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Efficacy and Toxicity of Oxytetracycline in Nubian Goats Infected with *T. evansi* and on *T. Evansi* in Phosphate Glucose Buffer Solution (PGS)

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Abstract

Eighty of Sudanese Nubian goats divided into eight groups each of ten, seven groups were infected with *T. evansi* goats in groups 3, 4, and 5 was given Oxy tetracycline (OTC) Intramuscularly (I/M.) at the rate of 20 mg / kg, 50 mg / kg, and 100 mg / kg respectively. Goats in group 6 and 7 were each given the drug I/M weekly at dose rate of 20 mg / kg for three successive weeks or twice/ week for two weeks respectively; while goats in group 8 were given the drug I/M at dose rate of 20 mg / kg daily for 8 days. Goats in groups 1 and 2 were not given the drug but used as control negative and control positive respectively**.** The parasitaemia in groups 4, 5, 6 and 7 was slight, no death occurred except group 8goats were end with fatal event. The clinical signs, pathological lesions in goats of groups 4,5, 6 and 7 and goats in group 8 showed that they were severely suffered.

Results conclude that doses at 50, 100 mg/kg at single I/M and that of 20 mg/kg repeated weekly for weeks were tolerated by the animals and decrease the level of the parasite to a level that the body can overwhelm the infection. Doses of 20 mg/kg daily for 8 days aggravate the condition and appeared toxic and end fatal. At 18 days' post treatment the parasites were cleared from peripheral blood and liver.

The *in vitro* studies showed that OTC at concentration of 4-15µg/ml killed the parasites completely. Also the concentration at 4-15 cleared the parasite completely at 100% in the peripheral blood of the rats.

Keywords: Efficacy; Nubian goats; Oxytetracycline; Parasitaemia; Toxicity; *Trypansoma evansi*

Introduction

Sudan has an immense animal wealth, which satisfies all local needs of meat and produces an export surplus constituting 20% of foreign currency earnings. It satisfies 80% of total milk needs in Sudan [1].

In the Sudan people are dependent on animals as a first source of food, milk and foreign currency. Chemotherapy, by stopping the multiplication of the trypanosomes helps the immune system to overcome the infection. Treatment will be more effective in well-fed and rested animals, in which the immune system is not adversely affected by stress and lack of food. The management of African Animal Trypanosomosis (AAT) at farmer's level has been predominately dependent on the use of the trypanocidals drugs (Diminazene, Homidium and Isometamidium). It is estimated that, about 35 million doses per year are used in Africa to cure the disease [2].

There is another problem in the treatment of animal trypanosomosis, because of the variety of domestic livestock, which are susceptible to trypanosome infection and the diversity of trypanosome species, which are pathogenic to animals of economic importance.

The problems of chemotherapy and chemoprophylaxis are even more complicated and formidable than human trypanosomosis [3]. Other complimentary measures aimed at tsetse eradication using insecticides have been successful only to a limited extent. Despite the fact that chemotherapy is the major means of disease controls.

Trypanosomosis may sometimes be associated with other infections such as internal parasites and bacterial infection. Of the commonly used antibiotics, a tetracycline group is a board

spectrum intoxic, bacterio-static, have effect against virus, mycoplasma, protozoa of blood (*Thielirea, Anaplasma*) [4], and have high concentration in the kidney, spleen, lung and liver [5].

The tetracycline's were discovered as a result of a search for antibiotics active against a wider range of bacteria than penicillin. They are bacteriostatic acting on the bacterial ribosome and they have a chelating action in binding the metallic ions, calcium, magnesium and manganese. They also inhibit a number of essential enzyme systems. It is found in the market in the form of an odorless, yellow base, insoluble in water, soluble in hydrochloride. Solutions lose their activity in a few days. They inhibit a wider range of Gram-positive and negative bacteria and act against certain protozoa such the *Anaplasma, Mycoplasma* spp. and also act against, Reckettsia and Theileria. They may be given by mouth and take 3-4 days to be excreted [6].

Giovani and Warren [3] found tetracycline groups in significant amounts in the bile of most animals after treatment with tetracycline. It can be excreted by hepatic cells into bile and eventually pass into the intestines. A cycle (enterohepatic) can result in which the drug is continually re absorbed from the intestines after biliary secretion until enough of the drug passes through the liver to render it sufficiently water soluble for urinary excretion [4,5].

Drug concentration may accumulate in developing bones, teeth and other organs of the young, since their detoxification enzyme systems are not fully developed [6].

Materials and Methods

Adaptation period of goats

Eighty healthy male Nubian goats, 8-12 months old, weighing 9-11 kg were housed in pens at the Faculty of Veterinary Medicine - Khartoum University - Khartoum State. Each animal was fed daily on 3 kg lucerne (*Medicaqo sativae*) and 1.5 kg sorghum (*Sorghum vulgare*) and 2 kg millet (*Pearl millet*) were given once weekly with free access to water.

Drug

Oxyteracycline (Remacyline®) 20mg/kg(Coophavet-France).

The Parasite and Infection

The animals were divided randomly into eight equal groups each of ten designated group 1, 2, 3, 4, 5, 6, 7 and 8. Group 1 let as a control negative group. Animals in groups 2, 3, 4, 5, 6, 7 and 8 were injected intravenously with (0.75 ml) 5 x 10^5 trypanosomes obtained from Western Sudan after amplification in a goat for 17 days.

Treatment

Single Dose Treatment

Each goat in group 3, 4, and 5 was given the single i.m. dose of the drug at the rate of 20 mg / kg, 50 mg / kg, and 100 mg / kg respectively.

Multiple Dose Treatment

Goats in group 6 were each given the drug weekly at dose rate of 20 mg / kg for three successive weeks intramuscularly.

Each goat in groups 7and 8 was given the drug i.m. at dose rate of 20 mg / kg, twice/ week for two weeks or repeated daily for 8 days respectively.

Goats in-groups 1 and 2 were not given the drug but used as control negative and control positive respectively**.**

Blood Collection

Animals were bled to detect parasitaemia and progress of the clinical and pathological events from the jugular vein at day 1, 3, 7 and 10 post infection and after 1, 3, 24 hours and 3, 7 , 14 , 21 , 28 , 35 , 42 and 49 days post-treatment using two plain vacutainer test tubes. The first tube contained no anticoagulant and serum was collected and kept at - 20ºC until analyzed for serobiochemical investigations. The second tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) was used for hematological investigations.

Clinical Examination

Experimental goats were examined daily for body temperature, body weight and respiratory rate. The pulse rate and blood pressure of the femoral artery were examined daily using electronic apparatus (Digital Blood Pressure Meter, Seinex Electronics Ltd. - UK).

Parasitological Methods

The source, prepration and infection of the parasite were done as described by Youssif et al. [7]. *T. evansi* was detected in the peripheral blood (wet blood film) and in the whole blood (thin film, thick film, buffy coat technique) to asses the parasitaemia.

The liver impression smears were obtained when animals died or slaughtered. Parasitaemia was estimated by examining 40 microscopic field. Efficacy was expressd as described by Lumsden et al. [8].

Hematological Methods

The red blood cells count (RBC), White Blood Cells Count (WBC), differential WBC count, Hemoglobin (Hb) concentration,

retculocytes, platelets, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were done in 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), α-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). Serum aspartate amino transaminase (AST 2.6.1.1) and alanine amino transaminase (ALT 2.6.1.2) were measured using commercial kits (Randox Laboratories Ltd. U.K.). Serum was also analyzed for the concentrations of creatinine, urea, direct bilirubin, glucose, total proteins, albumin using commercial kits (Randox Laboratories Ltd. U.K.). Serum globulins were calculated by deduction of the albumin from the total proteins. Serum total bilirubin was measured using commercial kits (Boehringer Mannheim GmbH Diagnostics, West Germany). Serum concentrations of phospholipids, triglycerides, total cholesterol, chloride, magnesium, calcium, inorganic phosphorus, zinc, copper and iron were measured using commercial kits (Linear Chemicals, S. L.- Spain).

Serum samples were also assayed for the concentrations of sodium and potassium using flame photometer (400 flame photometer Corning – England). Serum manganese was measured using atomic absorption spectrophotometer (Corning EEL 197 Spectra- Evans Electroselenium Ltd., England) at wave length 279.5 nm.

Statistical Analysis

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

The effect of oxytetracycline on *T. evansi* in phosphate glucose buffer solution (PGS):

The stock: 0.59g of PGS

9.5ml (0.1%) NaOH

Complete to 100 ml with distilled water.

The buffer 29.4g sodium citrate

Complete to 100ml with distilled water.

Citric acid 21g citric acid.

Complete to 100 ml with distilled water.

Working solution: 6 ml of the stock, 32 ml of the buffer and 17.3 ml of citric acid and complete to 1 liter with distilled water.

The T*rypanosoma evansi* added to the working solution and preserved in liquid nitrogen.

Oxytetracycline (100%) added to the work solution when there is a live trypanosome in different concentration (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5 µg/ml) the live trypanosomes examined every hour for 72 hrs.

Results

Parasitaemia and Efficacy

Parasitaemia in peripheral blood

a)Post infection

T. evansi was detected in the peripheral blood of goats in group (2-8) where it was mild on day 4, moderate on days 5and 6 and severe on day7 these goats were died on day 11.

b) Post treatment

Goats in group (3) showed moderate parasitaemia (++) between days1-3, mild (+) on days4-8, moderate (++) between days9-11 and till they were died on days 10-11. Goats in group (4) showed moderate (++) days1-24, and mild (+) on days 25-56. Goats of group (5) showed moderate parasitaemia (++) on days 1-3, then mild parasitaemia until day 49. Goats in group (6) showed moderate parasitaemia (++) in the first four days followed by mild parasitaemia (+) till day14 -49. Goats in group (7) showed moderate parasitaemia (++) and mild parasitaemia till day 26-49. Goats in group (8) showed moderate parasitaemia (++) between day1-2. The detected parasitaemia in goats of groups 4,5 and 6 were moderate in the first for days' post-treatment then it become mild for other 5 days, then the level of parasitaemia increased modality for another 5 days followed by mild parasitaemia thereafter till animals were slaughtered on days 21, 28, 35, 42 and 49. However, it was noticed that parasitaemia was free in goats of groups 7 and8 between days 20-25 and 10-15 respectively before a mild relapse. Goats in group7 slaughtered on days 21, 28, 35, 42 and 49 while goats in groups8 died between days 16-18 post -treatment Table (1).

post treatment																															
Days Groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Week5 (35day)	Week6 (42day)	Week7 (49day)
Group (1)	-																														
Group (2)	*																														
Group (3) 20mg/kg	+	+	+	+	+	+	+	+	+	+	Died																				
	+	+	+						+	+																					
Group (4) 50mg/kg	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	+	+	+											+	+	+	+	+	+	+	+	+	+	+							
Group (5) 100mg/kg	+	+	+	-																		+	+	+	+	+	+	+	+	+	+
	+	+	+																												
Group (6) 20mg/kg weekly for 3weeks	+	+	+	+	+	+	+	+	+	+	+	++		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+							+			+																	
Group (7) 20 mg/kg twice/week for 2weeks	+	+	+	+	+	+	+	+	+	+	+	++		+	+	+	+	+	+	-						+	+	+	+	+	+
	+	+	+	+	+				+	+	+																				
Group (8) 20mg/kg daily for 8days	+	+	+	+	+	+	+	+	+	-				+	+	+	+	Died													
	+	+																													
+ = 1-4 parasites/field (mild), - = no parasite detected and * = animals in group (2 died on day 11 post infection.																															

Table 1: The parasitaemia in the peripheral blood of Nubian goats infected with *T. evansi* an given single and multiple dosages of oxy tetracycline.

Detection of the parasites in liver impression smears

Liver impression smears of the dead goats of groups (2 and 3) showed moderate parasitaemia, but it was mild in group (4) were mild during the five weeks of the slaughter program. Goats in groups (5 and 6) showed moderate parasitaemia (++) in the first slaughters, mild parasitaemia in slaughter 2and 3 then parasites free on slaughters 4and 5 and goats in groups (7 and 8) were parasite -free during the five weekly slaughters program Table (2).

Slaughter (week)					Groups
W5	W4	W3	W2	W1	
-ve					Group (1)
*					Group (2)
(++) Died on day 10					Group (3) 20mg/kg
+					sGroup (4) 50mg/kg
-ve	+	+	+	++	++	Group (5) 100mg/kg
-ve	+	+	+	++	++	Group (6) 20mg/kg weekly for3weeks
-ve					Group (7) 20 mg/kg twice/week
(-ve) Died on day18				-ve	Group (8) 20mg/kg daily
-ve = no parasite detected, += 1-3 parasites/field, ++ = 4-6 parasites/field, * = Died on day 11post infection and showed ++parasites/field. **= Died on days26-28 post infection. Week1 (w1)=14day post treatment (d.p.t), w2=21d.p.t, w3=28d.p.t, w4= 35d.p.t and w5=42d.p.t.					

Table 2: Detection of the parasites by the liver impression smears in Nubian goats infected with *T. evansi* and given single and multiple dosages of oxy tetracycline.

Clinical signs and clinical investigation

Table (1) summarizes the parasitaemia in the peripheral blood of the goats infected with *T. evansi* and treated with single and multiple dosages of OTC.

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, decrease in the pulse rate, convulsions and shivering. In the second week the lymph nodes and testes were hot and swollen and animals became off food, cachexic and recumbent with lateral curvature of the neck for 1-2days prior to death.

Goats in group (3) showed the same clinical signs as group (2). But these signs are mild in goats of groups (4and 5) and partially regained their appetite and weight. Animals in groups (6-8) showed weakness in the fore and hind limbs, colic, lacrimation, convulsions, shivering, decrease in appetite, emaciation and over reflection in hind limb which appeared next week post treatment. The blood pressure was generally decreased in all experimental goats compared to group 1. It was noticed that goats of groups 8 recorded least respiratory, blood pressure and pulse rate. Death occurred in goats of groups 2, 3 and 8 while the other groups were slaughtered at the other two animals/ week on days 21, 28, 35, 42 and 49. Table (3).

Parameter Groups	Body Temperature (^{o}c)	Respiratory Rate(/min.)	Pulse Rate (pulse/min)	Body weight (kg)	Blood pressure (mmHg)	Fate of the animals(days)
Group (1)	39.5^{a} ±0.01	26.1^{a} ±0.00	75.5^{a} ±0.02	9.5^{a} ±0.05	$125/75^{a}$ ±1.12/1.5	Two animals slaughtered/ week on day21,28,35,42 and 49
Group (2)	34.3^{a} ±0.02	38.5b ±0.01	60.5^{b} ±0.01	7.8^{a} ±0.04	$90/44^{b}$ ±1.11/1.3	Died on day11post infection
Group (3) 20mg/kg	36.5^{a} ±0.01	30.5^{a} ±0.08	63.0^{b} ±0.000	8.2^{a} ±0.21	$90/50^{b}$ ±1.5/0.2	Died between days10-11post treatment
Group (4) 50mg/kg	37.6^{a} ±0.02	35.2^{ab} ±0.08	65.5^{b} ±0.00	7.3^{a} ±0.11	$95/60^{b}$ ±1.3/0.4	Two animals slaughtered/ week on days 21, 28,35,42 and 49
Group (5) 100mg/kg	36.1^{a} ±0.12	28.5^{a} ±0.02	80.0^{a} ±0.01	8.31^{a} ±0.012	$105/60^{b}$ ±1.2/0.4	Two animals slaughtered/ week on days 21, 28,35,42 and 49
Group (6) 20mg/kg weekly for 3weeks	36.2^{a} ±0.12	30.0^{a} ±0.03	80.9^{a} ±0.02	7.5^{a} ±0.01	$95/55^{b}$ ±1.5/0.5	Two animals slaughtered/ week on days 21, 28,35,42 and 49
Group (7) 20 mg/kg twice/week for two weeks	36.2^{a} ±0.12	35.8^{b} ±0.03	65.5^{b} ±0.00	7.5^{a} ±0.01	$100/60^{b}$ ±0.6/0.2	Two animals slaughtered/ week on days21, 28,35,42 and 49
Group (8) 20mg/ kg daily	36.3^{a} ±0.05	15.5^{c} ±0.01	50.0^{c} ±0.02	8.5^{a} ±0.32	$80/46^{b}$ ±0.5/0.1	Died between days 16-18
Same letters(a, b and c) in one column showed no significant changes $p \leq 0.05$.						

Table 3: The body weight and temperature, respiratory rate, blood pressure and fate of the animals in Nubian goats infected with *T. evansi* and given single and multiple doses oxy tetracycline (M±SE).

Postmortem Findings

Table (4) summarizes the post mortem findings in goats infected with *T. evansi* and treated with single and multiple dosages of oxytetracycline LA. No gross findings were observed in goats of group (1). The gross pathological findings in groups (2, 3, and 8) were severe or moderate, while goats in groups (4and 5) had mild or no lesions. Lesions under investigation were congestion, oedema and haemorrhage, hyperaemia, froth on the trachea, and dilatation and flabbiness of the heart. Fatty change and/or necrosis was seen mainly in livers and kidneys in addition nephritis, gastroenteritis, peritonitis, hydropretoneum, spleenomegaly, myocitis at the site of injection, inflamed and swollen nerve, lymphadenitis, orchitis and the thyroid glands were inflamed, congested and changed in different groups. It was noticed that these lesions disappeared in the fourth week post treatment in goats of groups (5and 6). Goats in group (5-8) showed greenish bile with white granules. Goats in group (7) showed urine retention. Goats in groups (6-8) showed atrophy of the right cerebellum and stiff muscle.

		Groups							
Site	Lesion	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Brain	Congestion	-	++	+	+	-	-	+	+
	Haemorrhage	-	+	+	-	-	+	-	-
	Atrophy	-	-	-	-	-	++	+++	+++
	Hyperaemia	-	-	-	-	-	++	++	++
Nasal Cavity	Congestion	-	+	+	+	-	-	-	-
Trachea	Congestion	-	-	-	-	+	+	++	++
	Haemorrhage	-	-	-	-	-	-	-	-
	Froth	-	-	-	-	+	-	+	++
Thyroid gland	Enlargement	-	++	++	-	-	-	+	-
Lungs	Hepatization	-	++	+	-	-	-	+	++
	Congestion	-	+++	-	-	-	-	-	-
	Haemorrhage	-	++	-	-	-	-	-	-
	Emphysema	-	+	+	-	-	-	+	++
Heart	Congestion	-	-	+	-	-	+	+	+
	Haemoarrhage	-	+	+	+	+	+	+	+
	Flabiness	-	++	-	-	-	+	+	++
	Hydropericardium	-	+	-	-	-	++	++	++
Liver	Congestion	-	+++	+	-	-	+	+	+
	Haemoarrhage	-	-	+	+	-	+	+	+
	Oedema	-	+	+	-	-	+	+	+
	Fatty change and/ or Necrosis	-	+	+	-	-	+	+	+
Kidneys	Haemorrhage	-	-	+	-	-	-	+	+
	Congestion	-	-	+	-	-	+	++	+
	Fatty change and/or necrosis	-	++	+	+	+	+	+	+

Spleen	Congestion	-	++	+	+	-	+	-	-
	Enlargment	-	+++	+	-	-	+	-	+
Intestine	Congestion	-	+++	++	+	+	+	+	+
	Haemorrhage	-	++	++	-	-	-	-	+
	Enteritis	-	+	-	+	+	+	+	+
Peritoneum	Peritonitis	-	-	+	-	-	+	+	+
	Hydroperitoneum	-	-	+	-	-	++	+	+
Site of injection	Myositis	-	-	-	-	+	+	+	+
Peripheral nerves	Damage	-	-	-	-	-	+	+++	+++
	congestion	-	-	+	+	+	+	+	+
Lymph node	Enlargement/lymph adenitis	-	+	-	-	-	-	-	-
Testis	Atrophy and/or orchitis	-	+	-	-	-	-	-	-
- = no lesion , + = mild, ++ = moderate, +++ = severe.									

Table 4: Postmortem findings in Nubian goats infected with *T. evansi* and given single and multiple dosages of oxytetracycline.

Hematological Changes

Tables (5and 6) summarize the haematological change in goats infected with *T. evansi* and treated with single and multiple dosages of oxytetracycline. Significant decreases (p 0.05) were seen in the Hb concentration, PCV and RBC while, a significant increase (p0.05) in the reticulocyte, platelets in animals compared to group (1), although there was no significant changes in the WBC count, but an increase was observed in these animals. The differential count of WBC, a significant increase (p0.05) in the neutrophils and basophils while the lymphocytes significant decrease (p0.05) in goats of groups 2-7. No significant decrease was seen in neutrophils and in lymphocytes in goats in groups 7 and 8. No significant changes were seen in MCV, MCH and MCHC in goats of groups (2-8).

Parameter Groups	**Hb (g/dl)**	**PCV (%)**	**RBC ($x10^6$/m^3)**	**Retic ulocyte ($X10^6/m^3$)**	**WBC ($x10^6/m^3$)**	**Platelet ($x10^6/m^3$)**	**Eosin ophils (%)**	**Baso phils (%)**	**Mono cyte (%)**	**Neutr ophils (%)**	**Lymph ocyte (%)**
Group (1)	9.0^a ±0.24	32.3^a ±0.02	14.5^a ±0.21	5.7^a ±0.02	11.7^a ±0.32	410.7^a ±0.73	2.5^a ±0.00	0.00^a ±0.00	3.00^a ±0.00	55.1^a ±0.01	40.1^a ±0.01
Group (2)	5.7^b ±0.02	24.8^a ±0.21	10.7^b ±0.31	7.8^b ±0.02	13.8^a ±0.31	489.5^b ±0.71	3.6^b ±0.002	2.8^b ±0.001	5.0^b ±0.002	68.0^b ±0.41	20.6^b ±0.05
Group (3) 200g/kg	5.5^b ±0.04	28.0^a ±0.31	11.5^b ±0.31	7.3^b ±0.21	12.2^a ±0.21	520.3^b ±0.65	2.0^a ±0.00	3.5^b ±0.001	2.5^c ±0.001	60.0^b ±0.32	32.0^a ±0.01
Group (4) 50mg/kg	6.7^b ±0.02	27.2^a ±0.41	10.2^b ±0.22	8.4^b ±0.03	13.3^a ±0.22	510.4^b ±0.66	2.0^a ±0.001	2.7^b ±0.001	3.5^c ±0.001	61.7^b ±0.14	30.1^a ±0.02

Group (5) 100mg/kg	7.5^{b} ±0.01	26.5^{a} ±0.31	10.3^{b} ±0.12	7.5^{b} ±0.02	14.2^{a} ±0.21	490.9^{b} ±0.65	1.5^{a} ±0.001	3.0^{b} ±0.02	1.5^{a} ±0.013	66.5^{b} ±0.32	27.5^{b} ±0.01
Group (6) 20mg/kg weekly for 3weeks	7.0^{b} ±0.02	26.1^{a} ±0.25	9.1^{b} ±0.11^{b}	8.2^{b} ±0.01	13.1^{a} ±0.21	488.7^{b} ±0.71	2.5^{a} ±0.02	2.5^{b} ±0.001	0.00^{a} ±0.02	65.8^{b} ±0.31	29.2^{b} ±0.01
Group (7) 20 mg/kg twice/week for two weeks	7.0^{b} ±0.02	26.1^{a} ±0.25	9.1^{b} ±0.11	8.2^{b} ±0.01	13.1^{a} ±0.21	488.7^{b} ±0.71	2.5^{a} ±0.02	2.5^{b} ±0.001	0.00^{c} ±0.02	65.8^{b} ±0.31	29.2^{b} ±0.02
Group (8) 20mg/kg daily	6.3^{b} ±0.02	23.3^{a} ±0.31	10.3^{b} ±0.13	9.6^{b} ±0.00	15.6^{a} ±0.21	550.3^{b} ±0.62	0.5^{c} ±0.01	3.0^{b} ±0.03	2.0^{a} ±0.02	55.4^{a} ±0.23	39.1^{a} ±0.1
Same letters(a, b and c) in one column showed no significant changes p≤0.05.											

Table 5: The Hemoglobin concentration, Packed Cell volume, count of red blood Cell, reticulocyte, white blood Cell, Platelet, eosinophils, basophiles, monocytes, lymphocyte and Neutrophils in Nubian goats infected with *T. evansi* and given single and multiple doses oxytetracycline (M±SE).

Parameter Groups	MCV (fl)	MCH (pg)	MCHC (%)
Group (1)	22.2^{a} ±0.03	62.06^{a} ±0.04	27.8^{a} ±0.01
Group (2)	23.17^{a} ±0.00	53.2^{a} ±0.05	22.9^{a} ±0.03
Group (3) 20mg/kg	24.3^{a} ±0.01	47.8^{a} ±0.04	19.6^{a} ±0.01
Group (4) 50mg/kg	26.6^{a} ±0.03	65.6^{a} ±0.05	24.6^{a} ±0.01
Group (5) 100mg/kg	25.2^{a} ±0.01	72.8^{a} ±0.07	28.3^{a} ±0.08
Group (6) 20mg/kg weekly for 3weeks	28.6^{a} ±0.01	76.9^{a} ±0.03	26.6^{a} ±0.09
Group (7) 20mg/kg twice/ week for two weeks	28.6^{a} ±0.02	65.4^{a} ±0.05	26.8^{a} ±0.09
Group (8) 20/kg daily	22.6^{a} ±0.03	61.7^{a} ±0.07	27.03^{a} ±0.05
Same letter (a) in one column showed no significant changes p≤0.05.			

Table 6: The mean ± Standard Error of (MCV), (MCH), and (MCHC) in Nubian goats infected with *T. evansi* and given single and multiple doses oxy tetracycline (M±SE).

Serobiochemical changes

The serobiochemical changes are summarized in Table (7-9) in goats infected with *T. evansi* and treated with single and multiple dosages of oxytetracycline. There were no significant (p 0.05) changes in the serum concentration of sodium, potassium chloride, zinc, copper and iron in groups (2-8). Decrease in serum calcium and phosphorus concentrations and also significant (p0.05) increase in serum magnesium and manganese concentrations were also observed in some groups. These changes were most evidenced in goats of groups (7and 8).

Parameter/ Groups	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Calcium (mmol/l)	Phosphorus (mg/dl)	Magnesium (mg/dl)	Zinc (µmol/l)	Copper (µmol/l)	Manganese (µmol/l)	Iron (µmol/l)
Group (1)	145.0^{a} ±0.50	3.8^{a} ±0.02	125.4^{a} ±0.19	9.5^{a} ±0.04	4.9^{a} ±0.04	2.3^{a} ±0.08	12.6^{a} ±0.01	10.7^{a} ±0.06	3.2^{a} ±0.05	40.7^{a} ±0.212
Group (2)	149.8^{a} ±0.296	4.6^{b} ±0.03	134.0^{b} ±0.39	7.4^{b} ±0.06	3.1^{b} ±0.06	3.9^{b} ±0.04	10.1^{a} ±0.02	11.3^{a} ±0.05	5.0^{b} ±0.09	38.9^{a} ±0.28
Group (3) 20mg/kg	150.0^{a} ±0.21	3.8^{a} ±0.01	126.8^{a} ±0.21	7.5^{b} ±0.01	3.5^{b} ±0.01	3.5^{b} ±0.01	10.3^{a} ±0.01	11.5^{a} ±0.04	3.6^{a} ±0.01	41.2^{a} ±0.03
Group (4) 50mg/kg	148.4^{a} ±0.22	3.5^{a} ±0.04	128.7^{a} ±0.31	6.5^{b} ±0.03	3.8^{b} ±0.01	3.7^{b} ±0.01	11.2^{a} ±0.05	10.5^{a} ±0.05	3.5^{a} ±0.01	40.5^{a} ±0.03
Group (5) 100mg/kg	145.7^{a} ±0.13	4.0^{b} ±0.02	129.8^{a} ±0.31	7.0^{b} ±0.04	3.9^{b} ±0.06	3.5^{b} ±0.02	12.0^{a} ±0.05	10.5^{a} ±0.05	4.5^{ab} ±0.01	39.7^{a} ±0.03
Group (6) 20mg/kg weekly for 3weeks	146.3^{a} ±0.18	4.8^{b} ±0.01	130.7^{a} ±0.18	7.4^{b} ±0.05	3.0^{b} ±0.04	3.6^{b} ±0.04	11.5^{a} ±0.04		5.5^{b} ±0.01	38.5^{a} ±0.03
Group (7) 20 mg/kg twice/week for two weeks	146.3^{a} ±0.18	4.8^{b} ±0.01	130.7^{a} ±0.18	7.4^{b} ±0.05	3.0^{b} ±0.04	3.6^{b} ±0.04	11.5^{a} ±0.04	11.4^{a} ±0.02	5.5^{b} ±0.01	38.5^{a} ±0.03
Group (8) 20mg/kg daily	145.3^{a} ±0.45	5.5^{b} ±0.02	138.2^{a} ±0.02	6.3^{b} ±0.12	2.5^{ab} ±0.02	4.6^{b} ±0.01	10.3^{a} ±0.02	13.3^{a} ±0.012	6.5^{c} ±0.02	33.0^{a} ±0.32
Same letters (a, b and c) in one column showed no significant changes p≤0.05.										

Table 7: The sodium, potassium, chloride, calcium, phosphorus, magnesium, zinc, copper, manganese, and iron in Nubian goats infected with *T. evansi* and given single and multiple doses oxytetracycline (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^{a} ±0.001	0.29^{a} ±0.002	18.7 ±0.08	2.1^{a} ±0.08	8.5^{a} ±0.06	3.3^{a} ±0.03	5.2^{a} ±0.03	125.8^{a} ±0.06	52.4^{a} ±0.03	85.2^{a} ±0.04	8.5^{a} ±0.04
Group (2)	0.85^{b} ±0.012	0.49^{a} ±0.005	13.0^{a} ±0.09	1.6^{b} ±0.05	12.0^{b} ±0.26	5.9^{b} ±0.18	7.1^{b} ±0.08	141.5^{b} ±0.14	60.1^{a} ±0.09	102.5^{b} ±0.02	12.5^{b} ±0.08
Group (3) 20mg/kg	0.71^{b} ±0.01	0.37^{a} ±0.01	13.3^{a} ±0.01	1.9^{a} ±0.01	9.5^{a} ±0.04	6.5^{b} ±0.01	3.0^{c} ±0.03	135.1^{a} ±0.22	61.3^{a} ±0.09	95.0^{b} ±0.04	10.3^{ab} ±0.04

Group (4) 50mg/kg	0.61^{a} ±0.001	0.41^{a} ±0.02	14.5^{a} ±0.02	2.0^{a} ±0.01	9.8^{a} ±0.15	5.4^{b} ±0.11	4.4^{ac} ±0.04	130.2^{a} ±0.12	58.2^{a} ±0.08	99.7^{b} ±0.03	9.2^{a} ±0.01
Group (5) 100mg/kg	0.63^{a} ±0.01	0.34^{a} ±0.01	15.0^{a} ±0.01	1.6^{b} ±0.01	8.5^{a} ±0.02	4.5^{ab} ±0.01	4.0^{ac} ±0.04	131.3^{a} ±0.02	60.5^{a} ±0.06	89.4^{a} ±0.07	8.1^{a} ±0.02
Group (6) 20mg/kg weekly for 3weeks	0.58^{a} ±0.001	0.41^{a} ±0.03	15.6^{a} ±0.01	1.7^{b} ±0.01	9.0^{a} ±0.02	3.5^{a} ±0.01	4.5^{ac} ±0.01	129.4^{a} ±0.01	56.5^{a} ±0.05	90.5^{a} ±0.02	9.3^{a} ±0.04
Group (7) 20 mg/kg twice/week for two weeks	0.58^{a} ±0.001	0.41^{a} ±0.03	15.6^{a} ±0.01	1.7^{a} ±0.01	9.0^{a} ±0.02	3.5^{a} ±0.01	4.5^{ac} ±0.01	129.4^{a} ±0.01	56.5^{a} ±0.05	90.5^{a} ±0.02	9.3^{a} ±0.04
Group (8) 20mg/kg daily	0.85^{b} ±0.01	0.72^{b} ±0.01	13.5^{a} ±0.02	0.5^{b} ±0.001	10.0^{a} ±0.12	7.3^{b} ±0.12	3.7^{c} ±0.01	140.2^{b} ±0.02	65.2^{a} ±0.21	96.2^{b} ±0.02	11.3^{b} ±00.01
Same letters(a, b and c) in one column showed no significant changes p≤0.05.											

Table 8: The bilirubin, direct bilirubin, urea, creatinine, total protein, albumin, globulin, cholesterol, glucose, phospholipids and triglyceride in Nubian goats infected with *T. evansi* and given single and multiple doses oxytetracycline (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)	326.3^{a} ±0.38	33.4^{a} ±0.13	31.6^{a} ±0.08	35.5^{a} ±0.14	14.00^{a} ±0.09	87.0^{a} ±0.37	27.2^{a} ±0.14	102.8^{a} ±0.11	315.6^{a} ±0.621
Group (2)	337.0^{a} ±0.49	35.0^{a} ±0.124	40.4^{a} ±0.44	40.3^{a} ±0.14	15.5^{a} ±0.07	76.3^{b} ±0.49	33.1^{b} ±0.25	95.39^{b} ±0.09	242.4^{b} ±0.6
Group (3) 20mg/kg	315.7^{a} ±0.41	30.2^{a} ±0.21	38.8^{a} ±0.32	43.6^{a} ±0.03	16.3^{a} ±0.01	85.5^{a} ±0.31	30.3^{a} ±0.22	98.5^{b} ±0.05	270.3^{c} ±0.63
Group (4) 50mg/kg	319.4^{a} ±0.31	31.4^{a} ±0.22	35.4^{a} ±0.23	36.4^{a} ±0.01	15.2^{a} ±0.04	86.4^{a} ±0.41	28.2^{a} ±0.21	99.4^{a} ±0.11	290.2^{c} ±0.51
Group (5) 100mg/kg	321.2^{a} ±0.43	26.5^{a} ±0.25	32.8^{a} ±0.35	34.2^{a} ±0.11	14.5^{a} ±0.02	79.3^{b} ±0.14	26.1^{a} ±0.23	103.3^{a} ±0.12	316.1^{a} ±0.52
Group (6) 20mg/kg weekly for 3weeks	322.2^{a} ±0.41	27.3^{a} ±0.32	31.0^{a} ±0.32	36.5^{a} ±0.11	15.5^{a} ±0.04	83.1^{a} ±0.31	25.3^{a} ±0.32	102.2^{a} ±0.01	315.4^{a} ±0.16
Group (7) 20 mg/kg twice/ week for two weeks	322.2^{a} ±0.41	27.3^{a} ±0.32	31.0^{a} ±0.32	36.5^{a} ±0.11	15.5^{a} ±0.04	83.1^{a} ±0.31	25.3^{a} ±0.32	102.2^{a} ±0.01	315.4^{a} ±0.16
Group (8) 20mg/kg daily	300.5^{b} ±0.35	25.3^{a} ±0.12	36.4^{a} ±0.33	44.2^{a} ±0.12	17.3^{a} ±0.11	71.0^{b} ±0.30	34.4^{b} ±0.21	90.3^{b} ±0.52	254.2^{c} ±0.13

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

Table 9: The lactate dehydrogenase, creatinine kinase, pyruvate kinase, (GOT), (GPT), alkaline phosphatase, succinate dehydrogenase, amylase and lipase in Nubian goats infected with *T. evansi*. and given single doses of oxytetracycline (M±SE).

Generally no prominent changes were observed in serum concentration of urea, creatinine, cholesterol, glucose, phospholipids and triglyceride in goats in groups 2-7.

No apparent changes were detected in serum activity of LDH, CK, PK, GOT, GPT, ALP, SDH, amylase and lipase in the experimental goats specially goats in group (2-7). Significant (p0.05) decrease were observed in many parameters tested in goats of group8.

The effect of oxytetracycline on *T. evansi* in Phosphate Glucose Buffer Solution (PGS):

Table (10) summarizes the effect of oxytetracycline on *T. evansi* in phosphate glucose buffer solution (PGS)and in rats. The effect of oxytetracycline (100%) in different concentrations (0.1-1and 1.5 - 15.5 µg/ml) on *T. evansi* was examined every hour for 72 hr. The working solution after examination inoculated in rats. No trypanosomes were detected in the solution or rats when 4.4-15.0 µg/ml were added while, in the concentrations of 0.1-0.9 µg/ml the trypanosomes were still alive and 50% of the trypanosomes died when the concentration of 1-3 µg/ml examined.

OTC concentration (µg/ml)	Trypanosomes in PSG	Trypanosomes in rats
0.1-0.9	++	++
1-3	+	+
4-15	-ve	- ve
- ve= negative no parasite detected, + = 50% of the parasite added to PSG and ++ = all the parasites added to PSG		

Table 10: The effect of oxytetracycline on *T. evansi* in phosphate glucose buffer solution (PGS).

Discussion

Treatment with OTC-LA at 100mg/kg, 20 mg/kg twice a week for two weeks and 20mg/kg daily for 8days showed period of free parasitism in the peripheral blood followed by a relapse of mild parasitaemia. Dosages of daily 20mg/kg for 8days have fatal end 18 days post treatment. This result is supported by liver impression smear where the livers were cleared 21 days post treatment and after cessation of the drug. Youssif, [9] found that the oxytetracycline decreased parasitaemia in Nubian goats by 75%, death occurring by 7days later. The relapse in trypanosomosis might be attributed to maturation of immature stages of the trypanosome [10]. Relapse also depends upon the concentration of the drug or its metabolites in the blood circulation acting on certain stages of the parasite. The present study also indicates that the animals can tolerate single dosage up to 100 mg/kg and also can tolerate multiple dosages of 20 mg/kg weekly for 3weeks or twice a week for two weeks, but dosages of daily 20 mg/kg for 8days result in fatal ends.

Goats which received the daily therapeutic dose for 8days didn't tolerate the drug, and the animals dying on day 18 this may be due to toxic effect of the drug in concommted with the infection.

Sharp decrease in pulse rate, blood pressure and respiratory rate in goats which receiving the drug at 20 mg/kg repeated for 8 days and with fatal end may be attributed to the pneumocardiopathy which end with circulatory insufficiency.

Increase in WBC counts might be due to the irritant effect of the drug at site of injection [5]. Decrease in the lymphocytes may be accounted for by the fact that trypanosomes have an immunosuppressant effect. But, the increase in platelet and reticulocyte counts may indicate of haematocrisis stimulation.

The slight increase in serum of potassium, chloride in addition to decrease of calcium, phosphorus as well as the pathological changes in some groups infected with *T. evansi* and treated with either single or repeated dosages indicate renal and/or metabolic disorders and acid-base imbalances.

Both oxytetracycline and tetracycline are eliminated unchanged primarily via glomerular filtration. Patients with impaired renal function can have prolonged elimination half-lives and may accumulate the drug with repeated dosing. These drugs apparently are not metabolized, but are excreted into the GI tract via both biliary and nonbiliary routes and may become inactive after chelation with fecal material [11].

It is noticed that goats which received the daily program recorded least phosphorus value and recorded high manganese serum level and Melvin and Swenson, (1995) reported that higher level of manganese interfere with the retention by bile, also Myra et al., [12] mentioned that manganese ions antagonism has been observed in baby pigs and a level of 50-150ppm of manganese with a diet interfere with haemoglobin formation.

Also it is noticed that slight decreases were observed in iron in repeating dosing program this may cause iron deficiency anaemia. The death may be due to anaemia or vital organs. Curtis and John [13] mentioned that tetracycline and chlortetracycline is a drug associated with the development of a plastic anaemia, also the same author mentioned that tetracyclines is one of cyanobiotic that developed specific coagulation factors inhibitor (Von

willebrand) factor. Also the same author mentioned that OTC-LA has cardiotoxicity manifested by negative entropic effect through decreasing Ca-ions.

Amodu and [14] elucidated the mechanism of development of anaemia in rabbits experimentally infected with *T. brucei* and concluded that the anaemia is possibly a result of an auto reaction together with anaemia and specific organ damage, a wider range of immunological abnormalities appear to characterize the pathology of the disease.

Slight increase was observed in GOT, which might be attributed to the degeneration in muscle, liver, kidneys, heart…. etc. because it is not a liver specific enzyme [15].

The drug at single I/M 50-100mg/kg or 20 mg/kg twice a week for two weeks reduced trypanosomosis in the blood, liver tissues 75% and kept the animals alive survived with mild parasitaemia for 49 days post treatment. This might justify the use of OTC by the Vets in the Sudan with a view to overcome the immunosuppressant effect of trypanosomes concomitant with conventional trypanocides.

It is noticed that in all groups, infected or infected treated, the body weight is not retain to that of group (1) until the end of experiment and its also noticed that most of these groups showed triglycerideaemia, phospholipidaemia and also an slight increase in cholesterol and glucose levels. In addition, liver and kidneys showed slight fatty changes.

Like the findings of Benzo [16] OTC-LA increased hepatic lipids and depressed body weight. Soback et al., [17] studied the pharmacokinetic changes of several antibiotics in children inducing fatty liver and found positive.

Since all treated groups get with mild or moderate parasitaemia, no parasites entered the liver tissues it is clear that OTC affection *T. evansi* so, we recommended OTC in *T. evansi* infection. Combination with other trypanocidals were suggested.

The parasites which had been detected in the peripheral blood or in the liver tissue might be due to the parasite which had been sequestered in the brain tissue. This phenomenon is supported by Susen and Donald [18] they mentioned that only small quantities of tetracycline and oxytetracycline are distributed to the CSF and therapeutic levels may not be attainable. Also Varma and Paul [19] reported a very slow rate of absorption of OTC-LAfrom I\m site which slightly attributed to the greater ionized fraction of the drug at pH of the body fluids, since it is the ionized fraction of the drug that usually passes across the cell membrane. Davey *et al*., [20] mentioned that the long acting formula of OTC gave a lower serum concentration and showed longer half-life as compared with the conventional formulation. The efficacy of the drug at 20 mg\kg intramuscularly and reported daily for 8 days remove the parasite completely from the peripheral blood and the liver tissue but, unfortunately terminated with death of the animals. The recommended therapeutic dose of OTC which had been given once I\M at the rate of 20 mg/kg failed to overcome the parasitaemia and trypanosomosis complication and terminated with the death of the animals at the same period to that of the control uninfected untreated group.

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