Tea 32(2) 2011, 69-74

EFFECTS OF TEA ON SURVIVAL RATES AND LIVER PATHOLOGY: AN ANIMAL MODEL STUDY

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ABSTRACT

The effect of different types of Kenyan tea extracts on the survival rates and liver pathology in an animal model of male Swiss albino mice infected with *Trypanosoma brucei brucei* isolate (KETRI 2710) was determined *in vivo*. Tea had a significant ($P \leq 0.05$) effect on the survival rate of mice. Additionally, in tea treated but infected mice, there was a reduction in infiltration of inflammatory cells into the periportal and parenchymal regions as well as hepatocyte cell damage compared to the infected untreated mice. Green and white teas were superior in the said effects while black and oolong teas had least effects. However, Kenyan teas were superior to Japanese and Chinese teas. Tea was more efficacious than dexamethasone (on established anti-inflammatory drug in prolonging the life of infected animals, thereby demonstrating its potential as a therapeutic agent. It was concluded that tea can act as an adjunct therapeutic agent in management of diseases having hepatic inflammation, including trypanosomiasis and also in prolonging life.

INTRODUCTION

Tea contains a wide variety of biologically active compounds such as polyphenols, methylxanthines, essential oils, proteins, vitamins and amino acids [2,7,17]. The biological activity of tea is however ascribed to the polyphenolic fraction, namely, tea catechins [5]. Tea polyphenols include: epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) in green tea while Theaflavins (TFs) and Thearubigins (TRs) are mainly 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 [13]. 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 continuc to show that tea has beneficial effects on human health by boosting immunity [8,19,20].

Substantial 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. Chen *et al.* [6] 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 this 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. These diseases have 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 has been reported [9]. 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. The main objective of this 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 albino mice 6–8 weeks old and weighing between 24-30 g were used. These were housed in standard mice cages in a controlled environment and provided with food *ad libitum.* (unrestricted) food (Mice Pellets) and water with or without tea extracts. Animal 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 and maintained in clean Swiss White mice a few days before the commencement of the research.

Tea samples

A set of eight commercial Kenyan teas including black, semi-oolong, green and white tea from different tea factories in Kenya were analyzed for polyphenolic composition as outlined in British Standard ISO document [1]. Actual values were determined from a standard curve generated using gallic acid, and were expressed as per cent (%) by mass of gallic acid (GAE) equivalent, expressed on a dry matter basis.

Experimental design

A total of 105 Swiss white mice were randomly divided into seven equal groups (n=15). Four groups were treated with green tea, black tea, oolong tea and white tea at 20g/L as described by Karori *et al* [11]. There were three control groups consisting of mice treated with 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 intraperitoneally infected with 10^4 *Trypanosoma brucei brucei* as previously described [9]. The mortality in mice was monitored.

Mice were sacrificed every seven days for four weeks, liver section collected and immediately stored in 10% phosphate–buffered formalin. The liver sections were trimmed, processed for histology and stained with hematoxylin 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.

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).

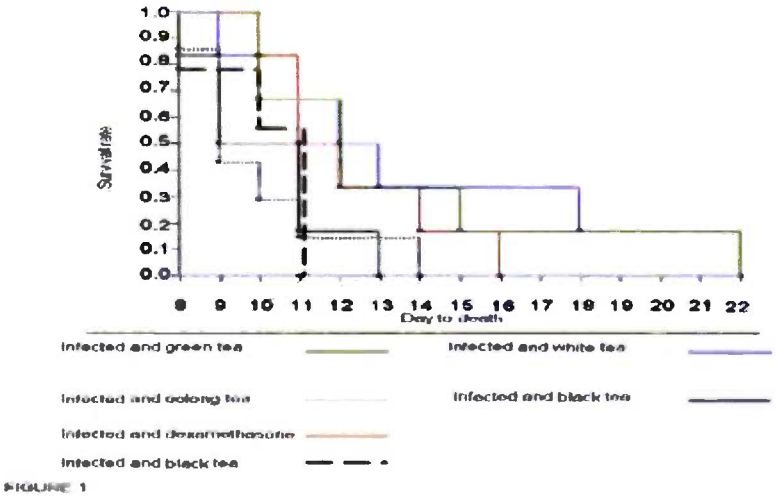

Figure 1: *Kaplan-Meir survival curves to compare survival rate in Trypanosoma brucei brucei mice treated with tea and dexamethasone with the infected control mice given water only.*
The survival rates are significantly (P≤0.05) different from the control group.

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. The survival rates were longer in infected mice treated with green, white and black teas in descending order and least in mice treated with oolong tea.

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 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 reduction in parenchymal infiltration (Figure 2A) compared to the control group (Figure 2B). Inflammatory cell infiltration was intense in mice treated with green tea than in black tea, and least in those treated with white tea.

FIGURE 2B

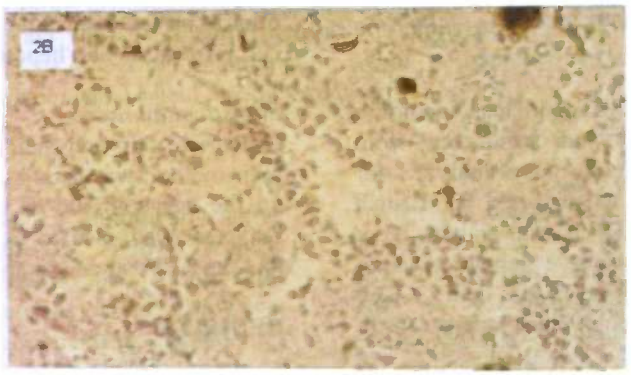

FIGURE 2A

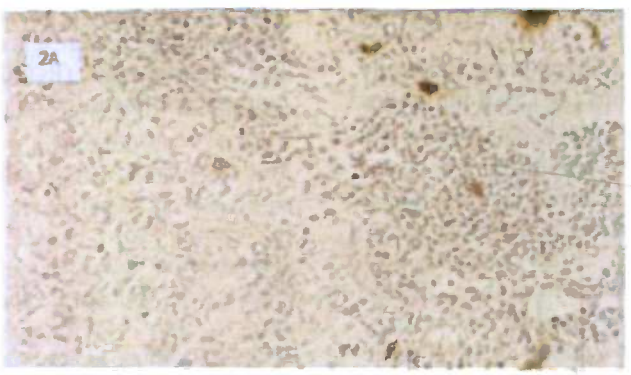

Figure 2B & 2A: *Representative liver sections showing histopathological profiles of mice infected using Trypanosoma brucei brucei and given; (A) water only (top) mag x3000 and (B) green tea (bottom) mag x1875 as seen on 7 DPI.*

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 Figure 3B).

FIGURE 3B

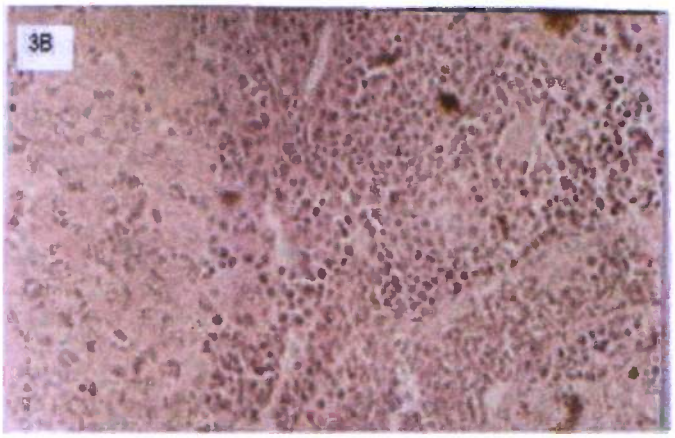

FIGURE 3A

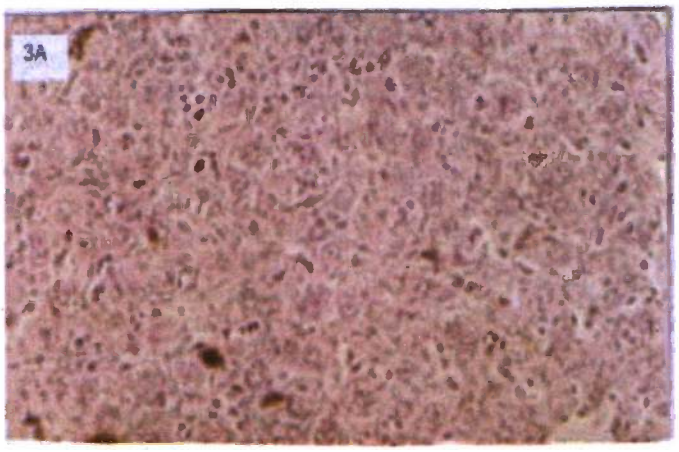

Figure 3B & 3A: *Representative liver sections showing histopathological profiles of mice infected using Trypanosoma brucei brucei and given; (A) water only (top) and (B) white tea (bottom) mag x1875 as seen on 11 DPI.*

In descending order, inflammatory cell infiltration was low in white tea, green tea, oolong tea and high in black tea. At 21 DPI, the same trend was observed with the reduction in cellular infiltration being marked in the liver of mice treated with white tea followed by green, black, and oolong tea, respectively. Figure 4 (A and B) shows a comparison of liver sections from white tea and black tea-treated mice. Overally, 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 4A

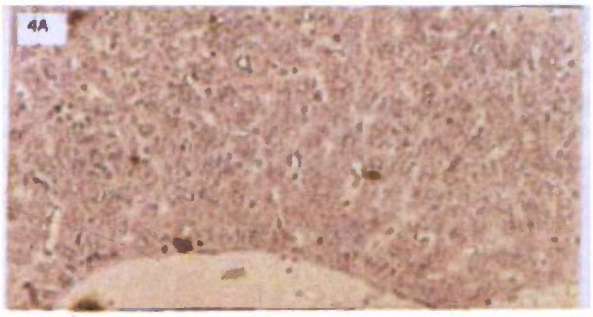

FIGURE 4B

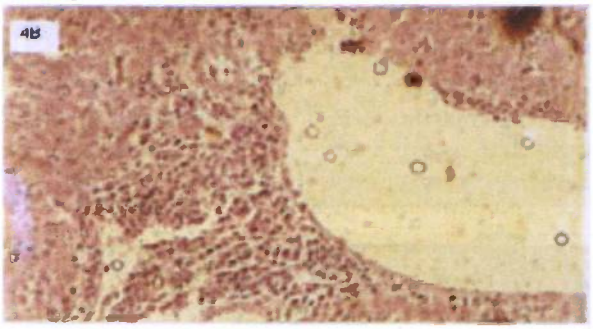

Figure 4A & 4B: *Representative liver sections showing histopathological profiles of mice infected using Trypanosoma brucei brucei and given (A) white tea (top) and (B) black tea (bottom) mag x1875 as seen on 21 DPI.*

DISCUSSION

Mice treated with tea significantly ($P \leq 0.05$) prolonged the survival of the infected animal better than the anti-inflammatory drug, dexamethasone which is used as an adjunct in the treatment of sleeping sickness. The pathogenesis of trypanosomosis 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 [12,14,18]. The prolongation of survival period in the mice treated with tea could be due to the ability of tea flavonoids to counter the trypanosomosis induced inflammatory reaction and aiding antioxidant defense system [6, 18]. Results from this study corroborate those of an earlier study on acute phase response [4] suggesting that tea extracts are promising 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 [15,16]. In this study, Kenyan tea reduced the severity of liver damage as observed in the minimal degree of cellular infiltration into the periportal and parenchymal regions and ultimately in 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 [6] though the mechanism underlying this protective effect on liver damage is unknown. Some studies have shown that hepatocellular inflammation in trypanosomiasis is caused by oxidative stress, production of pro-inflammatory cytokines (e.g. TNF and NO) and activation of M1 monocytic cells [3]. 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 high levels of catechins and performed better than black tea in reducing liver damage [10,11]. Since tea can be consumed over long periods of time without any obviously known side effects, its possible role as adjunct therapeutic agent in treatment of hepatic inflammatory disease deserves consideration.

ACKNOWLEDGMENTS

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

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