Phytochemical Screening And Effect Of Senne-Siamea Methanolic Leave Extract Against Trypanosoma Brucei Brucei In Albino Rat In-Vivo


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ABSTRACT: A two week study was carried out on the anti-trypanosomal activities of the leave extract of senna siamea which was obtained using Soxhlet extraction technique. The in vitro study of anti-trypanosomal activity of the crude extract was conducted using Atawodi S.E, 2005 method on albino rats. Two groups of infected rats were treated with 2mg/Kg of the methanolic extract of the leave of senna siamea resulted in the reduction parasite without any weight lostmaking the extract a potent trypanocidal agent while another group was treated with 1.5mg/Kg of diminazene aceturate which served as a positive control, one group served as a negative control method and the last group was uninfected and untreated. The study also investigated the qualitative phytochemical constituents of the methanolic extract of the leave of Senna siamea and the phytochemicals present are: tannin, saponins and alkaloid.

Key word: Senna Siamea,Methanolic extract,Trypanosoma brucie brucie

INTRODUCTION
The commonest and the most economically important animal protozoan disease in Africa are caused by trypanosoma species (T. congolense, T.vivax, T. simiae and T. brucei). Trypanosomes are flagellates that live in the blood and tissues of vertebrates [9]. Almost all animal species and man, except poultry, are affected. African trypanosomes cause a fatal disease commonly called “sleeping sickness” in humans and Nagana in animals [4, 5]. They are commonly transmitted cyclically by tsetse fly (genus glossina). Example of trypanosoma disease that is transmitted non-cyclically (mechanically) are biting insects in “sura” in horses and camels which caused by T. evansi [3, 9]. Chagas disease which occurs in central and South America, is caused by T. cruzi with bugs of the reduviidae family as vectors. T. equiperdum, this causes a venereal disease, dourine in horses and donkeys, is transmitted directly during coitus. Trypanosomes can also be spread by formites including surgical instruments, needles and syringes [2, 8]. Trypanosoma brucei brucei is a species of animal parasite called salivery trypanosome that causes African Trypanosomiasis, known also as ‘sleeping sickness’ in humans and Nagana in animals. T.brucie has traditionally been grouped into three subspecies: Trypanosoma brucei gambiense and trypanosoma brucei rhodesiense and trypanosoma brucei brucei. [21]. It is a complex debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse transmitted protozoan parasites of the genus trypanosone [3]. The most important species responsible for the disease is commonly known as “Nagana” in livestock includes, trypanosome brucei, T. congolense and T. Vivax. African animal trypanosomiasis represents one of the most serious veterinary problems in the world [19].

EXPERIMENTAL METHOD
PLANT COLLECTION AND IDENTIFICATION
The plant samples were collected from ‘Area A’ quarters of Ahmadu Bello University and authenticated at Biological Department of Ahmadu Bello University, Zaria. The plant leaves were dried and grinded into fine powder and kept in an air-tight container with proper labeling.

PREPARATION OF PLANT MATERIAL
The plant leaves were airdried to a constant weight and placed in the oven at a temperature of about 110°C for 15minutes. The dried plants were grinded into coarse form using a pestle and a mortar and then reduced to a powdery form using a mill.
EXTRACTION OF PLANT MATERIAL
Exactly 30g of the powdery plant material was weighed extracted using the Soxhlet extractor apparatus for 24hours. Extract was concentrated and kept in an air-tight container in the laboratory until needed.

PHYTOCHEMICAL ANALYSIS OF THE PLANT.
Phytochemical screening of the crude extract of the plant was performed by the method of Sofowora, (1993) as describe by Edeoga et al., (2005). Test for presence of alkaloids, saponins, tannins, flavonoids were carried out.

TRYPANOSOME PARASITE
Trypanosoma brucei brucei (Federi strain) used for the research was obtained from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The parasite was maintained by continuous passage in other donor rats. Parasitaemia was monitored daily by preparing a wet mount and viewing under x400 magnifications (Herbert and Lumsden, 1976).

INOCULATION OF PARASITE FROM ALBINO RATS
Parasite infected blood was obtained from the tail vein of trypanosoma infected donor rats at peak of Parasitaemia.

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Albino Rat</th>
<th>Invitro Screening</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Extract treated 2mg/Kg</td>
<td>1</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 Pos.</td>
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</tbody>
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<thead>
<tr>
<th>Group 2</th>
<th>Albino Rat</th>
<th>Invitro Screening</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Extract Treated with 4mg/Kg</td>
<td>1</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2 Pos.</td>
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</tbody>
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<tr>
<th>Group 3</th>
<th>Albino Rat</th>
<th>Invitro Screening</th>
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<tbody>
<tr>
<td>Diminazene aceturate 2 mg/Kg</td>
<td>1</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 Pos.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3 Pos.</td>
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</tbody>
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<tr>
<th>Group 4</th>
<th>Albino Rat</th>
<th>Invitro Screening</th>
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</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1</td>
<td>3 Pos.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 Pos.</td>
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<tr>
<th>Group 5</th>
<th>Albino Rat</th>
<th>Invitro Screening</th>
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<tbody>
<tr>
<td>Uninfected and Untreated</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Key: Pos. = +

The collected infected blood was used to maintain parasite suspension in normal saline. The suspension containing four to eight trypanosomes per field under ×400 magnifications (about 10⁶ parasites/ml of blood) was inoculated into the peritoneal cavity of the uninfected rats.

IN-VIVO TEST
Fifteen albino rats were grouped into 5 groups of three per group from 1-5 were having 3,3,3,3 and 3 rats respectively. The method used by Atawodi, 2003 was adopted. The animal used were group as follow:

- Group 1: Received plant extract 2mg/Kg
- Group 2: Received plant extract 4mg/Kg
- Group 3: Received standard drugs (positive control)
- Group 4: Received no treatment (negative control)
- Group 5: Untreated and uninfected (Reference group)

THERAPEUTIC MONITORING
Parasitaemia of each rat was monitored every 24hrs during the administration of the treatment. Blood samples were collected from treated rats and a smear was prepared and viewed under a light microscope to check the effectiveness of the extract at different concentrations.
DISCUSSION
Phytochemical screening of methanolic extract of senna siamea revealed the presence of alkaloids, tannins and saponin. The trypanocidal property of the extract may be due to the action of one or more constituents present in the plant. Several workers have either identified or isolated tannins, flavonoid and alkaloid in plants that showed trypanocidal activities. The result of trypanocidal activity of the leaves extract of senna siamea is shown in table 2. The extract immediate mortality when given at a dose of 4mg/150ml distilled water by oral route indicating that the extract is relatively effective in curing trypanosomiasis in albino rats. A relative decrease was observed in the body weight of rats in group 4 (untreated) at day 3 of the experiment when compared to day 1 and mortality occurred at day 3 and 4. This is consistent with the findings of (Ali et al., 2009) which showed that infection with T. brucei brucei was associated with weight loss and mortality. The increase in body weight and no mortality observed in animals treated with Diminazene Aceturate demonstrated that Diminazene Aceturate protected the rats from trypanosome-induced parasite. The effect of Diminazene Aceturate on the body weight and no mortality on the rats may be due to the trypanocidal effect of Diminazene that reduced the parasite burden in the body. The relative increase in the survival time of rats treated with 4mg/150ml distilled water of the plant extract suggests that the extract could be useful in the management of African Trypanosomiasis.

CONCLUSION
Administration of methanolic extract of senna siamea increased survival period of rats infected with T. brucei brucei. It also reduced the level of parasitaemia in infected rats. However, further study is required to determine the active constituent in the extract and its mode of action,

REFERENCES


