Trypanocidal effects of *Balanites aegyptiaca* Del. (Zygophyllaceae) Leaf Extract and Suramin on *Trypanosoma evansi* Experimental Infection in Albino rat

*[1]Abdullahi, A. M., [2]Malgwi, K. D., [3]Onyiche, E. T., [4]Bukar, K. B., [5]Kassu, M., [6]Muhammad, S. and [2]Daniel, N

[1]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri
[2]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri
[3]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri
[4]Department of Animal Health and Production, Mohamet Lawan College of Agriculture
[5]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto
[6]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto

* *Author for Correspondence: adamamusa12345@gmail.com*

ABSTRACT

Trypanosoma evansi, is one among several species of Trypanosomes responsible for a major protozoan parasitic disease of domestic animals. The current study aimed to investigate the trypanocidal effects of aqueous and methanolic leaf extracts of *Balanites aegyptiaca* Del (Zygophyllaceae) and suramin on albino rats infected with *Trypanosoma evansi*. In total, 35 Swiss albino rats randomly separated into seven (7) groups (A- G) of 5 rats each were intraperitoneally infected with 0.5 ml of blood containing 1.0×10^6 trypanosomes and treated with the extracts and suramin for 7 days and single dose respectively by day 12 post infection (p.i). Clinical parameters and haematological indices were evaluated. Preliminary phytochemical screening revealed that *Balanites aegyptiaca* contained the following metabolites: flavonoids, tannin, saponins, alkaloids, glycosides, steroids and volatile oils. There was marked initial reduction in Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cells (RBCs) counts in the infected/untreated group (Group A) but following treatment with *B. aegyptiaca* leaf aqueous and methanolic extracts, these values were reversed. Furthermore, there was reversion of leukopenia after treatment of the rats individually with methanolic leaf extracts (150 mg/kg of *B. aegyptiaca;* Group B) from $3.47\pm1.49 \times 10^3/mm^3$ by day 12 p.i. to 9.03 ± 2.18 by day 40 p.i., similar to the pre-infection value. In conclusion, the infection caused clinical and haematological changes that were ameliorated following treatment with methanolic and aqueous leaf extract of *B. aegyptiaca*.

Keywords: *Balanites aegyptiaca; Trypanosoma evansi;* Suramin; Trypanocidal.

INTRODUCTION

Tryanosoma evansi, the cause of "surra" is the first mammalian trypanosome to be described originally in the blood of equines and camels before subsequent description in other mammalian hosts (Desquesnes *et al*., 2013). This pathogen is responsible for major economic loss in camels and horses (Baldissera *et al*., 2017), and the pathogen is transmitted mechanically by biting flies of the genera *Tabanus*, *Lyperosia*, *Stomoxys* and *Atylotus* (Brun *et al*., 1998). However, vertical transmission (transplacental) as well as animal infection through the eating of *T. evansi* infected tissue have been documented (Campigotto *et al.,* 2015). Moreover, accidental human infection from a syringe containing *T. evansi* infected blood and through a wound while butchering infected raw beef have also been reported in India and Vietnam, respectively (Truc *et al*., 2013; Chau *et al*., 2016). This disease is a wide spread wasting disease

affecting a range of wild species and domestic livestock population (OIE, 2012), in which it causes enormous economic losses (Hoare, 1972). Recently, *T. evansi* has emerged as potentially pathogenic for humans, with a few cases of human infection reported in different countries (Joshi *et al*., 2005; Truc *et al*., 2013). Surra can be acute or chronic and asymptomatic or present with fever, anaemia, general weakness, neurologic abnormalities, marked ataxia, blindness, cachexia and often abortion and/or death if left untreated (Rodrigues *et al*., 2009; Desquesnes *et al*., 2013). The disease is often fatal in camels, horses and dogs while it is mild or subclinical in cattle, buffaloes, goats and sheep, whereas pigs may serve as reservoirs (Dargantes *et al*., 2009; Habila *et al*., 2012). Several surra outbreaks and epidemics have occurred across Asia, Africa, Europe and South America with increasing economic losses reported and studies have revealed that *T. evansi* is the first leading cause of trypanosomosis in camels and horses followed by other

trypanosome species such as *T. vivax*, *T. congolense*, *T. brucei* and *T. simiae* (Mossaad *et al.*, 2017; Ramírez-Iglesias *et al.*, 2017).

Due to scarcity of vaccines, prevention and control of trypanosomosis depended on the use of chemotherapy and these trypanocidal drugs are rapidly developing resistance (Chitanga *et al.,* 2011; Feyera *et al.,* 2013). Medicinal plants have a vital role in the discoveries and development of novel alternative drugs. In recent past, a handful of studies have evaluated the potential of medicinal plants as possible alternatives to conventional chemotherapy which offers hope as possible novel remedies to combat trypanosomosis (Mbaya *et al.*, 2010; Biu *et al.*, 2022; Wasagwa *et al.*, 2022).

B. aegyptiaca, (L.) Del. belongs to the family *Zygophyllaceae*, and is well distributed in the Sudano-Sahelian region of Africa. Methanolic extract of B. *aegyptiaca*, have been shown to posses' anthelmintic activity (Shalaby *et al.,* 2016; Shalaby *et al.,* 2020). Therefore, this study was designed to ascertain the phytochemical properties of the methanolic and aqueous leaf extract of *B. aegyptiaca* and to further test them to determine their trypanocidal activity by evaluating the hematological parameters in albino rats experimentally infected with *T. evansi.*

MATERIALS AND METHODS

Collection of samples

Five hundred (500) grams of fresh *B. aegyptiaca* was collected from city campus, Usmanu Danfodio University, Sokoto state in August, 2019. Samples of the plant was sent to the Herbarium of Department of Biological Sciences Usman Danfodiyo Unversity, Sokoto for identification and authenticated by a pharmacologist in the Department of pharmacology and toxicology in Usmanu Danfodiyo University Sokoto, Nigeria

Preparation of Plant extract

The collected leaves were air dried at room temperature for 4 weeks in the laboratory and pulverized using wooden mortar and pestle into fine powder, sieved to remove excess coarse plant materials. The powered leaves were put in sealed plastic containers, labeled and kept at 4°c until required. Two hundred and fifty (250) grams of the powdered leaves was soaked in 1000 ml (1liter) of distilled water and another two hundred and fifty (250) grams soaked in 100ml of methanol and allowed to stand for 24 hours at room temperature. The liquid extract was then decanted and the process was repeated twice. The decanted liquid extracts were pooled together and was then filtered through a cheese cloth before further filtration using a Whatman filter paper size 2. The liquid extract was allowed to evaporate at room temperature under a fast stream of moving air (Ode *et al.,* 2011). The percentage yield for the extracts was then calculated using the formula below:

$$\% \ Yield = weight \ of \ extract \ / \ weight \ of \ pulverized \ material \times 100$$

Phytochemical analysis

The aqueous and methanolic *B. aegyptiaca* leaf extracts were screened as described by Trease and Evans (1989) and Sofowora (1993).

Source of Experimental Drug

Suramin used for the study was manufactured by Interchemie (Werken "De Adelaar" B.V. Metaalweg and venray, Holland), marketed under the trade name Veriben® and recommended for use at the dose rate of 3.5 and 7.0 mg/kg body weight by deep intra-muscular administration in animals and intra-peritoneally in rats.

Source of the Parasite (*Trypanosoma evansi*)

The trypanosoma parasite used for the study was *Trypanosoma evansi.* The organism was isolated from camels that have been slaughtered from the abattoirs in Sokoto State of Nigeria. The parasite was sent for identification to National Veterinary Research Institute (NVRI) Vom, Nigeria. It was identified based on its morphology and negative blood inhibition and infectivity test (parasitological) and molecular characterization. It was stabilized by four passages in rats before storage in liquid nitrogen (LN). Four donor rats were used to multiply the parasites and transported by road from Vom to the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto State, Nigeria. The parasites were then maintained in Albino rats by serial passage until used.

Experimental Animals

Total of Thirty-five (35) apparently healthy albino rats of both sexes and of different ages were used in the study. The animals were purchased from breeders in Sokoto market. On arrival, they were kept in clean and well-ventilated locally made wire mesh with timber saw dust as beddings in the experimental laboratory of Veterinary Clinic, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto, Nigeria. They were routinely screened for internal and external parasites using standard methods (Soulsby, 1982). The Rats were fed with commercial growers feed (Vital Feeds, PLC, Nigeria), and water provided *ad libitum.* They were allowed to acclimatize to laboratory condition for two weeks prior to commencement of the experiment.

In vivo Experimental Protocol

Thirty-five albino rats were randomly separated into seven (7) groups (A- G) of 5 rats each. At day zero, to establish the baseline data, all the animals in each of the two groups were bled for haematology, and also clinical parameters (rectal temperature) were recorded using mini digital thermometer, while general body condition and physical signs were also observed. Each rat in groups A, B, C, D, E, F was inoculated through intraperitoneal route with 0.5 ml of blood containing 1.0×10^6 *T. evansi* as quantified using serial dilution as reported by Herbert and Lumsden (1976), while group G was left as uninfected untreated controls. At an interval of 4 days, blood samples were collected for haematology up to the end of the experiment. At day 12 post infection, treatment was commenced. Group A was infected with 0.5 ml of *T. evansi* (untreated control), Group B was infected with 0.5 ml of *T. evansi* but treated with high dose of *Balanite aegyptiaca* methanolic extract at a dose rate of 150 mg/kg body weight for 7 days   intraperinoeal (IP) by day 12 post infection (p.i). Group C was infected with 0.5 ml of *T. evansi* but treated with low dose of *B. aegyptiaca* methanolic extract at a dose rate of 50 mg/kg body weight for 7 days IP by day 12 post infection. Group D was infected with 0.5 ml of *T. evansi* but treated with

high dose of *B. aegyptiaca* aqueous extract at a dose rate of 150 mg/kg body weight for 7 days IP by day 12 post infection. Group E was infected with 0.5 ml of *T. evansi* but treated with low dose of *B. aegyptiaca* aqueous extract at a dose rate of 50 mg/kg body weight for 7 days IP by day 12 post infection. Group F was infected with 0.5 ml of *Trypanosoma evansi* but treated with suramin (single dose). Group G was uninfected untreated control.

The experimental rats were monitored daily for the manifestation of clinical signs of trypanosomosis including morbidity and mortality. Initial detection of parasitaemia, was done every 4 days and the degree of parasitaemia was estimated by the rapid matching technique (Herbert and Lumsden, 1976). Also, establish parasitaemia, the blood samples were examined using thin smear, wet mount and haematocrit buffy coat methods (Murray *et al*., 1983).

Determination of haematological indices

Blood samples were collected via cardiac puncture with 2 ml syringe and 23 gauge needle and put in a sample bottle containing EDTA anti-coagulant. The blood samples collected were used to determine the packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), haemoglobin concentration (Hb) and differential leucocyte count (DLC). Blood samples were collected at day 0 before inoculation to establish baseline data, then sample collection continued at 4 days interval after inoculation until day 12 when treatment commenced. Blood sample collection continues at 4 days interval after treatment. The PCV was measured by micro haematocrit reader using capillary tube, while RBC and WBC were measured using Neubauer counting chamber, while Hb concentration was determined using cyanmethaemoglobin method (Brown, 1976) by the use of colorimeter. Differential leucocytes count was performed using the method of Schalm *et al* (1975).

Monitoring of Infected and Control Animals

The experimental albino rats were monitored daily for the development of clinical signs of trypanosomosis including pale mucous membrane, raised hair coat, respiratory distress, emaciation, pyrexia, morbidity and mortality. Initial detection of parasitaemia was done every 4 days (because of the prepatent period of 4 days) and the degree of parasitaemia was estimated by the rapid matching technique (Herbert and Lumsden, 1976). Wet blood films were prepared and examined. The number of parasites in each field under the microscope was matched with the standard reference pictures according to Herbert and Lumsden (1976). Each count per field was matched with logarithmic figures obtained from the reference tables. The logarithmic figures were converted to antilog and subsequently converted to absolute numbers, which reflected the number of trypanosomes per ml.

Statistical analysis

The data obtained from the *in vivo* studies were summarized as means ± standard deviations and differences between the means determined at 5% level of significance using the two-way analysis of variance (Graph Pad Instat, 2003).

Ethical Statement

Ethical clearance for the experimental procedure was granted by the Ethical Committee of the Faculty of Veterinary Medicine, Usmanu danfodio University, Sokoto with AUP No: 2020/RO-07.

RESULTS

Preliminary phytochemical screening revealed that *Balanite aegyptiaca* contained the following metabolites: flavonoids, tannin, saponins, alkaloids, glycosides, steroids and volatile oils.

***In vivo* anti-trypanosomal effect of the extract**

The present study evaluated the trypanocidal activity of *B. aegyptiaca* leaf aqueous and methanolic extract against *T. evansi*. After infection of albino rats with *T. evansi*, trypanosomes appeared in the blood of all the infected groups during day 2 post infection (p.i.).

Temperature

The temperature of the albino rats in all the treated groups (Group B-F), did not vary significantly (P>0.05) when compared with the infected/untreated control group (Group A). Nonetheless, there was mild variation in temperature in Group A, as the temperature increased from 37.74 ±0.38 at day 0 to 39.00±0.00 by day 40 p.i (Figure 1).

Haematology

The mean PCV of the infected treated rats using methanolic extract at 150 mg/kg (Group B) was modulated to almost the pre-treatment values by day 40 p.i., similar to those in the infected suramin treated rats at 3.5 mg/kg (Group F). Statistically significant difference (P<0.05) between groups A (infected/untreated control) vs B (treated with 150 mg/kg of *B. aegyptiaca* methanol extract) was observed from day 16 until the termination of the experiment at day 40 (Table 1). On the contrary, the mean PCV of rats in group C (treated with 50 mg/kg methanol extract) and E (treated with 50 mg/kg aqueous extract) decreased consistently throughout the experiment with the lowest value at day 40 p.i. Lastly, the lowest mean PCV values was observed in the infected untreated rats (Group A) while the uninfected untreated group remained fairly constant throughout the experiment (Table 1).

The results of the mean RBC ($\times 10^6$ /mm^3) count of albino rats experimentally infected with *T. evansi* and treated with aqueous and methanolic extracts of *B. aegyptiaca*, Suramin and control is presented in table 2. The mean RBC ($\times 10^6$ /mm^3) count of infected rats decreases in all the treatment groups following infection with lowest values at day 12 p.i. The mean RBC values was most significant (P<0.001) at day 20 p.i. for group B. Interestingly, these values improved to almost pre-infection levels in majority of the groups (B, C, E & F) by day 40 p.i. (Table 2).

The results of the Hb concentration of albino rats infected with *T. evansi* and treated with aqueous and methanolic extract of *Balanite aegyptiaca*, Suramin® and their controls are presented in Table 3. Marked decrease in the Hb concentration was most notably in all the infected groups by day 16 p.i. with the exception of the Suramin treated rats (Group F). Statistically significant difference (P<0.001) was observed most notably with the Suramin treated rats (Group F) compared to the control.

At the end of the experiment, all the treatment groups had a Hb concentration similar to pre-infection values with the exception of two groups (Groups B and D) (Table 3). The mean WBC count decreases rapidly by day 4 p.i. in all the infected groups (Table 4). For the Suramin treated rats, the WBC counts modulates to pre-infection values by day 16 p.i. and varied significantly (P<0.001) with the infected untreated control (Group A). Similarly, the WBC count in the extract treated rats (Group B), appreciated to the pre-infection levels by day 36 p.i. until the end of the experiment (Table 4).

Table 1: Packed cell volume (%) of albino rats infected with *Trypanosoma evansi* and treated with various dosages of *B. aegyptiaca* leaf aqueous and methanolic extracts, Suramin® and their controls

Groups	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	DAY 32	Day 36	Day 40
Group A	40.50±1.29[a]	39.25±0.96[a]	34.50±1.92[a]	22.33±2.08[a]	20.00±3.00[a]	18.33±3.06[a]	15.67±3.06[a]	13.33±2.08[a]	17.00±2.65[a]	12.00±0.00[a]	12.00±0.00[a]
Group B	40.00±2.94[a]	36.75±2.06[a]	31.50±1.29[a]	20.67±1.16[a]	26.25±1.50[b]	29.75±2.50[c]	33.50±2.65[c]	35.00±2.16[c]	35.00±2.16[c]	37.00±2.45[c]	37.00±2.45[c]
Group C	39.25±3.30[a]	37.50±0.58[a]	34.00±1.41[a]	24.67±2.08[a]	22.50±2.08[a]	20.67±2.08[a]	18.33±1.53[a]	18.67±0.58[a]	18.67±0.58[a]	18.50±2.12[a]	18.50±2.12[a]
Group D	41.75±2.75[a]	36.00±1.41[a]	33.75±2.50[a]	27.00±4.58[a]	18.67±3.22[a]	17.00±3.61[a]	17.00±3.61[a]	16.67±3.22[a]	17.00±5.29[a]	20.00±0.00[a]	20.00±0.00[a]
Group E	43.50±2.65[a]	33.75±2.22[b]	26.67±3.22[c]	19.50±0.58[a]	18.33±1.16[a]	18.00±2.65[a]	18.00±2.00[a]	18.00±2.00[a]	18.00±0.00[a]	15.00±0.00[a]	15.00±0.00[a]
Group F	42.00±2.44[a]	33.25±1.50[d]	23.00±4.24[c]	25.67±2.08[a]	31.00±2.16[c]	35.25±4.57[c]	36.75±3.50[c]	39.00±2.74[c]	40.25±2.22[c]	39.75±1.71[c]	39.75±1.71[c]
Group G	41.00±2.16[a]	41.00±2.45[a]	42.60±2.70[c]	42.60±2.70[c]	43.40±2.70[c]	43.00±1.58[c]	44.60±1.82[c]	45.40±1.67[c]	41.20±2.39[c]	40.80±1.92[c]	40.25±1.70[c]

Superscripts between groups in columns differed significantly with respect to infected/untreated control (Group A) Group A: infected/untreated, Group B: methanolic extract 150 mg, Group C: methanolic extract 50 mg, Group D: aqueous extract 150 mg, Group E: aqueous extract 150 mg, Group F: suramin ® 3.5mg/kg, Group G: uninfected control; [b] (*p*<0.05) ; [c] (*p*<0.001); [d] (*p*<0.01)

Table 2: Red Blood Count (× 10^{6} /mm^{3}) of albino rats infected with *Trypanosoma evansi* and treated with various dosages of *B. aegyptiaca* leaf aqueous and methanolic extracts, Suramin® and their controls

Groups	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	DAY 32	Day 36	Day 40
Group A	6.74±0.97[a]	3.83±1.27[a]	3.21±0.73[a]	2.97±1.72[a]	2.34±0.40[a]	1.76±0.45[a]	2.20±0.28[a]	3.18±1.49[a]	2.52±1.27[a]	5.25±0.00[a]	5.25±0.00[a]
Group B	6.98±0.97[a]	3.57±1.23[a]	3.36±0.94[a]	2.40±0.72[a]	2.03±0.33[a]	8.19±4.02[c]	4.14±1.22[a]	5.88±0.95[a]	5.88±0.95[b]	5.79±2.36[a]	5.79±2.36[a]
Group C	6.32±0.55[a]	3.65±1.00[a]	3.78±1.98[a]	2.16±0.87[a]	2.55±0.62[a]	3.13±0.73[a]	5.13±1.07[a]	4.13±0.54[a]	3.13±0.54[a]	6.53±1.24[a]	6.53±1.24[a]
Group D	7.93±0.70[a]	3.16±0.93[a]	3.77±1.16[a]	2.04±0.99[a]	2.30±0.81[a]	2.37±1.00[a]	4.83±1.26[a]	6.00±1.41[a]	4.50±2.78[a]	5.00±0.00[a]	5.00±0.00[a]
Group E	6.65±1.12[a]	3.58±0.49[a]	3.12±0.58[a]	1.67±0.58[a]	2.00±0.70[a]	2.79±1.23[a]	5.89±1.26[a]	5.89±1.26[a]	6.69±0.44[b]	8.68±0.00[a]	8.68±0.00[a]
Group F	6.87±0.92[a]	3.28±0.81[a]	2.22±0.59[a]	2.49±0.67[a]	7.33±0.74[d]	5.55±2.17[b]	6.15±0.245[d]	6.15±0.25[a]	6.23±1.78[b]	7.15±2.16[a]	7.15±2.16[a]
Group G	7.19±1.09[a]	3.70±1.45[a]	5.61±4.23[a]	7.17±2.49[d]	5.77±2.14[b]	8.47±1.48[c]	6.15±0.241[d]	6.15±0.24[a]	4.79±1.96[a]	8.21±1.56[a]	8.21±1.56[a]

Superscripts between groups in columns differed significantly with respect to infected/untreated control (Group A) Group A: infected/untreated, Group B: methanolic extract 150 mg, Group C: methanolic extract 50 mg, Group D: aqueous extract 150 mg, Group E: aqueous extract 50 mg, Group F: suramin ® 3.5mg/kg, Group G: uninfected control; [b] (*p*<0.05) ;[c] (*p*<0.001); [d] (*p*<0.01)

Table 3: Haemoglobin (g/dL) concentration of albino rats infected with *Trypanosoma evansi* and treated with various dosages of *B. aegyptiaca* leaf aqueous and methanolic extracts, Suramin® and their controls

Groups	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	DAY 32	Day 36	Day 40
Group A	14.02±2.37[a]	10.42±2.20[a]	12.86±1.75[a]	13.81±2.26[a]	3.31±0.44[a]	2.75±1.38[a]	7.87±1.61[a]	6.91±3.55[a]	7.58±2.91[a]	8.00±0.00[a]	8.00±0.00[a]
Group B	12.72±1.09[a]	11.12±2.49[a]	11.48±1.54[a]	5.39±2.64[c]	5.22±2.52[a]	10.60±3.15[c]	9.79±1.77[a]	7.97±3.49[a]	6.72±3.39[a]	7.86±1.96[a]	7.86±1.96[a]
Group C	13.38±1.07[a]	14.53±2.31[a]	10.00±3.16[a]	3.22±1.26[c]	4.39±2.04[a]	12.10±1.15[c]	13.40±4.30[a]	13.40±4.30[b]	6.54±4.39[a]	9.16±5.44[a]	13.00±0.00[a]
Group D	11.02±1.01[a]	12.74±1.11[a]	8.00±2.58[a]	2.42±0.80[c]	4.06±2.68[a]	3.88±0.77[a]	10.21±3.96[a]	10.21±3.96[a]	10.21±3.96[a]	12.00±0.00[a]	8.66±4.73[a]
Group E	13.16±2.51[a]	10.26±2.84[a]	5.11±0.85[c]	3.76±0.32[c]	2.95±1.21[a]	2.82±1.08[a]	8.33±3.06[a]	8.33±3.06[a]	9.50±0.71[a]	13.00±0.00[a]	13.00±0.00[a]
Group F	11.66±1.49[a]	11.76±1.64[a]	5.49±2.55[c]	4.77±2.19[c]	12.09±2.84[c]	14.11±1.87[c]	13.60±1.72[a]	13.60±1.72[d]	12.35±3.52[a]	12.35±2.49[a]	12.35±2.49[a]
Group G	12.96±0.90[a]	12.40±1.52[a]	11.37±1.78[a]	14.32±2.86[a]	14.49±1.79[c]	12.80±3.01[c]	12.04±4.34[a]	12.04±4.34[a]	10.24±2.22[a]	10.24±2.63[a]	10.15±3.03[a]

Superscripts between groups in columns differed significantly with respect to infected/untreated control (Group A)
Group A: infected/untreated, Group B: methanolic extract 150 mg, Group C: methanolic extract 50 mg, Group D: aqueous extract 150 mg, Group E: aqueous extract 50 mg, Group F: suramin ® 3.5mg/kg, Group G: uninfected control; [b] (*p*<0.05) ; [c] (*p*<0.001); [d] (*p*<0.01)

Table 4: White blood cell (× 10³/mm³) of albino rats infected with *Trypanosoma evansi* and treated with various dosages of *B. aegyptiaca* leaf aqueous and methanolic extracts, Suramin® and their controls

Groups	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	DAY 32	Day 36	Day 40
Group A	8.61±0.75[a]	4.96±1.44[a]	2.84±1.71[a]	3.20±0.84[a]	3.26±0.72[a]	2.71±0.62[a]	3.07±1.75[a]	2.03±0.94[a]	4.50±0.99[a]	6.85±0.00[a]	6.85±0.00[a]
Group B	9.83±0.71[a]	2.91±0.79[a]	4.92±1.99[a]	3.47±1.49[a]	3.90±1.19[a]	13.01±1.39[c]	4.27±0.60[a]	5.50±2.42[a]	4.50±2.42[a]	9.03±2.18[a]	9.03±2.18[a]
Group C	10.91±2.54[a]	3.47±0.75[a]	3.78±0.68[a]	3.05±0.76[a]	2.22±0.75[a]	4.92±1.63[a]	3.23±0.92[a]	3.23±0.92[a]	6.23±1.36[a]	4.75±0.78[a]	4.75±0.78[a]
Group D	11.21±1.52[a]	3.48±0.32[a]	3.21±1.25[a]	2.37±0.98[a]	3.52±1.53[a]	2.95±0.36[a]	3.13±1.21[a]	5.10±2.59[a]	5.10±2.59[a]	6.00±0.00[a]	6.00±0.00[a]
Group E	11.82±2.19[a]	5.66±2.02[a]	2.67±0.31[a]	3.46±1.56[a]	3.38±0.54[a]	2.79±0.74[a]	2.75±0.66[a]	2.75±0.66[a]	4.00±1.41[a]	9.06±0.00[a]	9.06±0.00[a]
Group F	10.18±2.43[a]	4.84±1.52[a]	2.43±1.15[a]	1.95±1.56[a]	13.31±0.85[c]	8.06±5.76[d]	9.93±0.22[c]	9.93±0.22[c]	9.79±1.01[d]	10.87±3.17[b]	10.87±3.17[b]
Group G	10.75±2.17[a]	4.04±2.12[a]	12.20±1.59[c]	1.95±0.54[c]	13.18±1.96[c]	9.21±5.61[c]	7.53±3.53[b]	7.53±3.53[cd]	7.86±2.34[a]	9.02±1.67[a]	8.53±1.44[a]

Superscripts between groups in columns differed significantly with respect to infected/untreated control (Group A); Group A: infected/untreated, Group B: methanolic extract 150 mg, Group C: methanolic extract 50 mg, Group D: aqueous extract 150 mg, Group E: aqueous extract 50 mg, Group F: suramin ® 3.5mg/kg, Group G: uninfected control; [b] (*p*<0.05) ; [c] (*p*<0.001); [d] (*p*<0.01)

The mean eosinophil values fluctuated in the treatment groups reaching its peak by day 12 p.i. for Group C (methanol extract at 50 mg/kg), and day 16 p.i for Group B, D and E. Statistically significant difference (P<0.05) was observed by day 24 and 32 p.i. for group D with respect to the untreated control (Group A) (Figure 2). The mean lymphocyte values fluctuated significantly (P<0.05) across the different treatment groups which was most notably in group D by day 24 p.i. There was modulation of the mean lymphocyte count to its pre-infection values for groups C and E (Figure 3). Also, there was a steady decrease in the mean neutrophil values reaching its lowest values in some of the treatment groups (B, C and D) by day 8 p.i., and day 12 p.i. for groups (E and F). Statistically significant difference was observed at day 28 p.i. for treated rats in group E when compared with the untreated control (Group A) (Figure 4). Lastly, the mean monocyte values of majority of the treatment groups reached its peak by day 12 p.i. (Figure 5).
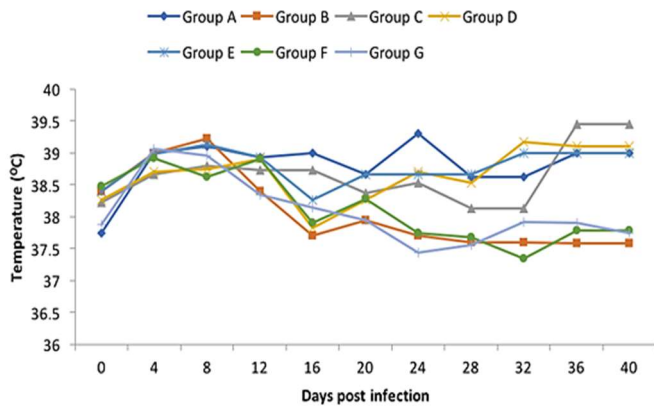
Figure 1: Mean temperature changes of albino rats infected with *Trypanosoma evansi* and treated with various dosages of aqueous and methanolic extract of *B. aegyptiaca*, Suramin® and their controls
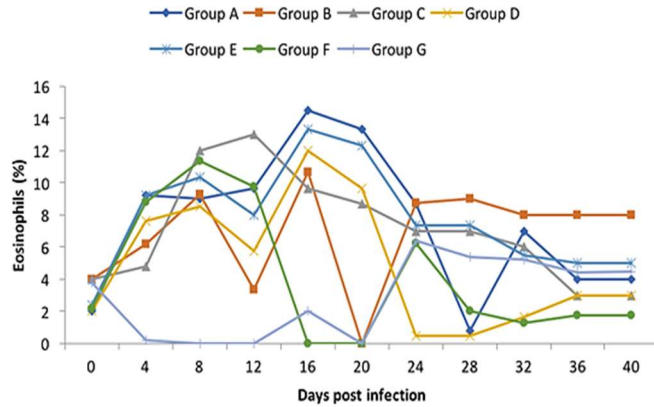

Figure 2: Mean eosinophil changes of albino rats infected with *Trypanosoma evansi* and treated with various dosages of aqueous and methanolic extract of *B. aegyptiaca*, Suramin® and their controls

disease in infected animals (Bashir *et al.,* 2015; Sivajothi *et al.,* 2015; Abo-Aziza *et al.,* 2017).

Figure 3: Mean lymphocytes changes of albino rats infected with *Trypanosoma evansi* and treated with various dosages of aqueous and methanolic extract of *B. aegyptiaca*, Suramin® and their controls
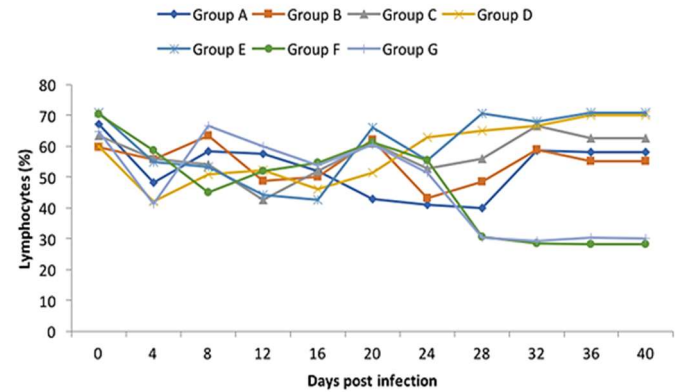

Figure 4: Mean monocytes changes of albino rats infected with *Trypanosoma evansi* and treated with various dosages of aqueous and methanolic extract of *B. aegyptiaca*, Suramin® and their controls
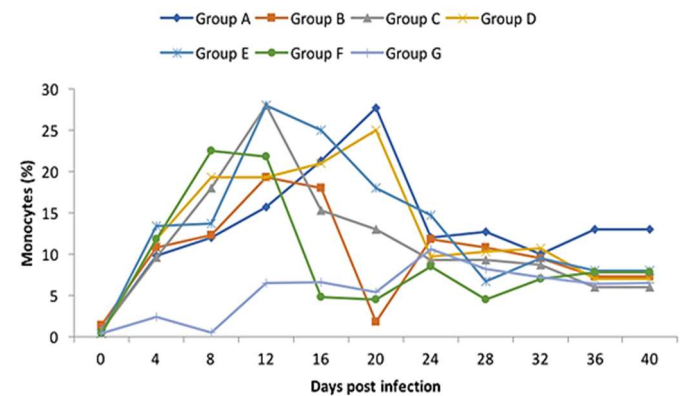
DISCUSSION

The challenge of trypanocidal resistance and increasing concern over drug residues in products from animal origin has led to resurgence of interest in the use of phytomedines, in the form of extracts containing a mixture of compounds (Ademola and Odeniran, 2017). This study investigates the efficacy of *B. aegyptiaca* and Suramin on *T. evansi* infected albino rats. Variations in rectal temperature as observed in the infected rats is typical of trypanosomosis (Taylor and Authie, 2004). Despite alterations in blood parameters, there was no conforming undulating pyrexia. It was documented in a similar study that pyrexia was absent in the experimental treated mice using extracts of *Brillantaisia owariensis* after infection with *T. brucei brucei*, and the observation was attributed to the likely presence of traces of Acetamide, N-(4-hydroxyphenyl)-N-methyl (Paracetamol) which is an antipyretic, (Ayawa *et al.,* 2021).

To ascertain the extent of deleterious effects of foreign agents on the blood constituents of mammals, hematological parameters are one of such important physiological indices that could be explored. Hematological changes are one of the principal clinical pathologic change characteristics of animal trypanosomosis and this change alters the pathogenesis of the

From our data, we observed that the PCV, Hb and RBCs counts were reduced in the infected groups, an indication of anemia due to increased susceptibility of the membrane of the red blood cells to oxidative damages as opined by researchers from previous studies (Taiwo *et al.,* 2003; Sivajothi *et al.,* 2015). Additionally, anemia resulting from infection of animals with *T. evansi* might be due to the depletion of erythrocytes due to chronic liver inflammation (Farghaly and Sadek, 2020). Following treatment with *B. aegyptiaca* leaf aqueous and methanolic extracts, there was improvement in the PCV, RBCs count and Hb concentrations.

Specifically, the PCV was markedly improved for the rats treated with 150mg/kg methanolic extracts (Group B), while treatment with 50mg/kg methanolic (Group C) and 50mg/kg aqueous (Group E) extracts had the most activity in improving the RBC count and Hb concentration. The reversal of anemia in the extract treatment rats could be attributed to the ability of the extract to decrease oxidative stress markers as previously proposed by Raish *et al.* (2016).

Furthermore, infection with *T. evansi* induced leukopenia with decrease in the total WBC counts, which was reversed considerably after treatment individually with methanolic and aqueous leaf extracts of *B. aegyptiaca*. The observed

leukocytosis evident by increase in the mean WBC count after treatment, is a good indicator that the extracts enhance and strengthen the immune system of the experimental rats by boosting the production of WBCs (Al-Otaibi *et al.,* 2019) and increasing mononuclear phagocytic system activity (Ahmadi-Hamedani *et al.,* 2014).

Additionally, it was observed that infected albino rats showed lymphopenia, neutropenia, monocytosis and eosinophilia before treatment commenced. The decrease in lymphocyte numbers of leucocytes and neutrophils suggest that the parasite causes marked antigenic stimulation leading to accelerated transformation of lymphocytes to plasma cells and transferred lymphocytes resulting to lymphopenia (Marrison *et al.* 1982). Similarly, the severe fall in neutrophil number in the infected rats might have been caused by marked depression of precursor cells and marked phagocytosis of neutrophil precursor cell in the bone marrow (Anosa *et al.* 1997) and spleen (Anosa, 1988). Eosinophilia was also observed in the treatment group before treatment. Anosa and Kaneku (1984) also reported eosinophilia in *T. brucei*-infected deer mice. The decrease in the number of eosinophils is due to the therapeutic effect of the extract and suramin which reduced the number of *T. evansi.* The number of monocytes (monocytosis) in the infected group before treatment increased compared to the uninfected control, until after treatment when the number of monocytes decreased. The monocytosis observed in rats infected with *T. evansi* before treatment may be due to increased demand for removal of particulate matter arising from severe pathology.

CONCLUSION

From the findings of the current study, it was concluded that both the methanolic and aqueous leaf extract of *B. aegyptiaca* had considerable antitrypanosomal activity which was dose-dependent and was effective in alleviating anemia. Therefore, this extract could be potentially useful for the control of animal trypanosomosis.

Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contributions

AMA designed the study, participated in the technical work, and wrote the manuscript. KDM and TEO reviewed and edited the manuscript and did the statistical analysis. MK and SM conducted the technical work, while KBB and ND did the final proof reading. All authors read and approved the final manuscript.

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