In vitro studies on the anti-trypanosomal effect of *Jatropha gossypii*olia var gossypii*olia*


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ABSTRACT

The anti-trypanosomal potential of aqueous stem extract of *Jatropha gossypii*olia var gossypii*olia* was investigated in vitro and the resultant effect on the infectivity of *Trypanosoma congolense* in Wistar rats was evaluated. Motility and mortality of the parasite was monitored over different incubation periods (0 min. - 1 hr 45 min at 37°C) with serial concentrations of the extract (6.25 mg/ml, 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100 mg/ml) prepared in phosphate buffered saline (PBS, pH 7.2). Activity was recorded at the higher concentrations when compared with the standard drug, isometamidium chloride (samorin Merial). This dose dependent activity delayed the onset/establishment of *T. congolense* infection in infectivity test in rats with resultant increase in prepatent period. This suggests possible anti-trypanosomal effect of this extract in vivo.

Key words: in vitro; infectivity test; Jatropha gossypii*olia*; Trypanosoma congolense; Wistar rats

INTRODUCTION

Trypanosomosis is a zoonotic disease caused by unicellular parasites (trypanosomes) which are transmitted biologically by bites of tsetse fly or mechanically by tabanids and *Stomoxys* (Zinsstag and Scholling, 2003; Kuzoe, 1993; Martins and Richard, 2004). Vaccination against this disease is futile due to antigenic variation properties exhibited by this parasite (Cross, 1996; David and Mar, 2004) and effective treatment is saddled with reported drug resistance and toxicity (Onyezili and Egwu, 1995; Geerts and Holmes, 1998). Sleeping sickness is currently treated with only 4 available drugs (pentamidine, melarsoprol suramin and eflornithine) as reported by Kuzoe (1993). However, only melarsoprol and eflornithine are effective against cerebral trypanosomosis remains diminazene aceturate (Berenil) and isometamidium chloride (samorin). While novidium and imidocarp are readily available and where available are relatively expensive (Onyekwelu and Okwuasaba, 2006). Trypanocidal regimen still in trial stages (Bacchi et al., 1998). There have been attempts to evaluate different chemicals agent/drugs for their trypanocidal activity (Bodley et al., 1995; Bacchi et al., 1998). While there are equally attempts to evaluate trypanocidal activity of some African plants with interesting in vitro outcomes (Igweh, and Onabanjo, 1989; Owolabi, et al., 1990; Freiburghaus et al., 1997 Atawodi et al., 2003; Abdulkadir et al., 2005).

Recent reports of some medicinal plants with anti-trypanosomal activity were reported (Atawodi et al., 2002, 2003; Shamaki et al., 2004; Abdulkadir et al., 2005). *Jatropha gossypii*olia is a naturalized
Asian plant in Africa, which was reported to possess anti-malarial activity (Gbessor et al., 1989), immune modulatory effect, (Lolade, 2005) and pain management (Morton, 1981).

In this work we present the in vitro anti-trypanosomal potential of *Jatropha gossypifolia var gossypifolia* on *T. congolense* and the resultant effect on the parasite’s infectivity in rats.

**MATERIALS AND METHODS**

**Plants material**

The stem and leaves of the plant was collected from Kaltungo in Gombe state and was transported to Jos (with other parts of the plant) for identification at the department of Botany, University of Jos, Nigeria.

**Trypanosomes**

*Trypanosoma congolense* (karu strain) was obtained from the Veterinary and Livestock studies Division of the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom. This was maintained in a donor Wistar rat by serial passage until required for the work.

**Animal**

Mature Wistar albino rats of both sexes, averagely weighing 102 g body weights, were obtained from the small animal units of the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom and kept in cages for the period of the study. They were fed with grower mash (Vital feed Ltd. Bukuru, Jos, Plateau State, Nigeria) and water *ad libitum*.

**Sample extraction and preparation**

Fresh stem of *Jatropha gossypifolia var gossypifolia* was collected and pounded in a laboratory mortar into small particles. Two hundred grams of the pulverized stem was weighed and extracted in 1,000 ml distilled boiling water (100°C) for 10 minutes. It was allowed to stand and cool for 24 hrs then filtered twice using muslin cloth and size 1 Whatman filter paper into a clean conical flask. The filtrate was dried in an electric drier at 40°C for 24 hrs as described by Abdulkadir et al. (2005). The dark brown paste (extract) collected at the end of the drying was kept in a clean closed glass until required for the experiment. One gram of the extract was dissolved in 10 ml of PBS (PH 7.2) to give 100 mg/ml which was used as a working stock. This was prepared just before the *in vitro* experiment commenced by preparing serial dilution of 100 mg, 50 mg, 25 mg, 12.5 mg and 6.25 mg/ml respectively. Isometamidium chloride (samorin, MERAL France) was used as a standard trypanocidal drug at dose of 10 mg/ml.

**In vitro test for anti-trypanosomal activity**

*In vitro* anti-trypanosomal activity of the aqueous extract was assessed in test tubes was set in duplicates (Atawodi et al., 2003). Parasitized blood was collected from a donor rat and diluted (1:4) in a phosphate buffered saline with glucose equal to 2.0 x 10⁶ *T. congolense/ml* of blood. This was kept in a separate test tube surrounded by ice cubes to maintain the inoculums. One ml of PBS was pipetted into 7 preset clean test tubes in duplicates. Test tubes 6 and 7 served as standard trypanocidal drug (isometamidium chloride) and PBS controls respectively. One ml each of the serially diluted extract was added into 5 of the test tubes. The 6th test tubes has standard trypanocidal drug. The 6th and 7th test tubes serve as controls. Zero point five ml of the blood containing approximately 2.0 x 10⁶ parasite/ml was added to each of the test tubes and were incubated in a water bath at 37°C over a period of 105 min and microscopic observations at x40 magnification was made at intervals of 5 min for motility and mortality of the parasites.
Infectivity test

Assessment of infectivity was performed (as a confirmation of in vitro test) using 21 Wistar rats weighing averagely 102 g. They were divided into 7 groups of 3 rats each. Zero point two ml of mixture from each of the in vitro test-tubes (1-7) was inoculated intraperitoneally into rats in different groups. Parasitaemia was monitored daily in blood obtained from the tail, pre-sterilized with methylated spirit, after 24 hrs of inoculation. Parasitaemia was determined microscopically at x40 magnification using the rapid matching method of Herbert and Lumsden (1976), until termination of the experiments. Behavioral changes were equally monitored.

RESULTS

The in vitro effect of Jatropha gossypiiifolia var gossypiiifolia on T. congolense is represented in Table 1, aqueous stem extract of Jatropha gossypiiifolia var gossypiiifolia harvested from Kaltungo, a savannah region of north-eastern Nigeria was evaluated for in vitro and activity against T. congolense parasites at concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml respectively. Complete elimination, or reduction in parasite motility was used as indices of trypanocidal activity. The extract resulted in complete cessation of motility at 30 min of incubation with 50 and 100 mg/ml of the extract, which was noted to be similar with the standard trypanocidal drug at 0.1 mg/ml at the termination of the experiments (105 min) only. The test tubes that contains lower concentrations of 12.5 and 6.25 mg/ml recorded sluggish motility throughout the study period. This dose dependent activity of the extract in vitro was observed in the in vivo infectivity test. Animals inoculated with 25-50 mg/ml showed delayed onset of parasitemia when compared with untreated control, while 100 mg/ml test tube group showed no parasitemia beyond 60 days observation period, which indicates the concentrations that gives lower and higher in vitro activity. Therefore, dose dependent activity of the extract in the studies, confirming further, in infectivity test, what was observed in vitro. While groups inoculated with mixture, containing 50 mg/ml, 25 mg/ml, had recorded longer prepatent period when compared to controls and the groups inoculated with inoculum containing lower doses of 12.5 mg/ml and 6.25 mg/ml had very short prepatent period as compared to the controls. Rats inoculated with mixtures containing standard trypanocidal drugs do not show parasitemia and the rats in these groups survived beyond 60 days.

Table 1. In vitro anti-trypanosomal activity of the aqueous stem extract of Jatropha gossypiiifolia var gossypiiifolia

<table>
<thead>
<tr>
<th>Incubation Time (minutes)</th>
<th>Parasitized blood (control)</th>
<th>Concentrations (mg/ml) of Extract (100-6.25) and samorin (0.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100  50  25  12.5  6.25  0.1</td>
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<tr>
<td>0</td>
<td>+++</td>
<td>+++  +++  +++  +++  +++  -</td>
</tr>
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<td>10</td>
<td>+++</td>
<td>-    +    +    +++  +++  -</td>
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<tr>
<td>15</td>
<td>+++</td>
<td>-    -    +    +++  +++  -</td>
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<td>30</td>
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<td>D    D    -    +++  +++  D</td>
</tr>
<tr>
<td>60</td>
<td>+++</td>
<td>D    D    -    +    ++  D</td>
</tr>
<tr>
<td>90</td>
<td>+++</td>
<td>D    D    D    +    ++  D</td>
</tr>
<tr>
<td>105</td>
<td>+++</td>
<td>D    D    D    +    +  D</td>
</tr>
</tbody>
</table>

"+++" = highly motile "++" = very motile "+" = motile "+ -" = sluggish, "- -" = very sluggish, "- - -" = highly sluggish, "D" = Dead/not motile
DISCUSSION

The results of the in vitro investigation of *Jatropha gossypifolia var gossypifolia* at 100 mg/ml recorded trypanocidal potential against *T. congolense* comparable to standard drug-isometamidium chloride, and prevented the establishment of *T. congolense* infection in rats. However, the trypanocidal effect of this plant appears to be dose dependent. This present study agrees with earlier reports of some selected African medicinal plants with similar interesting results against trypanosomiases in vitro (Freiburghaus et al., 1997 Owolabi et al., 1990; Abdulkadir et al., 2005; Atawodi et al., 2003). This present in vitro studies agrees with these earlier reports with absence of motility within 30 minutes of experiments which is same with standard drug. Plant showing potential anti trypanosomal activity has been reported to possess alkaloids. Earlier phytochemical studies of this plant revealed the presence of alkaloids and steroids (Shamaki et al., 2007). Although most plants may show promising trypanocidal activity in vitro, there may be difference in activity when orally administered (Freiburghaus et al., 1996).

The dose dependent response of this extract with complete loss of infectivity of *T. congolense* at 100 mg/ml and the delayed onset/establishment of infection at lower dose concentrations indicate some apparent effect on the parasites ability to multiply, however the mechanism of this activity, though unknown, needs to be investigated (Eghianruwa et al., 2004). Further work is needed to authenticate the possibilities of the extrapolation of this result with in vivo experiments.

In conclusion, the plant extract of *Jatropha gossypifolia var gossypifolia* showed some dose dependent trypanocidal activity in both in vitro and infectivity studies, with the highest dose of 100 mg/ml showing higher activity similar to standard trypanocidal drug, samorin (0.1 mg/ml), when compared to control, while lower doses of the extract of 12.5 mg/ml and 6.25 mg/ml showed the least trypanocidal activity when compared to control.

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REFERENCES


