

## Synergy of *Azadirachta indica* Seed and *Tridax procumbens* Leaf Extracts Induced Death of *Trypanosoma evansi*

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**Abstract:** *Trypanosoma evansi* (*T. evansi*) is the causative agent of trypanosomosis disease that affects animal productivity and health. The present study examined the combined administration of *Azadirachta indica* seeds (NSE) and *Tridax procumbens* (TP) extracts against *T. evansi*. In vitro activity of NSE (50 mg/mL) and TP (50 mg/mL) stopped parasite motility for 8 min and 6 min, respectively. The combined treatment with NSE (500 mg/kg/day) and TP (150 mg/kg/day) induced death of *T. evansi* in the infected animals on day 4 without parasitological relapse for 23 days post-infection. NSE was found to contain azadirachtin A and B while TP was found to contain antioxidant components. This study underscores the importance of combined therapy in trypanosome chemotherapy.

**Keywords:** trypanosomosis; *Trypanosoma evansi*; *Azadirachta indica*; *Tridax procumbens*; combined therapy.

### 1. Introduction

*Trypanosoma evansi* (*T. evansi*) is one of the species of trypanosome that causes trypanosomosis (Enwezor and Sackey, 2005). This disease is fatal and it affects a large number of wild

and domesticated animal species especially in Africa and is not yet combated by vaccination due to extensive antigenic variation by the parasite in the host (Dubois et al., 2005; Desquesnes et al., 2013). Several species of haematophagous flies are implicated with transferring the infection from host to host, thus acting as mechanical vectors (Berlin et al., 2009). The impacts of the disease have resulted in economic losses in affected areas (Shaw, 2009).

The parasite can also evade the immune system by modifying their surface membrane and as a result, the parasite undergoes rapid multiplication in the blood of the host producing waves of parasitaemia that characterize the disease (Nok et al., 2003; Habila et al., 2012; Desquesnes et al., 2013). The use of commercially available trypanocides, such as suramin, pentamidine, eflornithine, melarsoprol, quinapyramine and diminazene aceturate (Decampo and Moreno, 2003; Bacchi, 2009) has resulted in the appearance of strains resistant to these drugs (Kinabo, 1993; El Rayah, 1999). Thus drug resistance trypanosomes pose a great problem (Delespaux and De Kenning, 2007) especially in Africa therefore, highlighting the need for newer trypanocidal drugs.

*Azadirachta indica* (*A. indica*) is a plant of the mahogany family which possesses a wide range of medicinal properties (Brahmachari, 2004; Subapriya and Nagini, 2005). All parts of the plant have been used for various treatments of which more than 140 compounds have been isolated (Subapriya and Nagini, 2005). *Tridax procumbens* (*T. procumbens*) is commonly used as traditional medicine in the tropics for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhea and high blood pressure (Habila et al., 2010; Salahdeen and Murtala 2012). It has been reported to have anti-microbial activity against pathogenic organisms (Alka and Padma, 2013) and it also has anti-diabetic properties among many others (Mundada and Shivhare, 2010). Recently, we reported the trypanocidal potentials of *A. indica* against *T. evansi* in which *A. indica* was unable to clear the parasite at the late stage of the disease (Habila et al., 2011). As a follow up to that finding, we now focused our attention on combined therapy of *A. indica* seed extract and *T. procumbens* leaf extract against *T. evansi*.

## 2. Materials and Methods

### 2.1. Animals

Mature Wistar strain Albino rats (160-250 g) free from infection were obtained from the Animal House of National Research Institute for Chemical Technology, Zaria, Nigeria.

### 2.2. Plant Materials

*A. indica* seeds and *T. procumbens* leaves were harvested in Zaria then authenticated at the Herbarium in Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria with voucher

specimen numbers of TP 260 and *A. indica* 900151.

### 2.3. Infection of Rats with *T. evansi*

The parasite *T. evansi* STIB 731-AA (IL-2492) was obtained from the Department of Veterinary Parasitology, Ahmadu Bello University, Zaria, Nigeria. The parasite was passaged into rat by intraperitoneal inoculation and the parasitemia was monitored daily.

### 2.4. Sample Preparation

The *A. indica* seed extract was prepared using the seed kernel which was blended to powder then 40 g of the powder was defatted in petroleum ether (40 - 60 °C). The residue was dried at room temperature and refluxed using Soxhlet apparatus with 200 mL methanol. The methanolic extract was concentrated in a rotatory evaporator in which 1 g was dissolved in 10 mL normal dextrose-saline. This was referred to as the *A. indica* seed extract (NSE).

*T. procumbens* leaf was cleaned and air dried, then pulverized in a clean mortar into coarse form. The powdered plant sample (500 g) was weighed and placed in a Soxhlet extractor and defatted before extracted with chloroform. The extract was then concentrated using rotatory evaporator. This was prepared and subsequently referred to as *T. procumbens* extract (TP).

### 2.5. In vitro Studies

A 50 µL of blood containing the parasite was mixed with 20 µL of each extract. The assessment of in vitro anti-trypanosomal activity was performed in triplicates in a 96 well microtitre plate. The parasitemia was monitored every minute in which 1 µL of test mixture was placed on separate microscope slides and covered with cover slips. The drop in motility of the parasites in the blood treated with compared to that of parasite-loaded control blood without the extracts was taken as a measure of anti-trypanosomal activity. This was carried out for NSE and TP at concentration 50 mg/mL. To ensure that the effect monitored was that of NSE and TP alone, a set of positive control (PC) and negative control (NC) was set up. NC contained only the infected blood suspended in heparin and phosphate buffer saline glucose (PBSG). PC contained 6.25 mg/mL of DIMINAVETO® which served as reference drug.

### 2.6. In vivo Synergetic Studies

A total of 30 rats were divided into 6 groups (A, B, C, D, E and F) of five rats each. Group A, B, C, D and E were infected with *T. evansi* then treated with 150 mg/kg/day, 500 mg/kg/day, 500 mg/kg/day NSE plus 150 mg/kg/day TP, normal saline and 3.5 mg/kg/day with Diminveto®

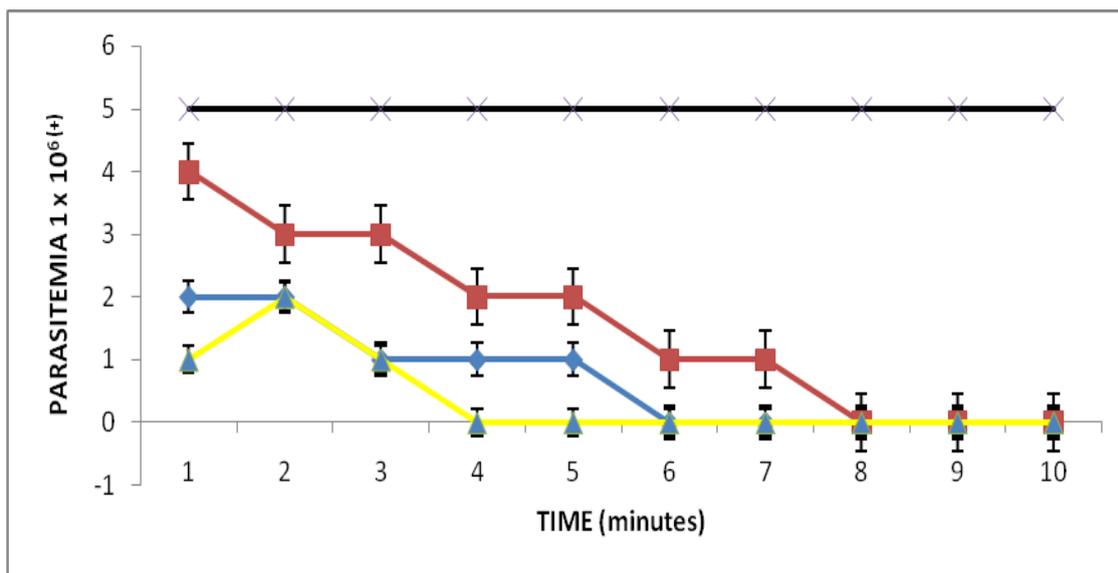
respectively. Group F was uninfected and untreated. Treatment was administered for 7 days and parasitemia was monitored daily for 21 days.

### 2.7. Spectroscopic and Chromatographic Analyses

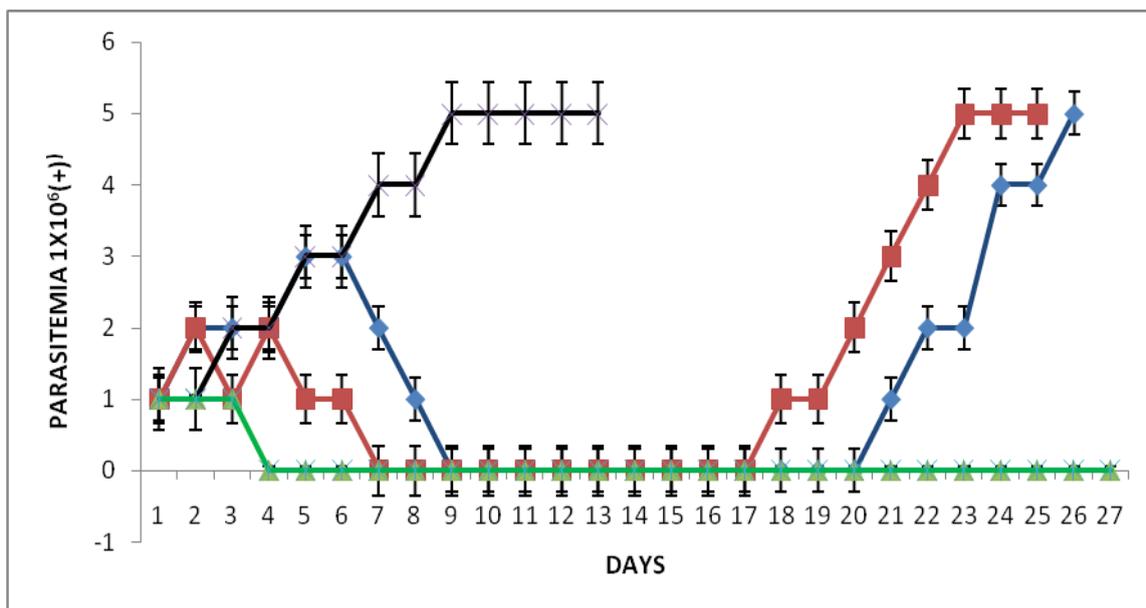
The NSE was subjected to high performance liquid chromatography (HPLC) in a Shimadzu HPLC prominence by isocratic method in spectrophotometric detector. The HPLC analytical conditions earlier reported (Habiba et al., 2010) are: flow rate, 1 mL/min; wavelength of detection, 215 nm; mobile phase, acetonitrile:water (35%:65%). While TP was subjected to Fourier transform infrared spectroscopy (FTIR) in a 10 scan system at a resolution of 2 (1/cm).

## 3. Results and Discussion

The result in figure 1 shows the in vitro anti-trypanosomal effect of TP and NSE against *T. evansi*. TP stopped parasite motility at about 6 min while NSE stopped parasite at about 8 min. Fig. 2 shows the in vivo anti-trypanosomal activity of both single and combined anti-trypanosomal activity of TP and NSE. The combination of TP and NSE administration was able to stop parasite motility from day 4 and the animals in the group survived without parasitological relapse for 24 days post treatment.



**Figure 1.** In vitro anti-trypanosomal effect of TP and NSE against *T. evansi*. TP (blue 50 mg/mL), NSE (red 50 mg/mL), Diminaveto® (yellow 6.25 mg/mL) and negative control (black).



**Figure 2.** Combined in vivo anti-trypanosomal effect of TP and NSE against *T. evansi*. TP (blue 150 mg/kg/day), NSE (red 500 mg/kg/day), TP + NSE (yellow), negative control (black) and Diminaveto® (green 3.5 mg/kg/day).

**Table 1.** High performance liquid chromatography and Flourier transformed infrared spectroscopy analyses of TP and NSE

Extract	Analysis	Component Present
NSE	HPLC	Azadirachtin A and Azadirachtin B
TP	FTIR	Phenolic, aliphatic, acids and hydrogen bonds.

The treatment of trypanosomosis has always been a major challenge for a very long time. This situation has been attributed to different factors such as the antigenic variation mechanism in which the parasite is able to change its outer coat by the variant surface glycoprotein (VSG) (Wang et al., 2010). From this study, we observed that, in vitro and in vivo anti-trypanosomal activity of TP and NSE had effect against *T. evansi*. The in vitro results revealed that both TP and NSE were able to stop parasite motility within few minutes. Ironically these extracts were unable to achieve same for in vivo studies when separately administered. TP and NSE were able to stop parasite motility for only a period of 11 days and 10 days respectively for single dose therapy then there was parasite relapse in the blood and the animals in these groups died subsequently. This could be due to the fact that the parasite on treatment disappeared from the blood stream and may have infiltrated into the organs or central nervous system (CNS) (Antoine-Moussiaux et al., 2008; Nok et al., 1993). On further administration of combined extract (TP and NSE), the parasites were cleared in the blood from day 4 to day 27. The animals in this group were monitored for 28 days and there was no parasitological relapse as observed

in the case of single dose therapy. The combined administration of TP and NSE was able to exert such effect possibly because the combined therapy prevented the parasite from escaping by VSG switch which is the major mechanism that *T. evansi* uses to evade trypanocides (Fairlamb, 2003).

The analyses of TP and NSE by HPLC and FTIR respectively revealed the presence of azadirachtin A and B for NSE (Habla et al., 2011) while the TP showed presence of phenolic and aliphatic components. Nok et al. (1993) have earlier reported the trypanosomal potentials of *A. indica* leaf against *Trypanosoma brucei* and the principal component in *A. indica* have also been reported to be azadirachtin (Schmutterer, 1992). Trypanosomes pathogenesis induces oxidative stress (Wolkmer et al., 2009) which contributes to serious disorders in infected animals, our observation that TP and NSE extract have trypanosomal activity could be related to the anti-oxidant activity of TP (Habla et al., 2010) and this could be useful for the likely mechanism of TP synergy with NSE.

#### 4. Conclusions

The advent of alternative medicine by combination therapy may therefore be a new facile in trypanosome chemotherapy. Our results show that TP and NSE have synergetic activity against *T. evansi* in vivo and this effect was able to eliminate parasite motility in the blood stream of infected animals without parasitological relapse.

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