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Different types of tea products attenuate inflammation induced in *Trypanosoma brucei* infected mice

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Abstract

An *in vivo* study was carried out to determine the effect of different types of Kenyan tea extracts on 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). Parasitemia levels in the untreated mice and those given different teas developed exponentially at similar rates reaching similar densities at the peak of parasitemia 8 DPI. Between 9 and 13 DPI parasitemia decreased more rapidly in tea treated compared to the untreated mice which indicated that tea lowered parasitemia level. Anaemia indicated by a fall in erythrocyte packed cell volume (PCV) occurred within 4 DPI and remained below the normal levels until the terminal stages of the disease. A significant difference ($P<0.05$) was observed 11 DPI between the tea treated and the untreated mice indicating that tea enhanced resistance to erythrocyte destruction. Mice treated with tea exhibited significantly ($P<0.01$) reduced parasite-induced hypoalbuminemia as compared to the untreated. Since albumin is a negative acute phase protein, it shows a decrease during inflammatory conditions and therefore its elevation in the mice given tea in this study clearly demonstrated that tea ameliorated inflammation induced by *T. b. brucei.* Although green and white teas were superior in most of these characteristics, black tea, which is the principle tea product from Kenya, displayed remarkable properties some even comparable to those of green tea. Interestingly, tea was more efficacious than dexamethasone an established anti-inflammatory drug, demonstrating its therapeutic potential.

Keywords: Tea; Inflammation; Trypanosomosis; Parasitemia; Packed cell volume (PCV); Albumin

1. Introduction

Africa and in particular Kenya are important producers of black tea. Despite this, the share of tea that is consumed locally has continued to decrease. For example, national per capita consumption in Kenya has declined from 0.8 kg made tea/person/year in 1989 to 0.4 kg made tea/person/year in 2004. During the same period the volume of tea consumed in Kenya declined from 17 million kg in 1989 to 14 million kg in 2004 [1]. 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. In other countries where tea (*Camellia sinensis*) is produced, the beverage has been widely marketed as a health product. In addition, tea has increasingly been put to other uses in products other than in food and drinks. Indeed, numerous environmentally friendly industrial cleaning agents, deodorizers and anti-microbial agents have been formulated using tea [2,3]. Data to support the view that tea is pharmacologically active has been generated particularly using green tea, which is widely consumed in Asia [4,5]. However, there is a dire paucity of information on the potential health benefits of black aerated/fermented tea, which is the principle type of tea product consumed in Kenya and the rest of the world. Therefore, there is a need to promptly initiate research on black tea to establish its beneficial effects on human health.

To investigate the potential health benefits of tea *in vivo*, a well established mouse model infected with *Trypanosoma brucei brucei* which is a tissue invasive parasite that causes the

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mice to develop extensive tissue damage similar to that which occurs in man was used [6]. Upon infection, the parasite leads to a severe inflammatory response, extensive tissue damage and untimely death when left untreated [7]. In response to bacterial and parasitic infections, the host mounts an acute phase response which leads to many systemic effects such as fever, cachexia and stimulation of hepatocytes-derived acute phase proteins [8–10]. This response is mediated by the release of pro-inflammatory cytokines such as Interleukin (IL)-1, IL-2, IL-6 and Tumour Necrosis Factor (TNF)-ά. The experimental approaches to study the role of cytokines in acute phase response in trypanosomosis are, however, hampered by their instability and transient presence in circulation [8,11].

A more effective, although, indirect means of demonstrating that cytokines have been activated during disease is quantification of their activity by measuring the plasma concentration of acute phase proteins (APPs). These proteins are recognized markers of inflammation with their plasma concentration increasing 2 and 1000 times for several days following infection [10]. The APPs consists of "negative" and "positive" proteins that show a decrease and an increase in levels, respectively, in response to a challenge [10]. The negative APP includes albumin, the most abundant and constitutive plasma protein in healthy individuals. The positive APPs are glycoproteins synthesized mainly by hepatocytes upon stimulation by pro-inflammatory cytokines and released into the blood stream. The positive APPs includes haptoglobin, C-reactive protein, serum amyloid-A and alpha-1-acid glycoprotein [10]. The levels of acute phase proteins in the blood may be related to the severity of the response to an infection, and thus may provide valuable quantifiable biochemical indicators of an inflammatory response [12]. The decrease in albumin levels during an acute phase response to trauma, inflammation or sepsis has been attributed to a decrease in the gene transcription rate of albumin mRNA. Previous studies using inflammation-induced rats showed a decrease in the rate of albumin synthesis [13,15]. A sustained inflammatory response in critical illness may lead to prolonged inhibition of albumin synthesis indicating that a decline of its synthesis could be used as a prognostic marker of inflammation [13–15].

In the present study, mice infected with *T. b. brucei* were given various Kenyan tea extracts with the objective of determining whether tea could efficiently and effectively down-regulate inflammation or effects of trypanosomosis during murine trypanosomosis. The murine model was chosen for this study because trypanosomosis caused by *T. b. brucei* mimics human trypanosomosis disease caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. The murine model therefore reflects all stages of human sleeping sickness and is now widely utilized as a model to study the disease and evaluate the efficacy of chemotherapeutic agents. Although *T. b. brucei* is not infective to man, it belongs to the same sub-genus (trypanozoon) as the human infective parasites species. The similarities include tissue invasiveness and causing chronic infection in domestic and laboratory animals with similar characteristics such as, the involvement of the central nervous system [6].

In this study, 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.

2. Materials and methods

2.1. Animals

Male Swiss albino mice 6–8 weeks old and weighing between 24 and 30 g were housed in standard mice cages in a controlled environment and provided *ad libitum* food and water with or without tea extracts. Animal care protocols and procedures used in the current study were reviewed and approved by the institutional animal care and use committee.

2.2. 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). For each concentration, the tea infusion was prepared by adding 1 L of boiling water to the weighed leaves of tea (*Camellia sinensis*) and extracted for 1 min. After cooling, the aqueous extract of the mixture was filtered and given to the mice.

The mice were acclimatized for 2 weeks during which each mouse was treated once using 0.1 mL of 1% Ivermectin (Ivomec®) equivalent to 1 mg per mouse during the first week in order to exclude any helminthes infestation. The animals were then randomly allocated into 5 groups each of six mice per group, with each group of mice 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 packed cell volume (PCV) determined using the standard microhaematocrit method. The animals were also weighed and monitored for any sign of disease.

2.3. Trypanosomes

Cryopreserved *T. b. 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.

2.4. Infection and treatment

A total of 105, eight weeks old male adult healthy Swiss albino mice weighing 24–30 g were used in all experiments. All control animals were age matched with experimental animals. 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 20 g/L, 0.1 mL of anti-inflammatory drug (dexamethasone) equivalent to 0.2 mg per mouse, water only (infected) and water only (non-infected/