Effects of crude extracts of Solanum nigrum on the Liver pathology and Survival time in Trypanosoma brucei rhodesiense infected mice

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ABSTRACT

Tissue inflammatory damage during trypanosomosis significantly affects the treatment and prognosis. The current study investigated the effects of water extracts of Solanum nigrum (SNE) on the liver pathology and survival of Swiss white mice infected with Trypanosoma brucei rhodesiense. Trypanosome infected mice treated with SNE had significantly (P<0.05) increased and dose dependent survival time and liver pathology. Mice treated with higher concentrations of SNE had minimal liver pathology with minimal infiltration by inflammatory cells compared with the dexamethasone treated and untreated mice which had massive infiltration suggesting that SNE could be superior to dexamethasone in reducing trypanosome mediated liver pathology. Therefore, SNE could be a better anti-inflammatory adjunct in the treatment of Human African trypanosomosis (HAT) and other inflammatory conditions such as hepatitis.

KEYWORDS: Liver pathology; Inflammation; Survival time; Solanum nigrum; Human African Trypanosomosis.

INTRODUCTION

Human African trypanosomosis (HAT) exerts an immense pressure on the health sector in Sub Saharan Africa, with an estimated 66 million people being at risk of infection (WHO, 2004). In Kenya, although the disease has been under control (Kagira et al., 2011), recent cases of T. b. rhodesiense sleeping sickness were reported in tourists visiting the Maasai Mara (Wolf et al., 2012; Clerinx et al., 2012). The disease is caused by Trypanosoma brucei rhodesiense and T. b. gambiense. The inflammatory changes observed during trypanosomosis are caused by tissue damage stimulated by the presence of the parasites in the body, leading to infiltration of the tissues by inflammatory cells and production of pro-inflammatory cytokines (Ngotho et al., 2011; Maina et al., 2004). The host inflammatory responses produce free radicals and reactive oxygen species (ROS) to control parasite invasion and proliferation but the enhanced immune responses induce collateral tissue damage (Stijlemans et al., 2007). Free radicals destroy tissues by lipid peroxidation and are common in many pathological conditions. Tissue damage occurs as a result of the interaction between ROS and/or other free radicals with polyunsaturated fatty acids and exacerbated by the presence of divalent metal ions such as Fe from haemoglobin breakdown (Novo and Parola, 2008). Increased hepatic iron-levels such as in trypanosomosis where there is lipid peroxidation of red blood cell membranes and the subsequent breakdown of haemoglobin may exacerbate oxidative stress by inducing mitochondrial damage and destabilizing lysosomal membranes (Ramm and Ruddell, 2005). Since the liver is involved in the synthesis and regulation of most biomolecules in the blood, detoxification and drug metabolism, hepatocyte damage such as in chronic inflammation may lead to decreased synthesis of essential biomolecules, waste accumulation and impaired drug metabolism. The drugs used in the treatment of trypanosomosis are highly toxic to the host. Some drugs like melarsoprol cause post treatment reactive encephalopathy (PTRE) which can be fatal encephalitis in up to 10% of the patients treated...
with this drug (Pepin and Milord, 1994). Therefore anti-inflammatory drugs such as dexamethasone and diazepam are used to prevent encephalitic syndrome (Jennings, 1993). Synthetic steroidal and non steroidal anti-inflammatory drugs are important in reducing inflammation during chronic diseases such as trypanosomosis. However, these drugs have more toxic effects when compared to the anti-inflammatory herbal remedies which have fewer side effects in addition to being cheaper and possibly with better anti-inflammatory activity (Ravi et al. 2009).

*Solanum nigrum*, commonly known as the black night shade, family Solanaceae, has a cosmopolitan distribution and occurs especially in tropical and warm temperate regions in South America, Australia and Africa. *Solanum* extracts contain polyphenol antioxidants. A study on the radical scavenging activities of medicinal plants found out that the leaves of *S. nigrum* have the highest antioxidant activity compared with other parts of the plant (Prakash et al., 2007). Solanum extracts protect the liver from damage caused by carbon tetrachloride (CCl₄) (Lin et al., 2008) and fibrosis induced by thioacetamide (Hsieh et al., 2008) and this protection is attributed to the presence of polyphenols such as flavonoids in the extract. The most potent flavonoids in protecting the body against oxidative tissue damage are flavones and flavonols due to their increased capacity to quench various radicals and reactive oxygen species (Harborne and Williams, 2000). The flavonoids synergize the endogenous scavenging antioxidants such as catalase and glutathione (Havsteen, 2002). The mechanism of protection by flavonoids is through interruption of the lipid peroxidation chain reaction thereby preventing glutathione depletion (Umar et al., 1999).

Considering the potential anti-inflammatory effects of *S. nigrum*, we evaluated the hepatoprotective activity of crude water extracts of *S. nigrum* in a mice model of trypanosomosis and the consequent lengthening of survival time in *T. b. rhodesiense* infected mice. To the best of our knowledge this is the first study of the on the anti-inflammatory and hepatoprotective activity of *S. nigrum* in trypanosomosis.

**MATERIALS AND METHODS**

**Collection and extraction of the plants**

The *S. nigrum* leaves were collected just before they flowered from Kapchuriai sub location, Nandi East district, Kenya identified by a plant taxonomist at the Department of Biological Sciences of Egerton University. A leaf sample was deposited at the botany herbarium (VN38). The leaves were air dried in the shade for a period of two weeks and ground into powder using a grinding mill and stored in water tight containers before extraction. Extraction was done as described by Lin et al., (2008) with modification in concentrating the extract which was done by freeze drying using Edwards Modulyo freeze dryer (EF4). The intake of the extract by mice was improved by adding 10% sucrose.

**Ethical approval and Toxicity studies**

All the animal experiments were carried out with ethical approval from and as per the Institutional Animal Care and Use Committee (IACUC) guidelines. The toxicity of the extract was tested in twenty five male Swiss white mice weighing between 24 to 28g which were randomly divided in five groups of five mice each. The toxicity of the extract was tested for 10 days *in vivo* and the mice monitored for a further 4 days without treatment with the extract for overt toxic responses. The doses of the extract are as shown below:

- Group i; 250mg/kg body weight (bwt)
- Group ii; 500mg/kg bwt,
- Group iii; 1000mg/kg bwt
- Group iv; 2000mg/kg bwt and
- Group v; was given water only as a negative control.

**Experimental mice**

Male experimental mice weighing between 24-28g were received from Kenya Medical Research Institute (KEMRI) and acclimatized in the laboratory for a period of two weeks. The mice were kept in standard rodent cages and were fed on mice pellets (Mice pencils®, Unga feeds Kenya) and water *ad libitum*. They were maintained at an ambient temperature of between 20 – 25°C, and wood shavings were used as beddings. The mice were dewormed with ivermectin (Ivermectin®, Anupco, Suffolk, England) at dosage of 300µg/Kg to clear any helminth and ectoparasitic infection.

**Ethical Approval**

All protocols involving the use of the laboratory animals were approved by the research ethics committee of TRC.
Trypanosomes

*T. b. rhodesiense* (KETRI 2537) initially isolated from a sleeping sickness patient was obtained from the trypanosome cryobank of the Trypanosomosis Research Centre (TRC) of the Kenya Agricultural Research Institute (KARI), Muguga.

Infection and treatment

KETRI 2537 parasites were propagated in two donor mice before the experiment after immunosuppression with 200mg/kg of body weight of cyclophosphamide for three consecutive days as described by Kagira et al., (2005). The experimental mice were randomly divided into 5 groups of 12 mice per group, and each group of mice housed separately. They were then infected with $10^4$ trypanosomes intraperitoneally. The infected groups were treated orally with decreasing concentrations of SNE so as to determine the effect of dose on the anti-inflammatory activity. They were treated according to table 1. The survival rates of the mice were then monitored. One mouse was sacrificed per group every 10 days up to day 50 post infection and their liver tissues preserved in 10% formalin solution. At the termination of the experiment on 97 dpi the surviving mouse was euthanized in chloroform and the livers harvested and preserved in formalin.

### Table 1: Treatment of trypanosome infected mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>10 mg/ml SNE</td>
</tr>
<tr>
<td>2</td>
<td>6.7 mg/ml SNE</td>
</tr>
<tr>
<td>3</td>
<td>3.3 mg/ml SNE</td>
</tr>
<tr>
<td>4</td>
<td>0.02mg dexamethasone</td>
</tr>
<tr>
<td>5</td>
<td>Water only</td>
</tr>
</tbody>
</table>

Liver histopathology

Liver histopathology procedures were carried out according to the protocol described by Keita et al., (1997) where approximately 1cm³ block portions of the fresh livers fixed in 10% buffered formalin were used. The tissue samples were dehydrated by passing the tissues through a series of increasing alcohol concentrations of 70%, 95% and 100% ethanol for 2 hours each. Ethanol was then cleared using 50:50 ethanol/xylene then pure xylene for 2 hours. The tissue were then transferred into a 50:50 xylene/paraffin followed by pure paraffin in an oven at 56-58°C for 1 hour then into a second container of melted paraffin for an additional 2 hours for the tissue block to be embedded in molten paraffin wax. The tissues were then placed into an embedding mold and molten paraffin poured into the mold to form a block. The blocks were then allowed to cool and sectioned into 5µm sections using a microtome and the processed tissues were mounted on a slide and stained with haematoxylin and eosin stain before microscopic examination for pathological alterations.

Data analysis

The Kaplan-Meier method was used for determination of survival distribution functions of the experimental and control mice. Rank statistics for testing homogeneity of survival curves were used to determine the effect of treatment of *T. b. rhodesiense*-infected and untreated control mice on early (during early phase of infection) and larger (during late phase of infection) survival times.

RESULTS

Effect of SNE on survival time

Figure 1 shows the survival distribution functions of *T. b. rhodesiense* infected mice treated with SNE and dexamethasone, and infected untreated controls.
The survival of the *T. b. rhodesiense*-infected mice ranged from 0-50, 0-85, 0-97, 0-68 and 0-60 days for 3.3, 6.7, 10mg/ml SNE, 0.2 mg dexamethasone and untreated infected controls respectively. The P-values associated with tests of homogeneity (Log-Rank and Wilcoxon) were less than 0.05. This indicates that the overall survival times of mice infected and treated with different concentrations of SNE were significantly different (*P* < 0.05) with SNE treated mice surviving longer than the infected untreated mice. The survival time was dependent on the concentrations of SNE taken by the mice. There was significant difference in time points in the death of mice following infection. At 60 dpi, 100% of the untreated mice and those treated with 3.3mg/ml SNE still survived while 20% of those treated with 0.2mg of dexamethasone and 10mg/ml SNE still survived at this time. Surprisingly, 80% of mice treated with 6.7mg/ml SNE still survived at 60 dpi. However, by 85 dpi all the experimental mice had died except 20% of those with 10mg/ml SNE which survived till the termination of the experiment on 97 dpi. The survival distribution functions for the mice infected and treated with 0.02mg/kg body weight of dexamethasone and the infected untreated controls showed no significant difference (Log-rank *P* >0.05) in the survival times.

**Figure 1**: Survival distribution function of *T. b. rhodesiense* infected male mice treated with different concentrations of SNE or dexamethasone and infected untreated controls.

**Effects of SNE on liver pathology**

General liver morphological changes induced during trypanosomosis were observed. These included degenerative changes of the hepatocytes and presence of inflammatory cells. Livers of infected mice showed progression of histological changes with time involving necrosis of hepatocytes and macrophage infiltration in liver parenchyma compared to the controls. The SNE treated mice had minimal histological changes over time. On day 30 dpi the infected SNE treated and dexamethasone treated mice had minimal infiltration of inflammatory cells (IC) compared to the infected untreated controls. There was more intensive degeneration of hepatocytes and presence of more macrophage inflammatory cells in infected dexamethasone treated and untreated mice on day 50 pi. The dexamethasone treated mice had moderate infiltration of macrophages compared to the infected untreated controls which had massive infiltration especially mononuclear macrophages (Figure 2). Infected mice treated with 10mg/ml SNE had minimal infiltration of mononuclear macrophages on 50 dpi compared to the infected untreated and the infected dexamethasone treated mice). One mouse treated with 10mg/ml SNE survived till 97 dpi when the experiment was terminated and histological sections of their livers had no lesions or cellular infiltrations.

DISCUSSION

This study showed that water extracts of *S. nigrum* ameliorated parasite induced inflammatory tissue damage prolonging host survival in trypanosome infected mice in a dose dependent manner. The liver of SNE treated mice had minimal infiltration by inflammatory cells while the dexamethasone treated and untreated mice had massive infiltration by the inflammatory cells. Tissue damage especially the liver, kidney and the heart tissues in trypanosomosis is a common occurrence in the infected mice especially in the late phase of the disease. Use of phytochemicals with antioxidant properties is one of the novel approaches to effective therapeutic option for the treatment or prevention of tissue damage (Vitaglione et al., 2004, Karori et al., 2008). Different phytochemicals such as those from tea have been used to ameliorate trypanosome induced inflammatory tissue damage and also have shown anti-trypanosomal activity against *Trypanosoma brucei brucei in vivo* (Karori et al., 2008, Mbuthia et al., 2011). Indeed phytochemicals from *S. nigrum* have been shown to ameliorate tissue damage from CCL4 induced tissue damage (Lin et al., 2008) which damages tissues by inducing inflammation in the same way as in trypanosomosis. This could be due to the effects of polyphenolic compounds such as flavonoids present in SNE which have been shown to have anti-trypanosomal activity against different developmental stages of *T. cruzi* and *T. b. rhodesiense in vitro* (Chataing et al., 1998; Tasdemir et al., 2006).

Tissue damage in trypanosomosis results from stimulation of inflammatory cytokines of the endotoxin-like molecules produced by the trypanosomes (Ngure et al., 2009). Cytokines activate inflammatory cells such as neutrophils, macrophages or monocytes, platelets and mastocytes which release large amounts of toxic oxidizing radicals. These radicals include nitric oxide (NO) and superoxide (O2-) which causes cellular injury via several mechanisms including the peroxidation of membrane lipids and the oxidative damage of proteins and deoxyribonucleic acid (DNA) (Bendich, 1996; Neviere et al., 1999). Indeed in the management of encephalopathic syndrome dexamethasone and diazepam are recommended as anti-inflammatory drugs (Jennings, 1993). However, these synthetic drugs induce toxic side effects such as insomnia, indigestion and allergic reactions.

Figure 2: Liver tissue of uninfected untreated control mouse; b) Liver tissue of infected SNE treated mouse showing minimal infiltration on 50 dpi; c) Liver tissue of infected dexamethasone treated mouse showing massive infiltration of macrophages on 50 dpi. d) Liver tissue of infected untreated mouse showing massive infiltration (Haematoxylin and Eosin Mag =×200).
Mice treated with *S. nigrum* had a longer survival time than those treated with dexamethasone, a known anti-inflammatory drug used in amelioration of inflammation during human African trypanosomosis. Amelioration of host inflammatory responses especially in the late stage of trypanosomosis is important since sustained inflammatory responses lead to tissue damage in vital organs such as the liver, heart, and kidneys and a focus on the mechanisms involved in the induction and/or prevention of pathology might provide new innovative combination therapy strategies (Bosschaerts et al., 2009).

The prolonged survival time of the mice treated with *S. nigrum* could be due to the effects of SNE flavonoids which ameliorates the inflammatory reactions due to their antioxidant activity (Havsteen, 2002, Ravi et al., 2009). The ability of the flavonoids to complex with parasite membranes thus disrupting them and this could be among the reasons for increased survival time for the mice treated with higher concentrations of SNE (Tsuchiya et al., 1996). The observed anti-inflammatory effects of SNE could be through down regulation of lipid peroxidation and enhancement of the antioxidant enzymatic activities such as superoxide dismutase and catalase (Manna et al., 2006). Earlier studies have shown that phytochemicals in tea ameliorates inflammatory liver damage during trypanosomosis (Mbuthia et al., 2011).

In conclusion this study showed that water extracts of *S. nigrum* increased the survival time of mice infected with trypanosomes with more enhanced anti-inflammatory activity than dexamethasone and is a candidate for use in prevention of trypanosome-induced liver damage. Further studies should be conducted to determine the anti-inflammatory effects of extracts from *S. nigrum* in controlling the post treatment encephalopathy caused by melarsoprol during treatment of second stage of HAT.

**Competing interests**
The author(s) declare that they have no competing interests.

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**REFERENCES**


