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Evaluation of In-vivo Curative and Haematopoietic Effect of Three Chinese Teas in Plasmodium bergheiInfectedMice

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Abstract:*The emerging resistance of Plasmodium species to currently available antimalarial drugs remains a public health problem, hence the need for new effective, safe and affordable drugs. The curative and haematopoietic activities of 200 mg/kg and 400 mg/kg body weight of crude ethanolic extract of three commercial Chinese green teas (BIA 849, TD 570 and GB/T19598) were assessed utilizing Rane's curative antiplasmodial assay in mice infected with Plasmodium berghei (NK65 strain). The impact of the extracts on weight of the creatures were also determined. 200 mg/kg bwt of BIA 849 and GB/T19598 were as potent as 5 mg/kg bwt of chloroquine, with 100 % curative activities at 3 days post-treatment. TD570 at 200 mg/kg bwt was more powerful in stifling plasmodium, having 100 % curative activity at 4 days post treatment. 400 mg/kg body weight of TD570 and GB/T19598 extracts were stronger than chloroquine having 100 % curative activities at 2 and 3 days post treatment. Antiplasmodial treatment with the extracts altogether increased WBC count of the groups. Reduction in RBC count of infected mice was resisted significantly by treatments with respect to the untreated gathering, while Hb concentration increased significantly. Weight changes were most significant with 400 mg/kg bwt of GB/T19598 treated group (3.18 % decrease) on curation in respect to 0 % and 1.75 % decrease in positive control group respectively. All in all, the green teas displayed high antiplasmodial and haematopoietic possibilities and are hence prescribed as contender for additionally screening as elective antimalarial drugs.*

Keywords:*curative, haematopoietic, antiplasmodial, plasmodium berghei*

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I. Introduction

Malaria is an endemic irresistible infection that is across the board in tropical and sub-tropical zones of the world and one of the six most imperative parasitic malady of human [1]. It is a noteworthy general medical issue in sub-Saharan Africa, where more than 85-90% of all worldwide weight of Malaria exists with up to half of all outpatient visit in territories with high Malaria transmission and 30-half of all doctor's facility confirmation are credited to Malaria[2]. In spite of the fact that a compelling vaccine is the best long haul control for malaria, ebb and flow investigate on immunization improvement is still at pre-clinical stage. In this way, the management of malaria predominantly centres on antimalarial drugs equipped for decreasing or disposing of parasites [3].

The rise of safe strains requires the presentation of intense new medications or drug combinations against malaria. Ideally, new medications ought to have novel methods of activity or be synthetically not quite the same as the medications in current utilize [4].

Medicinal plants, an approved wellspring of new drugs, have been utilized in basically all societies at one time or the other. It remains a wellspring of sheltered and compelling medications [5]. As a rich hotspot for new medications, a few vital antimalarial pharmaceuticals being used today were gotten from them [6].

Both quinine and artemisinin, antimalaria medicates being used, have been gotten from customary prescription and plant extracts [7]. The proposal of artemisinin, a plant extract derived by the World Health Organization as a component of the principal line treatment of malaria, ACT, has supported the sustained hunt for new antimalarial medications [8]. Besides, a few investigations have been embraced to assess not just the inhibitory impacts of different plant extracts on P. falciparum [9] utilizing in vitro culture, yet additionally in vivo antimalarial properties on Plasmodium berghei-infected mice [10]. One of such plants which scientists have created enthusiasm for of later is the tea plant.

Tea (Camellia sinensis) was first found as a drink and pharmaceutical in China around 2737 B.C. From that point forward, tea has turned out to be so prevalent making it the second drink to water regarding overall utilization [11]. World tea creation in 2006 achieved a record of 3.64 million tons, of which China, India and

Kenya were the best three greatest delivering nations [12]. The world tea generation has been consistently expanding; of which dark tea creation has been anticipated to develop at 1.9 % every year to achieve 3.14 million tons by 2017, while, the green tea generation has been anticipated to develop at yearly rate of 3.8 % yearly to accomplish around 1.57 million tons for a similar period [12].

A few constituents of Tea (counting flavonoids, caffeine and theanine) have been explored and associated with the medical advantages, for example, aversion of growths and cardiovascular maladies, diminishment the dangers of obesity and diabetes, and change of resistant framework of the immune system [13]. A few the study of disease transmission thinks about have announced the relationship between tea utilization and medical advantages [14]. In spite of the gigantic research that has been directed on the plant, there is still exceptionally restricted writing on the antimalarial action of tea plant. The present examination made an endeavour to investigate the conceivable antiplasmodial and haematopoietic possibilities of three Chinese green teas BIA 849, TD 570 and GB/T19598.

II. Material and Methods

Plant Materials

Three Chinese green teas brands, specifically BIA 849, TD 570 and GB/T19598, were sourced from Guangzhuo, China.

Preparation of Crude Extracts

40 g each of the three brands of Chinese green teas namely, BIA 849, TD 570 and GB/T19598 tea was micronised and exhaustively extracted in 800 ml 80 % ethanol at boiling point for 120 min [15]. The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator (Stuart RE300). Green coloured pastes were obtained and weighed prior to further analysis.

Experimental Animals

Healthy Swiss albino mice (14-22 g) of either sex were utilized for the study. The mice were obtained from the Animal House, College of Medicine, University of Lagos, Idi-Araba, Lagos. They were permitted to adapt to the new condition for a time of two weeks before the investigation. The mice, kept up on standard rat feed and water ad libitum, were housed in polypropylene confines at room temperature all through the examination and were kept up under standard states of dampness, room temperature and 12 h light/12h darkness cycle.

Rodent Parasite Strain

The rat parasite Plasmodium berghei NK 65 utilized in this investigation was gotten from National Institute for Medical Research (NIMR) Lagos, Nigeria and kept at Animal House of the, College of Medicine, University of Lagos, Idi-Araba, Lagos. The strain of parasite was kept up by persistent intraperitoneal passaging of the parasite into uninfected mice for two weeks. The contaminated mice were acclimatized to their environment and utilized for the investigation. Preceding the initiation of the examination, one of the infected mice was kept and seen to repeat malady side effects like human disease.

Experimental Design

The curative activity and haematological effects of the extracts was tested using in vivo anti-plasmodial effect against early infection models in chloroquine-sensitive Plasmodium berghei NK65-infected mice [16]. One hundred and eighty (180) mice (weighing 14-22 g) were divided randomly into nine groups of twenty mice each (Table 1).

Table 1 Treatment protocol

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

Ten mice from each group were treated using the curative models of antiplasmodial screening.

Antiplasmodial Screening

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.

Curative (Rane) Test

The curative capability of every one of the extracts was resolved by a technique portrayed by Iyiola et al. (2011)[17]. Ninety mice were arbitrarily isolated into nine groups and regarded as in table 1. The mice were infused intraperitoneally with standard inoculums of $1x10^7$ Plasmodium berghei NK 65 infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were treated with the drugs and tea extracts. The treatment was done once every day for 5 days and blood smears were gathered and inspected infinitesimally at an amplification of x100 to screen the parasitaemia level. Parasitaemia was expressed as 'parasites per microlitre' of blood and curative ability was established.

Haematological Analysis

From the respective groups, four mice were sacrificed 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 in heparinized sample bottles and submitted for haematological examination.The haematological analysis was carried out using an automated haematological analyser (HMX complete blood count analyser, Japan). Red blood cell count (RBC), Packed cell volume (PCV), Haemoglobin concentration (Hb), Mean Corposcular Volume (MCV), White blood cell count (WBC) were determined.

Weight Change Determination

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 after treatment in all the extract treated and control groups using digital sensitive weighing balance (Wigger Hauser). The mean body weight was calculated according to the following mathematical equation:

$$\text{Mean body weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}}$$ [18]

Statistical Analysis

Results were expressed as mean ± standard deviation (Mean ± SD) 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 $p< 0.05$.

III. Results

Curative Effect of Ethanolic Tea Extracts Against Plasmodium Berghei.

Figure 2 shows that parasitaemia increased 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 decline post treatment. At baseline (day 3), no group's parasitaemia was significantly lower compared to untreated control group (B). At day 4, treatment groups showed significant 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 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.

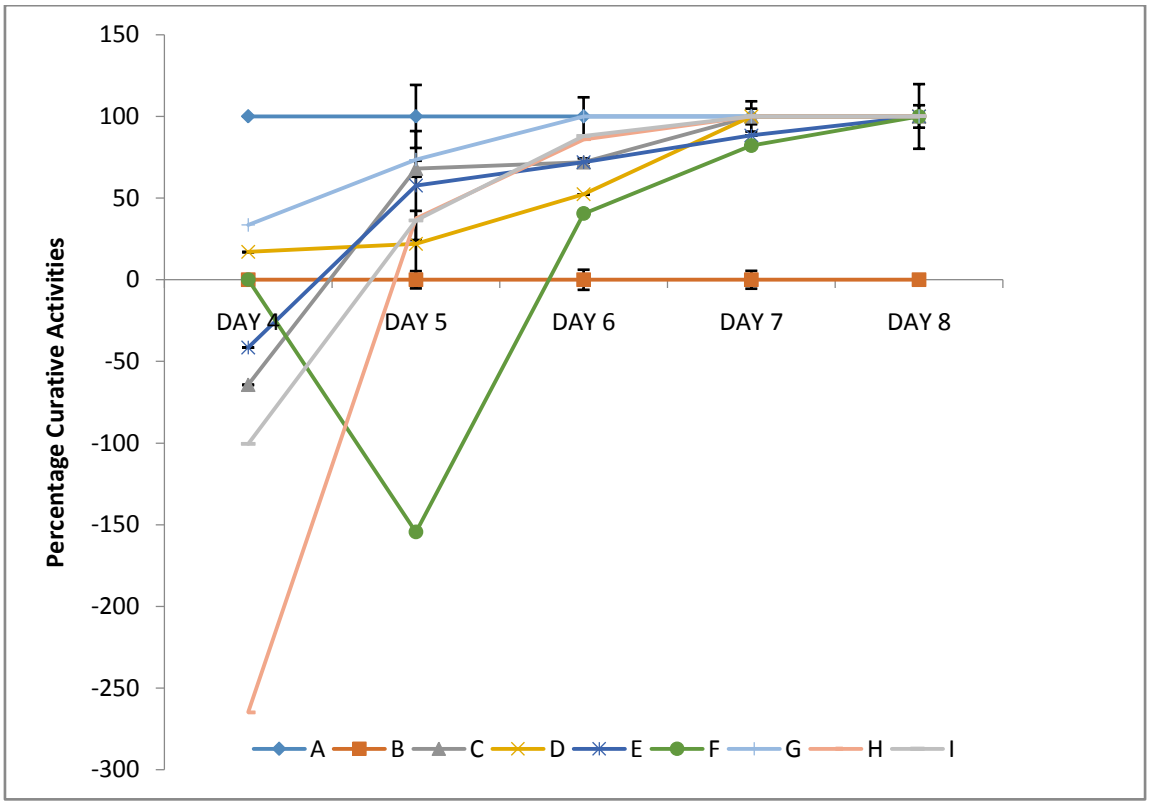

Figure 1: Curative effect of ethanolic tea extracts against plasmodium berghei.

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

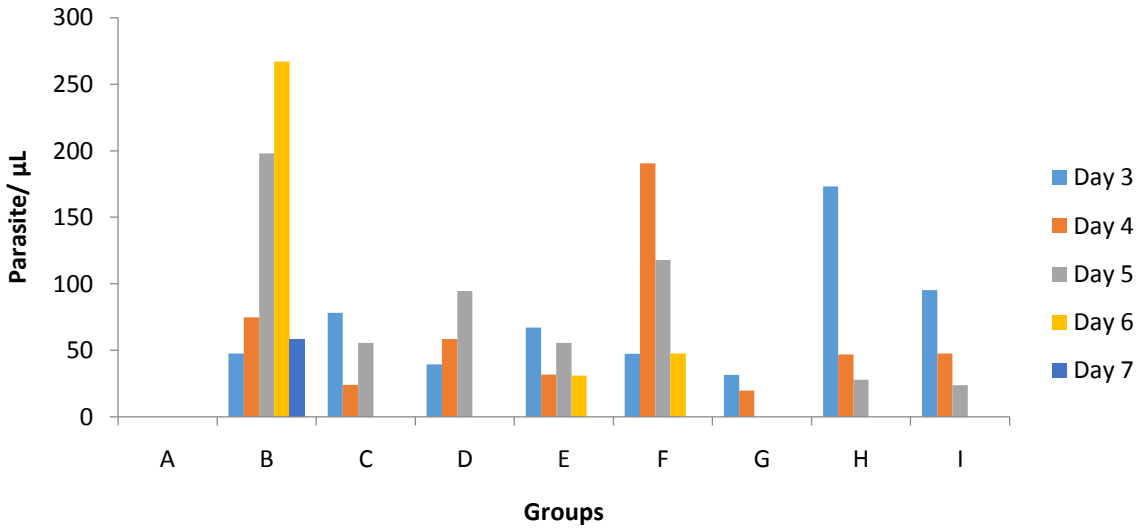

Figure 2:Parasitaemia count of ethanolic tea extracts against Plasmodium berghei using curative model.

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

Effect of malaria treatment using curative model on haematological parameters.

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 4.7, 4.8, 4.9 and 4.10 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 (41.7%) 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.

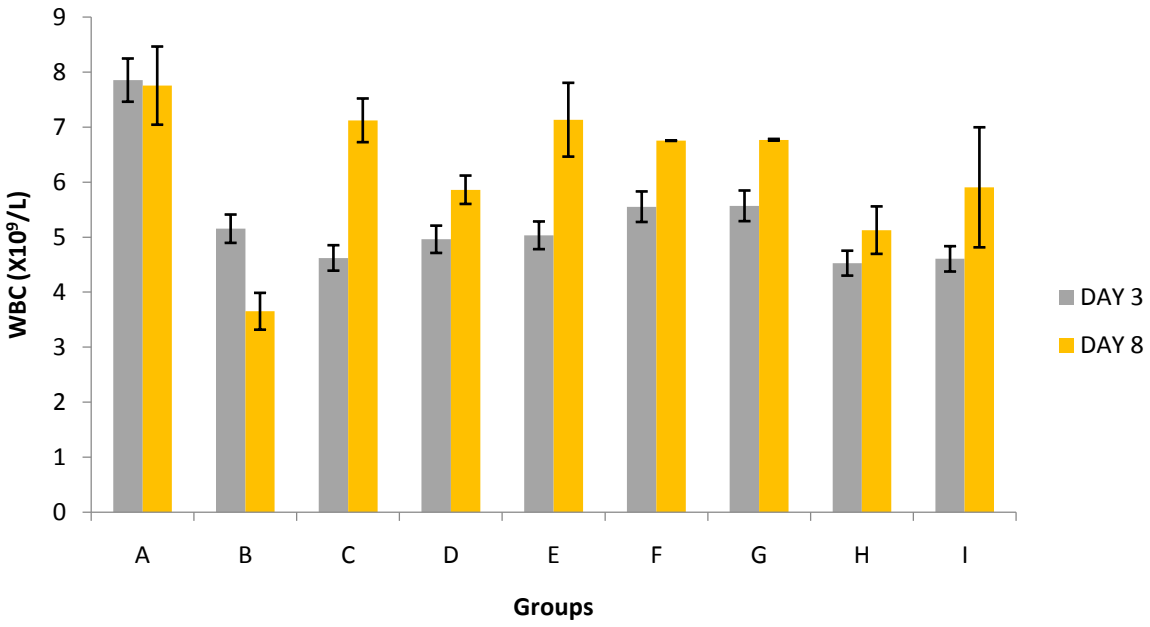

Figure 3 White blood cell count of P. berghei-infected mice treated with ethanolic tea extracts using the curative model.

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

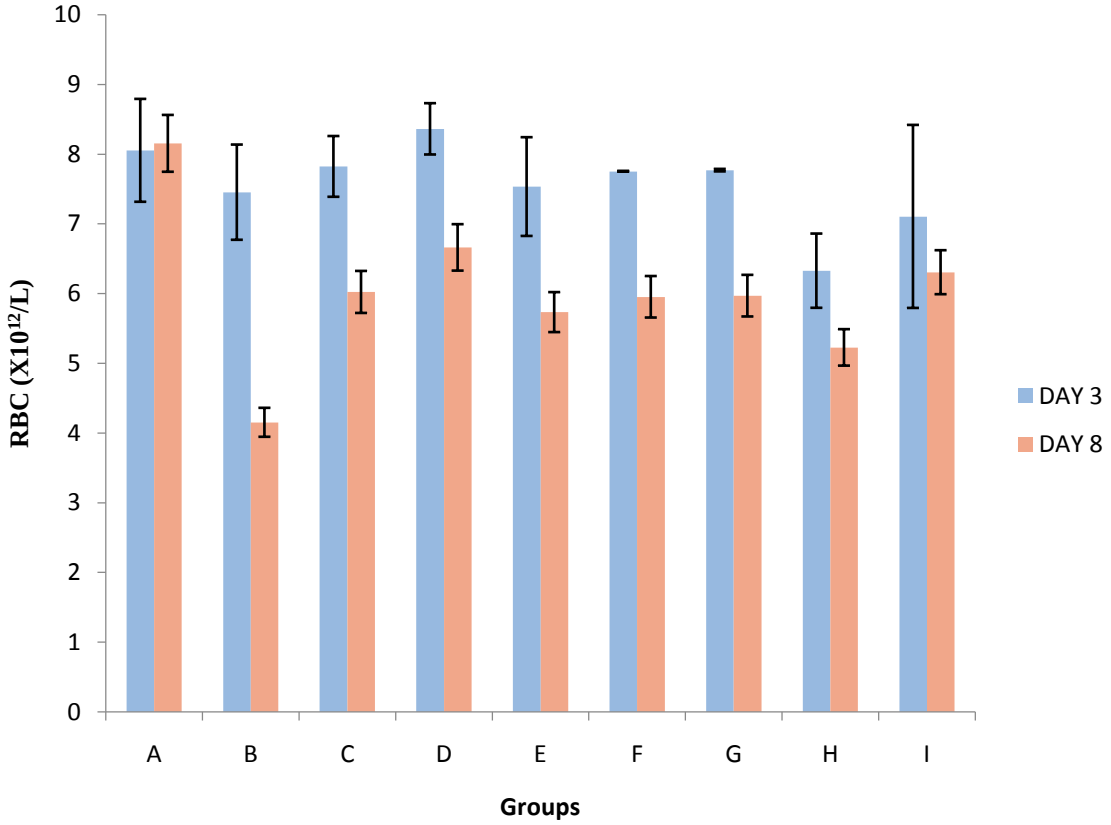

Figure 4 Red blood cell count of P. berghei-infected mice treated with ethanolic tea extracts using curative model.

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

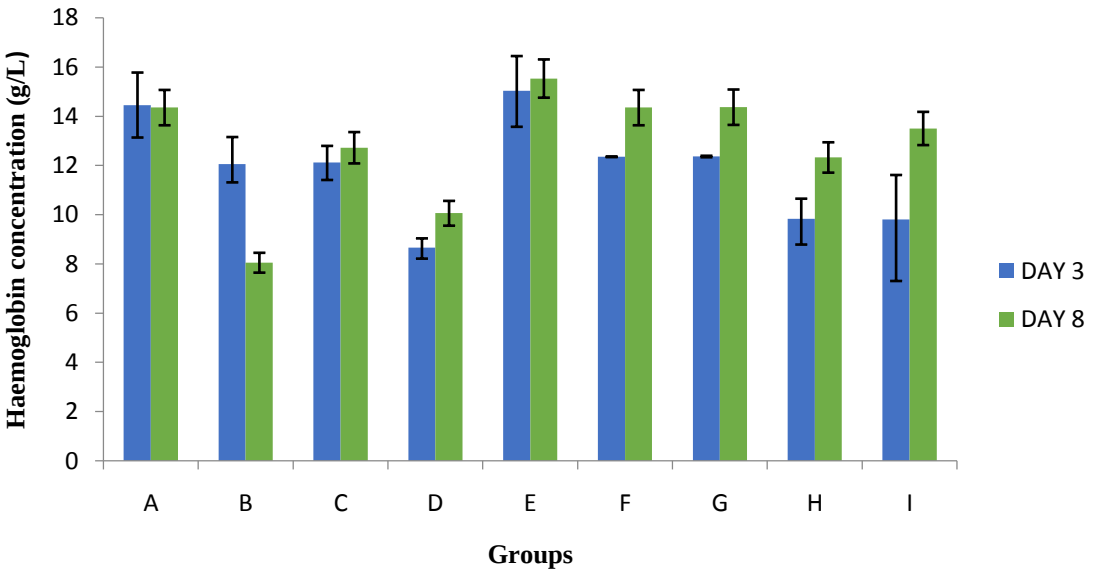

Figure 5Haemoglobin concentration of P. berghei-infected mice treated with ethanolic tea extracts using curative model.

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

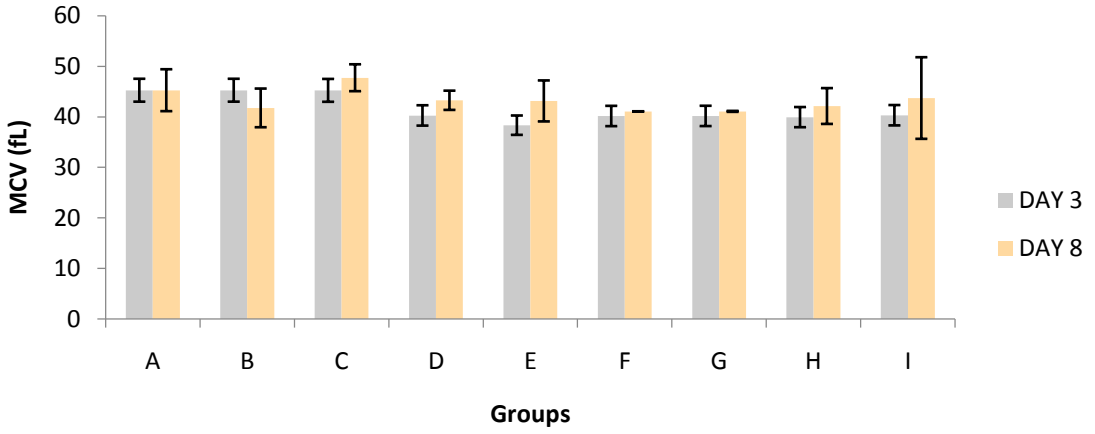

Figure 6 Mean corpuscular volume of P. berghei-infected mice treated with ethanolic tea extracts using curative model.

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

Effect of malaria treatment with ethanolic tea extracts using curative model on body weight.
Table 2 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±0.12 to 14.5±1.01) relative to the uninfected group in which an increase in mean body weight (15.2±0.08 to 16.0±0.22) 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 bod 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 ($p < 0.05$).

Table 2 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

V. Discussion

The in vivo antimalarial activities of the ethanolic crude extract of three Chinese teas were examined by assessing the curative properties utilizing standard animal model. In vivo models are typically utilized in antimalarial studies since they take perception of the conceivable pro-drug impact and plausible association of the safe framework in destruction of the pathogen[19].

Building up the antimalarial impacts of the crude extract of green tea in vivo speaks to an aftereffect of incredible intrigue and a novel undertaking. In fact, tea is a standout amongst the most prevalent drinks devoured around the world, boundless in the malaria endemic nations, shoddy, effortlessly open, safe and for all intents and purposes lacking of fundamental poisonous quality[20].

Crude ethanolic green tea extracts were seen to demonstrate characteristic antimalarial activities considering their healing impacts in examination with the standard drug, Chloroquine in Rane's curative test[17]. Treatment of Plasmodium berghei-infected mice with green teas TD 570 and GB/T T19598 indicated dosage dependent supression in examination with the untreated control gathering. The curative activities of the 200 mg/kg bw of GB/T19598 was practically identical to the impact of a 5 mg/kg bw of Chloroquine while treatment with 200 mg/kg of TD 570, and 400 mg/kg of TD 570 and GB/T19598 were more intense in stifling the parasite. This was like the outcome of Ajaiyeoba et al. (2006)[21] in which the activity of methanolic extract of Annona senegalensis relied upon the dose of the extract. The tea, BIA 849, anyway demonstrated malaria suppressive action yet not in a dose dependent way like outcomes gotten by Olorunniyi and Morenikeji (2014)[22] in their investigation of the antimalaria activity of aqeous leaf concentrate of Pyrenacantha staudtii.

These outcomes unequivocally exhibit that the three green teas, i.e. teas BIA 849, TD 570 and GB/T19598, firmly stifle P. berghei development in vivo. Be that as it may, it can be found that increasing the dose of the extract over 200 mg/kg body weight delivered no extra curative impact against malarial infection.

The curative data additionally demonstrated that parasite clearance was significantly more articulated on the seventh day. This might be ascribed to high concentration of the extract in the blood because of continued dosing and the extracts has gained entrance into the parasites and eliciting it effects.

Outstandingly, it was accounted for that the major polyphenols in tea, C-3 gallic acid esters of catechins, specifically ECG, EGCG, (-)- catechin gallate, and (-)- gallocatechin gallate, are powerful inhibitors of three imperative enzymes (FabG, FabZ, and FabI) associated with the fatty acid biosynthesis of P. falciparum[23]. Impedance with unsaturated fat biosynthesis may in this manner speak to an essential component by which the observed in vivo antimalarial impacts can be clarified. Another recent study has recommended that antimalarial impacts in vivo may be through EGCG impedance with cytoadherence forms[24].

The polyphenols present in this plant which have antioxidant effect may likewise add to the antimalarial action because of hindrance of haem polymerization[25-27].

Hematological indices have been accounted for to be solid for the appraisal of wellbeing status of animals, and seriousness of changes in these parameters relies upon the types of animals, physiological condition of the host and intensity or chronicity of contamination[28-31]. P. berghei-contaminated mice experience the ill effects of paleness in light of RBC devastation, either by parasite duplication or by spleen reticulo-endotelial cell activity, which includes the creation of numerous phagocytes by the spleen because of the nearness of numerous strange RBCs [32]. Result from this investigation demonstrates that the huge changes in the hematological profiles were obvious as parasitaemia bout increases.

Hematological profiles were standardized in the groups of mice infected and treated with compelling doses of ethanolic concentrates of Chinese green teas BIA849, TD 570 and GB/T19598 aside from the MCV values which did not vary significantly in the different groups of trial mice, an ordinary component of normocytic-normochromic pallor [33].

A decrease in WBC counts in the different experimental groupss of infected and treated mice was watched when contrasted with the trial control mice. This outcome is comparable to that seen by McKenzie et al. (2005) [34] and Taha et al. (2007)[35] who announced increment in WBC counts in treated malaria group. The decreased WBC check in infected group might be because of confinement of leukocytes from the fringe circulation and to the spleen and other minor pools as opposed to real exhaustion or statis as proposed by Ifeanyichukwu and Esan (2014)[36]. This perception is authenticated by a previous report about Typanosoma brucei rhodesiense expressing that the continuous decay of WBC counts are reflectors of tireless basic contaminations [37].

The loss of body weight saw in the extract treated mice was potentially because of hunger suppressant impact or the lipolytic impact of the unrefined tea extracts. This is in concurrence with that of a past report on different plants [32]. The aftereffect of the present examination on body weight, be that as it may, isn't in concurrence with that of Dikasso et al. (2006)[38].

These outcomes may prepare towards the improvement of green tea and constituent substances into compelling antimalarial operators.

VI. Conclusion

We deduced from our outcomes that the ethanolic extracts of the three Chinese green teas explored; BIA 849, TD 570 and GB/T19598, were found to curatively affect Plasmodium berghei development in Swiss albino mice. The tea extracts could be valuable contrasting options to antimalarial tranquilize or helpful in blend treatment, since they are significantly less expensive. The extracts of the teas additionally have haematopoietic impact against malarial anaemia and furthermore avoid weight reduction owing to the parasitic disease.

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