac puncture with a sterile needle and syringe (Bello *et al.,* 2009). Ninety mice were selected and eighty of them inoculated intraperitoneally with infected blood suspension (0.2ml) containing 1×10^7 infected erythrocytes. The mice were treated as shown in Table 1. The treatment started 1 hr after the inoculation on the first day (day 0). Treatment continued daily until the fourth day. On the fifth day (day 4 post-treatment), blood was collected from the tail of each mouse and smeared onto a microscope slide to make thick and thin films (Idowu *et al.,* 2010). The blood films were air-dried, fixed with methanol, air-dried again, stained with Giemsa at pH 7.2 for 45 min and observed under oil immersion for parasitaemia. Each slide was observed at different fields and the number of parasites relative to the number of leukocytes was calculated and expressed as 'parasites per microlitre of blood' using the mathematical expression:

$$\text{Parasites per microlitre of blood} = \frac{\text{Number of parasites counted} \times 8000}{\text{Number of leukocytes}}$$

(WHO, 2010)

The percentage suppression of parasitaemia was expressed as mean chemosuppression and this was calculated for each dose level by comparing the mean parasitaemia in infected untreated (negative) control with those of treated mice. The difference between the mean value of the control group (taken as 100%) and those of the experimental groups were calculated and expressed as percent reduction or activity using the following equation:

$$\text{Activity} = \frac{100 - \text{Mean parasitaemia treated} \times 100}{\text{Mean parasitaemia (-ve) control}}$$

(Olorunniyi and Morenikeji, 2014)

2.7.2 Curative (Rane) Test

The curative potential of each of the extracts was determined according to a method described by Iyiola *et al.* (2011). Ninety mice were randomly divided into nine groups and treated as in table 1. The mice were injected intraperitoneally with standard inoculums of $1x10^7$ *Plasmodium berghei berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were treated with the drug and extracts. The treatment was carried out once daily for 5 days and blood smears were collected and examined microscopically at a magnification of x100 to monitor the parasitaemia level. Parasitaemia was expressed as 'parasites per microlitre' of blood and curative activity was established as stated for chemossupression.

2.7.3 Collection of Blood and Determination of Haematological Parameters

From the respective groups, four mice were sacrificed by cervical dislocation from each of the groups on days 4 and 8 post infection, and days 3 and 8 post infection for suppressive and curative groups respectively. Blood samples were collected by ocular puncture via heparinzed capillary tubes, into labeled heparinzed bottles and haematological indices were assayed.

The haematological analysis was carried out using an automated haematological analyser (model-Sysmex KX21, Sysmex Corporation, Japan). Red blood cell count (RBC), Packed cell volume (PCV), Haemoglobin concentration (Hb), Mean Corposcular Volume (MCV), White blood cell count (WBC) were determined.

Statistical Analysis

Results were expressed as mean ± standard deviation (Mean ± SD) for triplicate measurements and subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (SPSS 20.0 Inc., USA). Statistical significance was considered at $p< 0.05$.

3.0 Results

Table 2 shows the effect of malaria treatment by suppressive model on body weights of *Plasmodium berghei* infected mice. Infection of mice with *Plasmodium berghei* caused a weight loss (15.4 to 14.5) relative to the uninfected group in which an increase in mean body weight (15.2 to 16.0) was observed. Treatment with 400 mg/kg of BIA 849 and TD 570 showed no significant loss in weight comparable to that shown by group treated with Chloroquine. Significant increase in body weight was shown by the group treated with 200 mg/kg bw of TD 570 (32 %). Group H treated with200 mg/kg bw of GB/T19598 suffered the greatest loss in weight (2.3 %) among treated groups which was still significantly different from that of the untreated-group (5.8%).

Table 3 shows the effect of treatment on body weight in *Plasmodium berghei* infected mice. Infection of mice with *Plasmodium berghei* caused a weight loss (15.4 to 14.5) relative to the uninfected group in which an increase in mean body weight (15.2 to 16.0) was observed. Treatment with 200 mg/kg bw of BIA 849 showed a weight loss of 1.95 % similar to that seen in group treated with Chloroquine (1.75 %) while better weight maintenances (0.70 and 0.57 % weight losses respectively) were observed in groups treated with TD 570 and GB/T19598. However, increase in dosage of tea extracts to 400 mg/kg body weight caused further loss in body weight of animals treated with BIA 849 (2.6 %), TD 570 (2.3 %) and GB/T19598 (3.18 %). All weight changes were significantly different from that observed in the untreated group ($p < 0.05$).

Figure 1 shows the chemosuppressive effect of the ethanolic extracts of three Chinese green tea on *P. berghei*. From the result, administration of standard drug, chloroquine and the tea extracts resulted in significant ($p<0.05$) decrease in the parasite load compared to the group adminstered water and the group that was not administered tea extract. Suppressive effects of the 200 mg/kg bw doses of tea BIA 849 and GB/T19598 (59 and 57.6 % respectively) showed no significant change ($p>0.05$) to that of the standard drug, chloroquine (57.5 %) while TD 570 showed a significant in chemo-

Table 2: Effect of suppressive malaria treatment with ethanolic tea extracts on body weight

Groups	Weights (g)*		Weight Change (%)
	Day 3	Day 8	
A	15.20±0.08	16.00±0.22	5.2[b]
B	15.40±0.12	14.50±1.01	-5.8[a]
C	17.10±0.06	17.10±0.39	0.0[a,b]
D	15.40±0.15	18.20±1.02	18.0 [a,b]
E	14.10±0.25	17.80±1.21	-0.07 [a,b]
F	18.20±0.02	18.80±0.12	32.0 [a,b]
G	17.00±0.06	17.00±1.04	0.0 [a,b]
H	17.60±1.03	17.20±0.91	-2.3 [a,b]
I	15.70±0.54	16.40±1.30	10.8 [b]

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg bw of ethanolic extract of Chinese green tea GB/T19598; * Values are presented as Mean±SD (n=4); [a] Significantly different from group A; [b] Significantly different from group B

Table 3: Effect of malaria curative treatment with ethanolic tea extracts on body weight

Groups	Weights (g)*		Weight Change (%)
	Day 3	Day 8	
A	15.2±0.08	16±0.22	5.20[b,a]
B	15.4±0.12	14.5±1.01	-5.80 [a]
C	17.1±0.06	16.8±0.83	-1.75 [b,a]
D	15.4±0.15	15.1±0.84	-1.95 [b,a]
E	19.1±0.25	18.6±1.52	-2.60 [b,a]
F	14.2±0.02	14.1±1.73	-0.70 [b,a]
G	17.0±0.06	16.6±0.71	-2.30 [b,a]
H	17.6±1.03	17.5±0.21	-0.57 [b,a]
I	15.7±0.54	15.2±1.05	-3.18 [b,a]

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; * Values are presented as Mean±SD (n=4); [a] Significantly different from group A; [b] Significantly different from group B

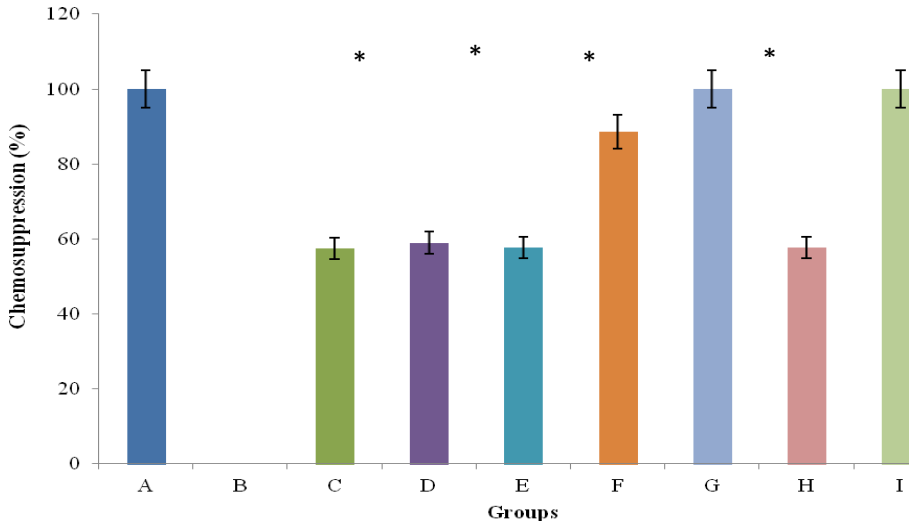

Figure 1: Chemosuppressive effect of ethanolic tea extracts against *Plasmodium berghei*.

Group A: Uninfected Control; Group B: Infected Negative control (administered 5 ml/kg bw. of distilled water); Group C: Positive control (administered 5 mg/kg bw.of chloroquine phosphate solution); Group D: Administered 200 mg/kg bw. ethanolic extract of Chinese green tea BIA 849; Group E: Administered 400 mg/kg bw. ethanolic extract of Chinese green tea BIA 849; Group F: Administered 200 mg/kg bw.ethanolic extract of Chinese green tea TD 570; Group G: Administered 400 mg/kg bw.ethanolic extract of Chinese green tea TD 570; Group H: Administered 200 mg/kg bw. ethanolic extract of Chinese green tea GB/T19598; Group I: Administered 400 mg/kg bw. ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicates; $^{*}p<0.05$ indicates a significant difference between the tests, negative and uninfected controls

suppressive action (88.9 %) at the same dose. 400 mg/kg bodyweight of Chinese green tea (TD 570 and GB/T19598) showed significant ($p<0.05$) increase in suppressive activity (100 %) against *Plasmodium berghei* and this appears to be the ideal dose from this study. Dosage of 400 mg/kg of BIA 849 was not significantly different from the 200 mg/kg bw.of the same tea compared with the controls.

Figure 2 shows that parasitaemia increased significantly ($p<0.05$) in the parasite-infected and untreated group (group B) daily until day 7 of infection. The treated parasite-infected groups had an initial increase in parasitaemia levels followed by a gradual significant decline post treatment. At baseline (day 3), no group's parasitaemia was significantly ($p\geq0.05$) lower compared to untreated control group (B). At day 4, treatment groups showed significant ($p<0.05$) decrease in parasitaemia compared to the previous day similar to what was obtained with the standard drug, chloroquine except groups D and F (treated with 200 mg/kg of tea BIA 849 and TD 570 respectively) which showed significant increase in parasitaemia. At day 5, parasitaemia level of group G, treated with 400 mg/kg bw of tea TD 570, was below detection limit while other treated groups including the group treated with standard drug had parasitaemia level significantly lower relative to the negative control group. At day 6, parasitaemia level of treated groups except groups E and F were below detection limit which was comparable to the result obtained with the standard drug. By day 7, parasite count of the untreated group dropped while all the treated groups showed 100 % parasite clearances which were comparable to that of the standard drug and statistically different from the untreated group (Figure 3).

The effects of treatment on haematological parameters; white blood cell count (WBC) red blood cell count (RBC), haemoglobin concentration (Hb) and mean corpuscular volume (MCV) are represented in Figures 4, 5, 6 and 7 respectively. All haematological

parameters measured reduced with increase in parasitaemia in the negative control. The reductions were statistically significant ($p < 0.05$) except in MCV where the decreased was statistically insignificant.

WBC count was highest in group D treated with 200 mg/kg bw of tea BIA 849 having 10.06 and 9.74 $x10^9$/L on days 4 and 8 respectively (Figure 4). WBC did not change significantly in treated groups from day 4 to day 8. Highest change with treatment (6.85 to 7.50± (10.1 %)) was recorded in group F treated with 200 mg/kg bw of tea TD 570 compared to a reduction in WBC from 5.15 to 3.66 ± $x10^9$/L (28.8 %) observed in the untreated group. Group D treated with 200 mg/kg bw of BIA 849 showed a decrease in WBC which was not significantly different from that shown by the standard drug, chloroquine. However, WBC counts were higher in all treated groups than in the group administered with Chloroquine standard after treatment. Changes in RBC count of treated groups did not change significantly from that of the uninfected group and from the chloroquine-treated group. Untreated group however showed significant ($p<0.05$) decrease in RBC from day 4 to day 8 (7.46 to 4.18).

Haemoglobin concentrations in all groups were significantly different from that of the uninfected group. The untreated group showed the highest decrease in Hb concentration from day 4 to day 8 (14.18 to 10.99). Highest increase in group Hb concentration from day 4 to 8 (9.40 to 12.51) was observed in group 'I' treated with 400 mg/kg bw.

Mean corpuscular volume was lower in all groups relative to the uninfected group. Treatment however, resulted in non-statistically significant increases from day 4 to 8 except in group E treated with 400 mg/kg bw of tea BIA849 which reduced from 41.18 to 38.85. The increases observed in treated groups were lower compared to that seen in the Chloroquine treated-group.

The effects of treatment on haematological parameters; white blood cell (WBC) and red blood cell counts (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV) are shown in figures 8, 9, 10 and 11 respectively. The blood parameters reduced with increase in parasitaemia before treatment at day 3 and increased with treatment except with RBC count whose further reduction was suppressed by treatment.

WBC count showed statistically significant increase after treatment with the increase reaching maximum in chloroquine treated group which increased from 4.62±0.01 to 7.12±0.02 (53%) followed by the group treated with 400 mg/kg body weight tea BIA 849 which increased from 5.03±0.04 to 7.13±1.02 at days 3 and 7 respectively.

RBC reduced drastically in the negative control group relative to the uninfected group. Reduction in RBC count was resisted significantly by treatment relative to the untreated group. Resistance to reduction by groups treated with 200 mg/kg and 400 mg/kg bw of BIA 849 (with 20.2 % and 23.7 % change respectively) and 200 mg/kg bw of TD 570 (with 23.1 % change) were similar to the resistance shown by Chloroquine (22.8 % change). Higher resistance was observed in groups treated with 200 mg/kg bw of TD 570 (16.9 % change) and GB/T19598 (11.1 and 11.5 % change by 200 mg/kg and 400 mg/kg respectively).

All treated groups showed statistically significant increase in Hb concentration ($p<0.05$) after treatment with the highest increase found in group treated with 400 mg/kg body weight of tea GB/T1959 which increased from 9.83±2.02 to13.52±0.84 (37.4%) at day 7. The increases in the tea-treated groups were higher than that in Chloroquine-treated except in group E treated with 400 mg/kg bw of BIA 849 in which a non-significant difference from that seen in the positive control group was observed. Mean corpuscular volume reduced with infection relative to the uninfected group. Treatment however, ameliorated the effect of infection on MCV. The increase in MCV after treatment was dose dependent and comparable in BIA 849 and GB/T19598 treated groups with the group treated with the standard drug. However, the changes were not statistically significant ($p>0.05$). Highest increase (38.30±1.64 to 43.13±4.04) was observed in group administered with 400 mg/kg bw of tea BIA 849.

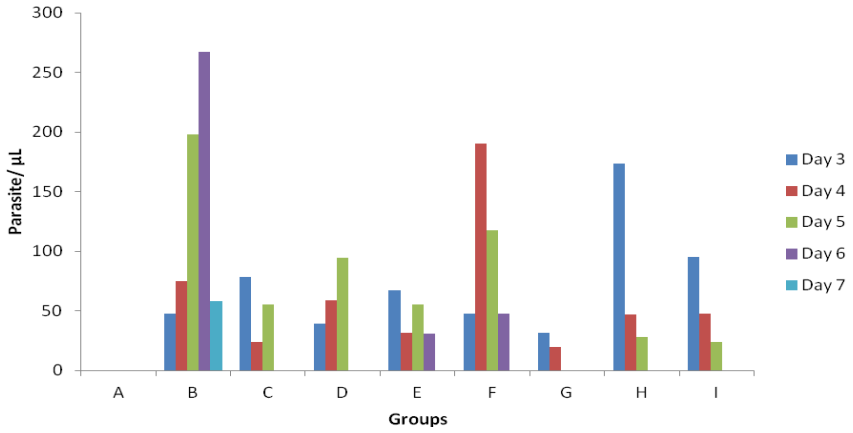

Figure 2: Parasitaemia count of ethanolic tea extracts against *Plasmodium berghei* using curative model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598

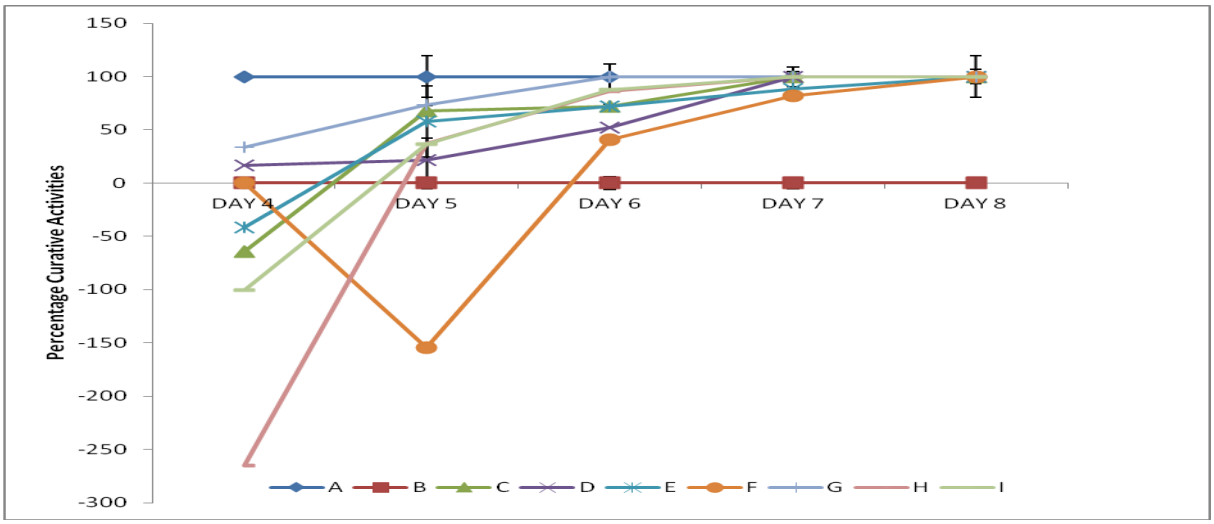

Figure 3: Curative effect of ethanolic tea extracts against *Plasmodium berghei.*

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598

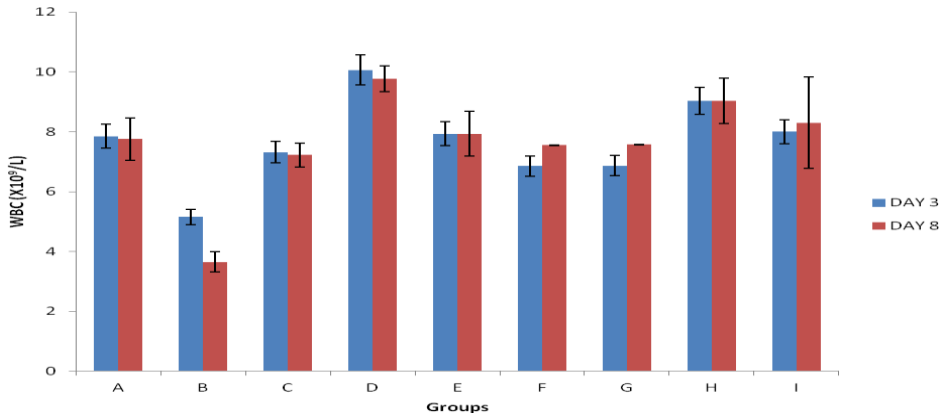

Figure 4: White blood cell count of *P. berghei*-infected mice treated with ethanolic tea extracts using the suppressive model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849
Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of chinese green tea GB/T19598; Values are mean ± S.D of triplicates; $^{b*}p<0.05$ indicates a significant difference between the tests and the controls

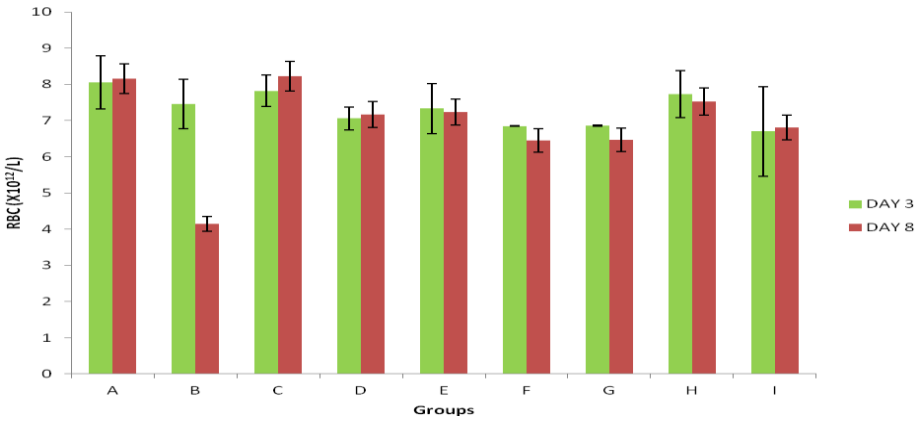

Figure 5: Red blood cell count of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicates; $^{b}p<0.05$ indicates a significant difference between the tests and the controls

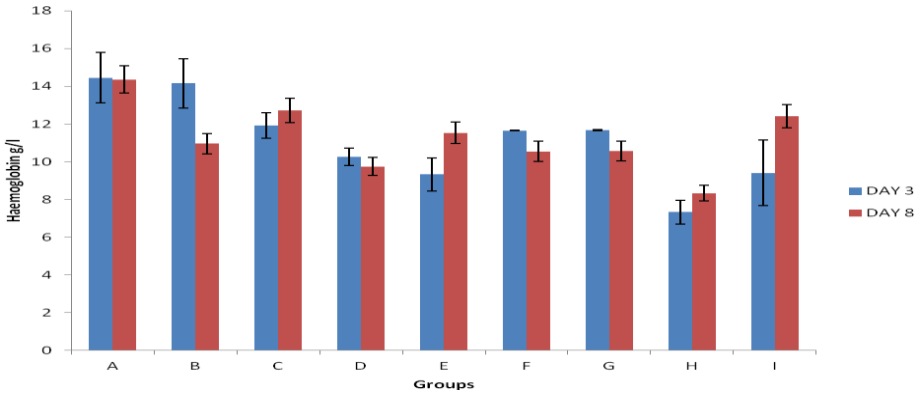

Figure 6**:** Haemoglobin concentration of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model.

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{b*}p<0.05$ indicates a significant difference between the tests and the control

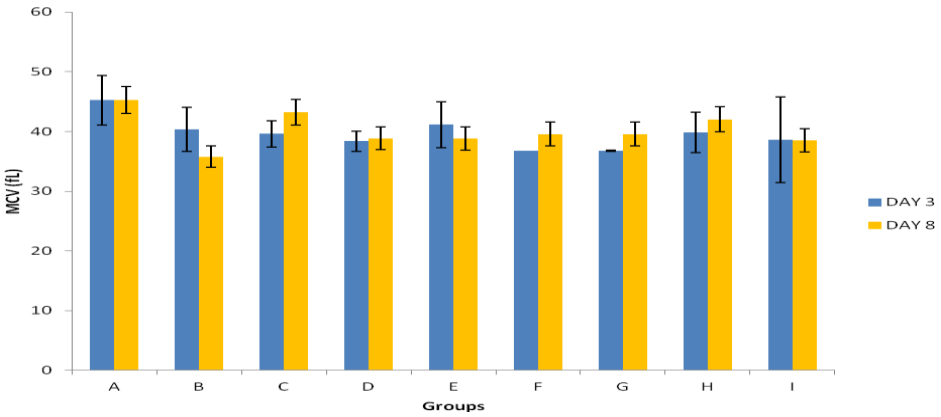

Figure 7: Mean corpuscular volume of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanol extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanol extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanol extract of chinese green tea TD 570; Group G: 400 mg/kg ethanol extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanol extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanol extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{*}p<0.05$ indicates a significant difference between the tests and the controls

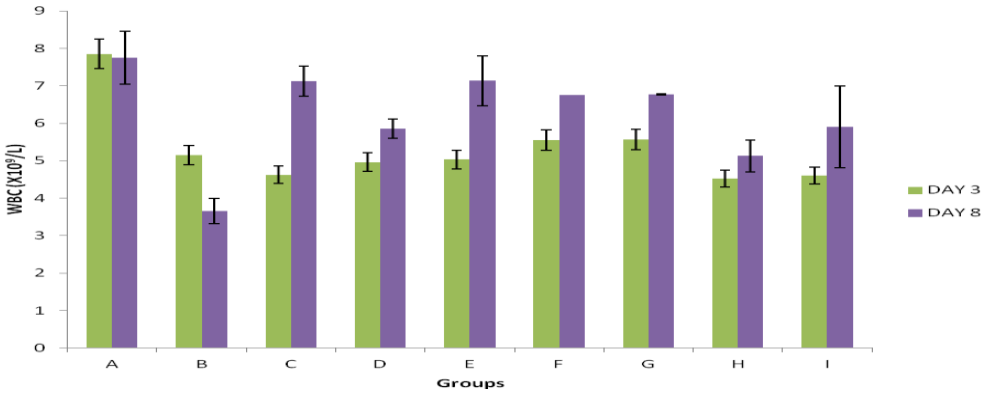

Figure 8: White blood cell count of *P. berghei*-infected mice treated with ethanolicteaextracts using the curative model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{b*}p<0.05$ indicates a significant difference between the tests and the controls

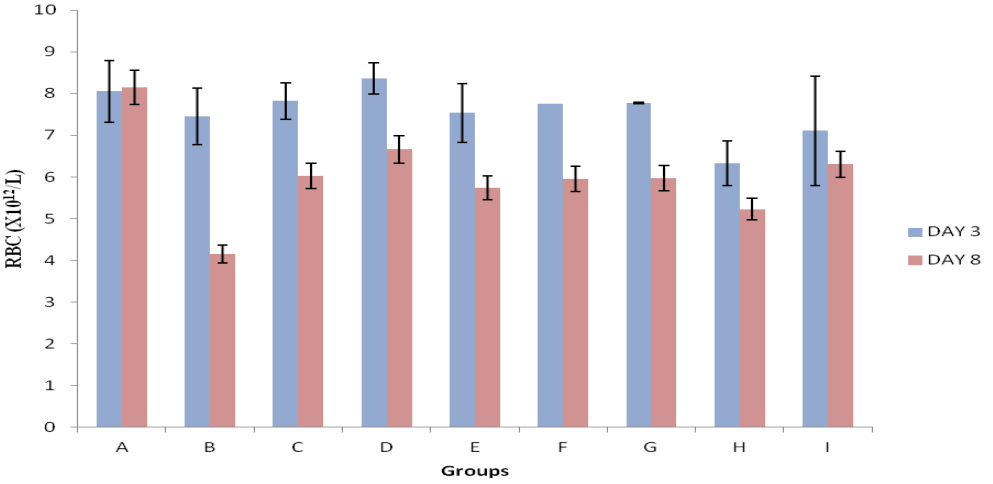

Figure 9: Red blood cell count of *P. berghei*-infected mice treated with ethanolic tea extracts using curative model**.**

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{b}p<0.05$ indicates a significant difference between the tests and the controls

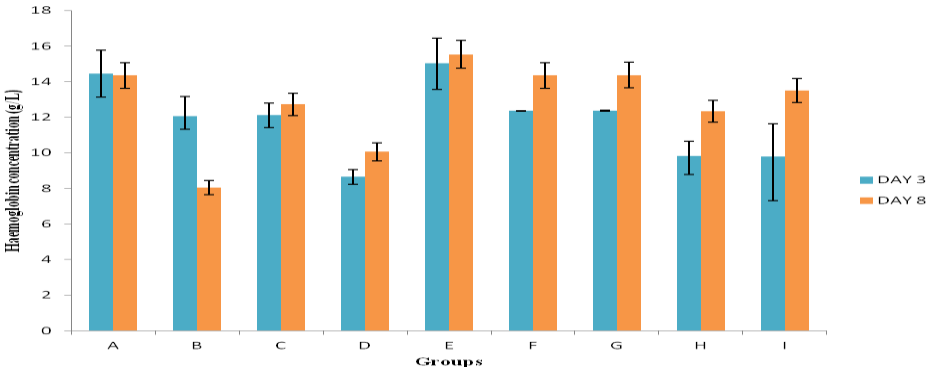

Figure 10: Haemoglobin concentration of *P. berghei*-infected mice treated with ethanolic tea extracts using curative model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{b}p<0.05$ indicates a significant difference between the tests and the controls

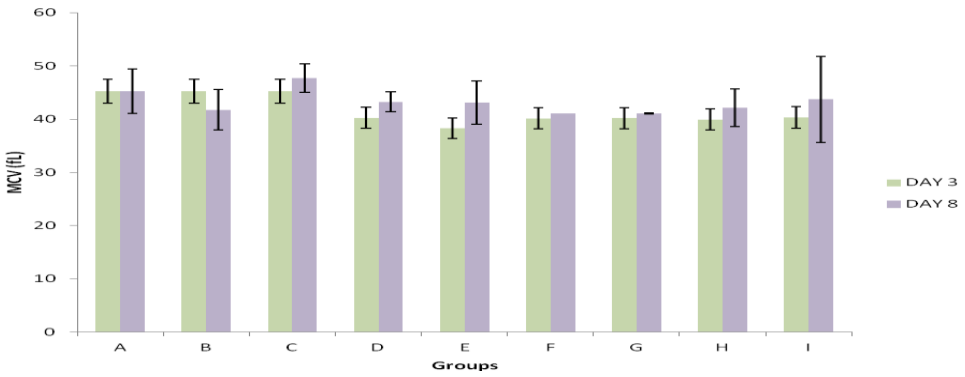

Figure 11: Mean corpuscular volume of *P. berghei*-infected mice treated with ethanolic tea extracts using curative model

Group A: Uninfected Control; Group B: Infected Negative control (5 ml/kg of distilled water); Group C: Positive control (5 mg/kg of chloroquine phosphate solution); Group D: 200 mg/kg ethanolic extract of Chinese green tea BIA 849; Group E: 400 mg/kg ethanolic extract of Chinese green tea BIA 849; Group F: 200 mg/kg ethanolic extract of Chinese green tea TD 570; Group G: 400 mg/kg ethanolic extract of Chinese green tea TD 570; Group H: 200 mg/kg ethanolic extract of Chinese green tea GB/T19598; Group I: 400 mg/kg ethanolic extract of Chinese green tea GB/T19598; Values are mean ± S.D of triplicate measurements; $^{b}p<0.05$ indicates a significant difference between the tests and the controls

4.0 Discussion

Results of the *in vivo* tests conducted in this study are consistent with the clinical efficacy of green tea in previous studies by Rath et al (2004) and Mueller *et al* (2004). We observed that crude ethanolic green tea extracts possess intrinsic antimalarial activity comparable to chloroquine. This is evident from the percentage chemo suppression and curative effects they exerted which favourably compared with the standard drug-Chloroquine. This suggests that the tea extracts contain bioactive constituents similar to chloroquine and perhaps other standard drugs employed in the treatment of malaria. It has been noted that synergistic interactions of compounds in tea such as flavonoids and saponins along with other active ingredients such as atermisin, quinine and quercetin which are known anti malarial bioactive constituents contained in Chinese tea might be responsible for the anti plasmodial actions observed in this study (Elford *et al*., 1987; Wilcox *et al*., 2004). Nafiu *et al* (2014) also observed the *in vivo* anti-malarial activities of aqueous extracts of *Tithonia diversifolia* and *Parquentina nigrescens* leaves in mice with the bioactive agents being saponins, alkaloids, flavonoids and cardiac glycosides in the two plants also implied to be present in our tested tea. Treatment of *Plasmodium berghei*- infected mice with green tea TD 570 and GB/T T19598 exerted significant ($p<0.05$) dose-dependent reduction in percentage parasitemia level, similar to the results of Ajaiyeoba *et al* (2006) in which the activity of methanolic extract of *Annona senegalensis* depended on the doses of the extract. These results demonstrate that the green tea (BIA 849, TD 570 and GB/T19598) strongly suppressed *P. berghei* growth *in vivo*. However, it can be deduced, that increasing the concentration of the extract above 200 mg/kg body weight produced no additional suppressive effect against malarial infection. Pre treatment of all groups increased the anti plasmodial activity of the tea extracts used possibly via a build up in the putative compounds in the animals prior to transfection, thus producing higher activity than when the animals were treated after transfection. Notably, it was reported that the major polyphenols in tea, C-3 gallic acid esters of catechins, namely ECG, EGCG, (-)-catechin gallate, and (-)-gallocatechin gallate, are potent inhibitors of three important enzymes (FabG, FabZ, and FabI) involved in the fatty acid biosynthesis of *P. falciparum* (Tasdemir *et al.,* 2006). Interference with fatty acid biosynthesis may therefore represent a primary mechanism by which the observed *in vivo* antimalarial effects can be explained. Another recent study has suggested that antimalarial effects *in vivo* might be through EGCG interference with cytoadherence processes (Dormeyer *et al.,* 2006).

The polyphenols present in this plant which have antioxidant effect may also contribute to the antimalarial activity due to inhibition of haem polymerization (Alexandru *et al.,* 2007; Taramelli *et al.,* 1999; Senanayake, 2013). WBC, RBC, HB and MCV concentrations were significantly reduced in the negative control. Malaria infection is usually characterised by destruction of blood cells by malaria parasites that depend on them for their metabolism hence haemolytic anaemia is one of the symptoms of severe malaria infection. However, the reversal in the reduction in the treated groups indicated high mortality or suppression of the activity of *P. berghei* due to the anti plasmodial activity of the bioactive constituents in the tea extracts.

Blood is a multi-component fluid with different cell types in suspension, circulating in virtually closed system of blood vessels to all other parts of the body. Haematological indices have been reported to be reliable for the assessment of health status of animals, and severity of changes in these parameters depends on the species of animals, physiological state of the host and acuteness or chronicity of infection (Jenkins and Facer, 1985; Obianime and Aprioku, 2011; Ohaeri and Eluwa, 2011; Saxena *et al.,* 2011). *P. berghei*-infected mice suffer from anaemia because of RBC destruction, either by parasite multiplication or by spleen reticulo-endotelial cell action, which involves the production of many phagocytes by the spleen as a result of the presence of many abnormal RBCs (Chinchilla *et al.,* 1998). Result from this study shows that the significant negative changes in the haematological profiles were evident as parasitaemia count increases as a result of the destructive actions of *P. berghei*

Haematological profiles were normalized in the groups of mice infected and treated with effective dosages of ethanolic extracts of Chinese green tea BIA849, TD 570 and GB/T19598 except for the MCV values which did not change significantly in the different groups of experimental mice, a typical feature of normocytic-normochromic anaemia (Menezes *et al.,* 2004).

The extracts prevented the reduction in WBC, RBC and Hb values, which are normally associated with malaria infection, in mice treated by the suppressive model and also caused an improvement in haematological indices lowered by already established infection. Anaemia is characterised by decreased values of RBC and Hb (Aleksandro *et al.,* 2009). In addition, a decline in WBC counts in the different experimental groups of infected and treated mice observed was similar to that observed by McKenzie *et al* (2005) and Taha *et al* (2007) who reported increase in WBC counts in treated malaria group. The reduced WBC count in infected group may be due to localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools rather than actual depletion as suggested by Ifeanyichukwu and Esan (2014). Ngotho *et al* (2011) had observed in *Typanosoma brucei rhodesiense* that the gradual decline of WBC counts are reflections of persistent underlying infections. Infection of mice with *P. berghei* caused weight loss in the mice relative to the uninfected group. It is known that malaria infection is usually characterised by weight loss due possibly loss of appetite by the individual suffering from malaria. The loss of body weight observed in the extract treated mice was possibly due to appetite suppressant effect or the lipolytic effect of the crude tea extracts. This is in agreement with that of a previous study on other plants (Chinchilla *et al.,* 1998). The result of the present study on body weight, however, contradicted the earlier observation by Dikasso *et al.* (2006). Based on the established findings, the use of these tea in folklore medicine is justified. However, there is a need to further isolate, purify, identify and characterise the specific bioactive constituents in these tea. Isolation of compounds such as quercetin, quinine and artemisin from the tea would imply that they could be explored as potential and novel sources of effective and affordable anti malarial bioactive ingredients that could be tapped for commercial therapeutic purposes. These results therefore might pave the way towards the development of green tea and constituent substances into effective antimalarial agents. Moreover interaction between EGCG or ECG constituents of tea leaves and artemisinin might be conveniently exploited to design new and/or more effective combination therapies.

In conclusion, the ethanolic extracts of Chinese green tea studied possess potent antiplasmodial and haematopoietic actions against severe malaria and associated anaemia. These tea brands on further screening may form a basis for development of anti malaria herbal medicines that are readily available in terms of cost and effectiveness. It is however recommended that further studies be carried out to establish the particular constituent of the tea responsible for the observed effects and the molecular interactions in the body leading to parasite clearance and amelioration of stress associated with anaemia during malaria infection.

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