Antitrypanosomal Effect of Neem (Azadirachta indica) Extract in rats infected with Trypanosoma brucei

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ABSTRACT
Antitrypanosomal investigation of medicinal plant Azadirachta indica traditionally used to treat malaria in Nigeria was evaluated for its in vitro activity against Trypanosoma brucei in albino rats. The selected plant was identified and extracted with water and ethanol and evaluated for antitrypanosomal activity against T. brucei. The rats were divided into four groups (I, II, III, IV) of three rats per group. Groups I, II and III were inoculated intraperitoneally with 0.5ml of 1.0 x 10³/ml of T. brucei diluted with phosphate buffered saline. Groups II and III were treated daily with 20.0mg/kg body weight with aqueous and ethanol extracts of A. indica respectively, while group I was not treated. Group IV served for control and was neither infected nor treated. The parasitaemia and weight of the rats were recorded at start of the experiment and subsequently at three days interval for a period of 27 days. There was no significant different (P>0.05) observed between the mean distribution of parasitaemia in treatment control when compared with the treated groups. There was a drop in the mean weight of the infected rats from day 3 to day 27 post infection, while the mean weight of non-infected group increased. The result of the study therefore suggests that Azadirachta indica had no curative effect against Trypanosoma brucei inoculated in the albino rats.

Keywords: Neem extract, antitrypanosome, rats, Trypanosoma brucei

INTRODUCTION
African trypanosomiasis is an infectious disease restricted to Africa which affects domestic and game animals including man. The causative agent is Trypanosoma- a protozoan parasite transmitted cyclically by different species of tsetse fly of the genus Glossina. Trypanosome vivax, T. congolense and to a lesser extent T. brucei, are the main species of Trypanosoma of importance in livestock (Abenga et al, 2002). These species cause Animal African Trypanosomisis (AAT). The disease constitutes a menace to the livestock industry leading to severe reduction in productivity as a result of failure of livestock to utilize available food as efficiently as healthy animals (Itard, 1989). Human African trypanosomiasis (HAT) in man is referred to as sleeping sickness, and it is caused by Trypanosoma brucei subspecies T. b. gambiense and T. b rhodesiense, while T. cruzi is the aetiology of South American trypanosomiasis (Chagas diseases). The World Health Organization estimates about 60 million people at risk of sleeping sickness in Africa (WHO, 2001) and only fraction of this is under surveillance in having access to health centre for screening and accurate diagnosis. It is estimated that 300,000 to 500,000 people are currently infected and about 100,000 people die annually (WHO, 2001). The disease causes abortion, sterility and gynaecological disorders in women while it is responsible for physical and intellectual growth retardation in children.
Majority of countries in the tsetse belt maintained a sustained low endemicity of trypanosomiasis for several years since 1960 (Fairlamb, 1990). However, due to severe budgetary and operational constraints faced by health services insecticide spraying were gradually phased out and subsequently villages were overgrown and infested with tsetse flies (Pepin and Milord, 1994). Drug treatment remains the only means of intervention. The current standard treatment in man for first stage disease is intravenous pentamidine or melarsoprol (for T. b. gambiense), or intravenous suramin for T. b. rhodensiense (Pepin and Mpia, 2006). In livestock isometamidium chloride, Homidium Chloride, Homidium bromide and suramin, are widely used drugs in prophylaxis and treatment of T. b. brucei infectious. Diminazene aceturate (Berenil) is effective against all three African animal trypanosomes (Onah, 1991).

Some factors like problem of drug resistance, adverse side effects and relapse development have limited the availability and choice of effective drugs (Egwu et al., 1993). These challenges have necessitated studies towards the development of more effective, safer and cheaper trypanocidal drugs (Atawodi et al., 2003; Ohaeri and Ezechukwu, 2010), especially from medicinal plants. The plant Azadirachta indica (Neem tree) is a member of mahogany family (Meliaceae) which includes a large array of tropical trees and shrubs native to both the old and New World (Conrick, 1994; Puri, 1999). The spreading deciduous Neem tree grows to a height of 40 to 80ft (12 to 25m). The leaves are dark green and slender with resin secreting glands on young leaves near the shot apex. The bark of young branches is green, but grey black on the main trunk (Puri, 1999). Extracts of Neem, often called "Nature's drugstore" have been used in medicine for over 2,500 years (Conrick, 1980) and perhaps much longer (Crane et al., 1994; Puri, 1999). Neem component like quinion has been used for cure of malaria for many years and have recently been in reach for cure and control of other protozoan blood parasite using various extract of which aqueous and ethanol extracts are involved in this current research works. The objective of the study is therefore to investigate the antitrypanosomal effect of A. indica extracts on Trypanosoma brucei infection in albino rats and to compare the efficacy of ethanol and water extract of A. indica on the infection.

MATERIALS AND METHOD

Collection and identification of plants
Fresh specimens of the plant Azadirachta indica (Plate 1) were obtained from Umudike, Umuahia Villages where they were ploughed out from branches of the tree. The plant was identified by consulting literature (Conrick, 1980) and was equally confirmed by Dr G. E. E. Osuagwu of the Department of Plant Science and Biotechnology, Michael Okapra University of Agriculture, Umudike, Abia State Nigeria. Neem was selected for this research work because of its medical properties in the field of medicine, agriculture and industries (Lake et al., 1990).

Preparation of plant extracts
Fresh plant materials were collected, washed with clean water and allowed to dry. Then the leaves and some part of the axial stems were milled with Arthur Thomas milling machine to obtain a fine powdery plant sample. The powdery plant sample was subjected to extraction.
For aqueous extract, 10g of the powder was soaked in 100ml distilled water (Uhegbu and Ogbuehi, 2004) and allowed to stand for 24 hours with occasional shaking. After 24 hours the resulting mixture was filtered using a muslin cloth and the resulting solution severed as the crude extract/stock solution. For ethanol extract, 10g of the powder was soaked in 100mls of 95% ethanol (Uhegbu and Ogbiehi, 2004) for 24 hours, after which the ethanol was evaporated completely. The left-over was then reconstituted with distilled water to equal concentration with the aqueous extract. Both aqueous and ethanol extracts were stored in a refrigerator to avoid spoilage of any kind.

**Determination of concentration of extract**

The actual amount of the substance in the extract (concentration) was determined by heating a known quantity of the solution to dryness and taking the weight of the residue left. The weight of the residue is expressed as a fraction of 1 ml to obtain the concentration (Ukegbi and Ogbuehi, 2004). The concentration of aqueous and ethanol extract were determined to be 10.3mg and 14.0mg per ml respectively.

**Experimental animals**

A total of twelve albino rats of mean weight 70.2g were used for the study. The rats were obtained from Department of Veterinary Parasitology and Entomology University of Nigeria Nsukka. The rats were left for two weeks for acclimatization before starting the experiment. They were fed with growers mash and clean water throughout the duration of the work.

**Source of the trypanosomes**

*Trypanosoma brucei* was obtained from the Department of Veterinary Parasitology and Entomology University of Nigeria Nsukka and was passaged in rats. The blood sample containing the parasites from an infected albino rat was diluted with phosphate buffered saline (pH7.4) to obtain the desired load of parasite for subsequent inoculation to other rats. Infection of experimental rats was by intraperitoneal inoculation with 0.5ml of blood containing $1 \times 10^3$ parasite per ml at 5th week old.

**Experimental design**

Before the start of the experiment, the albino rats were divided into four groups of three rats per group as follows: Group I: *T. brucei* infected rats with no extract treatment  
Group II: *T. brucei* infected rats with aqueous extract treatment
Group III: *T. brucei* infected rats with ethanol extract treatment

Group IV: Uninfected rats with no extract treatment

The weight of each rat was recorded at the start and at the end of the experiment and the mean weight of each group obtained by calculation. In the course of the experiment, the level of parasitaemia was monitored at 3 days interval together with the mean weight of the rats. Signs of the disease and mortality were recorded and the experiment was terminated at 4th week after commencement of treatment.

**Administration of extracts**

Administration of extract commenced three days post infection. The extracts were administered daily to the rats orally using a syringe, at a dosage 20.0 mg/kg weight of albino rat.

**Determination of parasitaemia**

Post infection parasitaemia was determined at three days intervals. The technique adopted for determination of parasitaemia was the rapid matching procedure as described by Herbert and Lumsden, (1976). A drop of blood was placed on a clean grease free slide and covered with cover slip avoiding air bubbles. The slide was then examined under the microscope using x 40 objective and the trypanosomes counted. The trypanosomes were detected in the wet mount by their characteristic movement. The number of trypanosomes in each field counted was matched with the log figures and absolute values of numbers of trypanosomes were obtained from the table. Data generated were analysed using Student $t$-test.

**RESULTS**

The results of this study showed that the weight of the *Trypanosoma brucei* infected rats decreased slightly ($P<0.05$) by the end of the experiment, while the non-infected rats increased in weight (Table 1). The neem extract treatment had no effect on the weight reduction of infected rats when compared with the control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial mean weight</th>
<th>Final mean weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected not treated</td>
<td>69.5 ± 0.31</td>
<td>60.33 ± 1.00</td>
</tr>
<tr>
<td>Infected treated with aqueous extract</td>
<td>69.83 ± 0.04</td>
<td>63.99 ± 0.23</td>
</tr>
<tr>
<td>Infected treated with ethanol extract</td>
<td>70.03 ± 0.10</td>
<td>62.12 ± 1.02</td>
</tr>
<tr>
<td>Not infected not treated</td>
<td>71.09 ± 0.02</td>
<td>79.23 ± 0.14</td>
</tr>
</tbody>
</table>

The antitrypanocidal effect of aqueous and ethanol extracts of *Azadiracheta indica* on the mean parasitaemic level of albino rats infected with *Trypanosoma brucei* are presented in Table 2. Generally, the parasitaemia recorded in the three groups of infected rats increased (log number of parasites per ml of blood) till end of experimental period, day 27 post infection. The extract treatment had no effect ($P>0.05$) on the parasitaemic levels of infected rats when compared with the untreated group. Statistically the analysis of the result showed that there was no significant difference ($P>0.05$) between the mean distribution of parasitaemia in the infected rats treated...
with aqueous and ethanol extracts. The level of parasitaemia increased progressively in all the infected groups throughout post infection period.

Records on symptoms of nagana and mortality of the experimental rats were kept, but no significant differences were established in the reactions to infection of treated and untreated control (Table 3). Mortality was observed in both treated and untreated infected groups as against uninfected control.

Table 2: Antitrypanocidal Effect of *Azadirachta indica* on the Parasitaemia (log Number of parasites per ml of blood) of Albino rats infected with *Trypanosoma brucei* (mean ±SE)

<table>
<thead>
<tr>
<th>Days</th>
<th>Infected not Treated</th>
<th>Treated with water extract</th>
<th>Treated with ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
</tr>
<tr>
<td>6</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
</tr>
<tr>
<td>9</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
</tr>
<tr>
<td>12</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
<td>&lt;5.4±0.00</td>
</tr>
<tr>
<td>15</td>
<td>5.5±0.10</td>
<td>5.6±0.17</td>
<td>5.4±0.07</td>
</tr>
<tr>
<td>18</td>
<td>5.8±0.17</td>
<td>5.7±0.60</td>
<td>5.5±0.00</td>
</tr>
<tr>
<td>21</td>
<td>6.2±0.20</td>
<td>5.9±0.45</td>
<td>6.0±0.29</td>
</tr>
<tr>
<td>24</td>
<td>6.4±0.70</td>
<td>6.7±0.10</td>
<td>6.1±0.10</td>
</tr>
<tr>
<td>27</td>
<td>7.4±0.38</td>
<td>7.7±1.22</td>
<td>7.2±0.20</td>
</tr>
</tbody>
</table>

Table 3: Mortality of albino rats infected with *Trypanosoma brucei*

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial number</th>
<th>Final number</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected not treated</td>
<td>3</td>
<td>2</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Infected treated with aqueous extract</td>
<td>3</td>
<td>1</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td>Infected treated with ethanol extract</td>
<td>3</td>
<td>2</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Not infected not treated</td>
<td>3</td>
<td>3</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Trypanosomiasis, which comprises of a group of widespread infectious conditions, produces grave manifestations of disease. Chief among these are the human infections with *Trypanosoma gambiense* and *T. rhodesiense* which cause sleeping sickness of Africa and the animal infections due to *T. brucei*, *T. vivax* and *T. congolense*. Of these organisms, *T. brucei*, which causes the nagana of wild and domestic animals of Africa was used as the experimental parasite. Experimental trypanosomiasis involving chemotherapeutic investigations provides a subject of immeasurable contributions to the literature. Chemotherapy is the only efficient and effective tool to cure and control trypanosomiasis, as efficacious vaccines against trypanosomes have not been developed so far. Indiscriminate use of synthetic antitrypanosomes in domestic animals has resulted in the development of resistance in the parasites. Added to this, adverse reactions, high cost, and inaccessibility to the rural farmers are problems associated with these agents. Consequently, there is an urgent need to develop newer, selective, and eco-friendly agents to
control trypanosome infections. Plant-based extracts offer an alternative to overcome some of these problems and they can be both sustainable and environmentally acceptable.

The result of this study indicates that both the aqueous and ethanol extract of Azadirachta indica had no antitrypanocidal effect with oral administration in rats. This is reflected in the increase in the level of mean parasitanemia in all the groups of infected albino rats with or without extract treatment, which continued till the end of experiment on day 27 post infection. This continuous increase in parasitaemic level could be responsible for weight loss in the Trypanosoma infected rats (Itard, 1989) as a result of parasite induced inappetance (Ivoke, 2005; Ohaeri, 2010). This increase in parasitaemia is an indication that extract of A. indica is not effective against trypanosomiasis. The aqueous and ethanol extract of the plant treatment of infected rats did not show any significant difference when compared with the infected untreated control (P>0.05). However, some infected animals treated and untreated with the extract equally died before end of experimental period. This further suggests that the neem extract had no antitrypanosomal effect on experimental rats. The inability of the extract to clear the parasite from the blood could be due to inability to reach the site of action or rapid metabolization (Dwivedi, 1997; Wurochekke et al., 2005).

The rats were counted each day after inoculation. A certain proportion of rats showing some of the signs of nagana infection died from 3 weeks after inoculation. At 2 weeks, they become emaciated and much weakened and ate but little. A number of infected animals in the same cage huddled together in one corner as if they were cold. The various signs of the infection were noted from 7th day after inoculation. These were bulging of the upper eyelids, swelling of the lips, redness and slight thickening of the base of the ears, breathing difficulties indicating lack of oxygen, convulsion and finally death. The death of the animals was not as a result of the extract treatment when compared with the untreated group, it could rather be as a consequence of the outcome of the infection, which could be intravascular obstruction of the circulation resulting in asphyxia. Regular active surveillance, involving case detection and treatment in addition to tsetse fly control, is the backbone of the strategy for control of trypanosomiasis.

CONCLUSION
From the findings of this research, it could be concluded that the aqueous and ethanol extracts of Azadirachta indica had no antitrypanocidal effect on rats infected with Trypanosoma brucei, despite its numerous other medicinal properties and values. It also implies that trypanosoma brucei infection reduces the weight of the experimental rats.

REFERENCES


