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EFFECTS OF KENYAN TEA ON INFLAMMATION: AN ANIMAL MODEL STUDY

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ABSTRACT

Emerging scientific data from pharmacological and physiological studies continue to show that tea has beneficial effects on human health. A number of *in vitro* studies have shown that tea helps the immune response by acting as anti-allergic, anti-viral and anti-bacterial agents. *In vivo* study was carried out to determine the effect of tea extracts on an animal model of male Swiss albino mice infected with *Trypanosoma brucei brucei* isolate KETRI 2710. The isolate produced a similar clinical picture after a pre-patent period of 5 days post infection (DPI). The levels of parasitemia in the control infected mice and those given different teas developed exponentially at similar rates reaching similar densities at the peak of parasitemia on 7 DPI. However, the decline on 9 to 13 DPI was significantly ($P<0.05$) different with that of treated mice decreasing more rapidly. This demonstrated that tea lowered parasitemia level. A fall in erythrocyte packed cell volume (PCV) occurred within 4 DPI due to the hemolysis of erythrocytes and consequent anaemia by the trypanosomes and this remained below the normal levels until the terminal stages of the disease. A significant difference ($P<0.05$) was observed on 11 DPI between the infected mice given tea and the infected untreated mice indicating that tea enhanced resistance to erythrocyte hemolysis signifying it could have a therapeutic role in cases of anaemia. The effect of tea on acute phase response and chronic inflammation was observed because tea produced a significant ($P<0.01$) elevation of parasite-induced hypoalbuminemia as compared to the infected untreated mice. Black tea, which is the principle tea product from Kenya, displayed remarkable properties some even comparable to those of green tea. Interestingly, tea used in this study was more efficacious than dexamethasone which is an anti-inflammatory drug thereby demonstrating its potential as a therapeutic agent.

INTRODUCTION

Kenya is an important producer of black tea. Despite this, the share of tea consumed locally has stagnated. This calls for urgent interventions to diversify black tea markets and more so, create a strong local demand in order to build the potential of increasing local consumption. Data to support the view that tea is pharmacologically active has been generated particularly using green tea, which is widely consumed in Asia [1, 2]. However, there is a dire paucity of information on the potential health benefits of black tea, which is the principle type of tea product consumed in Kenya and the rest of the world. Therefore, there is need to promptly initiate research on black tea to establish its beneficial effects on human health.

To investigate the potential health benefits of black tea *in-vivo*, a well established mouse model infected with *Trypanosoma brucei brucei* which is a tissue invasive parasite was used. The parasite causes a severe inflammatory response, extensive tissue damage and untimely death when left untreated [3] During inflammation, pro-inflammatory cytokines are activated leading to the release of acute phase proteins (APPs) which are

recognized markers of inflammation [4, 5]. A sustained inflammatory response in critical illness may also lead to a prolonged inhibition of synthesis of negative APPs such as albumin. The decline of albumin therefore could be used as a prognostic marker of inflammation [6].

In the present study, mice infected with *Trypanasoma brucei brucei* were given various Kenyan tea extracts with the objective of determining whether tea could down-regulate inflammation or the effects of murine trypanosomosis. Serum albumin levels were used as a marker of inflammation. Anaemia as measured by PCV was used as an indicator of disease severity and parasitemia levels were determined to ascertain whether tea had any anti-parasitic effect.

MATERIALS AND METHODS

Animals

Male Swiss albino mice 6-8 weeks old and weighing between 24-30 g were used. Animal care protocols and procedures used in the current study were reviewed and approved by the institutional animal care and use committee.

Consumption of tea extracts in water

Initially, it was tested whether the Swiss albino mice would voluntarily drink water supplemented with 10 g/L sucrose and various concentrations of green tea extract (GrTE) (0 – 20 g/L). The mice were acclimatized for 2 weeks during which each mouse was treated once using 0.1 ml of 1% Ivermectin to exclude any helminthes infestation. The animals were then randomly allocated into 5 groups each of 6 mice per group, with each group being housed separately. Over a period of 10 days, each group was subjected to either; (a). Water with 10 g/L sucrose (control), (b) water supplemented with 10 g/L sucrose + 5 g/L GrTE, (c) water supplemented with 10 g/L sucrose + 10 g/L GrTE, (d) water supplemented with 10 g/L sucrose + 15 g/L GrTE, (e) water supplemented with 10 g/L sucrose + 20 g/L GrTE. Daily consumption of water was monitored and PCV determined using the standard micro-haematocrit method. The animals were also weighed and monitored for any sign of disease.

Trypanosomes, infection and treatment

Cryopreserved *Trypanosoma brucei brucei* isolate (KETRI 2710) was obtained from Trypanosomosis Research Centre (TRC) trypanosome bank. The parasite was propagated and maintained in clean Swiss albino mice few days before the commencement of the research. A total of 105, eight weeks old male adult healthy Swiss albino mice were used in all experiments. The mice were randomly divided into seven equal groups (n =15 per group) and subjected to one of the following treatments: green tea, black tea, oolong tea, white tea at 20g/L, 0.1 ml of anti-inflammatory drug (dexamethasone) equivalent to 0.2mg per mouse, water only (infected) and water only (non-infected/placebo). Except for the placebo group, animals in other groups were infected with *Trypanosoma brucei brucei* isolate KETRI 2710. Inoculation was by intraperitoneal injection (*ip*) with approximately 10^4 trypanosome.

Parasitemia, blood sampling and determination of packed cell volume

To estimate the circulating parasite numbers in infected mice, two methods were used. The rapid "matching" method by Herbert and Lumsden [4] and the buffy coat technique as described by Murray *et al.*, [8]. Blood samples were obtained from three healthy animals prior to infection on day 0 and analyzed for baseline data. Subsequent data was obtained by serial sacrificing of 3 mice per group at each sampling time after every seven days except on day 11 when an early sampling was necessitated by death being experienced with the animals in the non-treated group. At each point, blood was taken by tail snip in 100μl microhaematocrit tubes for PCV determination. At time of sacrifice, the mice were anaesthetised using carbon dioxide (CO_2) and immediately blood for albumin assay collected from the heart by cardiac puncture. Serum was collected in sterile cryovials and stored at -20 ^{0}C until use.

Albumin assay

The concentration of serum albumin was measured using the BCG® Photometric colorimetric method as described by Mungatana *et al.*, [9].

Statistical analyses

Data was analysed using Statsview® Statistical programme (SAS) and significance of differences between means determined by ANOVA. A P value of < 0.05 was considered to be statistically significant.

RESULTS

Results on appropriate tea dosage determination using Gr TE on healthy mice indicated a significant difference ($P < 0.05$) on daily water intake but no significant difference on PCV for all the treatments. Twenty (20 g/L), the most consumed and tolerated concentration, was not significantly different from the control (Figure 1). This concentration was thus selected as a standard dosage for the main experiment since it ensured the best chance for maximal tea intake and thus activity when administered orally and also ensured absence of toxicity.

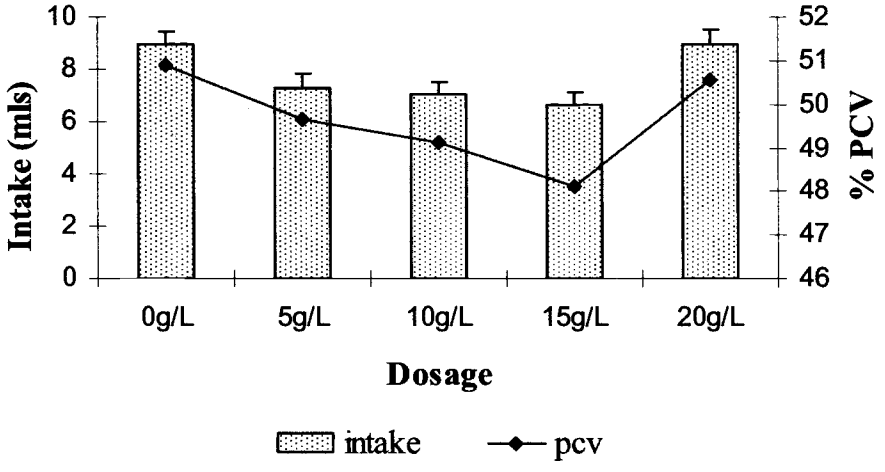

Figure 1: *Effect of oral administration of green tea extract on water intake and PCV on male Swiss albino mice. Data are means ± standard error of the means (SEM), n =6, P<0.05.*

Effects of tea on parasetemia, PCV and albumin levels

The *Trypanosoma brucei brucei* isolate 2710 stabilate produced a similar clinical picture in all infected animals after a pre-patent period of five days. Ultimately the animals developed anaemia, sleepiness and severe illness leading to death. Parasites in infected mice were observed on day five post infection which is in line with the parasites known incubation period of 5-10 days [7]. Levels of parasitemia in control mice and experimental mice developed exponentially at similar rates and reached similar densities at the peak of parasitemia on the same day, namely 8 DPI (Figure 2).

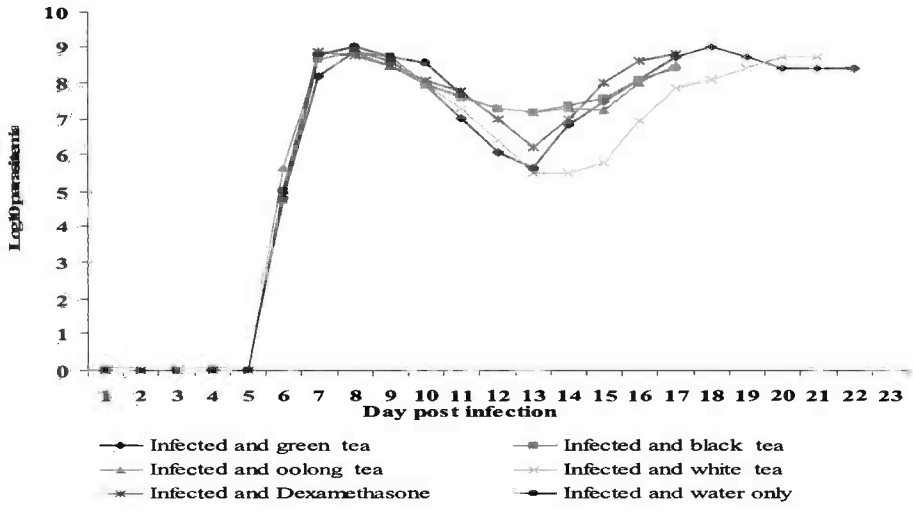

Figure 2: *Time course of Trypanosoma brucei brucei stabilate KETRI 2710 for different treatments plotted as Log10 of parasites per milliliter of blood; the scale is linear and ranges from 0-10*

Transient parasitemia decline after parasitemic peak on 11 DPI was significantly different ($P < 0.05$) between the various groups (Table 1). On day 11 post infection mice given tea had a significant reduction in parasitemia level compared to the ones infected and given water. However, no significant difference ($P > 0.05$) was observed between tea treatments (Table 1). Though tea was not able to eradicate the parasites, it significantly reduced the level of parasitemia enabling the mice to relapse and thus extending the mean survival time. At 13 DPI, a significant parasitemia reduction was evident, with green and white teas having the highest reduction in parasitemia and significantly different ($P < 0.05$) from other treatments including the drug dexamethasone (Table 1).

Table 1: *Values (Means ± SEM) of Log10 Parasitemia in mice infected with Trypanosoma brucei.*

Treatment	Day 11	Day 13
Infected and green tea	7.02 ±0.349 [a]	5.64 ±0.060 [a]
Infected and white tea	7.32 ±0.364 [a]	5.50 ±0.037 [a]
Infected and dexamethasone	7.80 ±0.092 [b]	6.20 ±0.080 [b]
Infected and oolong tea	7.62 ±0.120 [a]	7.20 ±0.010 [b]
Infected and black tea	7.65 ±0.276 [a]	7.20 ±0.010 [b]
Infected and water only	7.70 ±0.400 [b]	ND
Non infected and water only	No parasites	No parasites
	C.V 7.44, $P < 0.05$	C.V 5.38, $P < 0.01$

Treatments marked with the same letters are not significantly different at $P < 0.05$. ND- Not done since all mice in this group had died 11 DPI.

Accompanying the above events, the fall in PCV had occurred by 7 DPI (Figure 3).

To study the progressive reduction of PCV and the effect of various treatments over time, the mean change in PCV was analyzed on day 11 and 17 as shown in Table 2.

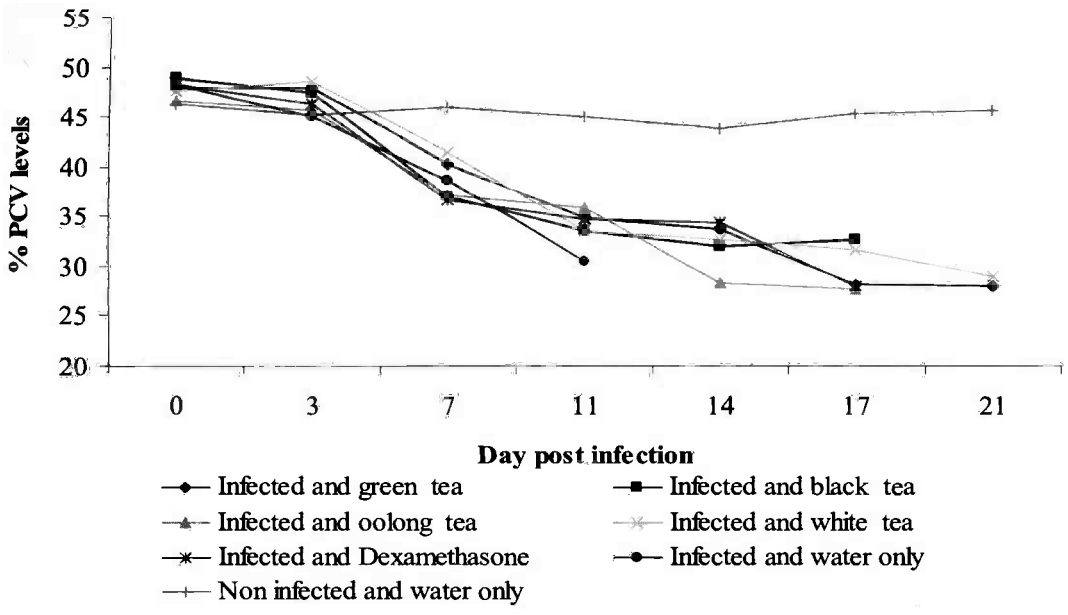

Figure 3: *Changes in PCV (Means ± SEM) during the period of study.*

On day 11 there was a significant PCV difference ($P < 0.05$) between animals treated using different teas and those infected and given water only. However, no significant difference was observed between the tea treatments even on day 17. These observations suggest that both teas could have a therapeutic role in cases of total packed erythrocyte volume reduction and the resulting anaemia.

Table 2: *Mean change (Means ± SEM) in PCV % of the treated animals and the control group from day 0 to day 11 and 17 post infection.*

Treatment	Day 11	Day 17
Infected and green tea	13.300 ± 1.263 [a]	21.750 ± 2.412
Infected and white tea	13.714 ± 1.510 [a]	14.000 ± 2.413
Infected and dexamethasone	15.333 ± 1.631 [a]	22.333 ± 2.786
Infected and oolong tea	13.000 ± 1.787 [a]	21.000 ± 2.876
Infected and black tea	15.333 ± 1.631 [a]	13.000 ± 2.786
Infected and water only	21.250 ± 1.988 [b]	ND
Non infected and water only	No change in PCV	No change in PCV
	C.V 6.97, $P < 0.037$	C.V 6.29, NS

Treatments marked with the same letters are not significantly different at $P < 0.05$. ND- Not done since all mice in this group had died 11 DPI.

The concentrations of serum albumin are shown in Figure 4. Treatment with various teas resulted in a significant amelioration ($P<0.01$) of parasite-induced hypoalbuminemia. The concentration in albumin was analysed on day 11 and 17 post infection to evaluate the effect of various treatments over time as shown in Table 3.

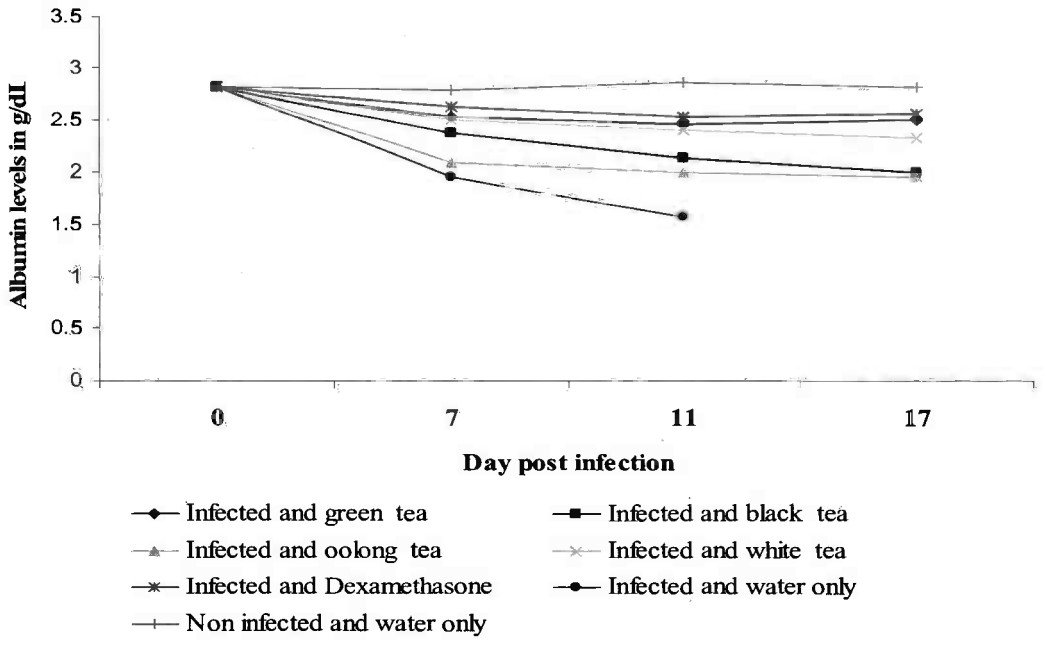

Figure 4: *Means ± SEM of albumin concentration post infection in various treatments.*

Table 3: *Albumin concentration g/dl (Means ± SEM) of the treated animals and the infected and non-treated animals on day 11 and 17.*

Treatment	Day 11	Day 17
Infected and green tea	2.467 ± 0.033 [a]	2.500 ± 0.115 [a]
Infected and white tea	2.400 ± 0.057 [a]	2.333 ± 0.145 [a]
Infected and dexamethasone	2.533 ± 0.089 [a]	2.567 ± 0.089 [a]
Infected and oolong tea	2.000 ± 0.200 [a]	2.067 ± 0.240 [b]
Infected and black tea	2.133 ± 0.120 [a]	2.000 ± 0.153 [b]
Infected and water only	1.567 ± 0.145 [b]	ND
Non infected and water only	2.867 ± 0.145	2.833 ± 0.082
	C.V 5.88, $P<0.01$	C.V 7.47, $P<0.01$

Treatments marked with the same letters are not significantly different at $P<0.05$. ND- Not done since all mice in this group had died 11 DPI.

The non infected (placebo) group had albumin concentration within the normal range throughout the duration of the experiment. On day 11 post-infection, only the animals given water showed a significant ($P<0.01$) reduction in albumin concentration. However, by 17 DPI mice given black and oolong teas had a significantly ($P<0.01$) lower albumin concentration than animals treated with green tea, white tea or dexamethasone.

DISCUSSION

The ability of tea to lower the level of parasitemia can be attributed to the toxic activity of polyphenols present in it. These compounds have the ability to complex with extracellular and soluble proteins and also parasite cell wall thereby disrupting the parasite cell membrane. Green and white teas were more effective in parasite reduction, since they contain catechins that are highly hydroxylated compared to black tea and oolong tea that have

oxidized polyphenols [5]. We can therefore speculate that without the host immunological assistance, high concentration of tea flavonoids would be necessary to reduce *Trypanosoma brucei brucei* in the host. This indicates the need for detailed mechanistic studies together with the development of parasite-specific drug formulations. This is due to the fact that the current treatment regimens, based on chemotherapy for these parasites are limited and not ideal since they are associated with severe side effects and development of drug resistance.

The loss of total packed erythrocyte volume early in the infection may be due to haemolysis which plays an important role in the generation of anaemia. This results from the direct binding of the trypanosomes antigens with specific receptors on the red blood cells giving rise to complexes which elicit the production of antibodies mainly IgM with a consequent lysis of red blood cells [5]. Infected mice given tea extracts in this study showed significantly higher levels of PCV compared to the infected mice given water only which can be ascribed to an enhanced resistance to erythrocyte haemolysis conferred by the tea. This demonstrates clearly that tea containing flavonoids possess *in vivo* ability to protect erythrocytes from haemolysis which can be attributed to flavonoids. In addition, erythrocytes have membranes with a high content of polyunsaturated lipids and a rich oxygen supply making them vulnerable to lipid peroxidation. Reactive oxygen species generated during infections like trypanosomosis can attack erythrocytes membrane, induce its oxidation and trigger haemolysis. However the antioxidant activity of tea [11] might have elicited a rise in plasma antioxidant capacity leading to a reduction in the susceptibility of erythrocyte membrane destruction. With these findings, we can hypothesize that ingestion of tea would reduce the risk of free radical induced oxidative damage to the erythrocytes.

Oral administration of tea extracts in this study had a significant ($P < 0.01$) prevention of albumin concentration reduction in *Trypanosoma brucei brucei* infected mice thereby indicating a decreased effect on inflammation induced by the trypanosome parasite. This effect can be ascribed to the presence of flavonoids. Tea flavonoids and evidence for their role in the prevention of many degenerative diseases is emerging [1]. The ability of tea flavonoids to prevent decline in albumin concentration and the resultant putative anti-inflammatory effects can be accredited to various properties. These ubiquitous compounds have the ability to exert strong antioxidant effects based in part on their structural characteristics especially the 3',4',-dihydroxylation of the B-ring in the catechol moiety.

These structural features of flavonoids represent the molecular basis for their radical-scavenging and reduction of reactive oxygen species, which have been implicated in the pathogenesis of inflammatory diseases [3]. Green tea contains flavan-3-ols or catechins which include epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epicatechn (EC) with EGCG being the major constituent and also the component with the highest antioxidant property. Catechins undergo major enzymatic biotransformation to form theaflavins and thearubigins which are the characteristic constituents in black tea but which have less antioxidant capacity [11]. During inflammation, toxic oxidants, including oxygen species are generated. The phenolic hydroxyl substitutions present mainly in EGCG act as potent radical scavengers, increasing the capacity of endogenous antioxidant defenses and thereby modulating the cellular redox state [15, 16]. This ability to strengthen the physiological antioxidant defense system helps improve the chronic inflammatory condition as observed in this study.

It is evident from this study that tea flavonoids elevated albumin concentration and this may be promising at least for tea as an auxiliary anti-inflammatory agent in chronic inflammatory diseases. Inflammation and several diseases often result from the effects of free radicals the most important ones being superoxide, hydroxyl, singlet oxygen and nitrites. The efficient radical scavenging property of tea extracts which is due to the presence of polyphenols is a property of great

importance in the management of degenerative diseases [11]. Consequently, the efficacy of tea polyphenols in preventing or ameliorating chronic disease is currently the subject of considerable scientific investigation. Although a number of mechanisms continue to be proposed for the beneficial effects of tea in different models of chronic disease, the radical scavenging and antioxidant properties of tea polyphenols remain the most frequently cited contributors. Much of the evidence supporting an antioxidant function for tea polyphenols is derived from assays of their antioxidant activity *in vitro* [11, 15]. However, evidence that tea polyphenols are acting directly or indirectly as antioxidants *in vivo* is more limited. Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of tea polyphenols to the physiological effects during oxidative stress. From results obtained in this and other previous studies, tea polyphenols could serve as models for the rationale design of synthetic analogues with higher *in vitro* and *in vivo* activities and more favorable chemical properties.

ACKOWLEDGMENTS

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