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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Anti-trypanosomal effects of chloroform extracts of *Artemisia herba-alba* against *Trypanosoma evansi* infection in rabbits: clinical, parasitological and haemato-biochemical responses

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Abstract

Haemato-parasitic infections are major causes of reduced productivity in livestock worldwide. For many centuries, chemotherapy has been used extensively, regardless their side-effects, on livestock production. Phytomedical options have been suggested as an alternative approach to treat parasitism and improve performance of livestock. However, scientific evidence on antiparasitic efficacy of most plant products is critically limited. The present study was conducted to validate the anti-trypanosomal properties of chloroform extracts of *Artemisia herba-alba in vivo*. A total of thirty rabbits (n=30) of approximately 5-6 months old, weighing about 1.5-2.5 kg were divided into six main groups of five animals each. The course of *Trypanosoma evansi* infection in rabbits was followed for 48 days post-infection. Animals were closely observed for clinical examination. Blood samples were collected for haematological and biochemical purposes. Animals of the infected group demonstrated depression and weakness, lethargy and pale mucous membranes. In these animals, parasites were detected from day 2 post-infection until the end of the experiment. Similarly, parasites were detected from day 2 post-infection in animals treated with chloroform extracts of *Artemisia herba-alba*. However, in these treated animals, elevating levels of parasitemia followed by intermixed and aparasitemic levels were detected. In contrast, animals of infected group showed reduction in the levels of blood indices (PCV, Hb, RBCs and WBC) and alterations in biochemical values. Unlikely, animals treated with chloroform extracts of *Artemisia herba-alba* revealed normal haematological and biochemical values thereby confirming their antiparasitic properties.

Keywords: chloroform; *Artemisia herba-alba*; *Trypanosoma evansi*; Parasitism; haematology; biochemistry; rabbits

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Introduction

Trypanosoma evansi is haematoparasitic agent that affects a wide range of mammalians and responsible for major production losses worldwide. Heavy burdens of this parasite can cause severe anemia and rapid death in affected hosts. The principal hosts, however, vary according to their geographical locations. Buffalo, cattle, camels and horses are particularly prone, although other animals, including wildlife, can also be infected (OIE, 2008). To date, these parasites are shifting their ranges in response to international trade of livestock. Many of cases have already been documented in Asia, Africa and Latin America (Luckins and Dwinger, 2004). Despite the scientific evidence published in the database, yet, *T. evansi* has not been included in the animal health conditions for international trade in many countries across the globe (Gutierrez et al., 2000). Controlling strategies have mostly been focused on the use of chemotherapy

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options as anti-trypanosomal medicines. However, extensive resistance of *Trypanosoma spp* to these drugs has developed. As an alternative, research is being conducted to identify plant products with anti-trypanosomal properties.

Artemisia herba-alba Asso, commonly known as white wormwood or desert wormwood (Arabic name chih), is a greyish-strongly aromatic dwarf shrub native to the Northern Africa, Arabian Peninsula, South Western Europe, and Western Asia. This plant belongs to the genus of Artemisia, an important source of biological compounds for insecticides and fungicides, antibacterial, allelopathic products (Chauhan et al., 2010). *Artemisia herba-alba* Asso, grows wild on nitrofilous and gypsum-rich substrata. In Libya, this taxon is abundant with the highest population density in the south-west Libya.

Numerous studies examined the chemical composition, biological activities, and other industrial characteristics of essential oils of *Artemisia sp.* (Bicchi etal., 1985; Salido et al., 2001). However, there is a lack of studies about the biological activates of *A. herba-alba.* Thus, this study investigated the potential value of this natural resource as anti-trypanosomal properties using rabbit model.

Materials and Methods

Plant material and extract preparation

The aerial parts of *A. herba-alba* were collected during the flowering and vegetative phase of the plant at different localities characterized by diverse geographic and climate conditions in Libya. The plants were cut at ground level and those portions from above ground were air dried for two weeks in a laboratory of the Botany Department, Omar Al Mukhtar University, Al Beida, Libya. The chopped, air dried plant material was stored in refrigerator, and awaited for extraction analysis. Thereafter, the choloride ethanolic extract was prepared at the Faculty of Science and Technology, University Kebangsaan Malaysia (UKM), Bangi. Dried plants of *A. herba-alba* were grounded into powder. Five hundred gram powdered plant material was extracted in a Soxhlet apparatus with 95% ethanol at a temperature of 50°C for 12 h. The residue was removed by filtration. The extract was concentrated in a rotary evaporator under reduced pressure at temperature of 40–50°C and then lyophilized to obtain a powdered extract and stored at 4°C until used (Ene et al., 2009).

Animals

All procedures described in this study were approved by the Ethics committee of UKM (UKMAEC) for the Care and Use of Animals. Experiments utilized 5-6 months old, New Zealand clinically healthy rabbits. Upon arrival, all rabbits (n=30) were screened for the presence of haemo-protozoan parasites using wet mount and Leishman stained. The animals were placed in large appropriate metal cages (1 per cage) and maintained in a fly proof isolation unit at the experimental animal house, Faculty of Biosciences and Biotechnology, University Kebangsaan Malaysia (UKM). Rabbits were provided *ad libitum* access to water and a stock chow pellets throughout the experimental period.

Stock of parasites and inoculum preparation

The *Trypanosoma evansi* isolate used for the experiment was obtained from the Parasitology Laboratory, Faculty of Veterinary, University Putra Malaysia (UPM). The parasite was originally isolated from a naturally infected deer from Perak state, Malaysia in 2007 (Adrian et al., 2010). The isolate is propagated by sub-passaging in mice. An inoculum of 5×10^5 parasites in 1 ml was then prepared by diluting the pooled mice blood with Alsever's solution.

Experimental procedure

At the start of the experiment, the rabbits were divided into six major groups of five rabbits in each group (Table 1). Animals of Group 1 were pre-treated for two days intraperitoneally with 20 mg/kg$^-$ of crude ethanolic extract (CEE) of the aerial parts of *A.herba-alba* before inoculation with *T. evansi* and thereafter treated for 6 days after infection. Rabbits of Group 2 were treated intraperitoneally with 20 mg/kg of CEE of *A. herba-alba* and concurrently infected with *T. evansi*. Rabbits of Group 3 were treated 6 days with 20 mg/kg of CEE of *A. herba-alba* for six days after establishment of parasitemia. Animals in Group 4 were positive control and were treated once with 3.5 mg/kg of diminazeneaceturate (Berenil®, Sigma Chemical Co. (St. Louis, MO, U.S.A.) after the establishment of parasitemia. Rabbits in Group 5 were only infected once intraperitoneally with 1 ml of 5×10^5 parasites. Animals of Group 6 served as reference group and were injected intraperitoneally with 1 ml of sterile Alsever's solution. The detailed information of the experimental design was summarized in Table 1.

Parasitemia estimation

All animals were sampled at two days interval during 48 days post-infection (pi), about 2 ml of peripheral blood were collected from the rabbit's marginal ear vein after shaving the area. Parasitemia count was determined by Micro-Haematocrit Centrifugation Technique (MHCT) (Woo, 1970). About 75 µl of fresh blood were taken with a heparinized capillary and centrifuged for 5 min at 12,000g. Capillary tubes were examined using a light microscope (100 or 400 X magnification) for detection and counting trypanosomes when the numbers were few

Table 1: Experimental design

Group	Category	Description	No. of animals	Dose of CEE (mg/kg)	Volume of inoculum of *T. evansi* (ml)	Route of administration
1	Pre-infection	Pre-treated with CEE of *A. herba-alba* before 2 days of *T. evansi* infection	5	20	1 ml of 5×10^5	I/p
2	Concurrent	Treated with CEE of *A. herba-alba* concurrently infected with *T. evansi*	5	20	1 ml of 5×10^5	I/p
3	Post-infection	Treated once after establishment of parasitemia.	5	20	1 ml of 5×10^5	I/p
4	Positive control	Treated with diminazeneaceturate after establishment of parasitemia.	5	3.5	1 ml of 5×10^5	I/m
5	Negative Control	Infected with *T. evansi* and remain untreated	5	*N/A	1 ml of 5×10^5	I/p
6	Reference	Uninfected but treated with Alsever's solution.	5	*N/A	*N/A	I/p

*N/A: Not applicable

around the buffy coat plasma interphase area (Woo, 1970). High parasitemia enumeration was undertaken using a *Neubauer* hemocytometer.

Collection of blood samples

Marginal ear venous blood samples were collected for haematological and biochemical analysis once every two weeks until 48 days post-infection. Blood samples for hematology were collected into tubes containing ethylene diaminetetriacetic acid (EDTA) as anticoagulant. Blood samples for biochemical analysis were collected into tubes containing no anticoagulant.

Clinical examination

The clinical observations presented by the diseased and non-diseased groups were regularly documented from the start of the study until the end point of the experiment. Because the scoring systems require capture of signs and symptoms since the beginning of the experiment, the information collected from the six groups was based on the individual presentation of the clinical signs. In summary, the clinical signs of six groups were scored in scale of 0-3 based on the presence of following parameters: loss of condition, oedema of the face, ocular discharges, encrustation of the lips and mortality rate. The score 0 represented no abnormality of clinical signs observed, 1 for mild (30% abnormality), 2 for moderate (60% abnormality), 3 for severe (more than 60% abnormality).

Estimation of haematological examination

The investigated haematological parameters include Packed cell volume (PCV), haemoglobin concentration (Hb%), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) and differential leucocytic count. They were measured in heparinized blood according to Jain (2000).

Estimation of biochemical assays

Changes of alkaline phosphatase (AP), alanine amino transferase (ALT), aspartate amino transferase (AST), and plasma glucose activities were measured using automated chemistry analyzer (HITACHI 902 Automatic Analyzer®, Japan).

Statistical methods

Evaluation of the obtained results were performed by using the statistical package Version JMP 9, SAS, where the values of Analysis of Variance (ANOVA) were used to detect the significant changes among the experimentally infected Rabbits.

Results

Parasitological examination

Daily examination of the blood of the five inoculated rabbits (1-5) by BCT revealed that *T. evansi* started to appear on the third day post-infection. The patent period was five days in all the inoculated rabbits. Maximum parasitemia was observed on the 6^{th}-8^{th} day post-infection (DPI). All five animals in each group were positive for *T. evansi.* After 10 days post-infection, periods of low parasitemia were intermixed with aparasitemia periods in rabbits of experimental groups 1-4 (Fig. 1). *Trypanosoma evansi* was not detected from the 15^{th} day post-infection until the end of the experiment at 48 DPI. The reference group showed no *T. evansi* during the experimental period. The infection in rabbits was slightly pathogenic in treated groups during the first two weeks DPI. These animals showed mild symptoms appearing from fist- ninth DPI. In contrast, animals in group 5 (negative control), parasitemia was recorded from day 3 post-infection, where animals remained with the high peaks of parasitemia between day 10-35 post-infection showing severe symptoms in the investigated parameters.

Clinical observation

The results obtained from the clinical observation were outlined in Tables 2-5. Animals served as negative control were severely affected after experimental infection with *T. evansi*. In these animals, the clinical

Table 2: Mean rank of poor condition between reference group, treated groups with Chloroform extract of *A. herba-alba* and negative group

	Parameters					
*DPI	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
2	$0.0^{a,x}$	$1.5^{b,x}$	$1.5^{b,x}$	$1.5^{b,x}$	$1.5^{b,x}$	$3.0^{c,z}$
10	$0.0^{a,x}$	$1.0^{a,y}$	$1.5^{b,x}$	$1.0^{a,y}$	$1.0^{a,y}$	$2.5^{c,z}$
20	$0.0^{a,x}$	$1.5^{b,x}$	$1.5^{b,x}$	$1.0^{a,y}$	$1.0^{a,y}$	$2.0^{b,y}$
30	$0.0^{a,x}$	$1.5^{b,x}$	$1.5^{b,x}$	$1.0^{b,y}$	$1.5^{b,x}$	$3.0^{c,z}$
40	$0.0^{a,x}$	$1.0^{b,y}$	$1.0^{b,y}$	$1.0^{b,y}$	$1.0^{b,y}$	$1.5^{b,y}$

*DPI: Days post-infection; [a-c]Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; [x-z]Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 3: Mean rank of edema between reference group, treated groups with Chloroform extract of *A. herba-alba* and negative group

	Parameters					
*DPI	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
2	$0.0^{a,x}$	$1.5^{b,y}$	$1.5^{b,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{c,z}$
10	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$	$1.5^{b,y}$	$2.0^{b,y}$
20	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	1.0	1.0	$2.0^{b,y}$
30	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$0.0^{a,x}$	$1.5^{b,y}$	$3.0^{c,z}$
40	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$

*DPI: Days post-infection; [a-c]Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; [x-z]Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 4: Mean rank of encrustation between reference group, treated groups with Chloroform extract of *A. herba-alba* and negative group

	Parameters					
*DPI	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
2	$0.5^{a,x}$	$1.5^{b,y}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{c,z}$
10	$0.5^{a,x}$	$1.0^{a,x}$	1.5	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{c,z}$
20	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$	$1.5^{b,y}$	$2.0^{b,y}$
30	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{b,y}$
40	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$

*DPI: Days post-infection; [a-c]Means with different superscripts within row differed significantly (P<0.05) due to treatment effect. [x-z]Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 5: Mean rank of ocular discharges between reference group, treated groups with chloroform extract of *A. herba-alba* and negative group

	Parameters					
*DPI	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
2	$0.5^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$	$1.5^{b,y}$	$1.0^{a,x}$	$2.5^{c,z}$
10	$0.5^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{c,z}$
20	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.0^{b,y}$
30	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$2.5^{c,z}$
40	$0.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.0^{a,x}$	$1.5^{b,y}$

*DPI: Days post-infection; [a-c]Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; [x-z]Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 6: Comparison of lethality rate between reference group, treated groups with chloroform extract of *A. herba-alba* and negative group

Reference Group			Treatment Groups (1-4)		Negative Group	
Time (weeks)	Number of animas died	Lethality rate (%)	Number of animals died	Lethality rate (%)	Number of animals died	Lethality rate (%)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	1	20
4	0	0	0	0	0	0
5	0	0	0	0	2	40
6	0	0	0	0	0	0
7	0	0	0	0	0	0
Total	0	0	0	0	5	60.00

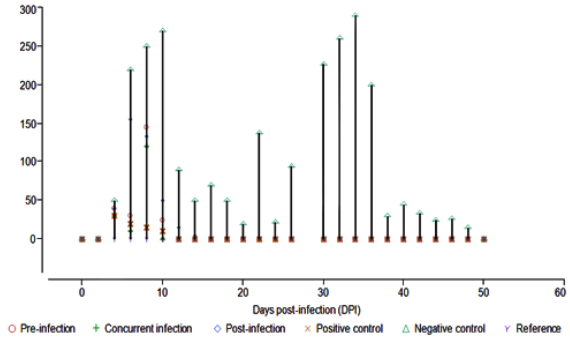

Fig. 1: Parasitemia of *T. evansi* from groups (1-6) that involved in the study

signs due to the infection of *T. evansi* appeared on day 3 post-infection where most of the animals exhibited typical clinical signs that included edema in the face, ocular discharges, poor condition and encrustation of the lips. The mean ranks of these tested parameters were significantly higher than all treated groups and control group (Tables 2-5). In contrast, these clinical signs were prevented by intraperitoneal administration of *A. herba-alba* in groups 1-3 throughout the experimental period. Similarly, animals treated with the standard drug (Bernil®) showed no clinical signs for the whole duration of the experimental period. No significant difference was observed between chloroform extracts treated and Bernil® treated groups.

Mortality rate

No death was recorded throughout the experimental period in all treated groups (1-4), treated either with CEE of *A. herba-alba* or with diminazeneaceturate. Similarly, animals served as reference group did not show any mortality rate in the whole duration of the experimental period. In contrast, animals within negative control (infected but not treated) started to die frequently where death of first animal (20%) observed on day 17th. Thereafter, another 2 animals (40%) died on day 34. The remaining 2 animals of the negative group survived throughout the experimental period making the total lethality (60%) (Table 6). Mortality rates were associated with severe parasitemia caused by the challenged strain *(T. evansi)*.

Changes in the haematological values

Haematologically, the characteristics of tested parameters in the different groups involved in the study are summarized in Tables 7-14. Of these groups, animals in group 5 (negative control) showed statistically a significant decrease in the tested values of PCV, Hb and RBC, with maximum mean values of 10.60±2.30, 74.94±7.07, 2.46±.33 respectively (Tables7-9). The values of these parameters started to drop from the first week of the study and remained lower than the values of control and treated animals until the end of the experimental period. On the other hand, the values PCV, Hb and RBC of treated groups (1-4) showed slight decrease in serum levels between day 3–5 post-infection. Thereafter, normalization of these values (PCV, RBC & Hb) was observed and remained unchanged throughout the experimental period. There were no significant differences observed in all tested parameters in animals served as reference group (Tables 7-14).

Changes in biochemical values

The mean levels of the tested blood biochemistry indices were summarized in (Fig. 2). Increased levels were observed in some of the indices of ALT and AST in group 5 (negative group) throughout all experimental period. However, this group (negative control) showed reduced levels in serum ALP during the experimental period. Moreover, animal in this group, serum albumin concentration decreased gradually concomitant to an increase in globulin levels leading to a decrease in the albumin–globulin ratio (data not shown). There was no significant differences observed in the values of total protein, bilirubin, and plasma glucose mean values between animals treated with Chloroform extract of *A. herba-alba* and other experimental units (data excluded). Similarly, animals treated with *A. herba-alba* and Bernil were not significant different than reference group in almost all tested biochemical parameters.

Discussion

Recently, studies concerning natural products with trypanosomal activity have been relatively gaining substantial increase in wide range of disciplines. In the context of *Artemesia*, most of the studies evaluated the phytochemical classes (Belhattab et al., 2013), the antioxidant properties (Lopez-lutz et al., 2008), the antiparasitic and the antimicrobial activities (Mighri et al., 2010). However, the current study examined, for the first time the *in vivo* trypanosmal activities of the aerial parts of *Artemisia herba-alba* cultivated in Libya. Our results indicate that manipulation of *T. evansi* led to the production of parasitemia in certain stages in all groups of 1-5 that involved in the study. The levels of the parasitemia observed in the early stages were significantly ($P<0.05$) associated with the tested clinical parameters; gradual loss of condition, facial edema and ocular discharges in almost all involved groups (1-5). These clinical signs are in agreement with those reported earlier in other studies of experimental nature (Losos and Ikede, 1972). High levels of parasitemia along with the rapid development of anemia were the characteristic of this early stage. This was in agreement with findings of Aquino et al. (1999) in dogs. In the later stage of the infection, the rabbits developed a

Table 7: The effect of Chloroform extract of *A. herba-alba on* PCV values of rabbits infected with *T. evansi*

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	36.00±1.73^{a,x}	23.50±5.17^{b,y}	26.10±3.71^{b,y}	26.57±4.56^{b,y}	28.33±.57^{b,y}	11.00±3.39^{c,z}
2	36.20±1.09^{a,x}	29.80±4.14^{b,y}	28.40±6.18^{b,y}	29.57±7.95^{b,y}	32.33±2.88^{b,y}	10.80±2.94^{c,z}
3	35.40±2.30^{a,x}	25.80±2.97^{b,y}	27.50±3.33^{b,y}	25.85±2.95^{b,y}	29.33±.28^{b,y}	12.40±3.84^{c,z}
4	35.80±1.64^{a,x}	26.10±3.61^{b,y}	28.50±3.80^{b,y}	23.66±5.20^{b,y}	26.75±6.84^{b,y}	10.60±2.30^{c,z}
5	35.95±1.82^{a,x}	25.60±2.07^{b,y}	24.10±3.47^{b,y}	25.33±3.14^{b,y}	27.87±1.03^{b,y}	13.60±4.03^{c,z}
6	36.17±1.77^{a,x}	24.60±2.04^{b,y}	27.60±2.70^{b,y}	24.66±2.87^{b,y}	25.75±3.94^{b,y}	15.61±9.65^{c,z}
7	35.28±1.77^{a,x}	25.00±1.87^{b,y}	24.80±5.84^{b,y}	22.16±1.83^{b,y}	25.87±2.75^{b,y}	15.54±4.39^{c,z}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 8: Hb values of rabbits treated with Chloroform extract of *A. herba-alba* and those not treated

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	133.00±2.54^{a,x}	125.80± 28.26^{a,x}	98.08±5.52^{b,y}	100.74±17.54^{a,x}	102.83±11.81^{a,x}	86.20±17.54^{c,z}
2	130.20±5.97^{a,x}	111.78±13.76^{a,x}	93.26±12.10^{b,y}	105.15±19.05^{a,x}	109.70±13.99^{a,x}	86.00±13.96^{c,z}
3	133.20±1.78^{a,x}	112.98±16.99^{a,x}	94.92±13.04^{b,y}	104.85±17.84^{a,x}	106.66±6.35^{a,x}	80.08±15.08^{c,z}
4	131.80±2.16^{a,x}	127.60±4.50^{a,x}	98.42±12.55^{b,y}	109.33±19.44^{a,x}	101.50±1.73^{a,x}	74.94±7.07^{c,z}
5	133.60±2.60^{a,x}	110.98±14.05^{a,x}	93.26±12.10^{b,y}	116.00±11.50^{a,x}	92.30±3.23^{a,x}	91.00±21.11^{b,y}
6	131.40±1.51^{a,x}	99.48±6.86^{b,y}	94.92±13.04^{b,y}	104.16±13.33^{a,x}	99.05±4.56^{a,x}	82.78±9.43^{c,z}
7	132.00±2.34^{a,x}	115.56±16.15^{a,x}	98.42±12.55^{b,y}	118.10±20.87^{a,x}	102.50±.57^{a,x}	78.54±13.01^{c,z}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect.
$^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 9: RBC values of rabbits treated with Chloroform extract of *A. herba-alba* and those not treated

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	4.43±.24^{a,x}	4.43±.24^{a,x}	3.84±.42^{a,x}	4.17±.56^{a,x}	4.35±.30^{a,x}	2.59±.85^{c,z}
2	4.15±.49^{a,x}	5.12±.29^{a,x}	4.52±.26^{a,x}	4.26±.48^{a,x}	4.66±.49^{a,x}	2.75±.70^{c,z}
3	3.78±.60^{a,x}	4.58±.51^{a,x}	3.84±.42^{a,x}	4.07±.32^{a,x}	4.35±.30^{a,x}	2.76±.98^{c,z}
4	4.23±.48^{a,x}	4.43±.24^{a,x}	3.82±.54^{a,x}	4.31±.71^{a,x}	4.89±.26^{a,x}	2.59±.83^{c,z}
5	4.28±.24^{a,x}	4.28±.24^{a,x}	4.16±.46^{a,x}	4.02±.77^{a,x}	4.88±.37^{a,x}	2.81±.77^{b,y}
6	4.53±.66^{a,x}	4.17±.47^{a,x}	3.82±.54^{a,x}	4.50±.37^{a,x}	4.32±.25^{a,x}	2.46±.33^{c,z}
7	3.92±.42^{a,x}	4.58±.51^{a,x}	4.16±.46^{a,x}	4.19±.54^{a,x}	4.89±.26^{a,x}	3.27±.35^{b,y}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 10: WBC values of rabbits treated with Chloroform extract of *A. herba-alba* and those not treated

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	4.02±1.56^{a,x}	4.13±1.71^{a,x}	3.85±.96^{a,x}	4.26±.97^{a,x}	4.10±1.83^{a,x}	4.44±1.45^{a,x}
2	3.07±.26^{a,x}	4.23±2.50^{a,x}	6.30±1.23^{b,y}	6.32±2.48^{b,b}	2.92±.00^{c,z}	6.98±1.63^{b,y}
3	3.24±.28^{a,x}	3.57±.96^{a,x}	4.12±1.58^{a,x}	3.67±1.09^{a,x}	2.92±.00^{c,z}	5.29±1.46^{b,y}
4	3.84±1.66^{a,x}	3.59±1.75^{a,x}	3.60±1.01^{a,x}	4.62±1.71^{a,x}	4.84±3.84^{a,x}	6.31±1.42^{b,y}
5	3.24±.28^{a,x}	4.96±2.55^{a,x}	4.15±1.58^{a,x}	2.92±.00^{c,z}	2.92±.00^{c,z}	5.23±2.15^{b,y}
6	3.24±.28^{a,x}	3.52±.98^{a,x}	4.12±1.58^{a,x}	2.99±.18^{a,x}	2.92±.00^{c,z}	6.47±2.35^{b,y}
7	3.24±.28^{a,x}	2.91±.60^{a,x}	3.60±1.01^{a,x}	2.88±.08^{a,x}	2.92±.00^{c,z}	4.47±2.12^{a,x}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

persistent and severe anemia while only a few parasites could be detected in the blood. This is explained by the chronic nature of the infection in these animals. Our findings revealed an initial and transient parasitemia when the condition is treated with the chloroform extract of *Artemisia herba- alba,* suggesting the anti-parasitic activities of *A. herba-alba* that is comparable to the exhibited by Bernil®, a standard trypanocidal drug for treatment of trypanosomosis in animals. This was in agreement with the findings of other researchers (Atawod et al., 2003; 2005).

The hematological findings in the tested animals indicated that haematological values decreased following development of parasitemia and were indicative of anemia. These observations were in accordance with earlier finding reported by Anene

Table 11: The effect of Chloroform extract of *A. herba-alba on* ALT values of rabbits infected with *T. evansi*

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	43.54±5.89^{a,x}	51.54±9.78^{a,x}	57.54±10.37^{a,x}	53.62±10.02^{a,x}	46.67±5.50^{a,x}	135.04±48.68^{c,z}
2	40.92±4.47^{a,x}	62.92±23.45^{b,y}	60.92±16.43^{b,y}	62.11±18.67^{b,y}	69.58±15.27^{b,y}	143.07±45.36^{c,z}
3	41.98±9.28^{a,x}	53.98±16.97^{a,x}	59.98±14.80^{a,x}	48.71±16.06^{a,x}	57.71±20.59^{a,x}	139.07±20.93^{c,z}
4	40.57±3.24^{a,x}	52.57±26.35^{a,x}	50.57±17.24^{a,x}	50.98±9.76^{a,x}	55.04±29.75^{a,x}	157.07±34.61^{c,z}
5	45.92±7.07^{a,x}	57.92±13.03^{a,x}	57.92±8.36^{a,x}	55.58±15.97^{a,x}	65.42±15.00^{a,x}	129.07±15.74^{c,z}
6	40.58±5.21^{a,x}	50.58±14.04^{a,x}	55.99±16.51^{a,x}	49.26±12.96^{a,x}	52.54±19.24^{a,x}	143.07±45.36^{c,z}
7	40.57±3.24^{a,x}	64.57±25.79^{b,y}	50.84±18.24^{a,x}	62.60±23.57^{b,y}	67.54±28.78^{b,y}	125.07±25.71^{c,z}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 12: The effect of Chloroform extract of *A. herba-alba on* AST values of rabbits infected with *T. evansi*

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	93.54±27.10^{a,x}	100.94±14.46^{a,x}	100.94±14.46^{a,x}	89.34±20.94^{a,x}	93.34±20.66^{a,x}	163.04±89.85^{b,y}
2	89.54±14.20^{a,x}	85.54±16.54^{a,x}	88.94±14.22^{a,x}	87.65±14.35^{a,x}	76.67±10.96^{a,x}	173.07±84.36^{b,y}
3	99.54±16.33^{a,x}	83.18±12.45^{a,x}	94.94±15.75^{a,x}	95.05±19.33^{a,x}	83.34±14.73^{a,x}	188.98±75.22^{b,y}
4	86.14±33.51^{a,x}	100.54±18.07^{a,x}	100.94±14.46^{a,x}	101.34±19.81^{a,x}	91.84±17.13^{a,x}	167.98±84.72^{b,y}
5	78.14±30.97^{a,x}	100.54±18.07^{a,x}	92.94±18.47^{a,x}	94.67±22.70^{a,x}	81.84±14.15^{a,x}	164.98±86.62^{b,y}
6	80.14±30.66^{a,x}	100.54±18.07^{a,x}	93.94±14.75^{a,x}	101.84±22.30^{a,x}	92.59±22.06^{a,x}	175.98±73.45^{b,y}
7	101.74±22.13^{a,x}	107.14±18.07^{a,x}	83.94±7.60^{a,x}	85.84±20.57^{a,x}	92.84±17.07^{a,x}	168.98±84.19^{b,y}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 13: The effect of Chloroform extract of *A. herba-alba on* ALP values of rabbits infected with *T. evansi*

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	61.54±15.35^{a,x}	55.34±12.30^{a,x}	51.74±4.82^{a,x}	58.34±13.67^{a,x}	49.67±.57^{a,x}	37.54±11.94^{b,y}
2	69.33±17.93^{a,x}	61.11±17.52^{a,x}	59.53±17.32^{a,x}	63.91±17.32^{a,x}	62.65±23.09	27.72±7.76^{b,y}
3	61.34±15.54^{a,x}	51.14±12.53^{a,x}	45.53±9.33^{a,x}	58.19±13.77^{a,x}	42.67±5.77^{a,x}	27.54±8.22^{b,y}
4	54.14±5.67^{a,x}	46.12±11.32^{a,x}	42.14±12.89^{a,x}	66.17±17.81^{a,x}	42.59±9.32^{a,x}	27.71±7.76^{b,y}
5	58.11±11.14^{a,x}	50.13±7.08^{a,x}	50.14±12.25^{a,x}	61.34±16.11^{a,x}	55.09±10.17^{a,x}	32.53±6.14^{b,y}
6	56.12±5.31^{a,x}	52.14±4.02^{a,x}	54.15±5.21^{a,x}	52.67±11.82^{a,x}	55.06±5.50^{a,x}	27.73±7.76^{b,y}
7	68.74±14.15^{a,x}	58.71±13.70^{a,x}	58.34±11.51^{a,x}	46.84±16.29^{a,x}	68.34±16.30^{a,x}	27.54±10.84^{b,y}

^{a,b}Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; ^{y,z}Means with different superscripts within column differed significantly (P<0.05) due to time effect.

Table 14: The effect of Chloroform extract of *A. herba-alba on* globulin values of rabbits infected with *T. evansi*

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative Control
1	3.01±.39^{a,x}	2.56±.98^{a,x}	3.14±.60^{a,x}	2.65±.78^{a,x}	2.34±1.15^{a,x}	2.94±.59^{a,x}
2	3.14±.60^{a,x}	2.56±1.07^{a,x}	3.05±1.41^{a,x}	3.17±.56^{a,x}	3.00±.68^{a,x}	3.00±.51^{a,x}
3	3.38±.50^{a,x}	2.43±1.02^{a,x}	2.90±.56^{a,x}	2.95±.90^{a,x}	2.34±1.15^{a,x}	2.44±.95^{a,x}
4	3.43±.46^{a,x}	3.15±.55^{a,x}	3.44±.40^{a,x}	3.23±.59^{a,x}	3.28±.70^{a,x}	3.36±.56^{a,x}
5	3.49±.39^{a,x}	2.95±1.02^{a,x}	2.93±1.38^{a,x}	2.96±.51^{a,x}	2.31±.94^{a,x}	3.14±.90^{a,x}
6	3.25±.45^{a,x}	3.40±.39^{a,x}	2.32±.64^{a,x}	2.69±.93^{a,x}	2.61±.89^{a,x}	2.61±.99^{a,x}
7	3.34±.40^{a,x}	2.36±.61	2.10±.31^{a,x}	3.23±.54	2.62±.49	2.62±.82^{a,x}

$^{a-c}$Means with different superscripts within row differed significantly (P<0.05) due to treatment effect; $^{x-z}$Means with different superscripts within column differed significantly (P<0.05) due to time effect.

(1987) and Mackenzie et al. (1978) that showed a progressively similar decrease in PCV and RBC in various animal species infected with trypanosomes. In this study, the tested haematological parameters improved following treatment with chloroform extract of *A. herba*-alba and Berenil® at 11 to 13 days after inoculation with *T. evansi*. This improved state of anemia is an indication of recovery from a state of cell toxicity arising from *T. evansi* infection.

Biochemically, a marked decrease in serum levels of ALP and albumin was observed in the negative control group. This could be attributed to possible hepatic damage caused by the haemato-parasite induced in these animals. Unlike, there was a considerable high level of globulin. This could be explained by possible antibody production. Hyperglobulinemia accompanied by hypoalbuminemia in the negative group was in agreement with the observations made in a variety of

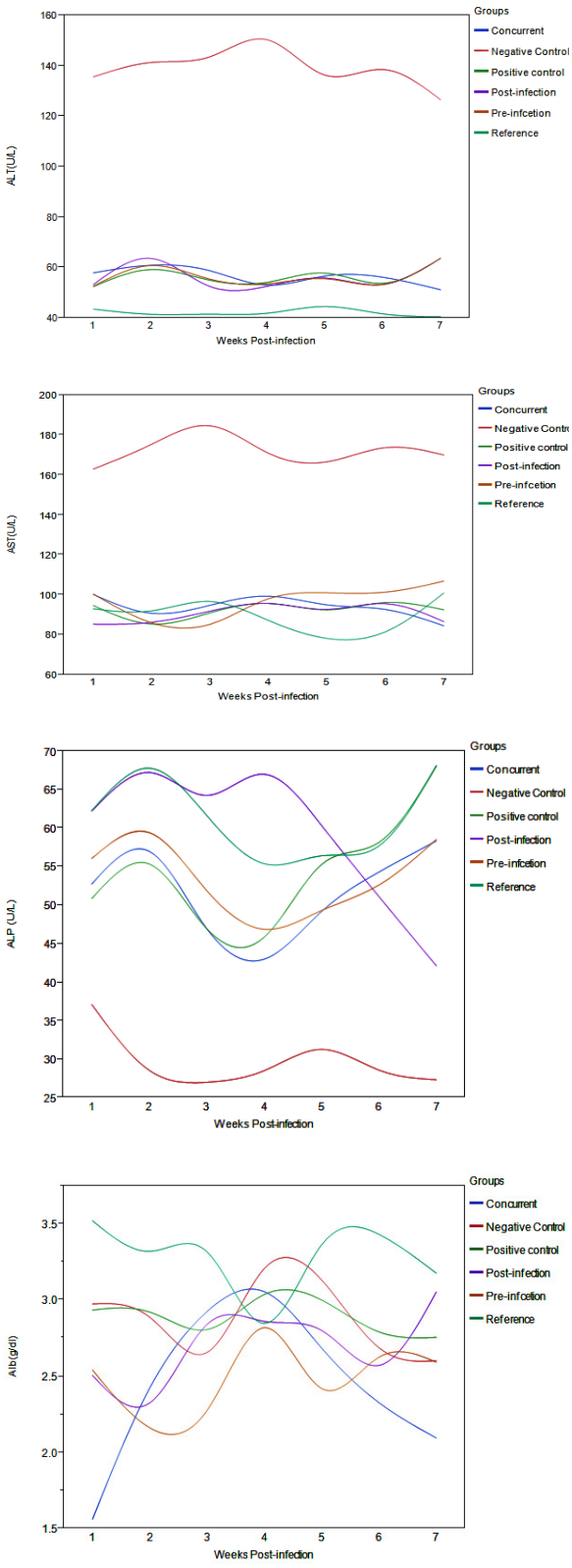

Fig. 2: Biochemical values of groups (1-6) of experimental infection of rabbits.

mammal hosts parasited by *T. evansi* (Singh et al., 1988; Monzon and Villavicencio, 1990; Soodan et al., 1996). A decreased serum albumin level has been observed in trypanosome infections (Katunguka-Rwakishava et al., 1992). The decreased levels could be attributed due to hemodilution. The high elevated values of ALT and AST shown by negative group in this study, may be related to the hepatic and cardiac damages.

Furthermore, the reduced levels in some of the blood chemistry indices like total protein and the transaminases AST and ALT in the treated groups compared to the untreated ones reflected the beneficial effects of the plant extract.

Within the treated groups, this study revealed that the extract of *Artemisia herba-alba* possessed no significant differences when tested pre, concurrent or post infection. Thus, there was no significant difference among animals treated with chloroform crude extract of *Artemisia herba-alba* in terms of the biochemical and haematological values. There were no significant differences in certain parameters such as total protein, bilirubin, and plasma glucose mean values between infected and treated rabbits. Blood parasites, such as trypanosomes, depend upon the host glucose for aerobic glycolysis. Apart from preventing glucose utilization by the parasites, diminazeneaceturate has been known to selectively block kinetoplast DNA replication. Chloroform crude extract of *Artemisia herba-alba* indicated to reduce blood glucose level as exhibited by diminazeneaceturate (Berenil®).

Conclusion

This study demonstrated the curative properties of *Artemisia herba-alba* by producing aparasitemic effect, reducing associated clinical signs of *T. evansi* and preventing severe high levels of AST and ALT and low values of serum alkaline phosphatase and albumin.

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