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J. Protozool. Res. 21, 1-7 (2011)

Effects of tea on survival rates and liver pathology of *Trypanosoma brucei brucei* infected mice

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ABSTRACT

The current study investigated the effects of different types of Kenyan tea extracts on the pathogenesis of *Trypanosoma brucei brucei* in a Swiss White mice model. Following infection with trypanosomes, the mice were monitored for survival and liver pathology. Tea significantly ($P<0.05$) enhanced the survival rate of tea treated mice. Additionally, in tea treated but infected mice, there was reduction in infiltration of inflammatory cells into the periportal and parenchymal regions as well as hepatocyte cell damage compared to the infected untreated animals. Green and white teas were superior in most of the above effects while black tea and oolong teas had the least effects. The tea extracts were more efficacious than dexamethasone in prolonging the life of infected animals. It is concluded tea can act as adjunct therapeutic agent in treatment of diseases having hepatic inflammation, including trypanosomiasis.

Key words: Inflammation; liver pathology; survival rate; tea; trypanosomiasis

INTRODUCTION

Tea, the dried leaf of the evergreen tea bush *Camellia sinensis* contains a wide variety of biologically active compounds such as polyphenols, methylxanthines, essential oils, proteins, vitamins and amino acid (Peterson *et al*., 2005; Bastos *et al*., 2006; Gallaher *et al*., 2006). Most of its biological actions are however ascribed to the polyphenolic fraction, namely, tea catechins (Cabrera *et al*., 2003). Tea polyphenols include: epigallocatechin gallate (EGCG), epicatechin, epigallocatechin and epicatechin gallate in green tea while theaflavins and thearubigins are present in black tea. Of these polyphenolic components of tea, EGCG is the major constituent and is also the component with the highest bioactivity in green tea. Catechins which belong to the flavan-3-ols family of polyphenols have recently received considerable attention because of their potential therapeutic effects. Emerging scientific data from pharmacological and physiological studies continue to show that tea has beneficial effects on human health by boosting immunity (Hu *et al*., 2004; Crespy and Williamson, 2004; Maeda-Yamamoto *et al*., 2004).

Considerable attention is currently being focused on the role of dietary and medicinal phytochemicals to inhibit, reverse or retard diseases mainly due to their radical scavenging properties. A study by Chen *et al.*, (2004) showed that green tea polyphenols reduced the severity of liver injury in toxin-induced hepatotoxicity mice using carbon tetrachloride. However, since the study focused on the role of a single catechin EGCG in preventing hepatic toxicity, it is important to point out that the overall protective effect of tea may require a combined action of several components in the beverage. Since tea can be consumed over long periods of time without any known side effects, its possible role as an adjunct therapeutic agent in inflammatory liver diseases deserves consideration.

In the present study, different types of whole tea extracts processed from green, black, oolong and white teas from Kenyan tea cultivars were given *ad libitum* to mice animal model infected with trypanosomes. African trypanosomes are protozoan parasites that cause sleeping sickness in humans and Nagana in domesticated cattle, diseases with a major health and economical impact on sub-Saharan Africa. The current trypanocidal drugs in use have a high level of toxicity and the development of drug resistant parasites is reported (Kagira and Maina, 2007). Trypanosomiasis is associated with severe inflammatory reaction in most body systems including the liver, and a focus on the mechanisms involved in the induction and/or prevention of pathology might provide new innovative ways of treatment (Bosschaerts *et al*., 2009). The main objective of the current study was to determine whether tea extracts could enhance survival rates and subsequently reduce the effect on liver injury.

MATERIALS AND METHODS

Animals

Male Swiss White mice 6-8 weeks old and weighing between 24-30 g were obtained from the Trypanosomiasis Research Centre laboratory animal breeding facility. The mice were housed in standard mice cages in a controlled environment and provided *ad libitum* with unrestricted food (Mice Pellets, Unga Ltd, Kenya) and water with or without tea extracts in feeders and drinking bottles, respectively. Rodent care protocols and procedures used in the current study were reviewed and approved by the Trypanosomiasis Research Centre Institutional Animal Care and Use Committee.

Trypanosomes

Cryopreserved *Trypanosoma brucei brucei* isolate (KETRI 2710) was obtained from Trypanosomiasis Research Centre (TRC) trypanosome bank. The parasite was propagated in gamma irradiated Swiss White mice. At the onset of parasitemia, the mice were euthanized, blood obtained from the heart and diluted to 10^4 trypanosomes per ml using phosphate saline glucose.

Tea samples

A set of commercial Kenyan tea samples teas that included fermented (black), semi fermented (oolong), non-fermented (green) and white tea were obtained from Kenya as previously described Karori *et al*., (2007). The samples had been manufactured in commercial factories using standard manufacturing conditions.

Experimental design

A total of 105 Swiss white mice were randomly divided into seven equal groups (n = 15 per group). Four groups were treated with green tea, black tea, oolong tea and white tea at 20 g/l. There were 3 control groups consisting of mice treated with 0.1 ml of anti-inflammatory drug (dexamethasone) equivalent to 0.2 mg per mouse, water only (infected) and water only (non-infected/placebo). Except for the placebo group, animals in other groups were intraperitoneally infected with 10^4 *T. b. brucei* as previously described (Kagira and Maina, 2007). The survival rates of the mice were then monitored.

Mice were sacrificed every 7 days for 4 weeks, liver section collected and immediately stored in 10% phosphate–buffered formalin. The liver sections were then trimmed, processed for histology and stained with haematoxylin and eosin dyes. Stained sections were observed under light microscopy to determine the degree of inflammatory cell infiltration and hepatic parenchymal damage.

Statistical analyses

Univariate survival analysis of data using Kaplan-Meir method was used to determine the effect of tea on the survival rate of infected animals. The log-rank test was used to examine the null hypothesis that the survival curves were identical. A P-value of < 0.05 was considered to be statistically significant.

RESULTS

Effect of tea extracts on survival rate

Infected mice given tea extracts or dexamethasone had significantly ($p<0.05$) longer survival rates compared to the infected untreated group (Figure 1). Mice treated with tea extracts had a longer ($p<0.05$) survival period than those administered dexamethasone. Infected untreated groups of mice died by 11 days post infection (DPI), whereas the last mice in the infected, tea-treated group died on 22 DPI. In descending order, the survival rates were longer in infected mice treated with green, white and black teas and shorter in mice treated with oolong tea.

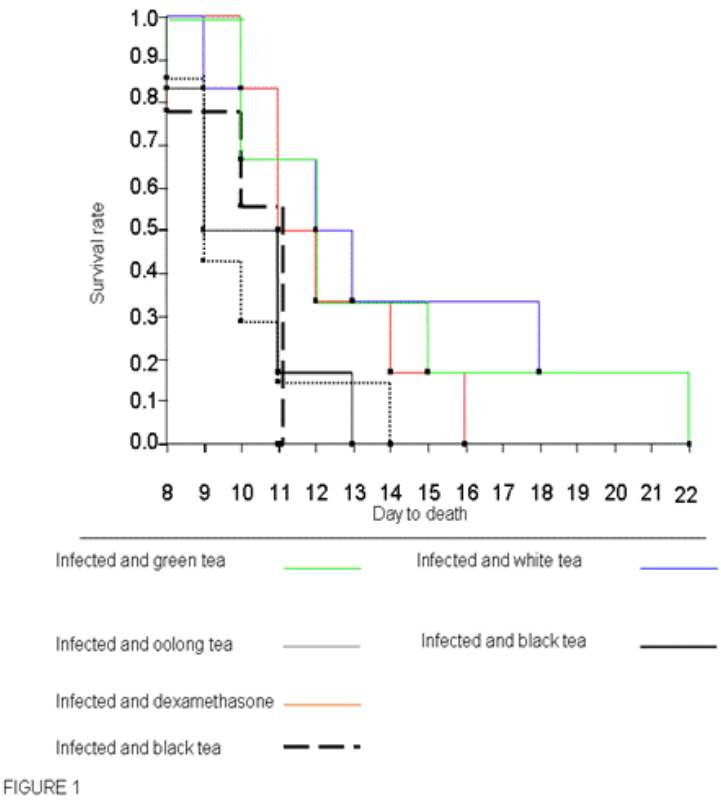

FIGURE 1

Figure 1. Kaplan-Meir survival curves comparing survival rate in *Trypanosoma brucei brucei* mice treated with tea and dexamethasone with the infected control given water only. The survival rates are significantly different from the control group, $P<0.05$ (Logrank test).

Effect of tea extracts on liver pathology

Following infection of mice, there was an infiltration of lymphocytes mainly at the periportal regions of the liver. However, as time progressed during the infection period, the lymphocytes were replaced by macrophages. In addition to the periportal infiltration, later stages of the infection were characterized by infiltration of inflammatory cells into the liver parenchymal tissue. This was accompanied by degeneration and necrosis of hepatocytes.

Comparison of the pathology between the liver of mice infected and treated using various tea extracts and infected untreated animals indicated a reduction in the pathology of tea treated animals. The effect was observed as a reduction in the cellular infiltration both at the periportal region and in the liver parenchyma. However, for green tea especially at the early stages (7 DPI), there was a marked increase in periportal inflammatory cell infiltration despite the effective reduction in parenchymal infiltration (Figure 2A) compared to the control group (Figure 2B). In descending order, at 7 DPI, inflammatory cell infiltration was more marked in mice treated with green tea and black tea and lowest in those treated with white tea.

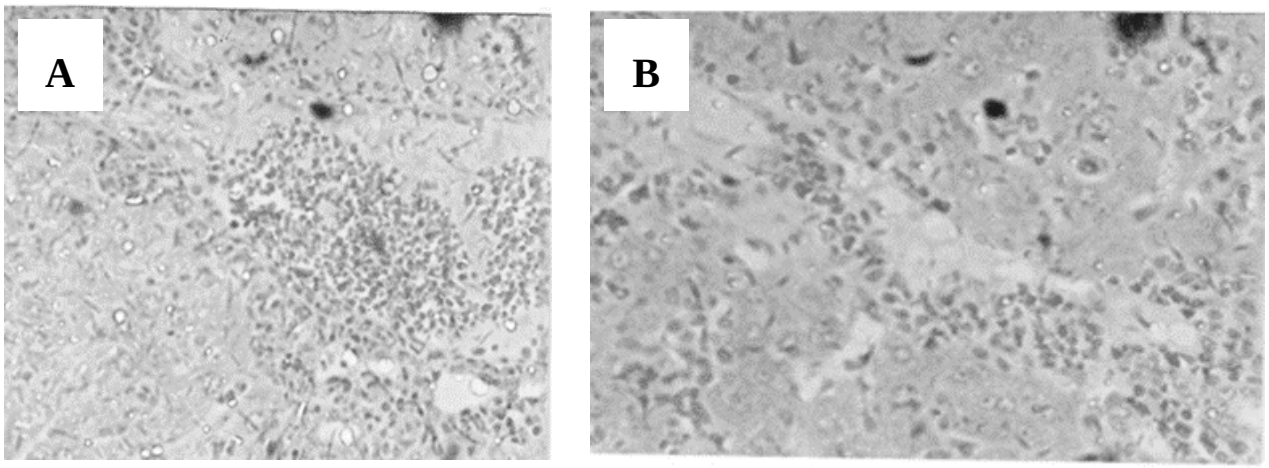

Figure 2. Liver sections showing histopathological profiles of mice infected with *Trypanosoma brucei brucei* and given; water only (A) x3,000 and green tea (B) x1,875 as seen on 7 DPI. The liver sections were stained with hematoxylin and eosin dyes.

At 11 DPI, mice treated with tea extracts had a reduction of periportal and parenchymal inflammatory cell infiltration compared to the control (Figure 3A and 3B). In descending order, inflammatory cell infiltration was lowest in white tea, green tea, oolong tea and highest in black tea. At 21 DPI, the same trend was observed with the reduction in cellular infiltration being more marked in the liver of mice treated with white tea followed by green tea, black tea and oolong tea, respectively. Figure 4 (A and B) shows a comparison of liver sections from white tea and black tea treated mice. In general, mice treated with green tea showed a reduced pathology throughout the infection period whereas white tea showed improved reduction in later stages of infection namely day 11 and 21. Black tea performed well at day 7 but showed reduced effect on day 11 and 21. Oolong tea showed intermediate effect at mid infection (11 DPI) but had little effect by day 21.

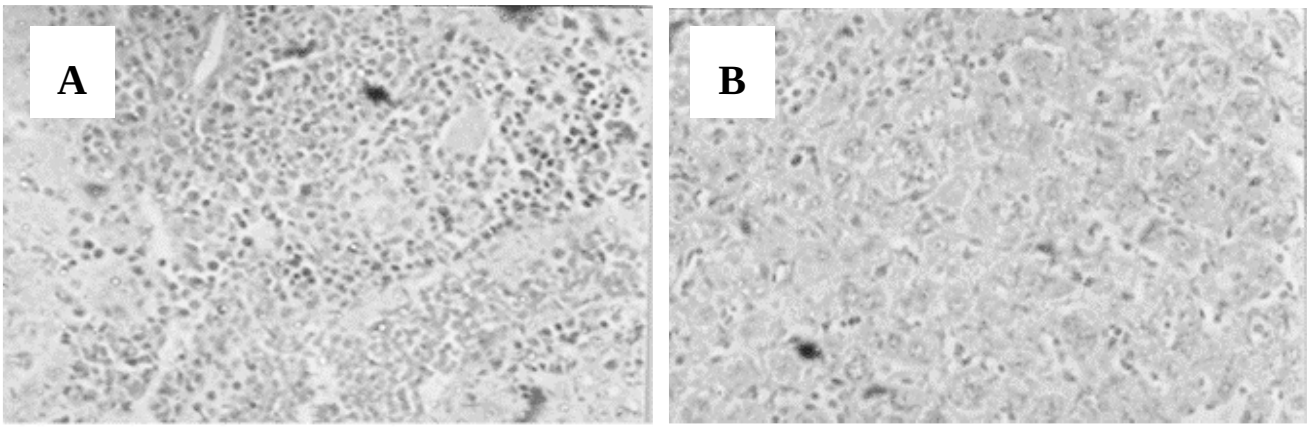

Figure 3. Liver sections showing histopathological profiles of mice infected using *Trypanosoma brucei brucei* and given; water only (A) and white tea (B) x1,875 as seen on 11 DPI. The liver sections were stained with hematoxylin and eosin dyes.

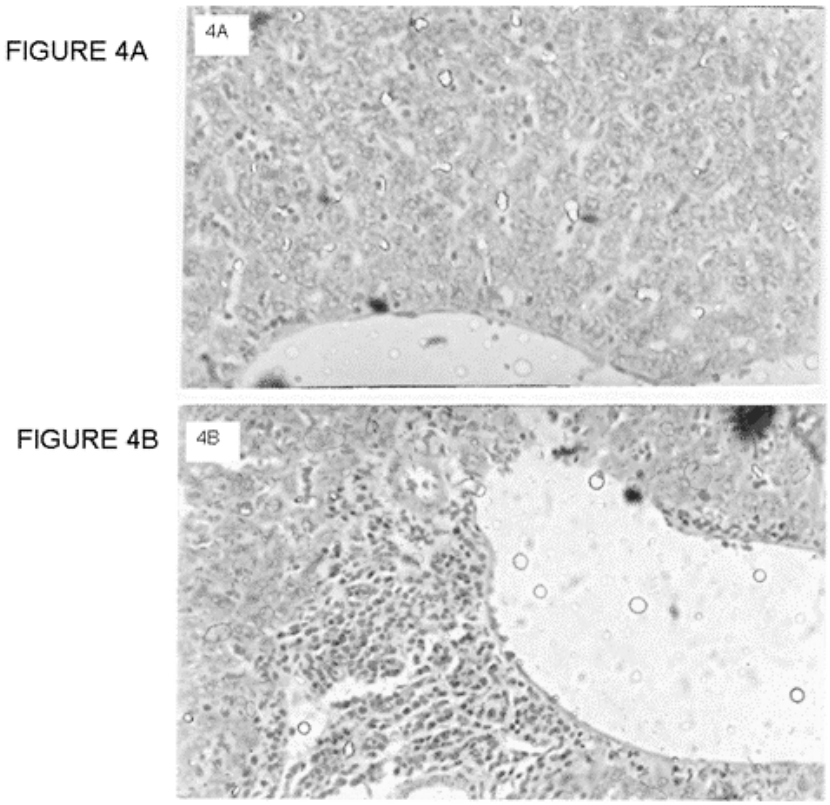

Figure 4. Liver sections showing histopathological profiles of mice infected using *Trypanosoma brucei brucei* and given white tea (A) and black tea (B) x1,875 as seen on 21 DPI. The liver sections were stained with hematoxylin and eosin dyes.

DISCUSSION

Mice treated with tea significantly prolonged the survival period of the infected animals even better than the anti-inflammatory drug, dexamethasone which is used an adjunct treatment of sleeping sickness. The pathogenesis of trypanosomiasis is associated with severe inflammation and production of radicals such as nitric oxide which affects the survival of the host. This production of nitric oxide is a disease-exacerbating factor and in murine trypanosomiasis, it causes damage to lymphocyte function of the host (Millar *et al*., 1999; MacLean *et al*., 2001; Tedeschi *et al*., 2004). The prolongation of survival period in the mice treated with tea could be to the ability of tea flavinoids to counter the trypanosomiasis induced inflammatory reaction and aiding antioxidant defence system (Chen *et al*., 2004; Tedeschi *et al*., 2004). Results from this study corroborate those of an earlier study on acute phase response (Bukowoski, 2004; Karori *et al*., 2008) and suggest that tea extracts may be promising at least as an auxiliary anti-inflammatory adjunct in the management of chronic inflammatory diseases. However, despite the observed effect on the survival rate, there is a scarcity of information on the mechanism involved through which tea is thought to prolong life.

Specific organ damage during trypanosomiasis is one of the major contributing factors to the disease pathogenesis and is characterized by a progressive inflammatory reaction in target tissues including the liver (Murray *et al*., 1974). In this study, tea reduced the severity of liver damage as observed in the minimal degree of cellular infiltration into the periportal and parenchymal regions and ultimately reduction of hepatic cell damage. The reduction in infiltration in tea treated mice is an indication that tea could have modulated the inflammatory response during the experimentally induced trypanosomiasis. Elsewhere, tea polyphenols have been shown to prevent toxin-induced hepatotoxicity in mice (Chen *et al*., 2004) though the mechanism underlying this protective effect on liver damage is not known. Some studies have shown that hepatocellular inflammation in trypanosomiasis is caused by oxidative stress, production of pro-inflammatory cytokines (eg., TNF and NO) and activation of M1 monocytic cells (Bosschaerts *et al*., 2009). It is possible that tea, being a potent radical scavenger and anti-inflammatory, could have limited pathogenicity by reducing the recruitment and activation of inflammatory monocytic cells. Indeed, white and green teas, which have with high level of catechins, performed better than black tea in reducing liver damage. In conclusion, the current study shows the potency of tea extracts in extending the survival and lessening the liver pathology in mice infected with trypanosomes and the possible role of tea as adjunct therapeutic agent in treatment of hepatic inflammatory disease should be investigated further.

ACKOWLEDGMENTS

We thank the Division of Research and Extension of Egerton University for funding. We thank the staff of pathology department in Trypanosomiasis Research Centre (TRC) for tissue preparation and Tea Research Foundation of Kenya (TRFK) for providing sample for analysis.

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