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Comparative assessment of Chemosuppressive activity of three Chinese teas on *Plasmodium berghei* infected mice

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ABSTRACT

Malaria, a hazardous infirmity caused by a parasitic malady of the red blood cells, is without question harming to the wellbeing. In the present investigation, the chemosuppresive and haematopoietic activities of 200 mg/kg and 400 mg/kg body weight of unrefined ethanolic concentrates of three Chinese green teas (BIA 849, TD 570 and GB/T19598) were assessed using the 4-day suppressive anti-plasmodial assay in mice *Plasmodium berghei* (NK65 strain) pre-infected mice. The effect of the extracts on weight of the animals was evaluated. It was observed that 200 mg/kg bw (body weight) of BIA 849 and GB/T19598 were as potent as 5 mg/kg bw of chloroquine, with percentage suppressions of 58.97 ± 5.04, 57.63 ± 5.62 and 57.50 ± 4.5, respectively. TD570 at 200 mg/kg bw was more effective in suppressing plasmodium. 400 mg/kg body weight of TD570 and GB/T19598 extracts were more potent than 5 mg/kg bw of chloroquine having 100 % chemosuppression. The chemosuppression of BIA 849 did not change altogether at 400 mg/kg bw. The haematological parameters, WBC, RBC and MCV did not significantly change in the groups treated with the tea extracts utilizing suppressive model of malaria treatment contrasted with the uninfected group and were comparable to those treated with chloroquine. Haemoglobin concentration nonetheless, varied significantly with respect to the uninfected group. Weight changes were most significant with 200 mg/kg bw of TD 570 treated group (32 % increase) on suppression. All in all, the green teas displayed high chemosuppressive and haematopoietic possibilities and are thusly prescribed as contender for additionally screening as elective antimalarial drugs.

Keywords: Antiplasmodial, Chemosuppression, Haematopoietic, Chloroquine.

INTRODUCTION

Malaria, a perilous ailment caused by a parasitic disease of the red blood cells, is without a doubt the absolute most damaging and hazardous irresistible specialist in the creating scene, transcendently tropical and subtropical areas, including parts of America, Asia and Africa [1]. Consistently 300 to 500 million new cases are breaking down and around 1.5 million people kick the bucket of the ailment [2]. The re-rising of jungle fever in numerous parts of the world is because of the fast increment of protection from the vast majority of the accessible hostile to malarial medications, and in addition resistance of vectors to insecticides [3]. Pharmaceutical safe strains of Plasmodium have been found in various endemic zones of the world and an extensive parcel of conventional anti-plasmodial medicines have been connected with treatment disillusionment. Advancement of drug resistant Plasmodium strains, trouble of making proficient immunizations and furthermore unfriendly reactions of the anti-malarial medications feature the critical requirement for novel, all around endured and compelling against malarial medications for both prophylaxis and treatment of malaria [4].

Plants have been an awesome wellspring of pharmaceutical valuable in the treatment of different infections including jungle fever [5]. Both quinine and artemisinin, antimalaria medicates being used, have been gotten from customary pharmaceutical and plant isolates [6]. The suggestion of artemisinin, a plant separate subordinate by the World Health Organization as a part of the main line treatment of malaria, ACT, has energized the proceeded with look for new characteristic item determined against malarial medications [7]. In addition, a couple of examinations have been grasped to survey not only the inhibitory effects of various plant extracts on *P. falciparum* [8] using as a piece of vitro culture, yet furthermore in vivo against malarial properties on Plasmodium berghei-infected mice [9]. One of such plants which analysts have created enthusiasm for of later is the tea plant.

Tea (*Camellia sinensis*) firstly was discovered as a refreshment and pharmaceutical around 2737 B.C in China. From that point forward, tea has turned out to be so prevalent making it the second refreshment to water regarding overall utilization [10]. Production of Tea in the world in 2006 accomplished a record of 3.64 million tons, of which Kenya, China and India were the best three biggest conveying countries. Tea making in the world has been constantly expanding; of which dark tea generation has been foreseen to

create at 1.9 % consistently to accomplish 3.14 million tons by 2017, while, the green tea age has been foreseen to create at yearly rate of 3.8 % consistently to achieve around 1.57 million tons for a comparative period [11].

Tea constituents including flavonoids, caffeine and theanine have been explored and connected to the medical advantages, for example, avoidance of malignancies and cardiovascular ailments, lessening the dangers of weight and diabetes, and change of insusceptible framework [12,13]. A few the study of disease transmission ponders have detailed the relationship between tea utilization and medical advantages [14]. Notwithstanding the tremendous research that has been directed on the plant, there is still exceptionally constrained writing on the antimalarial action of tea plant.

MATERIALS AND METHODS

Plant materials

Three brands of Chinese green teas, namely 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 extracted exhaustively in 800 ml 80 % ethanol at boiling point for 120 min [15]. The marc was separated with muslin material and dissolvable evacuated under diminished pressure in a rotating vacuum evaporator (Stuart RE300). Green shaded extracts were gotten and weighed before examination.

Experimental mice

Healthy Swiss albino mice (14-22 g) of either sex were utilized for the examination. The mice were acquired from the Animal House of the College of Medicine, University of Lagos, Idi-Araba, Lagos. They were permitted to adapt to the new condition for a time of two weeks preceding the investigation. The mice, kept up on standard rat feed and water not indispensable, 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 obscurity cycle.

Rodent parasite strain

The rodent parasite *Plasmodium berghei* NK 65 utilized as a part of this examination was acquired 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 maintained by continuous intraperitoneal passaging of the parasite into uninfected mice for two weeks. The infected mice were acclimatized to the environment and utilized for the examination. Preceding the initiation of the investigation, one of the tainted mice was kept and seen to duplicate ailment side effects like human contamination.

Experimental design

The chemosuppressive activity and haematological effects of the extracts was tested using *in vivo* anti-plasmodial effect against established 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 suppressive models of antiplasmodial screening.

Antiplasmodial screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was important to reject the likelihood of mice harbouring rodent species of plasmodium.

Chemosuppresive test

Assessment of suppressive capability of the extracts was carried out utilizing Knight and Peters 4-day suppressive test against mice infected with *P. berghei* [17,18]. Parasitized red blood cells were gotten from a donor contaminated mouse via cardiovascular cut with a sterile needle and syringe [19]. Ninety mice were selected and eighty of them inoculated intraperitoneally with infected blood suspension (0.2ml) containing 1 x 10^7 infected erythrocytes. The mice were treated as shown in Table 3.1. 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 4 post-treatment), blood was obtained from the tail of each mouse and spread onto a slide to make thick and thin films [20]. The blood films were air-dried, fixed with methanol, air-dried once more, coloured 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:

$$\frac{\text{Number of parasites counted x 8000}}{\text{Number of leukocytes}} = \text{parasites per microlitre} \quad [21].$$

The percentage suppression of parasitaemia was interpreted as mean chemosuppression and this was figured for each dosage level by looking at the mean parasitaemia in infected untreated (negative) control with those of treated mice. The difference between the mean estimation of the control (taken as 100%) and those of the experimental groups were computed and expressed as percent decrease or activity utilizing the accompanying equation:

$$\text{Activity} = 100 - \frac{\text{Mean parasitaemia treated}}{\text{Mean parasitaemia (-ve) control}} \times 100\ \% \quad [22].$$

Haematology

From the groups, four mice were relinquished from every one of the groups on days 4 and 8 post contamination, and days 3 and 8 post infection for suppressive. Samples of blood were gathered in heparinized anticoagulant bottles and subjected to hematological examination.

The hematological examination was done utilizing a hematological analyser (HMX complete blood count analyser, Japan). Red blood cell count (RBC), Packed cell volume (PCV), Hemoglobin concentration (Hb), Mean Corposcular Volume (MCV), White platelet tally (WBC) were resolved.

Weight change determination

To evaluate the viability of the extract in anticipating body weight loss 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:

$$\text{Mean body weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}} \quad [23].$$

Statistical analysis

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

RESULTS

Chemosuppressive effects of ethanolic tea extracts against *P. berghei*

Figure 1 shows the chemosuppressive effect of the ethanolic extracts of three different commercial Chinese green teas on *P. berghei*. From the result, administration of standard drug, chloroquine and the teas extracts caused reduction in the parasite activities compared to the untreated group. Suppressive effects of the 200 mg/kg bw doses of teas BIA 849 and GB/T19598 (59 and 57.6 % respectively) were comparable to that of the standard drug, chloroquine (57.5 %) while TD 570 showed a much higher suppressive effect (88.9 %). 400 mg/kg bodyweight of Chinese green tea TD 570 and GB/T19598 showed total suppression (100 %) against *Plasmodium berghei* while 400 mg/kg bw of tea BIA 849 did not show any significant variation from the 200 mg/k bw. The chemosuppressive effects were statistically significant at $p<0.05$.

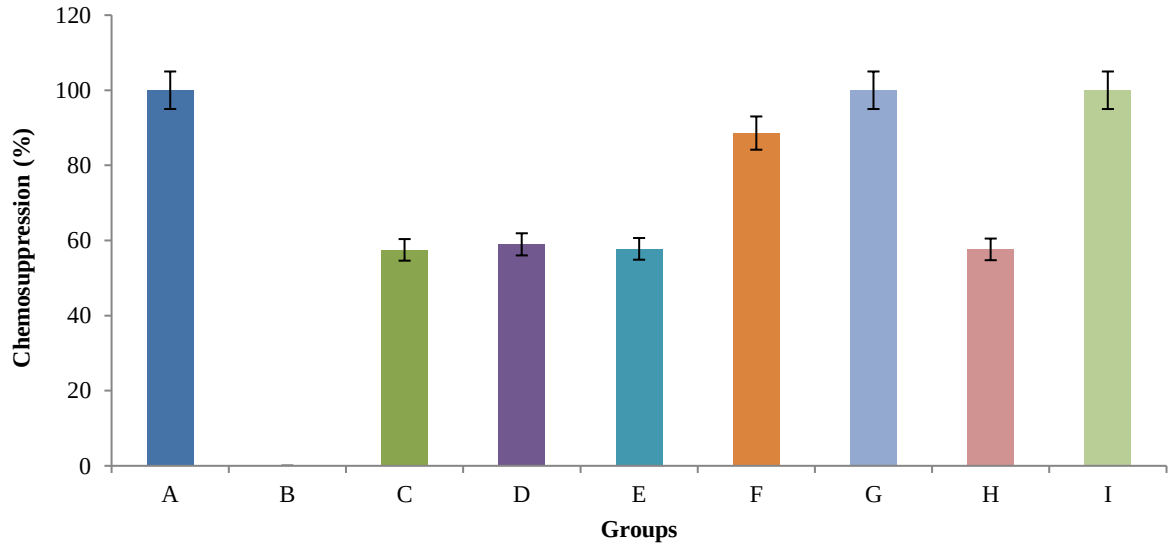

Figure 1: Chemosuppressive effect of ethanolic tea extracts against *Plasmodium berghei*

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

Effect of malaria treatment using suppressive model on haematological parameters

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 2, 3, 4 and 5 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 reduction was negligible.

WBC count was highest in group D treated with 200 mg/kg bw of tea BIA 849 having 10.06±1.0 and 9.74±1.1 $x10^9$/L on days 4 and 8 respectively (Fig. 2). WBC did not change significantly in treated groups from day 4 to day 8. Highest change with treatment (6.85±0.01 to 7.5±0.4 (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±0.12 to 3.66±0.22 $x10^9$/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 count red blood cells of treated groups did not differ essentially from that of the uninfected group and from the chloroquine-treated group. Untreated group however showed significant reduction in RBC from day 4 to day 8 (7.46±0.98 to 4.18±0.04).

Haemoglobin concentrations in all groups were significantly different from that of the uninfected group. The untreated group showed the highest reduction in Hb concentration from day 4 to day 8 (14.18±1.08 to 10.99±0.73). Greatest increase in group Hb concentration from day 4 to 8 (9.40±2.30 to 12.51±0.78) 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±2.3 to 38.85±1.6. The increases observed in treated groups were lower compared to that seen in the Chloroquine treated group.

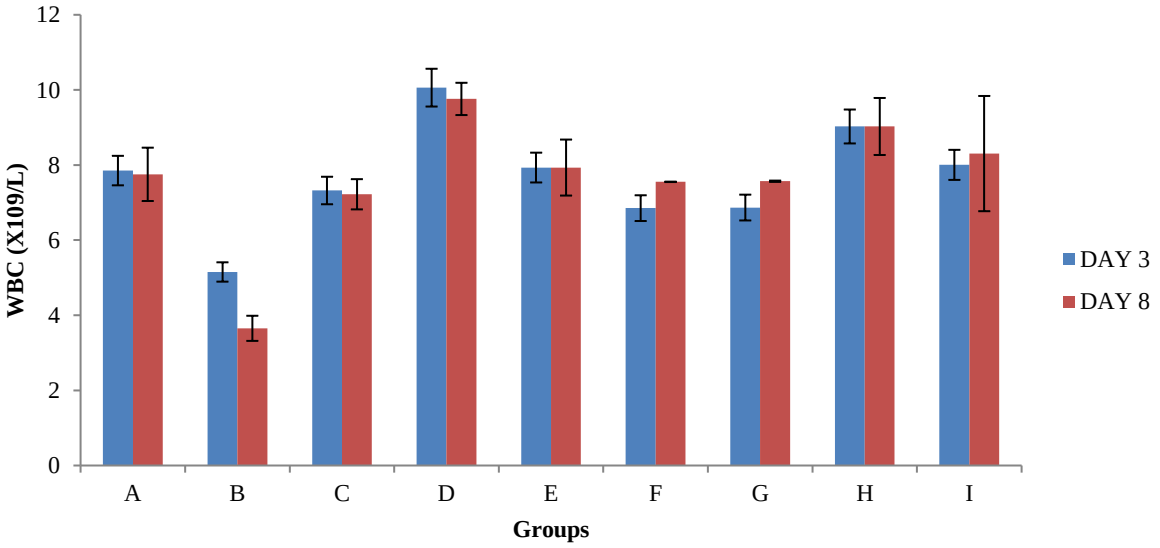

Figure 2: White blood cell count of *P. berghei*-infected mice treated with ethanolic tea extracts using the suppressive model

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

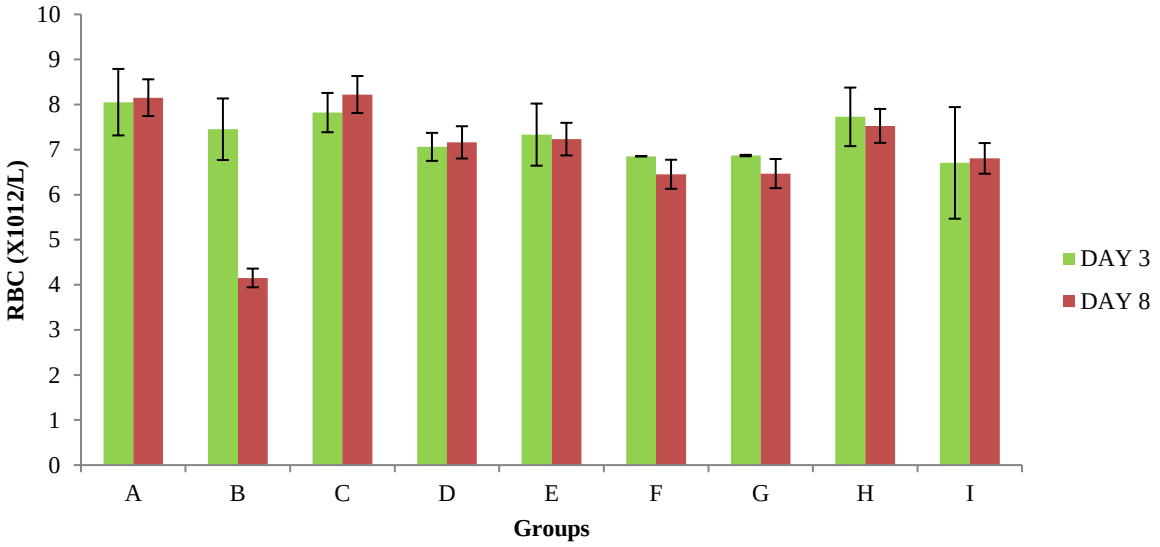

Figure 3: Red blood cell count of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model.

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

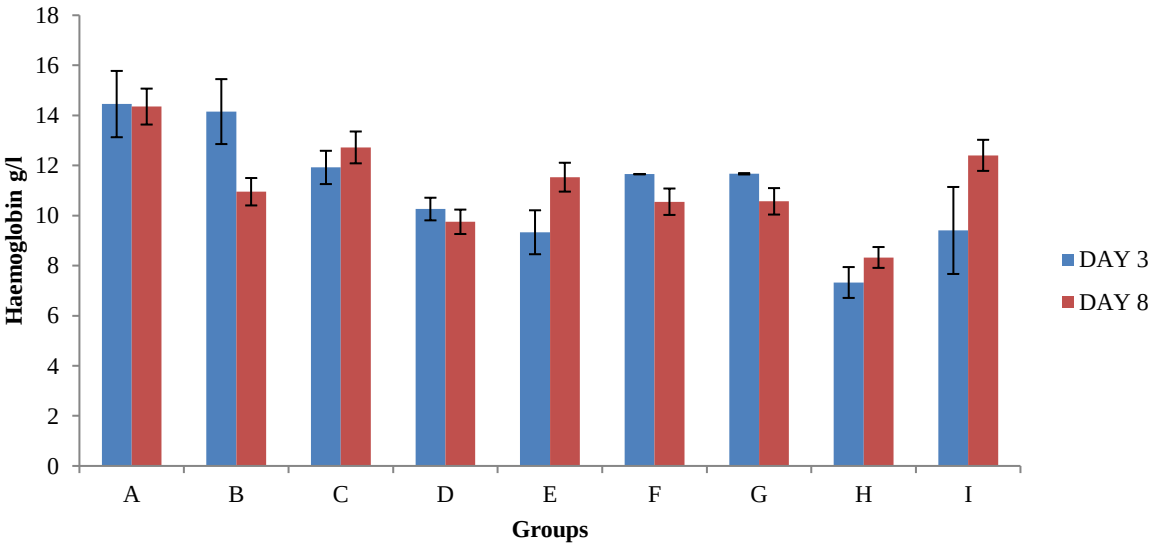

Figure 4: Haemoglobin concentration of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model

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

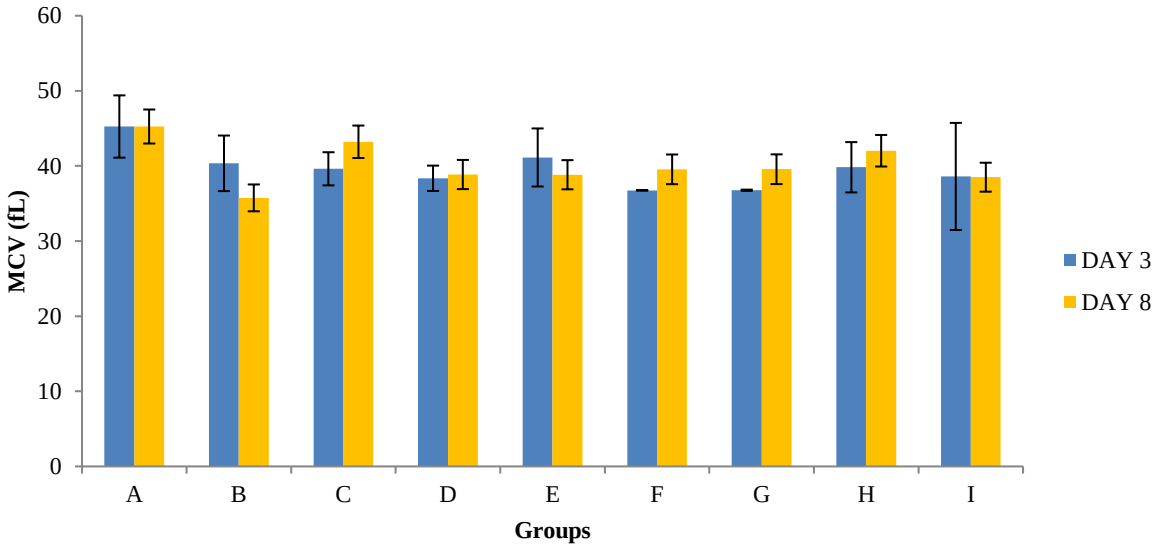

Figure 5: Mean corpuscular volume of *P. berghei*-infected mice treated with ethanolic tea extracts using suppressive model

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 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 suppressive model on body weight.

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±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 400 mg/kg of BIA 849 and TD 570 showed no significant loss in weight comparable to that shown by group treated with Chloroquine. Highest increase in body weight was shown b the group treated with 200 mg/kg 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 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 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

DISCUSSION

The high commonness of malaria in Africa and the regularly expanding drug resistance combined with the trouble of making proficient vaccines and unfavourable side-effects of the current anti-malarial medications feature the pressing requirement for novel, all around endured and financially savvy antimalarial drug.

Building up the antimalarial impacts of the crude extracts of green tea in vivo represent a consequence of extraordinary intrigue and a novel undertaking. In reality, tea is a standout amongst the most mainstream refreshments consumed around the world, across the board in the malaria endemic nations, shabby, effortlessly open, safe and for all intents and purposes lacking systemic toxicity [24].

Crude ethanolic green tea extracts were seen to indicate intrinsic antiplasmodial activity considering their rate chemosuppression in correlation with the standard medication, Chloroquine in 4-day suppressive test [17,18]. Treatment of Plasmodium berghei-contaminated mice with green teas TD 570 and GB/T T19598 shown dose-dependent chemosupression in correlation with the untreated control group. The suppressive action 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 potent in smothering malaria. This was similar to the outcomes of Ajaiyeoba *et al*. (2006) [25] where the action of methanolic extract of Annona senegalensis relied upon the doses of the extracts. The tea, BIA 849, indicated malaria chemosuppressive activity however not in a dose-dependent way like outcomes acquired by Olorunniyi and Morenikeji (2014) [22] in their investigation of the antimalaria action of aqeous leaf extract of *Pyrenacantha staudtii*.

These outcomes unequivocally show that the three green teas, i.e. teas BIA 849, TD 570 and GB/T19598, unequivocally smother *P. berghei* development *in vivo*. In any case, it can be contemplated that increasing the dose of the extract more than 200 mg/kg body weight conveyed no additional suppressive effect against malarial contamination.

Prominently, it was represented that the major polyphenols in tea, C-3 gallic destructive esters of catechins, particularly (-)- catechin gallate, and (-)- gallocatechin gallate, ECG, EGCG, are extraordinary inhibitors of three essential proteins (FabG, FabZ, and FabI) involved in biosynthesis of fatty acid of *P. falciparum* [26]. Impedance with biosynthesis of fatty acid may hence speak to an essential instrument in which the *in vivo* antimalarial impacts can be cleared up.

The polyphenols present in this plant which have antioxidant effect may likewise add to the antimalarial action because of hindrance of haem polymerization [27-29].

Hematological parameters have been accounted for to be solid for the assessment of health status status of animals, and seriousness of changes in these parameters relies upon the species of animals, physiological condition of the host and acuteness or chronicity of disease [30-33]. Result from this investigation demonstrates that the critical changes in the hematological profiles were apparent as parasitaemia session increments.

Hematological profiles were standardized in the groups of mice infected and treated with compelling dosages of ethanolic extracts of Chinese green teas BIA849, TD 570 and GB/T19598 aside from the MCV esteems which did not change essentially in the different groups of experimental mice, a typical component of normocytic-normochromic iron deficiency [34].

The extracts prevented the decline in WBC, RBC and Hb values, regular of the malaria infection, in mice treated by the suppressive model and furthermore caused a change in hematological indices brought down by officially settled infection. This perception is bolstered by a report expressing that iron deficiency is portrayed by diminished estimations of RBC and Hb [35]. What's more, a decrease in WBC tallies in the distinctive experimental groups of infected and treated mice was observed when contrasted with the test control mice. This outcome is like that observed by McKenzie et al. (2005) [36] and Taha et al. (2007) [37] who revealed increment in WBC tallies in treated malaria group. The reduced WBC count in infected group might be because of limitation of leukocytes from the fringe circulation and to the spleen and other negligible pools as opposed to real exhaustion or status as recommended by Ifeanyichukwu and Esan (2014) [38]. This observation is corroborated by a previous report on Typanosoma brucei rhodesiense expressing that the steady decrease of WBC tallies are reflectors of fundamental infections [39].

The weight loss seen in the extract treated mice was conceivably because of hunger suppressant impact or the lipolytic impact of the crude tea extracts. This is in concurrence with a past report on different plants [40]. The after-effect of the present report on body weight, in any case, isn't in concurrence with that of Dikasso *et al*. (2006) [41].

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

CONCLUSION

The ethanolic extracts of the three Chinese green teas investigated; BIA 849, TD 570 and GB/T19598, were able to suppress malaria due to *Plasmodium berghei* growth in Swiss albino mice and also have haematopietic effect against malarial anaemia and also prevent weight loss attributable to the parasitic infection. The use of the commercially available Chinese teas to suppress and cure malaria-associated symptoms may form a basis for development of herbal medicines that can be readily available to people without prompt access to modern anti-malarial agents, or those who choose not to use it.

Conflicts of Interest

Nil.

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