COMBINED PHYTOTHERAPEUTIC EVALUATION OF RECTAL TEMPERATURE AND SURVIVAL ON *TRYPANOSOMA EVANSI* INFECTED MICE

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Abstract

Survival rates and rectal temperatures of mice infected with Trypanosoma (T.) evansi were evaluated. A total of 20 male weaned albino mice infected with T. evansi were randomly grouped with 5 replicates per group using the completely randomized design. Group 1- infected untreated control, group 2- treated with 0.9mg/ml of combined antitrypanosomal plant oral therapy, group 3- treated with 1.8mg/ml of the combined plant therapy and group 4- treated with 3.6mg/ml of the combined plant therapy. Data collected were subjected to descriptive statistics, one way ANOVA and presented as histogram chart. Results of the rectal temperature revealed a significantly ($P<0.05$) lowered rectal temperature and higher survival time of group 3. The study revealed antipyretic properties of the 0.8mg/ml combined plant therapy and no cure of the oral aqueous plant therapy doses.

Keywords: Survival, temperature, T. evansi, therapy, aqueous.

Description of Problem

The urgent need for novel and safe therapeutic agents is currently geared towards metabolite derivatives from plant sources offering targeted anti parasitic mechanisms and evasion strategies (1). Diminazene aceturate as the most commonly used chemotherapeutic veterinary drug in treating Animal African Trypanosomosis (AAT) faces limitations due to emerging drug resistance and toxicity (2). This necessitates the rationale to explore combinatorial phytotherapy using the leaves of *Carica papaya* (Paw-paw), *Vernonia amygdalina* (Bitter leaf) and *Khaya senegalensis* (African mahogany), which have been proven to exhibit some levels of antitrypanosomal bioactive profiles in isolation (3, 4 and 5). Potent ethno medicinal alternatives in Managing *Trypanosoma* infection in animals could be possible when these plant leaves aqueous extract combined together are administered as a therapeutic treatment.

Trypanosoma evansi (T. evansi) is one of the most important *Trypanosoma* species and causative parasitic agent of the disease '*Surra*', which commonly affects camels and a host of domestic animals such as cattle, goats, sheep, pigs, dogs, cats and other wild animals. The infection of the haemoparasitic disease induces multi-systemic pathology characterized by progressive anaemia, immunosuppression, fatal hyperthermia and death (6). AAT remains a significant veterinary health challenge across Africa, with estimated livestock prevalence of 27.3%, causing substantial economic losses due to mortality and decreased productivity (7). Other symptoms linked to AAT also include high rates of abortions (61 – 65%) and neonatal deaths (82%) in camels and other livestock (8).There are few traditional formulations exploit synergistic effects of some plants, there remains a paucity of controlled clinical data on the triple plant therapeutic combinations effect against *Surra* (9).

This study aims at evaluating the survival rates and rectal temperature in mice experimentally infected with *T. evansi* and treated with aqueous

extracts of combined *C. papaya*, *V. amygdalina* and *K. senegalensis*.

Materials and Methods

Location of study area

The study was carried out at the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State with geographical coordinates of latitude 9°44'N and longitude 8°45'E (10).

Experimental animal management

Twenty healthy weaned male albino mice weighing between 15g to 24g were procured from the livestock small animal colony of NITR- Vom for the experiment. They were housed separately in metallic fabricated mouse cages embedded with dry litter wood shaving that was changed weekly. Each cage was fitted with drinker and feeder. The animals were fed with Chikun® Super starter and provided portable drinking water *ad libitum*. Seven days acclimatization period was observed and at the beginning of the trial, each mouse was inoculated intra peritoneally with 0.1ml inoculum containing 5 *T. evansi* per microscopic field view using wet film mount. Daily drop of blood was collected from the tip of the tail of each mouse by piercing it using a sterile lancet and blood was dropped on a microscopic slide, covered with cover slip, mounted on a compound microscope (olympus®) and viewed using × 40 objective for parasitaemia. At pre patency (period between inoculation and the day first parasite was sighted) of each mouse except the untreated control group, oral treatment using the test aqueous extract of the combined plants leaves were administered continuously for seven consecutive days.

Experimental design

Twenty male mice were randomly allotted to four treatments with five replicates using the Completely Randomized Design (CRD). Each animal represented a replicate. At the pre patent period, the mice were treated with aqueous extract concentrations of **T1** (5 albino mice infected with *T. evansi* as control), **T2** (5 albino mice infected with *T. evansi* and treated orally with a total of 0.9mg/ml of 0.3mg each of equal combinations of aqueous leaf extract from *Carica papaya*, *Vernonia amygdalina* and *Khaya senegalensis*), **T3** (5 albino mice infected with *T. evansi* and treated orally with a total of 1.8mg/ml of 0.6mg each of equal combinations of the aqueous leaf extracts of *Carica papaya, Vernonia amygdalina* and *Khaya senegalensis*) and **T4** (5 albino mice infected with *T. evansi* and treated orally with a total of 3.6mg/ml of 1.2mg each of equal combinations of the aqueous leaf extracts of *Carica papaya, Vernonia amygdalina* and *Khaya senegalensis*).

Ethical clearance

Ethical approval was obtained to use albino mice for the experiment from faculty of pharmaceutical science ethical committee, University of Jos, with reference number; UJ/FPS/F17-00379.

Rectal temperature and survival rate

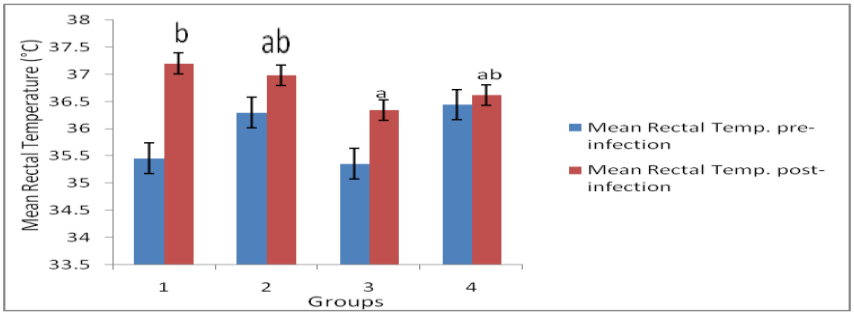
Weekly rectal temperature (°C) of each mouse was read using a digital thermometer (New Spring®). The duration of survival of each live mouse up to termination of the experiment was computed as the survival period.

Data analysis

Data generated were subjected to descriptive statistics and one way Analysis of Variance (ANOVA) at 95% confidence level using the Statistical Package for Social Sciences (SPSS) version 24. Fisher's Least Significant Difference (LSD) was used to separate the means where significant differences occurred.

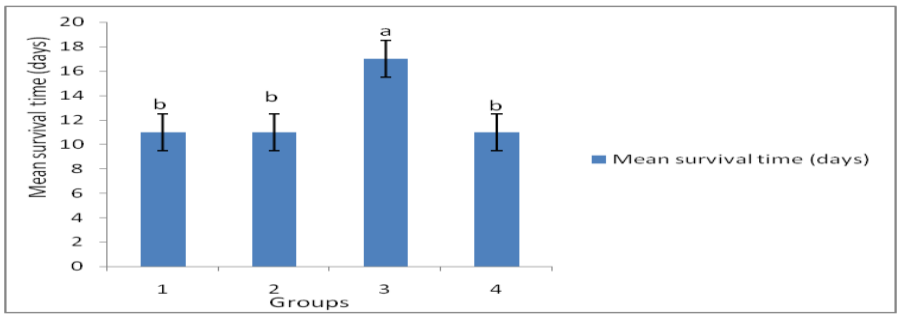
Results and Discussion

The result of mice rectal temperature as shown in figure 1 revealed that group 3 post infection mean values was significantly (P<0.05) lower than the control. The mean pre infection values were lower than the post infection values for all groups. These finding is contrary to the report of (10) where goats infected with *T. evansi* had insignificant (P>0.05) rectal temperature values. The significantly (P<0.05) lowered rectal temperature observed in group 3 post infection may be due to the optimum dosage of compounds found in *Carica papaya* reported to posses anti fever properties (11). The mean survival values (figure 2) observed in this study also showed group 3 to be significantly (P<0.05) higher than the other groups. The survival rate range of 11±1-17±1 days in this study was lower than 12±1-53±7 days reported by (12). The difference in the survival range may be attributed to the difference in sex of the mice used for the experiments.



a and b means are significantly different (LSD P<0.04)

Figure 1: Mean rectal temperatures of male albino mice infected with *Trypanosoma evansi* and treated with combined aqueous extract concentrations of *Carica papaya*, *Vernonia amygdalina* and *Khaya senegalensis*. Group1 infected untreated control, group 2 infected and treated orally with 0.9mg/ml(0.3mg each), group 3 infected and treated orally with 1.8mg/ml (0.6mg each), group 4 infected and treated with 3.6mg/ml (1.2mg each). I Standard error of Mean, LSD Fisher's Least Significant Difference.



a and b means are significantly different (LSD for 1 & 3 P<0.015; LSD for 2 & 3 P<0.02; LSD for 3 & 4 P<0.024).

Figure 2: Mean survival period of male albino mice infected with *Trypanosoma evansi* and treated with combined aqueous extract concentrations of *Carica papaya*, *Vernonia amygdalina* and *Khaya senegalensis*. Group1 infected untreated control, group 2 infected and treated orally with 0.9mg/ml(0.3mg each), group 3 infected and treated orally with 1.8mg/ml (0.6mg each), group 4 infected and treated with 3.6mg/ml (1.2mg each). I Standard error of Mean, LSD Fisher's Least Significant Difference.

Conclusions and Applications

1. The study revealed antipyretic properties at 1.8mg/ml (0.6mg each of *Carica papaya*, *Vernonia amygdalina* and *Khaya senegalensis*) combined oral therapy for male albino mice infected with *T. evansi*.
2. The combined oral plant therapy could not cure *surra* in the infected mice but the 0.8mg/ml aqueous extract plants combinations (P<0.05) increased survival than the other concentrations used.

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