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Full Length Research Paper

Residues of Cymelarsan^{R} in camels (*Camelus dromedaries*) and Nubian goats infected with *T.evansi* in Sudan

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Ninety out of hundred Nubian goats were experimentally infected with *Trypanosoma evansi* and twenty out of twenty-five camels naturally infected with the same parasite were used in this experiment. Single I / M doses of Cymelarsan were given to four groups at rates of 0.125, 0.25, 0.625 and 1.25 mg/kg. Other four groups were also given the drug via I / M route at rates of 0.125 and 0.25 mg/kg / week for two weeks and daily for 8 days. Three groups of camels were given Cymelarsan in single I / M doses at rate of 0.25, 0.612 and 0.125 mg/kg weekly for three successive weeks. Specimens of different tissues, serum, urine and bile were collected immediately after death or slaughter. A weekly slaughter program 14 days post cessation of the drug for surviving animals was conducted for successive five weeks (two goats/ week and one camel / week). Total arsenic of Cymelarsan^{R} residues detected in samples was measured. The concentration of arsenic was increased in serum, urine, bile and tissues of goats infected with *T. evansi* and given Cymelarsan in single I / M doses (0.125 - 0.625 mg/kg) and gradually returned to normal by the end of the experiment. In the remaining groups arsenic concentration in collected samples did not returned to normal level. In camels, the amount of arsenic in the different tissues, serum, urine and bile returned to normal by day 42 of last cessation of the drug. We conclude that camels tend to excrete arsenic from the body more rapidly than goats.

Key words: Nubian goats, camels, *Trypanosoma evansi*, cymelarsan, residues (arsenic).

INTRODUCTION

Trypanosomosis is by far the most important protozoan disease of camels and is probably the most important health problem of all. Death may reach 3%, declining milk production; abortion and chronic poor conditions are the classic symptoms of the disease in camels (Wilson, 1984).

Mornet (1954) considered that goats and sheep made up 4% of all the animals infected with *Trypanosoma congolense*. Lewis, (1949) reported that goats could be infected when kept close to infected cattle.

Cymelarsan® has a rapid trypanocidal activity. Trypanosomes are destroyed within a few hours; it achieves a peak plasma concentration by about 15 min post-injection, irrespective of the route of injection, after which plasma levels decline rapidly. The absolute bioavailability is a 100% when given by the intramuscular route (Bujon, 1990).

The product has a short half-life in horses and monkeys, and the toxicological profile or a residue is low. The proposed withdrawal time for meat, milk and edible tissue is provisionally recommended at 2 weeks (Raynaud et al., 1989). Cymelarsan^{R} in milk and meat was measured by determination of the total arsenic in infected camels in Kenya using recommended route (Sones, 1991). Groups of arsenical compounds (phenyl arsenoxides, arsphenamines, arsonic acids, acid-substituted phenylarsenoxides and inorganic arsenicals) in plasma were elucidated by detection of total arsenic in urine, stool, blood and tissues

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(Ralph et al., 1944). No significant difference between the pharmacokinetics and the routes of administration or the different formulation was reported (Toutain, 1991).

The aim of this study was to investigate the level of arsenic in serum, urine, bile and tissues of goats and camels infected with *Trypanosoma evansi* and given Cymelarsan at different dosages rate and frequency. Sudan has an immense animal wealth, which satisfies all local needs of meat and milk and produces an export surplus constituting 20% of foreign currency earnings (AOAD, 1992).

MATERIALS AND METHODS

Animals

Nubian goats

Hundred Nubian goats of both sexes, 8 – 10 month-old and weighing 9 - 10 kg, were purchased from Sheikh Abu Zaid Market, Omdurman, Khartoum State. Animals were housed in pens at the College of Animal Production and Veterinary Medicine, Sudan University, Hillat Koko. Each animal was fed daily on green forage consisting of 3 kg Lucerne (*Medicaqo sativa*), 1.5 kg sorghum (*Sorghum vulgare*) and 2 kg millet (*Pearl millet*) one time weekly as a concentrate, and had free access to water.

Twenty goats were chosen randomly. Group (1) was uninfected-untreated (control negative group), while group 2, infected with *T. evansi*, was infected-untreated (control positive group). The remaining animals were infected with *T. evansi* and were used for drug treatment. They were divided randomly into ten groups of 10 goats each.

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 / ml (five-times the therapeutic dose), respectively.

Each goat in groups 7 and 8 was given Cymelarsan® intramuscularly (I / M) at 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 the drug (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.

Camels (*Camelus dromedarius*)

Twenty-five one- humped camels (*Camelus dromedarius*) 1 – 3 years-old, of both sexes and weighing (350 - 400 kg) were obtained from El Gadarif State and were stabled in Elmewelh Market pens, Omdurman, Khartoum State. Each camel was fed daily on 4 kg Lucerne (*M. sativa*), 3.5 millet (*P. millet*) and 2.5 kg sorghum (*S. vulgare*); with free access to water. They were divided randomly into five groups of 5 camels each.

Group 1 was uninfected-untreated (control negative group), while group 2 was naturally infected-untreated (control positive group). The remaining animals were naturally infected with *T. evansi*.

Each camel in groups 3 and 4 was given a single intramuscular dose of Cymelarsan at the rate of 0.25 mg/kg (therapeutic dose), and 0.6125 mg/kg (two and half-therapeutic dose), respectively. Camels in group 5 were each given a single dose of the drug at rate of 0.125 mg/kg (half-therapeutic dose) weekly for three successive weeks. Fourteen days post cessation of the drug, a weekly slaughter programs for survival animals (2 goats and camel/week) was conducted for five successive weeks.

Parasite and infection

Albino rats of two months old, and weighing 250 g, were inoculated intraperitoneally with 0.2ml 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 of rat's blood containing 5 x 10^5 organisms. The parasites were activated by adding phosphate glucose solution (PGS) buffer before inoculation.

Collection and preservation of samples

serum

Each animal was bled from the jugular vein at 1, 3, 24 h and 3 days, 7, 14, 21, 28, 35, 42 days post-treatment in a test tube containing no anticoagulant. Samples were left to clot, centrifuged at 3000 rpm and were collected and kept in a deep freezer at -20°C for determination of residues of Cymelarsan.

Urine, bile and tissues

A weekly slaughter program for survival animals was conducted for successive five weeks (2 goats and camel / week) after cessation of the drug. Samples of urine and bile were taken in plain containers (Wols, U.K). Specimens of heart, brain (cerebellum and cerebrum), kidney, spleen, liver, lung, ovary, uterus, testis, muscle, fat and site of injection, were kept in plastic containers. Collected samples of urine, bile and tissues were kept in a deep freezer at -20°C for determination of residues of Cymelarsan.

Determination of total arsenic as residue of cymelarsan

Total arsenic residues of Cymelarsan were measured by AOAC, (1995) method using spectrophotometer (Unicam 8625 / U.V. Vis U.K.) at wavelength 450 nm.

Statistical analysis

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

RESULTS

Goats

The infected untreated goats group 2 died 11days post infection, while goats in groups 3 and 10, died 15 days post treatment (pt). Goats in groups 6, 7, 8 and 9 died 17 days pt, goats in groups 7 and 8 died 28 - 31pt and goats in group 9 died 22 days pt, and animals in groups 1, 4 and 5 survived till the end of the experiment (day 56 post treatment).

Table (1) summarizes the concentration of arsenic in serum, tissues, urine, and bile in goats infected with *T. evansi* and treated with single or multiple dosages of

Table 1. The concentration of Cymelarsan Arsenic in different organs (µg / g) and serum, bile and urine (µg / ml) in Nubian goats infected with *T. evansi* and given single or multiple dosages of Cymelarsan (M± SE).

Organs/ Dose	Serum	Liver	Kidneys	Heart	Lungs	Spleen	Cerebellum	Cerebrum	Fat	Site of injection	Leg Muscle	Uterus	Ovary	Testis	Bile	Urine
Group (1)	≤0.07[a]	**≤0.07**														
Group (2)	≤0.07[a]	**≤0.07**														
Group (3) 0.125mg/kg	0.10 ±0.14[b]	0.2±0.22 /≤0.07	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07	0.1±0. 02/ ≤0.07	0.2±0.0/≤ 0.07	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Group(4) 0.25mg/ kg	0.12±0.1 4[b]	3± 0.0/ 3.5±0.0/≤ 0.07	5±0.2/ 0.3±0.2/ ≤0.07	0.1± 0.01	≤0.07	≤0.07	≤0.07	≤0.07	0.2 ±0.01/ ≤0.07	0.5±0.1/ ≤0.07	≤0.07	0.7±0.0/ ≤0.07	0.1±0.0/ ≤0.07	0.2±0.0 / ≤0.07	1.5±0.0/ ≤0.07	≤0.07
Group (5) 0.625 mg /kg	0.15 ±0.19[b]	5±0.0/ 3.5±0.0/± 0.0/ ≤0.07	3±0.1/1± 0.2/0.5± 0.0/≤0.07	≤0.07	≤0.07	≤0.07	0.1 ±0.0 /≤0.07	0.5±0.02/ ≤0.07	0.1±0. 0/ 0.1±0. 0/ ≤0.07	0.5±0.2 /0.2±0.0/ 0.1±0.0 /≤0.07	≤0.07	0.5±0.0/ 0.5/±0.0 /0.2±0.00 1/ ≤0.07	0.2±0.1/ 0.1±0.02/ ≤0.07	1.5±0.0 1/ ≤0.07	1±0.1/ ≤0.07	≤0.07
Group (6) 1.25mg/kg	2.7 ±0.09[c]	30 ±1.1 /25±1.0	35±0.2/ 30 ±0.4	40± 0.1/33 ±0.9	20±1.0 1/25±1 .2	20±0.2/15 ± 0.1	10±0.4/ 1±0.1	15±0.1/ 10± 0.3	3±0.1/ 2± 0.0	5±0.01/ 5.5 ±0.2	25±0.2/ 20 ±0.5	10±1.0/ 8± 0.7	5±0.1/ 5± 0.0	15±0.2/ 20 ±0.3	10±0.01/ 15 ±0.02	5±0.01/50 .01
Group (7) 0.125 mg / kg twice / week for 2 weeks	2.5 ±0.02[c]	50±1.5/ 60.5± 1.3	70±0.5/ 85± 0.2	30±1.5/ 35 ±1.2	15±0.9 / 20±0.3	40±0.3/35 ± 0.5	10±0.4/ 15 ±0.1	5± 0.4/ 5± 0.7	50±0.4 / 45 0.5	80±0.2/ 95 ±0.3	40±0.3/ 30± 0.4	20±0.2/ 25± 0.4	10±1.0/ 15± 1.2	30±0.5/ 25 ±0.4	20±0.2/ 40 ±0.3	25±0.01/2 5 ±0.01
Group (8) 0.125 mg / kg daily for 8days	3.5±0.01[c]	105±2.2/ 140± 2.2	130 ±0.3 /110±0.2	90±1./ 95 ±1.4	30±0.5 / 40±0.7	80± 0.2/ 85 ±0.5	75±0.2/ 70± 2.5	45±0.8/ 40 ±0.3	105±0. 11/ 40±0.6	130±0.1/ 120 ±0.2	100 ±2.1 /105± 2.3	50±0.3/ 55 ±0.3	30±1.4/ 40± 1.1	60±0.6/ 75± 0.7	50±0.7/ 70± 0.5	25±0.0/ 25±0.01
Group (9) 0.25 mg / kg twice / week for 2 weeks	3.00 ±0.001[c]	100±2.4/ 105± 2.3	140±0.5/ 155± 0.6	160±0. 1/140± 0.7	85±1.2 / 801.9	120± 0.1/120± 0.2	115±3.4/ 180 ±3.5	160±0.8/150 ±0.4	200±3. 0/150 ±1.5	180±0.4/ 200± 0.5	250±2.3/ 300± 2.0	90±0.6/ 100± 0.8	110±1.3/ 120 ±1.5	120±0. 3/ 150± 0.4	100±1.1/ 110± 1.0	15±00.33/ 16±0.46
Group (10) 0.25 mg / kg daily for 8days	5.9±0.03[d]	250±1.9/ 300±2.5	300±1.7/ 305± 1.8	250±2. 4/300± 2.0	120±1. 1/130± 2.0	350±2.3/3 60± 2.7	350±3.1/ 250 ±3.2	150±0.1/160 ± 0.1	305±1. 1/ 355± 1.7	450±0.1/ 250 ±0.2	300±2.5/ 250 ±2.2	200±1.3/ 250± 1.4	150±1.1/ 180± 1.7	120±1. 2/ 200 ±1.5	180±1.2/ 200 ±1.5	25±00.37/ 30±0 0.7

The different letters in one columns show significant changes $p < 0.05$, Week 1 (w1) = 14 day post treatment (d.p.t), w2 = 21 d.p.t, w 3 =28 d.p.t, w 4 = 35 d.p.t and w 5 = 42 d.p.t.

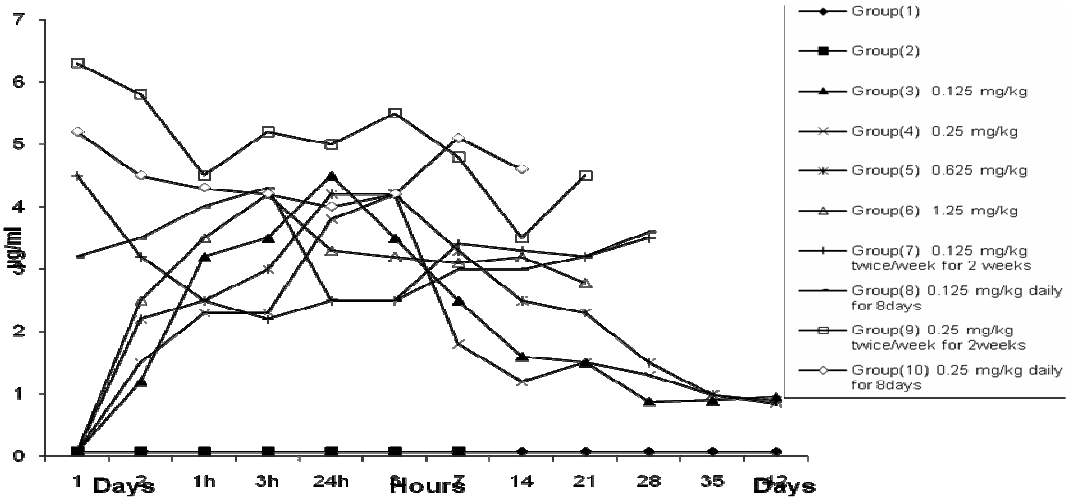

Figure 1. The mean of arsenic in serum of *T. evansi* infected Nubian goats and treated with single dosages or Cymelalarsan.

Table 2. The concentration of Cymelarsan arsenic in different organs (µg / g), serum and urine (µg / ml) in camels infected naturally with *T.evansi* and given single or multiple dosages of Cymelarsan within the withdrawal period (M ± SE).

Groups/Organs	Group (1)	Group (2)	Group (3) 0.25 mg / kg	Group (4) 0.625 mg / kg	Group (5) 0.125 mg / kg Weekly / 3weeks
Serum	≤0.07	≤0.07	2.3±0.01	8.3±0.02	5.6±0.00
Liver	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Lungs	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Kidneys	≤0.07	≤0.07	10 ±0.05 /3 ±0.01 /≤0.07	10 ±0.01 /5 ±0.02 /≤0.07	10 ±0.02 /4 ±0.00 /≤0.07
Heart	≤0.07	≤0.07	0.5 ±0.01 /0.6±0.00 /≤0.07	0.5±0.01 /0.5±0.00 /≤0.07	0.8±0.00 /0.3±0.00 /≤0.07
Spleen	≤0.07	≤0.07	0.1 ±0.02 /≤0.07	0.5 ±0.00 /≤0.07	0.5 ±0.01 /≤0.07
Cerebellum	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Cerebrum	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Fat	≤0.07	≤0.07	0.5 ±0.05 /≤0.07	0.5 ±0.00 /≤0.07	0.5 ±0.00/≤0.07
Site of injection	≤0.07	≤0.07	5 ±0.2 /≤0.07	5 ±0.02 /≤0.07	5 ±0.02 /≤0.07
Leg muscle	≤0.07	≤0.07	≤0.07	≤0.07	≤0.07
Uterus	≤0.07	≤0.07	2.2 ±0.01 /≤0.07	2.5 ±0.02 /≤0.07	2.02 ±0.01 /≤0.07
Ovary	≤0.07	≤0.07	0.5 ±0.02 /≤0.07	0.7 ±0.00 /≤0.07	0.8 ±0.00 /≤0.07
Testis	≤0.07	≤0.07	0.2 ±0.01 /≤0.07	0.5 ±0.01 /≤0.07	0.9 ±0.01 /≤0.07

Cymelarsan. Goats in groups 6-10 showed increased amounts of arsenic in the different tissues at levels up to (450 µg/g) while, urine(30 µg/ml), bile (200 µg/ml)and in serum(5 - 9 µg/ml) did not return to normal levels(≤ 0.07 µg/g) or (≤ 0.07 µg/ml) till the end of the experiment. The increase was found to depend upon the dose and the frequency of drug administration. Goats of groups (3 - 5) had values (≤0.07 µg/g) in tissues and (≤0.07 µg / ml) in serum (Figure 1), urine and bile by the end of experiment (day 42 pt) equal to that of control goats in groups 1and 2.

Camels

No death was observed in experimental camels naturally infected with *T. evansi* and treated with single or multiple dosage of Cymelarsan, till the end of the experiment but the infected untreated control group (group 2) died 22 days post infection (22 days after found the camels in the field). Table (2) summarizes the concentration of arsenic in tissues, serum, and urine in camels. All groups of treated animals tend to record ≤0.07 µg/ml arsenic in serum (Figure 2), urine and ≤0.07 µg / g in different tissues samples by the end of the experiment.

DISCUSSION

The purpose of the study was to investigate and compare the residues of the drug Cymelarsan (arsenical compound) in the different tissues, serum, bile and urine in

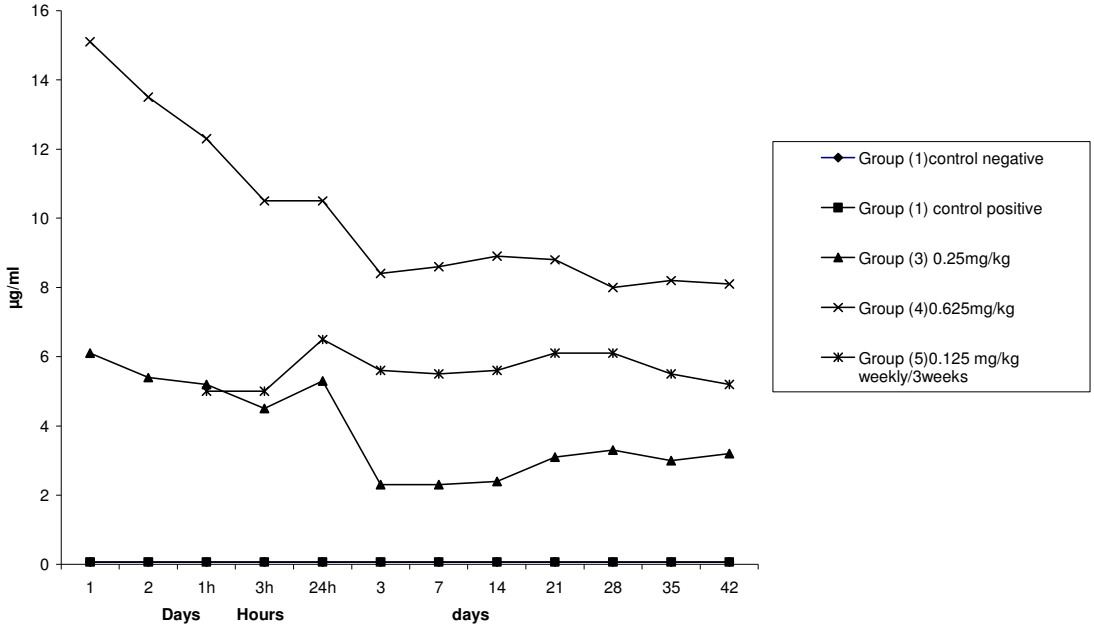

Figure 2. The mean of arsenic in serum of *T. evansi* infected camels and treated with single or multiple doses of Cymelarsan

Nubian goats and one-humped camels infected with *T. evansi*. The study also investigated the relationship between the accumulation pattern of the drug and its toxicity effect in the two species of animals.

Ralph et al. (1944) mentioned that the toxicity of arsenical compounds is primarily a function of the degree to which they are bound by tissues, and that the red blood cells form a valid model for the body tissues constituting a reliable index of systemic toxicity, as the sequence of events in the blood stream reflects what occurs in body tissues. After leaving the blood, toxic compounds were firmly bound by the organs and relatively high levels were found in the liver and kidneys 48 h after injection. This supports our findings that goats tissues such as liver, spleen, kidneys, heart and lungs, can accumulate considerable amounts of arsenic as these organs contain high concentrations of the enzymes containing thiol groups.

The accumulation of arsenic at high levels in the muscles and site of injection is comparable to the findings of Youssif, (2005) who studied the toxic effect of different dosages of Cymelarsan in Nubian goats characterized by irritating effect of arsenic on tissues.

It was noticed that in camels the serum, liver, kidneys, and site of injection contained the highest values compared to other organs. Sones, (1991) Studied the residues of As in infected camels in Kenya and treated with the recommended dose of Cymelarsan and found that the residues of total arsenic were detected in fat, liver and kidneys were less than 2 mg / kg with no residues in plasma of one camel 7 days and others slaughtered on days 14 or 21.

In goats which received half recommended therapeutic dose twice a week for two weeks or once weekly for three weeks in camels the picture is surprising where goats recorded higher values of As than that recorded in camels. This may be due to species difference. Cymelarsan with arsenical atoms, has a high affinity to bind to RBC in goats compared to that of camels; this was supported by the increased level of arsenic in goats especially those which received the recommended dose twice a week for two weeks and also by the high concentration of As in kidneys and urine in goats compared to that of camel. This might be attributed to unknown other route of excretion of arsenic. Excretion could be through saliva or faeces or As might be deposited in bones. Another possible explanation is that camels might have a high capacity to excrete arsenic through the kidneys rapidly in less than 28 days post treatment or in goats Drug accumulation might have taken place due to the fact that large and repeated doses are beyond the capacity of the vital organs responsible for the arsenic detoxification and excretion. The nervous system contains large amounts of fats and repeated administration of the drug revealed a tendency of the drug to accumulate in the fat and nervous tissues of the goats more than camels. The accumulative effect in goats is more evident than in camels which might explain why camels can tolerate five times the recommended therapeutic dose and also supports the findings of Bujon, (1990) who mentioned that camels can tolerate 12 - 20 times the recommended therapeutic dose. High doses of inorganic arsenical compound given to pregnant experimental animals produced various malformations in fetus and offspring.

However, such effects have not been noted in humans with excessive occupation exposure to arsenic compounds (Curtis and John, 2003).Joseph, (2001) mentionned that arsenic produces pathological effect on the reproductive system, which is confirmed by our findings that testis and the uterus have the tendency to accumulate as in large quantities.

We conclude that goats are more susceptible to accumulation of As in the tissues and express clinicopathological performance than camels. Further studies are needed to elucidate the physiological pathways that make camels more tolerable to excess accumulation of As than goats.

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