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# Efficacy of Aqueous and Methanolic Leaf Extract of *Phyllanthus Amarus* for *Anti-Trypanosomal* Activity in Mice Experimentally Infected with *Trypanosoma Congolense*

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## ABSTRACT

*Animal African trypanosomiasis negatively affects animal health and productivity. Chemotherapy, the main means of controlling AAT is under threat due to parasite resistance and toxicity of the anti-trypanosomal drugs. The study was carried out to investigate the efficacy of P. amarus in the treatment of Animal African Trypanosomiasis with Trypanosoma congolense strain as a case study. Leaves of Phyllanthus amarus were extracted using methanol and aqueous solvents. Forty five mice weighing between 20-35g were divided into 9 groups (group 1- 9) of 5 mice each. Each mouse was infected with Trypanosoma congolense parasites intraperitoneally with 0.2 ml of blood containing a specified number (2.5 x $10^6$) of trypanosomes and treated with various concentrations of 100, 250 and 500mg/bw of corresponding methanolic and aqueous leaf extract of P. amarus. Diminazine at standard concentration of 3.5mg/bw was used as control. Data collected were analyzed using analysis of variance (ANOVA) at $P \leq 0.05$ level of significance. The result showed that treatment with methanolic and aqueous leaf extract of P. amarus though concentration dependent significantly resulted in marked suppressed ($P < 0.05$) parasitaemia and extension of survival days of the treated mice for additional 22 days against the infected untreated group. There was also a corresponding increase in the mean weight and PCV values of the Trypanosoma congolense infected but treated groups. Thus, the plants extract can be used as an alternative means in the absence of synthetic chemical drugs to sustain lives of animals infected with Trypanosoma congolense.*

**Keywords**: *Phyllanthus amarus; weight; PCV values; Trypanosoma congolense.*

## INTRODUCTION

Trypanosoma congolense is among the species of trypanosomes which cause the disease African Animal Trypanosomiasis (AAT) or Nagana. They are single celled protozoa belonging to the family Trypanosomatidae, genus Trypanosoma. Trypanosoma congolense resides in the subgenus Nannomonas, a group of small trypanosomes with medium-sized marginal kinetoplasts, no free flagella, and poorly developed undulating membranes (Bengaly, 2002). In East Africa, T. congolense is considered to be the single most important cause of African Animal Trypanosomiasis. This trypanosome is also a major cause of the disease in cattle in West Africa. Sheep, goats, horses, and pigs may also be seriously affected. In domestic dogs, chronic infection often results in a carrier state. Among the species of trypanosomes responsible for trypanosomiasis in animals, T. congolense have been considered to be highly pathogenic for cattle, it is however the most important cause of AAT in West African cattle and are transmitted by insect vector of the genus Glossina. The trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (Dargie, 1979). Animal African trypanosomiasis has been reported to negatively impact animal health and productivity, livestock production and limits land utilization (Bengaly, 2002). Chemotherapy, the main means of controlling AAT is under threat due to parasite resistance and toxicity of the anti-trypanosomal drugs.

There is a urgent need for the development of alternative drugs against African Animal trypanosomiasis which are safe, environmenta- lly common, friendly and effective.

Phyllanthus amarus commonly called Wind Breaker or Carry Me Seed in the family Euph orbiaceae have been known for its high medicinal values. It has about approximately 800 species which are found in tropical and subtropical countries of the world (Tahseen et al., 2013). The study was therefore aimed at investigating plant of these medicinal values for its efficacy against Animal African Trypanoso- iasis with Trypano-soma congolense strain as a case study.

## Materials and Methods

### Collection and Identification of Plant Materials

Mature *P. amarus* was collected from its natural habitat in Ikot Nkim, Ibesikpo Asutan Local Government Area, Akwa Ibom State. The plant was identified by Prof. (Mrs) Margaret E. Bassey a Biosystematics/Taxonomist of Botany Department, University of Uyo, Akwa Ibom State, Nigeri-a.Voucher number: Udo, UUH3810 (Ikot Nkim Ibesikpo) was given. Voucher specimen was kept in Herbarium unit, Department of Botany University of Uyo, Akwa Ibom State, Nigeria. Leaves of the plant were used for the experiment. The collected plant specimens were taken to Federal University of Agriculture, Department of Biological Sciences' Laboratory for extraction and further experiments.

### Preparation of Methanolic and Aqueous Extracts

Leaves of the plant species were used for the study. Fresh leaves of the plant species were washed thoroughly using distilled water, to remove sand and other foreign materials and air dried on laboratory bench for two weeks. Methanolic and aqueous solvent was used for the extraction using cold maceration technique as describe by Evans (2002) and Sofowora (2006).

### Experimental Animals

Sixty five mice of both sexes were obtained from The Laboratory Animal Unit of Nigerian Institute for Trypanosomiasis Research (NITR), Vom, and Plateau State, Nigeria. The animals were maintained in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). They were kept for a period of 7 days to acclimatize the environment before the commencement of the experiments. They were allowed to have access to clean drinking water and feed (Vital Feed) ad libitum before and during the experiments.

### Trypanosome Stock

*Trypanosoma congolense* stock was obtained from Department of Vertrinary Parasitology and Entomology Faculty of Veterinary Medicine Ahmadu Bello University (ABU), Zaria, Nigeria under The Laboratory Technologist 1 in person of Usman Lawal. The parasites were inoculated into rats. The inoculated rats were monitored daily for parasitaemia by examining microscopically at x 40 magnification from a drop of blood collected from the tail.

## Evaluation of P. Amarus Extract for Antitrypanosomal Activity in T. Congolense Infected Mice

### Groupings and Infection of Mice

Forty five mice weighing between 20-35g were used for the experiment. They were divided into 9 groups (group 1- 9) of 5 mice each. Group 1 to group 8 (G1-8) were infected with Trypanosoma congolense parasites intraperitoneally with 0.2 ml of blood containing a specified number (2.5 x $10^6$) of trypanosomes / mouse. By 7 days post infection, parasitaemia was established in all the infected mice. By 9days post infection when parasitaemia was approximately log 7.8 (63 x $10^6$ parasites/ml) treatment with graded doses of both methanolic and aqueous leaf and extract of P. amarus (100, 250 and 500 mg/kg respectively ) were administered for 5 consecutive days to 6 different groups of mice (groups 1 - 6) orally. Diminazene diaceturate (Nozomil®) was given to mice in group G7 at a dose of 3.5 mg/kg intraperitoneally. Mice in group 8 were infected untreated controls while those in groups 9 were uninfected.

Detailed grouping of mice were as follows:

### Group1

*T. congolense* infected mice were treated with 500mg/kg of aqueous leaf extract of *P. amarus*

### Group2

*T. congolense* infected mice were treated with 250mg/kg of aqueous leaf extract of *P. amarus*

### Group3

*T. congolense* infected mice were treated with 100mg/kg of aqueous leaf extract of *P. amarus*

### Group4

*T. congolense* infected mice were treated with 500mg/kg of methanolic leaf extract of *P. amarus*

### Group5

*T. congolense* infected mice were treated with 250mg/kg of methanolic leaf extract of *P. amarus*

### Group6

*T. congolense* infected mice were treated with 100mg/kg of methanolic leaf extract of *P. amarus*

### Group7

*T. congolense* infected mice were treated with Diminazene Diaceturate (Nozom- il®) at standard dose (3.5 mg/kg) given intraper itoneally.

### Group8

Infected mice - non treated (positive control).

### Group9

Non infected – non treated (negative control).

### Determination of Live Weight

Mice in all the groups were weighed in grams (g) on a 3 day interval using weighing balance (sensitive electronic weighing balance with Model number: Labtech® BL2OOO1). Mean weights from each replication was also recorded to establish the growth rates against the expected normal growth curve of a mouse.

### Determination of Body Temperature

Body temperature was determined per rectum by the use of a digital clinical thermometer (MODE: GF-MT502). Before taking the temperature, the thermometer was properly disinfected using a cotton wool containing methylated spirit. The bulb end was thereafter lubricated with water to reduce friction against the mucous membranes during insertion. Once inside the rectum, the thermometer was slanted to make sure the bulb made contact with the rectal mucous membranes thus avoiding taking the temperature of the faeces and was allowed for 1minutes to make the "bib" sound approximately for 60sec before taking the reading.

### Quantification of Parasitaemia

The level of parasitaemia was monitored in each mouse every day by the rapid matching technique as described by Herbert and Lumsden (1976). Briefly, a drop of mouse tail blood was examined first at x10 then x40 magnification using a table microscope. The number of trypanosomes in each field was counted. Each counting per field was matched with log figures obtain from the reference table. The log figures were converted to antilog values which were subsequently converted to absolute numbers. This gave the number of trypanosomes per milliliter. Where no trypanosomes were seen in blood, the buffy coat layers were also examined.

### Packed Cell Volume (PCV) Determination

The microhaematocrit method of Coles (1968) was used for determination of PCV. Briefly, mice tail blood was collected into sodium heparinized micro-haematocrit capillary tubes to one third of the tube. The unfilled end of the tube was sealed with plastiseal by pushing the capillary tube down into it. The capillary tube was then centrifuged in a microhaematocrit centrifuge at 10, 000 revolutions per minute (rpm) for five minutes. The PCV was read off using a haematocrit reader.

### Analysis of Data

Data for every experimental variable including the parasitaemia levels, Temperature, body weight and packed cell volume were entered and managed using Microsoft Excel (version 2007). Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 21. Values of the data obtained was summarized and expressed as mean ± standard deviation. The significant differences of the mean of parasitaemia, body weight, packed cell volume of mice from the negative control group, Diminazene treated group and the extracts treated groups at different dosages were compared by One Way ANOVA. P values less than 0.05 were considered significant.

## RESULTS

### Effect of Treatment on Daily Live Weight of Mice Infected with T. Congolence and Treated with Different Concentrations of Aqueous and Methanolic Leaf Extracts of P. Amarus

The result of weight of mice infected with T. congolense and treated with P. amarus is presented in Table 1. Mice in groups did not show significant drop ($P < 0.05$) in their various body weight on Day 4 through Day 15 post infection days (7 days post treatment) when

compared with the control group (group9) but was significantly higher ($P < 0.05$) than the infected untreated group (group8). However, Mice in the treated groups, (group 1 – 7) exhibited a statistically insignificant decrease ($P > 0.05$) in their live body weight on day 8 of the experimental post infection days. There was however an increase in live body weight of mice on day 12 through Day 19 though the difference was not statistically significant at $P \leq 0.05$ when compared to the control group. On day 22 of the experimental days, the mean live weight of the treated mice was seen to increase in group 1, 4, and deminazene (group7) treated group 13 with mean values of 33.65±2.17, 26.30±1.76 and 30.10±1.35 respectively and the difference was statiscally significant ($P < 0.05$) from the infected not treated group. The mean value was however seen to reduce on 29 through day 34 of the experimental days. On the other hand, mice in group 2, 3 and group 6 were seen to exhibit a reduction in their various live body weight though this was not statistically significant ($P < 0.05$) when compared to the uninfected untreated group 9 in day 22 through the remaining experimental days.

There was however, a continuous reduction in the mean weight values of mice in group 8 which served as the infected not treated group from day 8 till the end of the experimental days. In contrast, there was a progressive increase in live body weight of mice all through the experimental days in group 9 which served as the uninfected control group.

**Table1.** *Mean Live Weight (grams) ( Mean±SD) of Mice Infected with T. congolense and Treated with Different concentrations of Aquoeus and Methanolic Leaf extracts of. P. amarus*

| | DAY 1 | DAY 4 | DAY8 | DAY 12 | DAY 15 | DAY 19 | DAY 22 | DAY 29 | DAY 34 |
|---|---|---|---|---|---|---|---|---|---|
| **Group1** | 31.75±1.66 | 31.65±1.78 | 32.02±0.61 | 33.40±1.35a | 33.80±1.56a | 33.65±2.19a | 35.30±1.56a | 34.52±0.00a | 32.15±0.00a |
| **Group2** | 28.36±0.97 | 28.43±1.66 | 28.93±1.33 | 29.70±1.84 | 29.85±2.19a | 30.50±0.00a | 28.60±1.41a | 26.30±0.00a | -*b |
| **Group3** | 26.40±1.21 | 26.23±1.10 | 25.56±1.24 | 26.60±1.30 | 27.06±1.67a | 26.60±1.27a | 24.20±0.00a | -*b | -*b |
| **Group4** | 23.16±1.16 | 25.30±0.65 | 99.80±12.2 | 26.70±0.43 | 26.53±1.07a | 26.30±1.76a | 27.33±1.65a | 28.19±1.29a | 25.54±4.01*ab |
| **Group5** | 26.60±2.09 | 28.33±2.87 | 27.63±2.17 | 29.50±1.63 | 28.90±2.43a | 29.50±2.17a | 30.20±1.58a | 27.21±6.94a | 28.10±0.00a |
| **Group6** | 24.80±4.36 | 24.17±4.31 | 23.80±4.48 | 24.20±4.26 | 30.07±2.34a | 22.15±3.04*a | 21.95±2.90*ab | 20.90±3.11*ab | -*b |
| **Group7** | 28.57±1.92 | 28.57±1.82 | 27.80±1.70 | 28.63±1.61 | 29.36±1.60a | 30.10±1.35a | 30.90±1.27a | 31.86±1.14a | 32.63±1.40a |
| **Group8** | 26.63±0.96 | 26.33±0.55 | 25.46±0.50 | 23.83±0.87 | -*b | -*b | -*b | -*b | -*b |
| **Group9** | 25.27±4.42 | 26.33±4.42 | 27.20±4.56 | 28.03±4.34 | 28.90±4.10a | 29.60±3.96a | 30.37a | 31.67±3.61a | 32.80±3.68a |

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at $P<0.05$ whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP7).

**Key:** *represent complete mortality of mice in the group.*

## Mean Group Temperature ($^{O}$c) ( Mean±SD) Of Mice Infected With *T. Congolense* And Treated With Different Concentrations Of Aqueous And Methanolic Leaf Extracts Of *P. Amarus*

The result of Temperature of mice infected with T. congolense and treated with P. amarus is presented in Table 2. An increase in mice body temperature was recorded in the mean values of mice across the infected group on day 8 of experimental days and the difference was statistically significant ($P < 0.05$) when compared with the uninfected control group (group 9). However, this persisted through day 5 of the experimental days.

On Day 19, a drop in mice body temperature ranging from (38.40±36.20±0.98) was recorded across the treated group, though this was only statistically significant ($P < 0.05$) in group 3 and 6 with mean values (37.25±0.35 and 36.80±0.99 respectively) (Table 2). Group 6 recorded a significant decrease ($P < 0.05$) (35.50±0.63) in temperature on day 22.

A continuous drop in body temperature of the treated mice across the groups was recorded from day 22 through the experimental days and was statistically significant ($P < 0.05$) in group 4 and 5 when compared with the uninfected control groups (group 9). In group 8 which served as the infected untreated group, mice in the group exhibited a rise in their body temperature which was statistically significant on day 8 and this however followed by a statistically significant ($P < 0.05$) drop in body weight on day 12 of the experimental period.

Mice in group 9 did not show any difference in their body temperature all through the period of the experiment.

**Table2:** *Mean group Temperature ($^oC$) ( Mean±SD) of Mice Infected with T. congolense and Treated with Different concentrations of Aqueous and Methanolic Leaf extracts of P. amarus*

| | DAY 1 | DAY 4 | DAY8 | DAY 12 | DAY 15 | DAY 19 | DAY 22 | DAY 29 | DAY 37 |
|---|---|---|---|---|---|---|---|---|---|
| **Group 1** | 38.16±0.15 | 39.37±0.20* | 38.92±0.47 | 39.22±0.34 | 39.20±0.00a | 37.90±1.56a | 38.10±1.31a | 37.20±1.84a | 38.80±0.00a |
| **Group 2** | 38.06±0.11 | 38.70±0.62 | 39.00±0.26 | 38.53±0.25 | 38.15±0.77a | 38.40±0.56a | 37.80±0.99a | 38.45±0.21a | 38.95±0.63a |
| **Group 3** | 38.06±0.11 | 39.33±0.15* | 39.53±0.05* | 38.60±0.45 | 38.55±0.63a | 37.25±0.35a | 36.10±0.00a | -*b | -* |
| **Group 4** | 38.00±0.00b | 38.96±0.32 | 39.03±0.35 | 38.66±0.28 | 37.80±0.62a | 38.40±0.20a | 38.06±0.80a | 36.56±1.50*ab | 35.23±2.28*ab |
| **Group 5** | 38.16±0.05 | 38.60±0.40* | 38.36±0.77 | 39.00±0.10 | 38.73±0.72a | 39.00±0.45a | 38.50±0.43a | 38.55±0.07a | 36.95±2.05a |
| **Group 6** | 38.10±0.10 | 39.13±0.72* | 38.73±1.02 | 38.66±0.58 | 39.05±0.21a | 36.80±0.99a | 35.50±0.63*ab | -*b | -* |
| **Group 7** | 38.33±0.15 | 39.56±0.11* | 39.20±0.26 | 149.00±191.00 | 38.76±0.15a | 38.20±0.20a | 38.50±0.30a | 38.26±0.25a | 38.06±0.11a |
| **Group 8** | 38.06±0.11 | 39.43±0.50* | 39.43±0.30* | 36.15±0.49 | -*b | -*b | -*b | -*b | -* |
| **Group 9** | 38.16±0.15 | 38.00±0.10ab | 38.03±0.05ab | 38.10±0.10 | 38.06±0.11a | 38.16±0.15a | 38.10±0.17a | 38.20±0.20a | 38.20±0.20a |

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP7).

**Key:** *represent complete mortality of mice in the group.*

## Mean PCV (%) ( Mean±SD) of Mice Infected with T. congolense and Treated with Different concentration of Aquoeus and Methanolic Leaf and Root extracts of P. amarus

The result of PCV of mice infected with T. congolense and treated with P. amarus is presented in Table 3. PCV in Mice in group 1 – 8 decrease steadily from day 8 ranging from 50.40 ± 0.78 to 48.93±0.60. This was however statistically significant (P < 0.05) on Day 13 when compared with group 9(uninfected control group).

Among all the treated groups, increase in PCV values was recorded in group 4 and group 7 with mean value of 43.03±2.95 and 49.20 respectively. However, group 1, 2, 3, 5 and group 6 on the other hand recorded a progressive decrease in PCV values from day 8 through 21 days of the experiment though this was significantly higher than the infected untreated control group (group 8). However, there was a progressive decrease in PCV values of the treated group including group 4 from Day 29 through the experimental days. There was however, a progressive increase in PCV values recorded in the deminazene treated group from day 21 through the experimental days. Mice in the infected untreated group 8 exhibited a progressive decrease in PCV values from say 8 through the experimental days, the difference was however, statistically significant (P < 0.05) when compared to group 9. Mice in group 9 which served as the uninfected control group did not show any variation in their PCV values all through the period of the experiment.

**Table3:** *Mean PCV (%) (Mean±SD) of Mice Infected with T. congolense and Treated with Different concentrations of Aquoeus and Methanolic Leaf extracts of P. amarus*

| | DAY 1 | DAY8 | DAY 13 | DAY 21 | DAY 29 | DAY 35 |
|---|---|---|---|---|---|---|
| **Group 1** | 50.75±1.79 | 50.35±1.06 | 44.19±3.22*a | 33.30±0.00*ab | 29.70±0.42*ab | 19.18±11.43*ab |
| **Group 2** | 51.40±1.27 | 49.40±01.27 | 40.27±1.97*a | 32.60±0.81*ab | 31.45±1.91*ab | 16.10±5.52*ab |
| **Group 3** | 51.70±0.60 | 49.86±0.77 | 39.60±0.36*ab | 29.70±2.26*ab | -*b | -*b |
| **Group 4** | 51.66±0.57 | 49.93±0.28 | 44.33±3.79*a | 43.03±2.95a | 37.15±2.33*ab | 26.40±2.83*ab |
| **Group 5** | 50.13±0.15 | 49.03±0.89 | 41.77±1.91*a | 33.25±2.02*ab | 30.20±2.97*ab | 18.78±2.18*ab |
| **Group 6** | 50.60±2.10 | 49.43±2.46 | 38.13±1.95*ab | 29.86±1.42*ab | 22.30±3.82*ab | -*b |
| **Group 7** | 51.93±0.81 | 50.40±0.78 | 46.00±1.32*a | 49.20±0.55a | 47.66±0.90*a | 49.03±1.26*a |
| **Group 8** | 52.33±0.28 | 50.03±1.02 | 30.83±1.35*b | -*b | -*b | -*b |
| **Group 9** | 52.20±0.26 | 52.26±0.37 | 52.54±0.25ab | 52.20±0.26a | 52.46±0.35ab | 52.13±0.15a |

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at P<0.05 whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b)

are statistically different compared to the Dimi group (GRP7).

**Key:** *Represent complete mortality of mice in the group.*

### Effect of Treatment on Parasitaemia of Mice Infected with T. congolense and Treated with Different Concentrations of Aqueous and Methanolic Leaf Extracts of P. Amarus

The parasitaemia of in mice infected with T. congolense and treated with P. amarus is presented in table 4. Parasitaemia was established in all the infected group on 7$^{th}$ day post infection days (Day 7). There was a decrease in Parasitaemia level across the treated group from Day 13 through Day 19 with significant reduction ($P < 0.05$) achieved on Day 19 across the treated groups. Group 4 achieved highest (2.11±0.53) reduction in mean Parasitaemia value.

However, group 8 (infected untreated group) recorded a significant higher (280.10±30.43) parasitaemia on Day 13 when compared with other groups. A complete reduction in parasitaemia was recorded on Day 19 in the Dimmazene treated group (Group 7). However, there was a relapse in parasitaemia across the extract treated group from day 21 which increase steadily, till the end of the experiment in the extract treated group. No relapse was seen in the Deminazene treated group (group7).

**Table4.** *Mean (Mean±SD) group Parasitaemia ($10^6$) of Mice Infected with T. congolense and Treated with Different concentrations of Aqueous and Methanolic Leaf extracts of P. amarus*

| | DAY 1 | DAY 7 | DAY 9 | DAY 13 | DAY 19 | DAY 21 | DAY 23 | DAY 35 |
|---|---|---|---|---|---|---|---|---|
| **Group 1** | 0 | 45.7±21.5 | 95.20±30.8* | 67.10±23.40*a | 10.97±2.10* | 43.62±14.23 | 261.30±14.40*ab | 404.84±4.34*ab |
| **Group 2** | 0 | 74.2±36.0* | 137.50±33.10* | 103.81±10.77*ab | 15.62±2.13* | 93.43±12.10*ab | 355.10±61.50*ab | 401.60±0.00 |
| **Group 3** | 0 | 51.90±32.3 | 156.25±6.77* | 104.24±8.32*ab | 75.40±17.30*ab | 183.50±25.60*ab | 443.90±74.10*ab | - |
| **Group 4** | 0 | 32.30±23.80 | 139.30±35.00* | 53.90±17.60*a | 2.11±0.53* | 49.03±2.77*ab | 113.80±24.10*ab | 335.40±56.00*ab |
| **Group 5** | 0 | 39.50±24.60 | 133.30±23.50* | 74.50±19.20*ab | 11.28±3.97* | 80.77±15.47*ab | 221.70±30.90*ab | 434.80±47.10*ab |
| **Group 6** | 0 | 49.90±27.70 | 141.00±34.20* | 99.57±10.79*ab | 37.28±15.02*ab | 169.80±27.40*ab | 306.40±78.00*ab | - |
| **Group 7** | 0 | 39.02±7.54 | 143.10±22.10* | 26.23±13.56a | 0 | 0 | 0 | 0 |
| **Group 8** | 0 | 28.80±24.3 | 159.10±86.40* | 280.10±30.4*b | - | - | - | - |
| **Group 9** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Values are presented as mean ± standard deviation (SD).Values indicated by asterisk down the group are statistically different compared to the Non- infected control (GRP9) at $P<0.05$ whereas all values indicated by the superscript (a) down the group are statistically different compared to the Infected control group (GRP8) and values indicated by superscript (b) are statistically different compared to the Dimi group (GRP7).

***Key:*** *represent complete mortality of mice in the group.*

## Discussion

The observed prepatent period of Trypanosoma congolense in mice was 7 days. In a similar experimental inoculation of T. congolense in mice, Nweze et al. (2011) recorded a prepatent period of 12 days and Nok (2002) recorded a prepatent period of just 3 days. Generally, T. congolense has a longer prepatent period than T. brucei. For example, Kubata et al. (2005) infected intraperitoneally balb/c mice with 2 x 106 T. b. brucei organisms and by two days post infection (PI) they were already parasitaemic. Length of prepatent period is determined by the strain of the parasite in question and host immune status. Different strains of T. congolense differ in their pathogenicity (Bengaly et al., 2002). Results of parasitaemia showed that the methanol extract of P. amarus had antitrypanosomal effect in the T. congolense infected mice at the tested doses.

The result of antitrypanosomal effect of P. amarus on mice infected with T. congolense revealed an insignificant ($P > 0.05$) decrease in body weight of infected mice on 8$^{th}$ day post infection followed by a corresponding rise in weight following the administration of methanolic and aqueous leaf extract of P. amarus. This could be attributed to the deleterious effect of T. congolense in this study as earlier mentioned, loss of appetite a secondary sign of trypanosomis is May reduced the food intake thus resulting in reduced growth. This result agrees with the ones in the reviewed literature.

During the first two weeks of the experiment there was no significant difference in mean weight gains of all the groups. By the third week of the experiment, mean group weight gain was significantly higher in the 500 mg/kg-treated group than in 250 mg/kg-treated and control groups. By the fourth week of the study, mean weight gain in the 500 mg/kg-treated group was higher than those of all the other groups. Weight

gain was fluctuating in all the groups and may have had nothing to do with infection or treatment. Emaciation is a feature of chronic infections of animals with trypanosomes. Infected animals suffer from anaemia and emaciation and most die if untreated (Brun and Lun, 1994).

A corresponding rise in temperature is also attributed to the intermittent fever which normally occurs in trypanosoma infection and the subsequent low temperature could likely be related to the compromised action of the immune system in the infected mice.

Animals infected with trypanosomes characteristically exhibit fever in addition to other non-specific host defence mechanisms (Kluger, 1986). Zwart et al. (1990) documented high body temperature to be detrimental to the trypanosomes.

The elevation in body temperature has also been reported to results in an enhancement of the immune response by increased mobility and activity of the white blood cells (Zwart et al., 1990).

Pyrexia in trypanosomosis is caused by trypanolytic crisis which enhances red blood cells damage and destruction leading to anaemia (Anosa, 1988). This was shown in this study by the concurrence of febrile peaks with onset of parasitaemia and fall in packed cell values. Other workers have also reported anaemia in animals as a result of T. congolense infection (Abenga et al., 2005; Bengaly et al., 2002; Dargie et al., 1979).

The tested extract reduced the parasitaemia in T. congolense in the blood though the reduction was dose dependent. At 500 mg/kg there was a significant clearance ($P < 0.05$) of the trypanosomes in the blood by the 12th day post-treatment (Day 21). However, by the $14^{th}$ (Day 23) there was a relapse of infection.

The ability of the extract to extend survival period of infected mice was dose-dependent, indicating the anti-trypanosomal activity of the extract and that it can be improved by using a higher dose or by using isolated active compounds in the extract. These findings are in agreement with a previous study which reported that compound with poyphenols exhibit encouraging in vivo trypanocidal activity with a reduction in the level of T. brucei parasitemia in mice (Mbaya et al., 2010). This anti-trypanosomal effect may be attributed to specific limonoid and flavonoid constituents of P. amarus. Anti-trypanosomal effect of P. amarus extract against T congolese infection and can further be deduced from the weight status of the extract-treated animals as compared to the negative control (infected untreated) animals. The body weight improvement was consistent with the observation made on parasitaemia among the extract treated animals. This observation indicates that the extract treated animals could feed better (as a result of their improved physical state) than those in the negative control group and resist weight loss that is usually associated with trypanosomiasis.

Similarly, methanolic and aqueous leaf extract of P. amarus prevented weight loss and decline in PCV of T. congolense infected mice as compared to negative control. This could be attributed to the reports on isolation of compounds from the root and leaf P. amarus which revealed the presence of limonoids, polyphenols secondary metabolites which are the active components in the tested extracts.

Also the methanolic leaf extracts had higher activity than the aqueous leaf extracts. These results are not surprising since Sukumar et al (1991), reported that the activities of phytochemical compounds on target species vary with respect to the solvent used for extraction, among other factors. It is presumed that methanol enhanced the extraction/release of the active component(s) in cases where its extracts demonstrated higher activity than their aqueous counterparts.

## CONCLUSION

Both methanolic and aqueous phyllanthus amarus extract can be used as alternative means in the absent of synthetic chemical drugs to sustain lives of animals infected with Trypanosoma congolense incase of disease outbreak till when the synthetic drug is available.

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