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Full Length Research Paper

Efficacy and toxicity of cymelarsan® in Nubian goats infected with *Trypanosoma evansi*

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Nine groups of Nubian goats were infected with *T. evansi*. Groups 1 and 2 were used as negative and positive control respectively, groups 3, 4, 5 and 6 were given a single intramuscular dose of Cymelarsan® at the rate of 0.125, 0.25, 0.625, 1.25 mg/kg, respectively. Goats in groups 7 and 8 were each given intramuscular Cymelarsan® at the rate of 0.125 mg/kg twice/week for two weeks and 0.125 mg/kg daily for 8 days, respectively. Goats in groups 9 and 10 were each given the drug intramuscular at the rate of 0.25 mg/kg twice/week for two weeks and 0.25 mg/kg daily for 8 days respectively. Goats in group 2 showed clinical signs of trypanosomosis while infected goats of groups (4 - 5) signs disappeared. Goats of group 3 responded to the treatment but relapse occurred. Goats of groups (6 -10) were parasite free but expressed clinical signs of arsenic toxicosis. The haematological and serobiochemical changes were correlated with the clinical signs and pathology. Single dosages of 0.25–0.625 mg/kg wee recommended for treatment of *T. evansi*. Single dosage of 1.25 mg/kg and the multiple dosages were toxic and fatal. The drug has accumulative effect.

Key words: Nubian goats, Cymelarsan, efficacy, toxicity, *T. evansi*, Sudan.

INTRODUCTION

Trypanosomosis (Surra) encompasses about 9 – 10 million square kilometers of Savannah area (Atang, 1982) and is widespread in East Africa and West Africa, Middle East, India and Asia (Wilson, 1984) . The disease is well known by camel's herder but not for small ruminants (Rötcher and Heising,1980).

Treatment in camels is dependent on one of two drugs suramin and quinapyramine (Bujon, 1990). However, suramin has become less effective (Gad-el Mwla and Fayed, 1979) . In addition, suramin has disadvantages related to its route of administration (Zelleake et al., 1989) and quinpyramine sulphate is no longer available from the original manufacturer. On the other hand, isometamidium chloride (samorin) only removes the parasites from the blood-stream for 21 h followed by relapse and causes some serious adverse effects (Ali et al., 1986) . Diminazine aceturate (Berenil) was found to be toxic at a dose of 10 or 20 mg / kg in camels (Leach and Robert, 1981).

Mwambu (1975) re-evaluated the value of ethidium for the treatment of *T. brucei* subgroup infection in cattle and concluded that the use of this drug as a curative in *T. brucei* infection of cattle should be restricted. Ethidium bromide was recorded as carcinogenic and mutagenic drug.

Extensive use of these compounds led to the appearance of drug resistance, which is becoming more widespread (Leach and Robert, 1981). This drug resistance poses a great problem thus highlighting the need for newer trypanocidal drugs. Since 1961 no additional drugs for use against animal trypanosomosis have gone beyond the experimental stage (Mahmoud and Gray, 1980; Youssif, 2000). Cymelarsan® is a new trypanocide for treatment of camel trypanosomosis presented in the market by Rhône Mérieux – France, it is a trivalent arsenical compound formed by conjugation of one equivalent of melarsenoxide and two equivalent of cystamine and is indicated for the treatment of acute, sub-acute and chro-

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nic *T. evansi* infections, and which are resistant to other drugs (Bujon, 1990). At the recommended dose Cymelarsan® has been well tolerated (Bujon, 1990; Youssif, 2000). the objective of this study is to investigate the efficacy and toxicity of Cymelarsan® in diffeent doses in *T. evansi* infected goats.

MATERIALS AND METHODS

Drug

Cymelarsan® 0.25 mg / kg (Rhône – Mérieux – France).

Animals

hundered Nubian goats, of both sex, 8 - 12 months and body weight 9 - 11 kg were housed in pens at the College of Veterinary Medicine – Khartoum University, Khartoum State. Each animal was fed daily on 3 kg lucerne (*Medicaqo sativa*), 1.5 kg sorghum (*Sorghum vulgare*) and 2 kg millet (*Pearl millet*) once weekly, and had free access to water.

Animals were divided randomly into ten groups, each of ten goats and were kept 14 days for acclimatization. During this period, health examinations had been done daily for determination of normal base-line data.

Two groups were chosen randomly and usd as controls. Group1 was uninfected-untreated (negative control), while group 2 was infected-untreated (positive control). The remaining animals were infected with *T. evansi* and divided randomly into 8 groups each of ten.

The Parasite and infection

Albino rats of two months old, weighing 250 gm, were inoculated intraperitoneally with 0.2 ml camel's blood containing 3 - 5 parasite/field, (These camels were infected naturally with *T. evansi* strain Gad trip (1) which was obtained from El Gadarif State, Eastern Sudan). When parasitaemia developed in rats, each goat (except goats in group 1) was injected intravenously with 0.75 ml rat's blood containing 5×10^5 organisms. The parasites were activated by adding phosphate glucose solution (PGS) buffer before inoculation.

Experimental design

Treatments

Each goat in groups 3, 4, 5 and 6 was given single intramuscular (I/M) dose of Cymelarsan® at the rate of 0.125 mg / kg (half-therapeutic dose), 0.25 mg/kg (therapeutic dose), 0.625 mg/kg (two and half times the therapeutic dose), 1.25 mg/kg (five-times the therapeutic dose), respectively. Each goat in groups 7 and 8 was given (I/M) a dose of Cymelarsan® at the rate of 0.125 mg/kg (half-therapeutic dose) twice/week for two weeks, 0.125 mg/kg (half-therapeutic dose) daily for 8 days, respectively. Goats in groups 9 and 10 were each given Cymelarsan (I/M) at the rate of 0.25 mg / kg (therapeutic dose) twice/week for two weeks and 0.25 mg/kg (therapeutic dose) daily for 8 days, respectively.

Goats in groups 1 and 2 were not given the drug but were used as control negative and control positive respectively. A weekly slaughter program for surviving animals (two goats / week) was conducted on days 14, 21, 28, 35 and 42.

Blood collection

All experimental animals were bled from the jugular vein at day 1 and then three days post infection. All animals were bled after 1, 3, 24 h and 3, 7, 14, 21, 28, 35 and 42 days post-treatment. Two plain vaccutainer test tubes were used for each animals, (Becton and Sons- France). The first tube containing no anticoagulant and was left to clot, centrifuged at 3000 rpm and serum was collected and kept at -20^oC until analyzed for serological investiation. The second tube containing anticoagulant(EDTA) was used for haematological investigation.

Clinical examination

Experimental goats were examined daily for body temperature, body weight, respiratory rate according to methods described by Kelly (1986). The pulse rate and blood pressure were examined daily using Electronic Apparatus (Digital Blood Pressure Meter, Seinex Electronics Ltd. – UK).

Parasitological methods

T. evansi was detected daily in the peripheral blood (wet blood film), whole blood (thin film, thick film, buffy coat technique) and in the liver (liver impression smears) as described by Soulsby (1982).

Haematological methods

The red blood cells counts (RBC), white blood cells counts (WBC), differential WBC count, haemoglobin (Hb), packed cell volume (PCV), and erythrocytic indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were measured at Khartoum North Human Hospital using Cell-DYN®, 1700; Division Abbott (Laboratories Abbott Park, IL 60064 USA).

Serobiochemistry

Serum samples were analyzed for the activities of serum lactate dehydrogenase (LDH 1.1.1.27), creatine kinase (CK 2.7.3.2 C), pyruvate kinase (PK 2.7.1.40 C), alkaline phosphatase (ALP 3.1.3.1), - amylase (3.2.1.1 C), lipase (3.1.1.3) and succinate dehydrogenase (SDH 1.3.99.1) using commercial kits (Linear Chemicals, S.L.-Spain).

Activities of serum asparate aminotransferase (AST, 2.6.1.1) and alanine aminotransferase (ALT, 2.6.1.2) were measured by commercial kits (Randox Laboratories Ltd. U.K.).

Also serum was analyzed for the concentrations of creatinine, urea, direct bilirubin, glucose, total protein, albumin by commercial kits (Randox Laboratories Ltd. U.K.). Serum globulin was obtained by subtracting the albumin value from that of total protein. Serum total bilirubin was measured by commercial kits (Boehringer Mannheim GmbH Diagnostics, West Germany). Serum phospholipids, triglycerides and total cholesterol were measured by commercial kits (Linear Chemicals, S.L.-Spain).

Serum samples were also analyzed for the concentrations of sodium and potassium by flame photometer (400 flame photometer Corning, England). Serum concentrations of chloride, magnesium, calcium, inorganic phosphorus, zinc, copper and iron were measured by commercial kits (Linear Chemicals, S.L.-Spain).

Serum manganese concentration was measured according to the method of Jeffery et al., 1989) by atomic absorption spectro-metry (Cornning EEL 197 Spectra, Evans Electroselenium Ltd., England) at wave length 279.5 nm or 1 - 4 air acetylene/l (fuel lea).

Statistical analysis

All data were computerized using MSTAT-C program (Michigan State University), for the analysis of variance and for means separation.

RESULTS

Parasitaemia and efficacy

Peripheral blood

Post- infection: *T. evansi* was detected in the peripheral blood of goats in group (2) which was mild on days 4-5, moderate on day 6 and severe on days 7-10 until the animals died on days 9 - 11. Goats in groups (3-10) also showed mild parasitaemia on day 4 and the severity gradually increased till day 11.

Post treatment: Parasitaemia disappeared post treatment in goats of groups (4- 6, 9 and 10) until they died or were slaughtered, but it appeared 3 - 4 days post treatment in goats of groups (7 and 8) then disappeared completely until they died or were slaughtered. The parasitaemia in goats of group (3) was mild until animals died on days 13 - 15 post treatment.

Liver impression smears

Goats of group (2) showed moderate parasitaemia throughout the period of 9 - 11 days post infection. No parasites were detected in goats of group (4-10) until died or were slaughtered at day 14 post treatment. Livers of goats in group (3) showed mild parasitaemia until they died.

Clinical signs

Table 1 illustrates changes in the body weight, body temperature, pulse rate, respiratory rate, blood pressure and fate of the animals infected with *T. evansi* and treated with single and multiple doses of Cymelarsan. No clinical signs were observed in goats of group (1). Goats in group (2) showed 4 - 7 days post infection hypothermia, watery lacrimation, frothy salivation, mucopurulent conjunctivitis, mucopurulent nasal discharge, decrease in appetite, severe diffuse alopecia, diarrhoea, depression, apathy, muscle tremors, slight increase in the respiratory rate, and decrease in the pulse rate, shivering and convulsions. In the second week, the lymph nodes and testes were hot and swollen, and animals became off food, cachexic, recumbent with lateral kink of the neck for 1 - 2 days then the animals died.

Post treatment (pt), in the first week, all goats showed swelling at the site of injection, which disappeared within 1 - 2 days in goats of groups (3 - 5) and never disappeared in other groups. Goats in group (3) showed mild clinical signs as that of group (2) and there was keratitis while goats of group (4) showed nasal discharge and mild diarrhoea. Goats of group (5) showed no apparent clinical signs except diarrhoea, which stopped on day 3 pt. In contrast, goats in groups (6-10) showed watery lacrimation with mucopurulent conjunctivitis, accompanied with blindness in goats of groups (6 and 7) and in 75 % of goats in groups (8-10), mucopurulent nasal discharge, frothy salivation, fever, depression, apathy, pressing the head against objects, watery diarrhoea for 3 - 4 days then changed to bloody diarrhoea, bloody urine, muscle tremors, decrease in appetite, slight increase in the respiratory rate, decrease in the pulse rate. In the second week, groups (8 - 10) showed hot and swollen lymph nodes and testes, severe diffuse alopecia which was slight in the other treated groups, with no shivering or convulsions, paralysis of the forelimb, sternal recumbency, off food, emaciation and anuria. These signs continued until death.

Goats in groups (6 - 10) showed skin lesions in the second week as red 3 mm raised concave nodules which increased in size to 5 mm then changed to a brown scab. These scabs when hand removed left white to yellowish ulcers, with greasy white yellowish hard material was seen and remained until death. The body weight was increased in goats of groups (4 - 5) and decreased in groups (6 - 10).

Hematological changes

Table 2 summarizes the haematological changes in Nubian goats infected with *T. evansi* and treated with single or multiple dosages of Cymelarsan. The haemoglobin concentration, packed cell volume and red blood cell count decreased significantly in goats of groups (2, 3, 6 - 10) but were within reference interval in groups (4 and 5) compared to the control group (1). Slight and statistically insignificant increase was observed in white blood cell counts in all groups. Significant increases were observed in platelets count, neutrophils and eosinophils of goats in groups (2, 6 - 10), but they were within reference interval in groups (3, 4 and 5) compared to control group (1). Basophils were absent in control group (1) but showed an increase in other experimental groups. However, lymphocytes were decreased significantly in all experimental animals compared to control group (1) and goats of groups (4 and 5). Significant increase was observed in the MCV of all groups tested except goats in groups (1, 2, 4 and 5) while the MCH decreased moderately in group (6) and sharply in group (3) and insignificant in the other groups. Although there was a decrease in the MCHC values in goats of groups 2 and3 an increase in goats of groups 4 - 10 was observed, but they were insignificant compared to that of control group (1).

Serobiochemical changes

Tables 3 and 4 show some serobiochemical changes in

Table 1. Clinical parameters and fate of *T. evansi* infected Nubian goats and treated with *single* or multiple dosages of Cymelarsan (M±SE).

Parameter/Groups	Body temperature (^{o}C)	Respiratory rate (/min.)	Pulse rate (/min)	Body weight (Kg)	Blood pressure (mmHg)	Fate of the animals(days)
Group (1)	39.5±0.01 ^{a}	26.1±0.00 ^{a}	75.5±0.02^{a}	9.5±0.05^{a}	125/75±1.12/1.5^{a}	Two animals slaughtered /week on days14, 21,28,35and 42
Group (2)	34.3±0.02 ^{b}	38.5±0.01 ^{b}	60.5±0.01^{b}	7.8±0.04^{b}	90/44±1.11/1.3^{b}	Died on day11post infection
Group(3) 0.125mg/kg	36.5±0.03 ^{b}	25.8 ±0.05 ^{a}	68.0 ±0.02^{ab}	7.0±0.01^{b}	87/42±0.9/1.4 ^{b}	Died between days13-15 post treatment
Group (4) 0.250mg/kg	39.5±0.01 ^{a}	26.5 ±0.01 ^{a}	80.3±0.02^{a}	11.2±0.05^{a}	117/58±0.8/1.2 ^{a}	Two animals slaughtered /week on days14, 21,28,35and 42
Group (5) 0.625mg/kg	38.7±0.01 ^{a}	26.5 ±0.02 ^{a}	81.3 ±0.03^{a}	12.5±0.04^{a}	125/65 ±0.5/1.3^{a}	Two animals slaughtered /week on days14, 21,28,35and 42
Group (6) 1.25mg/kg	38.4±0.05 ^{a}	32.0 ±0.25 ^{b}	85.3 ±0.05^{a}	6.2±0.01^{b}	75/40±0.4/0.1 ^{b}	Died between days14-15 post treatment
Group (7) 0.125mg/kg twice/week for 2weeks	39.5±0.03 ^{a}	20.0 ±0.04 ^{a}	75.5±0.01 ^{a}	7..5±0.05^{a}	80/50b±0.8/0.5^{c}	Died between days26-28 post treatment
Group (8) 0.125mg/kg daily for 8 days	41.3±0.20 ^{a}	15.5 ±0.01 ^{c}	70.5 ±0.08^{a}	9.4±0.05^{a}	70/40±0.9/0.8^{c}	Died between days25-28 post treatment
Group (9) 0.250mg/kg twice/week for 2weeks	40.0±0.4 ^{a}	15.5±0.03 ^{c}	75.5 ±0.01^{a}	7.7±0.11^{a}	65/40±1.5/1^{c}	Died between days20-21 post treatment
Group (10) 0.250mg/kg daily for 8 days	41.2±0.22 ^{a}	15.0±0.01 ^{c}	60.0±0.02 ^{b}	6.2±0.16^{bc}	60/35±1.2/1.2^{c}	Died on days14 post treatment

Same letters (a, b and c) in one column showed no significant changes p 0.05.

serum of Nubian goats infected with *T. evansi* and treated with single or multiple dosages of Cymelarsan. The serum sodium concentration increased slightly but not significantly in all groups except goats of group (6) which showed a significant decrease. Serum potassium concentration showed moderate increases in groups (2, 5, 7 - 10) but slight decreases in groups 3 and4 and sharp decrease in group (6). The serum chloride concentration increased in groups (2, 3, 6, 7, 9 and 10) but not in groups (1, 4, 5 and 8). There was slight decrease in serum concentration of Ca and P in all groups compared to that of group (1) but, sharp decrease was depicted in the serum concentration in goats of groups (3 and 6) however, an increase was observed in the serum magnesium concentration in goats of groups (2, 3, and 6 - 10). The serum zinc concentration was decreased in groups (2 and 3) and increased in groups (7, 8 and 10). The serum copper and manganese concentrations were increased only in groups (2 and 3) but, the manganese concentration decreased sharply in group (9) and no changes were observed in the remaining groups while, the serum iron was decreased in groups(2, 3, 5, 6, 8, 9 and 10) and no changes were observed in group (4) Table 3.

Significant increase was noticed in serum concentration of bilirubin and direct bilirubin of goats in groups (2, 6-10). Another significant increase was also observed in serum cholesterol concentration except in groups (1, 4 and 6), however, the triglyceride and phospholipid serum concentration increased in all the experimental animals except goats of groups (4 and 5) compared to the control group (1). The changes in

Table 2. Haematological changes in *T. evansi* infected Nubian goats and treated with single or multiple doses of Cymelarsan (M±SE).

Parameter Groups	Hb (g/dl)	PCV (%)	RBC ($x10^6/mm^3$)	Reticulocytes ($10^6/mm^3$)	WBC ($x10^3/mm^3$)	Platelet ($x10^3/mm^3$)	Eosinophils (%)	Basophils (%)	Monocytes (%)	Neutrophils (%)	Lymphocyte (%)
Group (1)	9.0±0.24 a	32.3±0.02 a	14.5±0.21 a	5.7±0.02 a	11.7±0.32 a	410.7±0.73 a	2.0±0.00 a	0.00±0.00 a	3.00±0.00 a	55.0±0.01 a	40.0±0.01 a
Group (2)	5.7±0.02 b	24.8±0.21 b	10.7±0.31 b	7.8±0.02 b	13.8±0.31 a	489.5±0.71 b	3.6±0.002 b	2.8±0.001 b	5.0±0.002 b	68.0±0.41 b	20.6 ±0.05 b
Group(3) 0.125mg/kg	3.4±0.01 c	16.7±0.22 c	11.4±0.21 b	11.4±0.13 c	13.2±0.18 a	520.3±0.74 c	2.1±0.05 a	1.5±0.05 a	3.5±0.01 a	75.7±0.15 b	17.2 ±0.02 b
Group (4) 0.250mg/kg	9.2±0.01 a	33.1±0.21 a	13.4±0.20 a	6.1±0.01 a	12.5±0.17 a	415.1±0.74 a	2.5±0.04 a	1.0±0.04 a	3.5±0.00 a	55.3±0.14 a	37.7 ±0.3 a
Group (5) 0.625mg/kg	8.5±0.01 a	31.7±0.21 a	13.8±0.19 a	5.5±0.1 a	13.9±0.18 a	419.9±0.82 a	2.0±0.04 a	2.5±0.02 b	3.5±0.01 a	60.2±0.24 a	31.8 ±0.1 a
Group (6) 1.25mg/kg	4.4±0.12 bc	15.4±0.01 c	9.5±0.20 b	5.7±0.02 a	15.3±0.14 a	530.1±0.79 c	7.5±0.01 c	1.0±0.01 a	4.0±0.01 b	70.0±0.32 b	17.5 ±0.1 b
Group (7) 0.125mg/kg twice/week for 2weeks	6.1±0.02 b	15.0±0.01 c	10.0±0.21 a	13.7±0.12 c	15.5±0.72 a	490.7±0.40 b	3.5±0.00 a	1.5±0.00 b	3.5±0.00 a	75.0±0.20 b	16.5 ±0.09 b
Group (8) 0.125mg/kg daily for 8 days	5.0±0.01 b	14.1±0.09 c	9.5±0.22 a	13.0±0.31 c	12.2±0.52 a	520.4±0.50 b	6.5±0.02 b	1.5±0.00 b	2.5±0.00 b	70.0±0.40 b	19.5 ±0.1 b
Group (9) 0.250 mg/kg twice/week for 2weeks	5.5±0.10 b	13.1±0.02 c	8.4±0.31 ab	12.8±0.41 c	13.4±0.74 a	495.7±0.20 b	5.5±0.0 0 b	2.5±0.00 b	1.5±0.00 c	77.0±0.40 b	13.5 ±0.02 b
Group (10) 0.250 mg/kg daily for 8 days	5.8±0.10 b	16.2±0.00 c	8.3±0.21 ab	15.1±0.42 c	13.8±0.54 a	532.9±0.22 bc	6.5±0.01 b	0.00±0.00 a	0.00±0.00 c	70.5±0.01 b	23.0 ±0.03 b

Same letters (a, b,and c) in one column showed no significant changes p 0.05

serum glucose concentration increased in groups (2, 3, 7, 9 and 10), Table 4.

An increase in total protein was noticed in goats of groups (2, 3 and 6), however, the globulin concentration decreased in groups (7 - 10) and increased in groups (2 and 3). Generally, there were decreases in the concentrations of urea and creatinine in most experimental groups compared to control group (1), Table 4.

Table 5 shows the enzymes activities in serum of Nubian goats infected with *T. evansi* and treated with single or multiple dosages of Cyme-larsan. An increase was noticed in the activities of SDH and AST and decreases in ALP and amylase in most of the experimental animals. It was noticed that serum lipase activity decreased in groups (2 - 6) and increased in groups (7 - 10). There was slight increase in serum activities of ALT, PK, CK and LDH in all groups compared to group1 but, statis-tically insignificant increase was observed in groups 2 and 9, Table 5.

Table 3. Changes in serum concentration of serum Sodium, Potassium, Chloride, Calcium, Phosphorus, Magnesium, Zinc, Copper, Manganese, and Iron in Nubian goats infected with *T. evansi* and given single or multiple dosages of Cymelarsan (M±SE).

Parameter / Groups	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mg/dl)	Zinc (mol/l)	Copper (mol/l)	Manganese (mol/l)	Iron (mol/l)
Group (1)	145.0±0.50^{a}	3.8±0.02^{a}	125.4±0.19^{a}	9.5±0.04^{a}	4.9±0.04^{a}	2.3±0.08^{a}	12.1±0.01^{a}	10.7±0.06^{a}	3.2±0.05^{a}	40.7±0.212^{a}
Group (2)	149. ±0.2968^{a}	4.6±0.03^{b}	134.0±0.39^{b}	7.4±0.06^{b}	3.1±0.06^{b}	3.9±0.04^{b}	10.1±0.02^{a}	11.3±0.05^{a}	5.0±0.09^{b}	38.9±0.28^{a}
Group(3) 0.125mg/kg	146.06±0.11^{a}	3.5±0.011^{a}	135.7±0.07^{b}	2.9±0.03^{c}	2.0±0.09^{c}	4.4±0.03^{b}	10.2±0.02^{a}	12.5±0.02^{a}	3.9±0.02^{a}	35.9±0.06^{b}
Group (4) 0.250mg/kg	144.6±0.11^{a}	3.1±0.01^{a}	127.8±0.08^{a}	6.5±0.03^{b}	4.2±0.03^{a}	2.5±0.03^{a}	11.3±0.12^{a}	9.9±0.02^{a}	3.4±0.02^{a}	39.4±0.20^{a}
Group (5) 0.625mg/kg	144.0±0.51^{a}	4.3±0.02^{b}	128.2±0.11^{a}	6.9±0.04^{b}	4.4±0.02^{a}	2.5±0.01^{a}	13.9±0.01^{a}	11.5±0.12^{a}	3.7±0.01^{a}	36.7±0.17^{b}
Group (6) 1.25mg/kg	103±0.81^{b}	1.8±0.02^{c}	139.9±0.11^{b}	2.9±0.03^{c}	1.5±0.03^{c}	4.5±0.02^{b}	12.0±0.02^{a}	9.7±0.02^{a}	3.4±0.12^{a}	31.5±0.051^{b}
Group (7) 0.125mg/kg twice/week for 2weeks	149.7±0.03^{a}	5.9±0.02^{ac}	133.5±0.08^{b}	7.8±0.23^{a}	4.4±0.02^{a}	2.8±0.01^{a}	13.0±0.20^{a}	10.1±0.02^{a}	3.3±0.02^{a}	39.9±0.09^{a}
Group (8) 0.125mg/kg daily for 8 days	152.9±0.13^{a}	5.6±0.03^{ac}	131.7±0.14^{a}	10.8±0.02^{a}	5.2±0.05^{b}	2.5±0.03^{a}	14.2±0.05^{a}	9.4±0.0 3^{a}	3.9±0.02^{a}	33.3±0.10^{a}
Group (9) 0.250mg/kg twice/week for 2weeks	153.3±0.24^{a}	4.6±0.04^{a}	138.1±0.07^{b}	5.2±0.03^{b}	3.4±0.02^{a}	2.4±0.01^{a}	11.9±0.02^{a}	10.9±0.01^{a}	1.9±0.00^{c}	33.4±0.14^{a}
Group (10) 0.250mg/kg daily for 8 days	134.3±0.04^{a}	4.3±0.07^{b}	136.5±0.159^{b}	5.7±0.08^{b}	3.7±0.07^{a}	3.2±0.02^{b}	14.5±0.10^{a}	10.8±0.02^{a}	4.2±0.06^{ab}	30.9±0.17^{a}

Same letters (a, b and c) in one column showed no significant changes p 0.05.

DISCUSSION

The present study showed that the parasitaemia reached a peak at 7days and death occurred on day 11. Mutayoba et al. (1988) reported parasitaemia reach the maximum on days 8 - 12, and the death occurred 17 weeks post-infection. Also Youssif (2000) studied the parasitaemia in infected *T. evansi* goats, and found the parasitaemia, reached maximum on day 7**,** death occurred on day 5**.** However, death occurred after 33–49 days as described by Arowlo et al. (1988) when the parasitaemia was studied in goats infected with *T. evansi*. Elmalik (1983) found that Nubian goats infected with *T. evansi*, the parasitaemia appeared 2 - 30 days post-infection, the death rate was10%. We believe that the variation might be due to the difference in species of the strains or animals. Also, no effect was observed ascribable to sex of the animals in this study.

It is well known that Cymelarsan inhibits the metabolism of the cells by an enzyme which is central to the regulation of the thiol/disulfide redox balance in the parasite and absent from the host (Bujon, 1990) . The therapeutic dose cleared the parasite from the peripheral blood within 4 days post-treatment but relapse was observed. When

Table 4. Serobiochemical changes in *T. evansi* infected Nubian goats and treated with single or multiple doses of Cymelarsan *(M±SE).*

Parameter / Groups	Bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)
Group (1)	0.56±0.001^{a}	0.29±0.002^{a}	18.7±0.08^{a}	2.1±0.08^{b}	8.5±0.06^{a}	3.3±0.03^{a}	5.2±0.03^{a}	125±0.06^{a}	52.4±0.03^{a}	85.2±0.04^{a}	8.5±0.04^{a}
Group (2)	0.85±0.012^{b}	0.49±0.01^{b}	13.0±0.09^{b}	1.6±0.05^{a}	12.0±0.26^{b}	5.9±0.18^{b}	7.1±0.08^{b}	141.5±0.14^{b}	60.1±0.09^{a}	102.5±0.02^{b}	12.5±0.08^{b}
Group(3) 0.125mg/kg	1.05±0.01^{b}	0.61±0.005^{c}	11.2±0.05^{b}	1.6±0.01 ^{a}	12.8±0.04^{b}	4.5±0.01^{ab}	8.3±0.03^{b}	129.3±0.07^{a}	61.2 ±0.04^{a}	101.2±0.14^{b}	11.9±0.01^{b}
Group (4) 0.250mg/kg	0.60±0.01^{a}	0.295±0.01^{a}	18.0±0.000 ^{a}	2.0±0.01^{b}	9.0±0.07^{a}	3.5±0.07^{a}	5.5±0.00^{a}	126.0±0.01^{a}	56.0±0.01^{a}	83.7±0.11^{a}	9.0±0.000^{a}
Group (5) 0.625mg/kg	0.58±0.02^{a}	0.33±0.01^{ab}	17.3 ±0.01^{a}	1.9 ±0.04^{a}	9.1±0.04^{a}	3.9±0.01^{a}	5.2±0.03^{a}	138.1±0.01^{b}	54.1±0.01^{a}	85.7±0.14^{a}	8.5±0.12^{a}
Group (6) 1.25mg/kg	1.02±1.20^{b}	0.57±0.01^{c}	8.2±0.01^{c}	0.5 ±0.04^{c}	14.9±0.06^{c}	8.4±0.05^{c}	6.5±0.01^{a}	128.4±0.09^{a}	56.0±0.01^{a}	85.4±0.17^{a}	15.9±0.18^{c}
Group (7) 0.125mg/kg twice/week for 2weeks	0.99±0.003^{b}	0.50±0.002^{b}	15.0±0.07^{a}	2.3±0.08^{a}	10.8±0.07^{a}	6.9±0.05^{b}	3.9±0.02^{a}	141.3±0.17^{b}	60.0±0.01^{b}	103.1±0.001^{b}	11.8±0.12^{b}
Group (8) 0.125mg/kg daily for 8 days	0.12±0.013^{c}	0.73±0.004^{b}	11.0±0.04^{b}	1.5±0.04^{b}	10.9±0.13^{a}	9.9±0.04^{b}	1.1±0.08^{c}	143.7±0.14^{b}	55.9±0.15^{a}	108.2±0.04^{b}	11.1±0.14^{b}
Group (9) 0.250 mg/kg twice/week for 2weeks	1.04±0.005^{b}	0.58±0.005^{b}	14.0±0.08^{a}	1.8±0.09^{b}	10.3±0.06^{a}	7.9±0.01^{b}	2.4±0.005^{a}	144.0±0.19^{b}	60.8±0.14^{b}	104.0±0.02^{b}	10.5±0.12^{b}
Group (10) 0.250 mg/kg daily for 8 days	1.24±0.02^{b}	0.74±0.005^{b}	10.1±0.17^{b}	1.7±0.09^{b}	10.8±0.16^{a}	9.2±0.05^{b}	1.6±0.11^{c}	140.8±0.11^{b}	60.9±0.10^{b}	105.0±0.02^{b}	12.1±0.04^{b}

Same letters (a, b and c) in one column showed no significant changes p 0.05.

the drug was administered at two and a half the recommended therapeutic dose the blood was cleared immediately and the animals survived but, death occurred 15 days post-treatment with five times the therapeutic recommended dose. In groups (7 - 10) the peripheral blood and the liver were cleared from the parasite but animals died. This might be due to the toxic effect of the drug on the affected vital organs. Furthermore, results obtained by liver impression smears in group (3) agree with that of peripheral blood or whole blood indicating the existence of the parasite that

Table 5. Enzymes activities in *T. evansi* infected Nubian goats and treated with single or multiple doses of Cymelarsan (M±SE).

Parameter / Groups	LDH (U/L)	CK (U/L)	PK (U/L)	GOT (U/L)	GPT (U/L)	ALP (U/L)	SDH (U/L)	Amylase (U/L)	Lipase (U/L)
Group (1)	326.6±0.38^{a}	33.4±0.13^{a}	31.6±0.08^{a}	35.5±0.14^{a}	14.0±0.09^{a}	87.0±0.37^{a}	27.2±0.14^{a}	102.80±0.11^{a}	315.6±0.621^{a}
Group (2)	37.0±0.49^{a}	35.0±0.124^{a}	40.4±0.44^{b}	40.3±0.14^{a}	15.5±0.07^{a}	76.3±0.49^{b}	33.1±0.25^{b}	95.39±0.09^{b}	242.4±0.6^{b}
Group(3) 0.125Mg/kg	315±0.13.^{a}	40.8±0.03^{a}	39.4^{b}±0.63	42.6±0.07^{a}	17.3±0.07^{a}	63.0±0.01^{b}	36.1±0.03^{b}	81.4±0.06^{b}	266.3±0.47^{b}
Group (4) 0.25Mg/kg	321.3±0.23^{a}	40.7±0.11^{a}	36.6±0.12^{a}	40.6±0.08^{a}	15.9±0.26^{a}	63.0±0.35^{b}	32.2±0.16^{b}	48.8±0.27^{c}	279.0±0.73^{b}
Group (5) 0.625 Mg/kg	318.3±0.13^{a}	38.5±0.02^{a}	36.9±0.09^{a}	40.8±0.04^{a}	13.6±0.05^{a}	69.0±0.36^{b}	36.2±0.09^{b}	101.5±0.03^{a}	267.2±0.37^{b}
Group (6) 1.25 Mg/kg	321.2±0.252^{a}	39.9±0.05^{a}	36.7±0.11^{a}	40.1±0.11^{a}	15.5±0.02^{a}	66.7±0.23^{b}	39.1±0.18^{b}	96.9±0.07^{b}	246.4±0.27^{b}
Group (7) 0.125 mg/kg twice/week for 2weeks	318.0±0.11^{a}	36.8±0.02^{a}	35.0±0.09^{a}	36.2±0.03^{a}	14.1±0.03^{a}	66.9±0.35^{b}	32.4±0.07^{a}	98.5±0.08^{b}	343.7±0.46^{c}
Group (8) 0.125mg/kg daily for 8 days	315.9±0.11^{a}	35.2±0.19^{a}	37.7±0.09^{a}	43.6±0.14^{a}	14.4±0.03^{a}	60.7±0.27^{b}	32.5±0.52^{a}	98.0±0.03^{b}	349.9±0.47^{c}
Group (9) 0.25mg/kg twice/week for 2weeks	369.4±0.00^{b}	39.3±0.06^{a}	36.8±0.12^{a}	35.4±0.23^{a}	14.3±0.35^{a}	59.5±0.21^{c}	35. ±0.521^{a}	97.2±0.09^{b}	330.4±0.51^{b}
Group (10) 0.250mg/kg daily for 8 days	315.9±0.19^{a}	34.2±0.08^{b}	36.0±0.11^{a}	36.3±0.26^{a}	17.1±0.11^{a}	63.0±0.36^{b}	38.2±0.18^{a}	104.3±0.23^{a}	491.6±0.87^{d}

Same letters (a, b, c, and d) in one column showed no significant changes p 0.05.

caused death of animals. The present study indicates therapeutic activity of Cymelarsan at doses of 0.25 - 0.625 mg/kg. Also, the half and the recommended therapeutic dose at twice/week for two weeks or administered daily for 8 days is toxic and fatal. Although the parasite was cleared, the drug has accumulative effect. Radostits et al. (2000) mentioned that the trivalent arsenical compounds cause severe syndrome and arsenic is a general tissue poison combining with and inactivating sulfhydryl groups in tissue enzymes. The same author also mentioned that nervous signs caused by organic arsenic as a result of inhibition of dehydrogenase enzyme system, e.g. pyruvate, ketoglutarate system) causing de generative changes while poisoning with arsenilic acid compounds the lesions are mostly in optical nerve causing blindness. In the poisoning with phenyl arsonic group, the nerves of the limbs appear to be affected most. Radostits et al. (2000) mentioned that local skin lesions include initial hyperaemia followed by necrosis and sloughing, leaving indolent ulcers, which are extremely slow to heal, increase in the respiratory rate, heart rate

and the pulse is small in amplitude. Also Bentram and Anthony, (1993), and Joseph (2001) mentioned that the trivalent As absorbed from gastrointestinal tract, skin, respiratory system, and all mucous surfaces causes acute gastroenteritis, nausea, vomiting, colic, diarrhoea (rice water), capillary damage, dehydration, shock. Chronic form causes skin damage, loss of hair, bone marrow depression, anaemia, weak pulse, ruminal atony, prostration and high morbidity and mortality rate, while in the subacute form watery bloody diarrhea, depression, dehy-dration and anuria are prevalent.

The present study showed that the effect of the high dose of the drug was apparent especially in goats of groups (6 - 10) and the fatalities, are due to the toxic effect of the drug which contained arsenical molecule, specially for the skin lesion, blindness, bloody urine, gastroenteritis manifested by colic, bloody diarrhoea and nervous signs.

It is well known that cardiac muscle is rich in mitochondria. Curtis and John, (2003) mentioned that arsenic inhibits the energy -linked functions of mitochondria. In the light of this information, our results showed blood pressure decrease in groups (6 - 10) which migh indicate weakness of cardiac muscle with terminal cardiovascular failure. This finding is comparable with that of Smith et al. (2001) and Rubaiul et al. (2004). The single dose at 0.25 - 0.625 mg/kg is safe and can therefore be used efficiently in this animal species. The
study shows that doses higher than 0.625 mg/kg can be effective in treatment of trypanosomes of the CNS, cross the blood- brain barrier but unfortunately such doses proved to be fatal.

Horst, (1996) mentioned that the immunosuppressive action of trypanosome exceed the actual course of trypanosomosis, and secondary infections from other pathogens are activated.

We suggest that gastroenteritis may be due to immunosuppressive effect that activates secondary infection. Curtis and John, (2003) mentioned that arsenic is one of the xenobiotics which are capable of immunosuppression.

In the present study in goats of groups (4 and 5) the Hb concentration, PCV % and the RBC count were within the reference interval, but they were decreased in goats of groups (2, 3 and 6 - 10). It is well known that trypanosomosis causes anaemia attributed to erythrophagocytosis as a result of stimulation and expansion of the mononuclear phagocytic system throughout the reticuloendothelium system and to the mechanical cell and tissue damage caused by the active mechanical invasion of the extraordinary strong and mobile pathogens (Horst 1996).

In this study anaemia accompanied by reticulocytosis in parasitaemic goats was even more prominent in goats that received high dosages of Cymelarsan. The slight increase in MCV and the decrease in MCHC indicate that the anaemia is macrocytic and hypochromic. Kenneth et al. (2003) mentioned that in regenerative anaemia the bone marrow is actively responding to anaemia by increased production of erythrocytes, reticulocytosis in addition to monocytosis and hypochromasia.

In the goats which received multiple dosages of Cymelarsan, reticulocytosis and increased MCV, was more prominent indicating regenerative anaemia. Steven and Michael, (2002) and Kenneth et al. (2003) mentioned that trypanosome spp. causes haemolytic anaemia of unknown pathogenesis and caused spleenomegaly, monocytosis, thrombocytosis and hyperproteinaemia suggesting that the anaemia is of an extravascular type. In goats which received the drug in high doses, the anaemia may be haemolytic and of intravascular type though it is mild to moderate. Where there is slight increase in the total bilirubin and the unconjugated bilirubin, this might mean that the liver is still capable of removing the bilirubin from plasma. In addition the increase of the MCV and MCHC indicate cell membrane deformity as glutathione reductase, one of the enzymes which contain thiol group, was possibly inactivated by the arsenic molecules present in the drug. Further toxic substances released when the parasite is destroyed within the circulatory system have been reported to damage the lining of the blood vessels.

Platelets, also referred to as thrombocytes, increased mildly in the present study in parasitaemic groups. The present study showed that experimental animals expressed cellular defense mechanisms (through neutrophilia). Goats receiving high dosages of the drug showed immunosuppressive effects of humoral nature as the globulins were decreased significantly with concurrent decrease in the lymphocytes and monocytes.

The present study showed that the changes in serum sodium, potassium and chloride concentrations in the experimental animals are within reference intervals. Also there is a decrease in the PCV, high total proteinaemia and albuminaemia, and the loss of body weight are indicative of dehydration especially in parasitaemic group and those receiving high doses of Cymelarsan. The dehydration might be attributed to loss of appetite or inability of the animals to move towards water points as a result of recumbency and/or weakness of the limbs.

Kenneth et al. (2003) mentioned that normonatraemia and hypernatraemia can occur when there is diarrhoea and renal diseases. The same authers mentioned that hyperkalaemia results in cases of diarrohea, marked muscle exertion and massive tissue necrosis while, hypokalaemia can result where there are anorexia (specially herbivores), loss of gastrointestinal fluids, abomasal stasis and diarrhoea or through increased renal loss. Steven and Michael, (2002) mentioned that hyper-chloraemia typically occurs when there is hyper-natraemia or when there is a decreased (HCO^-3) and it is well seen in cases of water deficit, meta-bolic acidosis.

The present study showed that serum calcium and phosphorus follow a declining trend, though within the reference interval, and we belief that this decrease can be attributed to the prolonged anorexia and recumbency in addition to the gas-troenteritis which might hinder mineral

absorption, or might be due to renal excretion. Respiratory alkalosis and metabolic acidosis might have played a role in the calcium- phosphorus picture.

Also the present study showed no significant changes in the serum concentration of urea and creatinine, ALT activity while there were slight increases in the activities of serum AST and SDH in most of experimental groups. Kenneth et al. (2003) mentioned that significant renal disease may be present in the absence of clinical signs or laboratory abnormalities due to the extensive reserve capacity of the kidney, and urea excretion in ruminants is governed by nitrogen intake where animals that are on a nitrogen –deficient diet or that have severe anorexia excrete almost all blood urea via the gastrointestinal tract and very little via the kidneys. Therefore, urea can be within the reference intervals in some ruminants with severe renal disease. Decreased urea production via decrease hepatic urea cycle function occurs in cases of hepatic insufficiency. The same author mentioned that creatinine is freely filtered by glo-merular and tubular reabsorption.

Thus serum creatinine is a more accurate measurement of GFR and the creatinine concentration is a more sensitive indicator of renal diseases in cows and horses than BUN because in these species the potential for gastrointestinal excretion of creatinine is limited in contrast to urea. Steven and Michael, (2002) mentioned that SDH indicates hepatocytic damage, AST is a common marker of hepatocytes damage, but muscle damage, haemolysis and other processes also increase serum AST activity.

The slight increase in the serum activity of LDH and CK is attributed mostly to muscle damage (mostly skeletal, occasionally cardiac, rarely smooth).

Also it is noticed that the fluctuation in the serum activity of AMS (amylase) and LBS (lipase) is within reference intervals and might be due to the dehydration.

Conclusion

The present study recommendes treatment of *T* . *evansi* infection in Nubian goats at single I/M do-sages at rates of (0.25 - 0.625 mg/kg).

Further studies shall be done to investigate the efficacy and toxicity of the drug in other animals species and other trypanosomes species. Further studies are also needed to investigate the residual effect of the drug.

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