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Research Article

Therapeutic Activity of Crude Ethanol Extracts of *Rosmarinus Officinalis* against *Trypanosoma evansi* in Experimental Models

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Abstract

Natural products are still major potential sources of innovative therapeutic agents for various conditions, including infectious diseases as they represent an unmet source of chemical diversity. This study was conducted to determine the antitrypanosomal properties of *Rosmarinus officinalis* crude ethanol extract (CEERO) following intraperitoneal exposure to rabbits. New Zealand white rabbits were divided into six major groups; namely pre-infection (Group1), concurrent (Group 2), post-infection (Group 3), positive control (Group 4), negative control (Group 5) and reference group (Group 6). Animals in Group 1- were treated intraperitoneally with 100 mg kg^{-1} of extracts two days before *T. evansi* infection until 4 days after infection. Group 2- rabbit was infected and concurrently treated with 100 mg kg^{-1} of extract for 6 days; Group 3- rabbit was treated with 100 mg kg^{-1} of extract for 6 days after detection of parasitemia; Group 4 (positive control) was treated via intramuscular with 3.5 mg kg^{-1} Berenil® after establishment of parasitemia following *T. evansi* infection; Group 5-(negative control) was not treated but infected; Group 6- served as reference group and treated interperitoneally with Alsever's solution and not infected. The course of *T. evansi* infection in rabbits was followed for 48 days post-infection (DPI). Rabbits were closely observed for clinical examination. Blood samples were taken to measure haematological and biochemical parameters. Rabbits in group 5 showed prominent clinical signs that were characteristic of *T. evansi* infection, which included anemia, poorer general condition, oedema of the face, ocular discharges, encrustation of the lips and mortality rates. The isolate also induced alterations with increased levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and decreased levels of blood indices; notably packed cell volume (PCV), haemoglobin (Hb) and red blood cells (RBC). Mortality rates were also significantly ($p<0.05$) recorded among the infected rabbits. These data suggest the crude ethanol extracts of *R. officinalis* possess relatively antitrypanosomal activity, and reinforce the use of this plant as alternative remedy for *T. evansi* infection in traditional medicine.

INTRODUCTION

The World Health Organization reported that up to 80% of the world's population relies primarily on traditional medicine (WHO, 2012) and a major part of the traditional therapies involve the use of plant extracts or their active constituents. The local use of natural plants as primary health remedies is due to their pharmacological properties. It is known that a large number of plant species contain different bioactive compounds that may have health-beneficial properties such as anti-oxidative, anti-inflammatory, antibacterial and antiparasitic effects, and their preventive and therapeutic use is gradually increasing. Various plant products have been already tested in different animal models infected with parasite for the development of new preventive and curative therapies.

Rosemary (*Rosemarinus officianals*) is a common household plant belonging to the family lamiaceae and is a pleasant smelling perennial shrub that grows in several regions all over the world (Bakırel et al., 2008; Hernández-Hernández et al., 2009). It is a well-known valuable medicinal herb that is widely used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, diaphoretic and useful for urinary ailments (Mahmoud et al., 2005; Labban et al., 2014), Furthermore, the plant has also been used as carminative, rubifacient, stimulant and as flavouring agent for liniments, hair lotions, inhaler, soaps and cosmetics (Kokate et al., 2010). *Rosemarinus officianals* is available in different forms such as dried whole herb, dried powdered extract, volatile oil and preparations made from fresh or dried leaves.

Infectious diseases such as Trypanosomosis represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Therefore, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years, especially due to the emergence of resistant microorganisms.

Trypanosomosis, an important blood protozoan zoonotic disease, is caused by flagellate parasites of the genus *Trypanosoma*. In Africa, the estimated losses as a result of the disease in agricultural production amounted to 3 billion pounds annually (Hursey, 2001). Resistance to current trypanocides is on the increase as reported in endemic regions globally. Chemotherapy is the most widely used means of controlling the trypanosomiasis. The few registered trypanocides are often associated with severe side effects (Atawodi et al., 2003; Yeboah and Osafo2017) and require lengthy parenteral administration, lack efficacy and are unaffordable in most cases (Legros et al., 2002). There is an urgent need to source for new, cheap and safe alternative chemotherapy against trypanosomiasis from natural origin.

The discovery of potent antitrypanosomal extracts from plant has recently increased the great potentials of plant species to provide lead compounds for the development of new natural drugs for effective treatment of Trypanasomiasis.

Until recently, few studies have so far analysed the effects of rosemary or its essential oil as antiparasitic. Therefore, this study was designed to examine the antitrypanasomal effects of crude ethanol extracts of *Rosemarinus officianals* in vivo models.

MATERIALS AND METHODS

Plant material

Fresh *Rosemarinus officianals* were purchased from Libya during the flowering and vegetative phase of the plant at different localities characterized by diverse geographic and climate conditions. The plant was dried under shade at 25 °C, and the dried leaves of the plant were grounded with a blender. The powdered part was kept in nylon bags in a deep freezer until the time of use. Subsequently, the crude ethanolic extract was prepared at the Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Bangi. Dried plants of *Rosemarinus officianals* grounded into powder.

Preparation of plant extract

The powdered leaves were extracted in soxlet with 95% ethanol at a temperature of 50°C for 12 h. five hundred gram powdered plant material was extracted and the residue was removed by filtration. Under reduced pressure at temperature of 40-50°C, the extract was concentrated in a rotary evaporator and then lyophilized to obtain a powdered extract and stored at 4°C until used (Ene *et al.*, 2009).

Animals

The rabbits used for the experiment were purchased from New Zealand and they were 5 - 6 months old, clinically healthy rabbits. All rabbits were kept 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, Universiti Kebangsaan Malaysia (UKM). They were fed with a standard died and water ad libitum. The rabbits were kept in those conditions for a7-day period of acclimatization prior to start of the experiment. Sixteen hours before the experiments, they were screened for the presence of haemo-protozoan parasites upon arrival by using wet mount and leishman stained blood films and were all confirmed negative. The experimental protocol has been approved by the Ethics committee of UKM (UKMAEC) for the Care and Use of Animals.

Stock of parasites and inoculum preparation

The *trypanosoma evansi* isolate used for the experiment was provided by the Parasitology Laboratory, Faculty of Veterinary, Universiti 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

As shown in Table 1, the rabbits were divided into 6 major groups of 5 rabbits in each group at the start of the experiment. Rabbits of Group 1 were pre-treated for two days intraperitoneally with 20 mg kg^{-1} of crude ethanolic extract (CEE) of the aerial parts of *Rosemarinus officianals* before inoculation with *T. evansi* and thereafter treated for 6 days after infection. Animals of Group 2 were treated intraperitoneally with 20 mg kg^{-1} of CEE of *Rosemarinus officianals* and concurrently infected with *T. evansi*. Animals of Group 3 were treated 6 days with 20 mg kg^{-1} of CEE of *Rosemarinus officianals* for 6 days after establishment of parasitemia. Rabbits in Group 4 were positive control and were treated once with 3.5 mg kg^{-1} of diminazeneaceturate (Berenil®) 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

About 2 ml of peripheral blood were collected from the rabbit's marginal ear vein at two days interval during 48 days post infection (pi) after shaving the area. By using Micro- Haematocrit Centrifugation Technique (MHCT) (Woo, 1970), parasitemia was counted. Around 75 µl of fresh blood were taken with a heparinized capillary and centrifuged for 5 min at 12,000g. By using a light microscope (100 or 400 X magnification), capillary tubes were examined for detection and counting trypanosomes when the numbers were few around the buffy coat plasma interphase area (Woo, 1970). By using *Neubauer* hemocytometer, high parasitemia enumeration was under taken.

Collection of blood samples

To analyse haematological and biochemical reactions, marginal ear venous blood samples were collected once every two weeks until 48 days post-infection.Tubes containing ethylene diaminetetriacetic acid (EDTA) as the anticoagulants were used for blood samples collection for hematology and tubes without anticoagulant for biochemical analyses.

Clinical examination

From the start of the study until the end point of the experiment, the clinical observations presented by the

Table 1: Experimental design.

Group	Category	Description	No. of animals	Dose of CEE (mg kg^{-1})	Volume of inoculum of *T. evansi* (ml)	Route of administration
1	Pre-infection	Pre-treated with CEE of *Rosemarinus officianals* before 2 days of *T. evansi* infection	5	20	1 ml of 5×10^5	I/p
2	Concurrent	Treated with CEE of *Rosemarinus officianals* 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

Table 2: Mean rank of loss of condition between reference group, treated groups with CEERO and negative group.

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

*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

diseased and non-diseased groups were frequently recorded. The information obtained from the 6 groups was based on the individual presentation of the clinical signs, because the scoring systems require capture of signs and symptoms from the beginning of the experiment. In conclusion, the clinical signs of 6 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

Blood samples were collected and analysed at the Haematology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) using an Animal Blood Counter and the serum samples were analysed using a 902 Automatic Analyser (Hitachi®, Japan). The examined parameters included haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), differential leucocytic count. For PCV, an aliquot of blood with anticoagulant from each animal was collected in micro-capillary tubes and then centrifuged for 10 min at 14,000 rpm in an IEC MB Micro Haematocrit centrifuge (Damon/IEC Division). After centrifugation, samples were analysed for Packed Cell Volume (%) using a micro-capillary reader (Damon/IEC Division). The plasma protein was determined using spectrophotometric methods. The MCV and MCHC were calculated using the standard formulae. These parameters were measured according to protocols as described by Jain (2000).

Estimation of biochemical assays

Automated chemistry analyzer (HITACHI 902 Automatic Analyzer®, Japan) was used to measure the changes of alkaline phosphatase (AP), alanine amino-transferase (ALT), aspartate amino-transferase (AST), and plasma glucose activities.

STATISTICAL METHODS

Data was analysed using JMP 9, SAS, and the Values of Analysis of Variance (ANOVA) were used to detect the significant changes among the experimentally infected Rabbits.

RESULTS

Parasitological Examination

Elevated levels of parasitaemia was observed in treated groups during the second week of post-infection followed by intermixed pattern of aparasitemia. The course of infection was characterized by presence of parasitaemia in which the treated

Table 3: Mean rank of oedema between reference group, treated groups with CEERO and negative group.

*DPI	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
2	0.0^{a,x}	1.0^{a,x}	2.5^{c,z}	2.0^{b,y}	1.5^{b,x}	2.5^{c,z}
10	0.0^{a,x}	1.5^{b,y}	1.0^{a,x}	1.5^{b,y}	1.0^{a,y}	2.0^{b,y}
20	0.0^{a,x}	2.5^{c,z}	1.5^{b,y}	1.0^{a,x}	1.0^{a,y}	2.0^{b,y}
30	0.0^{a,x}	1.5^{b,y}	1.5^{b,y}	2.0^{b,y}	1.5^{b,x}	3.0^{c,z}
40	0.0^{a,x}	1.0^{b,x}	1.0^{b,x}	2.5^{c,z}	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 4: Mean rank of encrustation between reference group, treated groups with CEERO and negative group.

*DPI	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
2	0.0^{a,x}	1.0^{a,x}	2.5^{c,z}	2.0^{b,y}	1.5^{b,x}	2.5^{c,z}
10	0.0^{a,x}	1.5^{b,y}	2.0^{b,y}	1.5^{b,y}	1.0^{a,y}	2.5^{c,z}
20	0.0^{a,x}	2.5^{c,z}	1.5^{b,y}	1.0^{a,x}	1.0^{a,y}	2.0^{b,y}
30	0.0^{a,x}	2.0^{b,y}	1.5^{b,y}	1.5^{b,y}	1.5^{b,x}	2.5^{b,y}
40	0.0^{a,x}	1.0^{b,x}	1.0^{b,x}	2.5^{c,z}	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 5: Mean rank of ocular discharges between reference group, treated groups with CEERO and negative group.

*DPI	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
2	0.0^{a,x}	1.0^{a,x}	2.5^{c,z}	2.0^{b,y}	1.5^{b,x}	2.5^{c,z}
10	0.0^{a,x}	2.5^{c,z}	2.0^{b,y}	1.5^{b,y}	1.0^{a,y}	2.5^{c,z}
20	0.0^{a,x}	1.5^{b,y}	1.0^{a,x}	2.5^{c,z}	1.0^{a,y}	2.0^{b,y}
30	0.0^{a,x}	2.0^{b,y}	1.5^{b,y}	1.0^{a,x}	1.5^{b,x}	2.5^{c,z}
40	0.0^{a,x}	1.0^{b,x}	1.0^{b,x}	1.5^{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 6: Comparison of mortality between reference group, treated groups with crude ethanol extract of *R. officinalis* (CEERO) and negative control group.

Reference & Berenil® Groups			Treatment Groups (1-3)		Negative Group	
Time (weeks)	Number of animas died	Mortality (%)	Number of animals died	Mortality (%)	Number of animals died	Mortality (%)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	1	20	2	40
4	0	0	0	0	0	0
5	0	0	2	40	2	40
6	0	0	0	0	0	0
7	0	0	0	0	0	0
Total	0	0	3	60	4	80

Table 7: Comparison of PCV values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	36.00±1.73^{a,x}	21.50±5.17^{b,y}	26.10±3.71^{b,y}	24.57±4.56^{b,y}	29.33±.55^{b,y}	11.00±3.39^{c,z}
2	36.20±1.09^{a,x}	19.80±4.14^{b,y}	18.90±4.14^{b,y}	28.50±2.00^{b,y}	32.33±2.88^{b,y}	10.80±2.94^{c,z}
3	35.40±2.30^{a,x}	28.50±2.00^{b,y}	15.61±9.65^{c,z}	26.60±2.04^{b,y}	29.33±2.28^{b,y}	12.40±3.84^{c,z}
4	35.80±1.64^{a,x}	18.10±3.61^{b,y}	28.50±3.80^{b,y}	18.90±4.14^{b,y}	35.80±1.64^{a,x}	10.60±2.30^{c,z}
5	35.95±1.82^{a,x}	20.60±2.07^{b,y}	19.80±4.14^{b,y}	18.50±4.00^{b,y}	27.87±1.03^{b,y}	13.60±4.03^{c,z}
6	36.17±1.77^{a,x}	15.61±9.65^{c,z}	27.60±2.70^{b,y}	15.54±4.39^{c,z}	25.75±3.94^{b,y}	15.61±9.65^{c,z}
7	35.28±1.77^{a,x}	21.00±1.87^{b,y}	23.80±5.84^{b,y}	28.16±1.83^{b,y}	30.87±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: Comparison of Hb values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	133.00±2.54^{a,x}	118.±28.26^{a,x}	88.08±5.52^{b,y}	124.60±4.50^{a,x}	122.83±11.81^{a,x}	86.20±17.54^{c,z}
2	130.20±5.97^{a,x}	99.78±1376^{a,x}	100.98±14.05^{a,x}	102.15±19.05^{a,x}	109.70±13.99^{a,x}	86.00±13.96^{c,z}
3	133.20±1.78^{a,x}	100.98±16.99^{a,x}	81.98±14.05^{a,x}	99.85±17.84^{a,x}	118.66±6.35^{a,x}	80.08±15.08^{c,z}
4	131.80±2.16^{a,x}	125.60±4.50^{a,x}	96.42±12.55^{b,y}	86.33±19.44^{a,x}	127.60±4.50^{a,x}	74.94±7.07^{c,z}
5	133.60±2.60^{a,x}	110.98±14.05^{a,x}	87.26±12.10^{b,y}	91.00±11.50^{a,x}	110.98±14.05^{a,x}	91.00±21.11^{b,y}
6	131.40±1.51^{a,x}	90.48±6.86^{b,y}	101.98±12.05^{a,x}	100.16±13.33^{a,x}	110.98±14.05^{a,x}	82.78±9.43^{c,z}
7	132.00±2.34^{a,x}	113.56±16.15^{a,x}	112.98±14.05^{a,x}	97.10±20.87^{a,x}	130.20±5.97^{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: Comparison of RBC values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean ± SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
z	4.43±.24^{a,x}	4.33±.24^{a,x}	3.77±.42^{a,x}	3.92±.42^{a,x}	4.35±.30^{a,x}	2.59±.85^{c,z}
2	4.15±.49^{a,x}	3.22±.29^{b,y}	4.20±.26^{a,x}	4.26±.48^{a,x}	4.66±.49^{a,x}	2.75±.70^{c,z}
3	3.7 ±. 60^{a,x}	4.58±.51^{a,x}	2.81±.77^{b,y}	3.27±.35^{b,y}	4.35±.30^{a,x}	2.76±.98^{c,z}
4	4.2 ±. 48^{a,x}	3.92±.42^{a,x}	4.50±.26^{a,x}	4.22±.26^{a,x}	4.89±.26^{a,x}	2.59±.83^{c,z}
5	4.28±.24^{a,x}	2.75±.70^{c,z}	3.56±.46^{a,x}	4.13±.26^{a,x}	4.88±.37^{a,x}	2.81±.77^{b,y}
6	4.53±.66^{a,x}	4.11±.47^{a,x}	3.82±.54^{a,x}	4.11±.37^{a,x}	4.32±.25^{a,x}	2.46±.33^{c,z}
7	3.92±.42^{a,x}	3.27±.35^{b,y}	4.16±.46^{a,x}	4.00±.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: Comparison of WBC values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	4.02±1.56^{ax}	4.44±1.71^{ax}	4.75±.96^{a,x}	4.71±.97^{a,x}	3.57±.96^{a,x}	4.44±1.45^{a,x}
2	3.07±.26^{a,x}	5.23±2.50^{ax}	6.22±1.23^{b,y}	6.88±2.48^{b,b}	3.57±.96^{a,x}	6.98±1.63^{b,y}
3	3.24±.28^{a,x}	3.99±.96^{a,x}	4.73±1.58^{a,x}	3.73±1.09^{a,x}	2.92±.00^{c,z}	5.29±1.46^{b,y}
4	3.84±1.66^{ax}	5.23±2.15^{by}	3.99±1.01^{a,x}	4.69±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^{ax}	4.47±2.12^{a,x}	4.47±2.12^{a,x}	3.57±.96^{a,x}	5.23±2.15^{b,y}
6	3.24±.28^{a,x}	3.52±.98^{a,x}	3.57±.95^{a,x}	5.23±2.15^{b,y}	3.57±.96^{a,x}	6.47±2.35^{b,y}
7	3.24±.28^{a,x}	3.91±.60^{a,x}	3.90±1.01^{a,x}	3.88±.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

Table 11: Comparison of ALT values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	43.54±5.89^{a,x}	99.54±9.67^{b,y}	77.54±1.87^{b,y}	80.62±10.02^{a,x}	46.67±5.50^{a,x}	135.04±48.68^{c,z}
2	40.92±4.47^{a,x}	107.92±23.68^{c,z}	106.92±7.93^{c,z}	88.11±18.67^{b,y}	49.58±15.07^{b,y}	143.07±45.36^{c,z}
3	41.98±9.28^{a,x}	102.98±16.36^{c,z}	87.92±7. 55^{b,y}	67.71±16. 63^{b,y}	45.92±7.00^{a,x}	139.07±20.93^{c,z}
4	40.57±3.24^{a,x}	88.57±26. 79^{b,y}	79.92±7. 67^{b,y}	79.98±9. 58^{b,y}	45.92±7.00^{a,x}	157.07±34.61^{c,z}
5	45.92±7.07^{a,x}	69.92±13. 66^{b,y}	111.54±5.83^{c,z}	101.58±15.36^{c,z}	45.92±7.00^{a,x}	129.07±15.74^{c,z}
6	40.58±5.21^{a,x}	122.58±14.36^{c,z}	109.54±5.77^{c,z}	95.26±12.69^{b,y}	52.54±19.24^{a,x}	143.07±45.36^{c,z}
7	40.57±3.24^{a,x}	98.57±25.79^{b,y}	95.54±5.67^{b,y}	89.54±5. 78^{b,y}	57.54±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: Comparison of AST values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	93.54±27.10^{a,x}	119.94±14.46^{a,x}	127.94±14.26^{b,y}	123.34±20.22^{b,y}	93.34±20.66^{a,x}	163.04±89.85^{b,y}
2	89.54±14.20^{a,x}	116.54±16. 36^{b,y}	133.94±14.66^{b,y}	117.65±14.57^{b,y}	76.67±10.96^{a,x}	173.07±84.36^{b,y}
3	99.54±16.33^{a,x}	188.98±75.22^{b,y}	177.98±75.22^{b,y}	99.05±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}	121.94±14.58^{b,y}	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}	139.54±18.56^{b,y}	115.94±18.75^{b,y}	97.67±22.70^{a,x}	81.84±14.15^{a,x}	164.98±86.62^{b,y}
6	80.14±30.66^{a,x}	111.54±18.07^{a,x}	99.94±14.75^{a,x}	114.84±22.30^{a,x}	92.59±22.06^{a,x}	175.98±73.45^{b,y}
7	101.74±22.13^{a,x}	145.14±18.36^{b,y}	168.98±75.22^{b,y}	139.84±20.39^{b,y}	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: Comparison of ALP values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	61.54±15.35^{a,x}	40.34±12.76^{b,y}	47.74±4.82^{a,x}	55.34±13.67^{a,x}	61.34±15.54^{a,x}	37.54±11.94^{b,y}
2	69.33±17.93^{a,x}	27.73±7.76^{b,y}	55.53±17.32^{a,x}	39.91±17.32^{a,x}	61.34±15.54^{a,x}	27.72±7.76^{b,y}
3	61.34±15.54^{a,x}	39.14±12.26^{b,y}	34.34±15.54^{a,x}	60.19±13.77^{a,x}	42.67±5.77^{a,x}	27.54±8.22^{b,y}
4	54.14±5.67^{a,x}	35.12±11.32^{a,x}	29.34±15.54^{a,x}	48.17±17.81^{a,x}	60.59±9.32^{a,x}	27.71±7.76^{b,y}
5	58.11±11.14^{a,x}	47.13±7.08^{a,x}	36.14±12.25^{a,x}	58.34±16.11^{a,x}	55.09±10.17^{a,x}	32.53±6.14^{b,y}
6	56.12±5.31^{a,x}	33.34±15.54^{a,x}	41.15±5.21^{a,x}	42.67±11.82^{a,x}	55.06±5.50^{a,x}	27.73±7.76^{b,y}
7	68.74±14.15^{a,x}	51.34±15.54^{a,x}	39.34±11.51^{a,x}	49.84±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: Comparison of Alb values of rabbits treated with CEERO with those not treated (reference, positive & negative control groups) (Mean±SD).

Time (Weeks)	Groups					
	Reference	Pre-infection	Concurrent	Post-infection	Positive control	Negative control
1	3.01±.39^{a,x}	2.97±.98^{a,x}	3.00±.60^{a,x}	2.95±.78^{a,x}	2.99±1.87^{a,x}	2.94±.59^{a,x}
2	3.14±.60^{a,x}	2.83±1.07^{a,x}	3.12±1.41^{a,x}	2.54±.56^{a,x}	3.00±.68^{a,x}	3.00±.51^{a,x}
3	3.38±.50^{a,x}	3.40±.39^{a,x}	2.81±.56^{a,x}	3.08±.60^{a,x}	3.14±.60^{a,x}	2.55±1.87^{a,x}
4	3.43±.46^{a,x}	3.11±.55^{a,x}	2.92±.40^{a,x}	3.23±.59^{a,x}	3.28±.70^{a,x}	3.11±.56^{a,x}
5	3.49±.39^{a,x}	2.75±1.02^{a,x}	3.14±.60^{a,x}	2.99±.51^{a,x}	3.31±.94^{a,x}	2.77±1.32^{a,x}
6	3.25±.45^{a,x}	2.88±.39^{a,x}	3.11±.69^{a,x}	2.39±.93^{a,x}	2.89±.89^{a,x}	2.87±.36^{a,x}
7	3.34±.40^{a,x}	2.69±.32^{a,x}	3.28±.75^{a,x}	3.87±.74^{a,x}	3.62±.56^{a,x}	2.43±1.02^{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

groups were not significantly different from negative group. In comparison, animals in group 5 (negative control), parasitemia was recorded from day 3 post-infection and the animals remained with the high peaks of parasitemia between day 10-35 days post-infection (DPI) showing severe symptoms in the investigated parameters (data were excluded).

Tables 2-5 showed the results obtained from the clinical observation. Animals in the negative control group were severely affected after experimental infection with *T. evansi*. In these animals, the clinical signs appeared on day 3 post-infection due to the infection of *T. evansi* where most of the animals showed typical clinical signs such as oedema in the face, ocular discharges, and loss of condition along with encrustation of the lips. The mean ranks of these tested parameters in the negative groups were not significantly ($p>0.05$) higher than all treated groups (Tables 2-5). In comparison, the clinical signs of positive control were prevented by intraperitoneal administration of Berenil® whereby the clearance of the infection was very low in treated groups with CEERO of *R. officinalis* throughout the experimental period. Within treated animals, there was no statistical difference observed. Similarly, no statistical significant ($p>0.05$) difference observed between negative control and those treated with CEERO. All animals challenged were significantly ($p<0.05$) different in comparison to those served as reference group.

Mortality

The mortality recorded in treated groups with CEERO was (60%). No death was recorded throughout the experiment in positive control. Similarly, there was no mortality documented in animals served as reference group throughout the experimental period. In comparison, animals in negative control (infected but not treated) started to die where death of the first two animals (40%) observed on day 21. Then, another 2 animals (40%) died on day 35 while the remaining animals of the negative group survived throughout the experimental period making the total mortality (80%) (Table 6). Mortality was associated with severe parasitemia due to *T. evansi* infection.

Changes in the haematological Values

The results of the haematological values in the different groups involved in the study were outlined in Tables 7-10. Of these groups, animals in group 5 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) and (2.46±.33) respectively (Tables 4, 5). From the first week of the study, the values of these parameters started to drop 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 considerable decrease in their levels between day 4-7 DPI. Thereafter, normalization of these values (PCV, RBC and Hb) was not observed and remained decreased throughout the experimental period. There were significant ($p<0.05$) differences observed in all tested parameters of treated groups in comparison to those served as reference group (Tables 7-10).

Changes in Biochemical Values

Tables 11-14 summarize the mean levels of the tested blood biochemistry indices. In group 5 (negative group), increased levels were observed in some of the indices of ALT and AST throughout all experimental period. However, during the experimental period, this group (negative control) revealed decreased levels in serum alkaline phosphatase (ALP). Furthermore, albumin concentration decreased slowly simultaneously in the negative group. In comparison, treated groups were not significantly different from the negative group. In contrast, animals treated with CEERO were significantly ($p<0.05$) different from both positive control and those served as reference group.

DISCUSSION

To the best of our knowledge, this study evaluates, for the first time, the antitrypanosomal effect of *Rosmarinus officinalis* in a rabbit model. The results revealed that manipulation of *T. evansi* lead the production of intermixed levels of parasitemia in all the four groups that treated with CEERO. All treated individuals showed levels of the parasitemia throughout the experiment which were significantly ($p<0.05$) associated with the tested clinical parameters; gradual loss of condition, facial oedema and ocular discharges.

The rabbits developed a persistent and severe anemia while only a few parasites could be detected in the blood in the later stage of the infection and this was due to the chronic nature of the infection in these models. The findings showed an initial and relatively persistent parasitemia when the condition was treated with the ethanol extract of *R.*

officinalis. This could be attributed to the antiparasitic activities of the CEERO but was not comparable to the exhibited by Berenil®. It seems that our study were not consistent to the findings as reported by Atawodi et al. (2005), which could be related to variation species and experimental period used.

The haematological findings in the current study revealed that there is a reduction in terms of haematological values in both negative controls as well as treated groups. Anene (1987) and Mackenzie et al. (1978), reported decrease in PCV and RBC in various animal species infected with trypanosomes similar to the current findings. Following treatment with CEERO at 11 to 13 DPI, the tested haematological parameters did not improve with any restoration in the tested variables.

Biochemical results showed that there was a marked decrease in serum levels of alkaline phosphatase and albumin in the negative control group as well as treated groups. This could be suggested as a result of possible hepatic damage which is caused by the isolate used in the experiment. Decreased serum albumin level has been reported in trypanosome infections (Katunguka-Rwakishava et al. 1992). The decreased levels could be attributed due to hemodilution. The increased values for ALT and AST shown by negative group and treated with CEERO could be related to the hepatic and cardiac damages. Furthermore, the elevated levels observed in the blood chemistry determinations in some of the indices such as AST and ALT in the treated groups compared to the untreated ones reflected the absence of potentially therapeutic effects of the CEERO. This study revealed that treatment with the extract of *R. officinalis* has no significant differences when tested pre, concurrent or post infection within the treated groups. Hence, there was significant

difference in terms of the biochemical and haematological values among animals treated with crude ethanol extract of *R. officinalis* (CEERO) and those served as negative group.

CONCLUSSION

In our present study reports the main and important therapeutic effects of *R. officinalis* following intraperitonaial inoculation of *T. evansi* in experimental animals (Rabbits). There is currently little scientific evidence with conflicting nature on the biological activities of *A. R. officinalis* in the context of their curative properties against parasitic diseases. One reason for this conflict is the lack of relevant experimental evidence from field trails. A further reason is that most supplementation studies have been designed to investigate the antiparasitic effects of a given component alone without taking into account their potential detrimental effects on host performance if used extensively. The antiparasitic properties of this plan; *R. officinalis* on parasitised host performance should be considered and evaluated simultaneously. As relatively few plants have shown considerable activity against blood parasites within sheep and goats, there is still a need to look further for plants with higher levels of antiparasitic activity. Plant products that have showed high activity against haemato-arasites *in vivo* or *in vitro* need to be evaluated and tested in actual hosts. Finally, additional investigations dealing with the study of active components of *R.officianalis* are critically required in order to support the valorization of these popular medicinal plants in human as well as animals.

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