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Effect of intramuscular administration of oxytetracycline on serum kinetics of diminazene aceturate in healthy male Sahel goats

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Abstract

Serum kinetics of diminazene aceturate following intramuscular (IM) administration of diminazene aceturate alone at 3.5 mg/kg and its combination with oxytetracycline at 20 mg/kg were evaluated in 6 healthy male Sahel goats to ascertain the effect of oxytetracycline on serum concentration and pharmacokinetic parameters of diminazene aceturate. Two groups (A and B) of three goats each were used, and oxytetracycline was administered 30 minutes prior to diminazene aceturate administration. Blood samples were collected at various intervals (0.17 h – 72 h) post-drug administration, and diminazene serum concentrations were measured using the method of Klatt and Hadju. Kinetic determinants were calculated employing a two-compartment open model. Mean serum concentrations of diminazene aceturate of 2.43 ± 0.95 µg/ml and 1.73 ± 0.44 µg/ml at 0.17 h were measured in groups A and B, respectively. These serum concentrations were found to increase until a peak concentration of 6.91 ± 0.59 µg/ml and 7.55 ± 1.20 µg/ml were reached at 2.0 h in groups A and B, respectively. The peak concentrations subsequently decreased at 72 h post diminazene aceturate administration with serum concentrations of 0.00 ± 0.00 and 0.32 ± 0.28 µg/ml in groups A and B, respectively. Pharmacokinetics parameters like the volume of distribution (Vd), elimination half-life (T½β), concentration maximum (Cmax), absorption rate constant (α), and area under the curve from 0 to 72 h (AUC_{0-72}) were significantly higher in goats treated with diminazene aceturate and oxytetracycline combination while total body clearance (Cl), and elimination rate constant (β), were significantly higher in goats treated with diminazene aceturate alone. The mean residence time (MRT) of diminazene aceturate increased from 19.70 ± 2.53 h in diminazene aceturate treatment to 25.11 ± 1.81 h in diminazene aceturate and oxytetracycline treatment. Oxytetracycline was therefore found to alter the elimination pattern of diminazene aceturate in oxytetracycline pre-treated goats.

Keywords: Diminazene aceturate, oxytetracycline, pharmacokinetic parameters, serum kinetics, Sahel goats

Introduction

Diminazene aceturate (diminazene) is a potent curative antitrypanosomal and antibabesial drug used routinely in the control of African trypanosomosis and babesiosis in domesticated animals (Milne *et al.,* 1955; Verma *et al*., 1973; Van Miert *et al.,* 1978; Onyeyili & Onwualu, 1991). It is one of the conventional trypanocides available for the treatment of trypanosomosis in Nigeria. Diminazene administered to rats subcutaneously at the dose rate of 25 mg/kg produced a blood level of 11.4 µg/ml (Raether *et al*., 1972). Klatt & Hadju (1976) observed that, following intramuscular diminazene administration to cattle at 2.5 mg/kg, two-phase elimination occurred and the elimination half-life from the blood was 63 h. Onyeyili (1982) noticed the presence of diminazene aceturate in the blood of lactating healthy goats for 36 h after a single intravenous dose of 3.5 mg/kg. Also, 69.1% of the drug was recovered in the urine within seven days.

Gilbert & Newton (1982) reported that the administration of bisphenyl^{14}C-labelled diminazene at the dose of 3.5 mg/kg resulted in a peak blood level of 1.1 µg/ml within 1 h of intramuscular injection with detectable concentrations in the blood for six days. Odika *et al*. (1995) reported significantly higher concentrations of diminazene in the organs of *T. brucei brucei* infected rats compared to the non-infected rats treated with the drug at the dose of 3.1 mg/kg. Concurrent administrations of lithium chloride with diminazene significantly increased the level of diminazene in the brain of treated rats (Odika *et al.*, 1995). In lactating goats and sheep 60-90 % of diminazene was reported to be plasma protein bound (Aliu *et al*., 1984). Aliu *et al*. (1993) using the HPLC method observed that diminazene administered to cows intravenously produced a biphasic distribution. Mamman *et al*. (1993) compared the pharmacokinetics of diminazene in non-infected and *Trypanosoma congolense* infected cattle after a 3.5 mg/kg diminazene intramuscular injection. They observed a few pharmacokinetic differences in drug concentration and its metabolites in physiological fluids like plasma. However, the maximum concentration of diminazene in plasma was significantly higher in cattle with acute infection compared to those with chronic infection and non-infected cattle. Onyeyili & Anika (1989; 1991) determined the influence of *T. brucei brucei* and *T. congolense* on the disposition of diminazene in dogs. They obtained a biphasic drug elimination process, irrespective of infection; however, the infection significantly shortened the absorption half-life of the drug from 0.17 h to 0.12 h.

Oxytetracyclines are broad-spectrum antibacterial agents, with activity also against intracellular chlamydiae, mycoplasma and rickettsiae (Chopra & Roberts, 2001). Oxytetracyclines have also been used for the effective treatment and prophylaxis of malaria due to *plasmodium falciparum* (Roberts, 2003). The agents have activity against other protozoan parasites including *Entamoeba histolytica, Giardia lamblia, Toxoplasma gondii and Trichomonas vaginalis* (Chopra & Roberts, 2001). Oxytetracyclines and tetracyclines act by binding reversibly to the 30s ribosomal subunit of the bacterial organisms to inhibit the translation process during protein synthesis (Boothe, 2016). This study is however aimed at estimating the effect of oxytetracycline on serum concentration of diminazene and the effect of oxytetracycline on pharmacokinetic parameters of diminazene in healthy male Sahel goats.

Materials and Methods

Study area

The study was carried out in the large animal clinic of Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, Borno State of Nigeria.

Experimental animals

Six clinically healthy male Sahel goats between 1 and 2 years old, weighing between 13 and 15 kg were used for the study. Fly proof environment was provided as housing while the goats were fed with groundnut hay and wheat offal. Tap water was given *ad libitum*. The goats were screened for ecto and endo parasites and allowed to acclimatize for two weeks before the commencement of the experiment. Animals were handled following the international guiding principle for biomedical research involving animals developed by the Council for International Organization of Medical Sciences (CIOMS, 1985). Ethical approval and clearance were obtained from the University of Maiduguri Animal Research Ethics Committee (with approval reference No. UM/FVM/VPP/3.2.2. 552/AUP – R141).

Blood collection

Blood samples were collected from each goat 15 min. before drug administration (0 h) which served as the control. After the administration of the drugs, 3 ml of blood samples were obtained at these periods 0.17, 0.33, 0.5, 1, 2, 3, 6, 9, 12, 24, 36, 48, 60, and 72 hours (h) respectively. All blood samples were centrifuged at 4000 rpm for 10 min to obtain the serum.

Diminazene assay

Total Diminazene aceturate in serum was determined using the chemical method as described by Klatt & Hadju (1976). The procedure is based on the hydrolysis of diminazene and coupling of the diazonium salt of the drug with N-1-naphthyl ethylenediamine to form a pink colour. Treatment of diminazene aceturate with hydrochloric acid and sodium nitrite solutions results in the formation of the diazonium salt from the diminazene present. The excess nitrous acid present is destroyed by the addition of ammonium sulphamate. The diazonium salt thereafter is coupled with N-1-naphthyl ethylenediamine. The colour produced was measured using UV-Spectrophotometer at 540 nm. One millilitre (1ml) of 10 % trichloroacetic acid (TCA) was added to 1 ml of serum in a centrifuge tube, thoroughly mixed and left to stand for 15 min. Thereafter, it was centrifuged for 10 min. at 4,000 rpm, and then 1 ml of the clear supernatant solution was obtained to which was added 1 ml of 1 M HCl in a test tube. This was then diazotized with 0.2 ml of 0.5 % sodium nitrite solution. After 3 min., 1 ml of 1 % ammonium sulphamate solution was added and the mixture was shaken thoroughly. After 3 additional min., 1 ml of 2 % N-1- naphthyl ethylenediamine solution was added and shaken. The colour change was then read at 540 nm using a UV-Spectrophotometer. The limit of detection was 0.0625 µg/ml. The linear calibration curve of diminazene aceturate in serum, with the range 1 = 10 µg was obtained by plotting percentage absorbance against drug concentrations. The correlation coefficient (R^2) was greater than 0.94. The concentration of diminazene in serum was calculated using the formula below

Drug Conc. = Conc. of STD × Optical Density of Drugs ÷ Optical Density of STD

Where:

Conc. = Concentration

STD = Standard

Calculations of pharmacokinetic parameters

Pharmacokinetics is the mathematical description of drug concentration changes within the body with respect to time. For the drug diminazene aceturate, the decline in concentrations in serum with time was examined under a two-compartment open model (Onyeyili & Anika, 1991). Pharmacokinetic analysis of experimental data from serum was performed using a standard procedure (Baggot, 1977). The following parameters and constants were determined using methods previously described (Gibaldi & Perrier, 1975; Watson *et al*., 1987; Bauer, 2006).

i. Area Under the Curve (AUC) = D/CL (D=Dose administered, CL=Total body clearance
ii. Total body clearance (CL) = D/AUC or Vd× β (Vd=Volume of distribution, β=Elimination rate constant)
iii. Maximum concentration of drug in serum (C_{max})
iv. Time at which maximum concentration of the drug occurred in serum (T_{max})
v. Volume of distribution (Vd) = CL/β
vi. Absorption half-life ($T½_{\alpha}$) = 0.693/α
vii. Absorption rate constant (α) = 0.693/$T½_{\alpha}$
viii. Elimination rate constant (β) = 0.693/$T½\beta$
ix. Elimination half-life ($T½\beta$) = 0.693/β
x. Mean Resident Time (MRT)
xi. Mean Absorption Time (MAT)

Data analysis

The data were presented as Mean ± Standard deviation (SD). Test for significance between the parameters in respect of diminazene treatment alone and diminazene plus oxytetracycline treatment were performed using paired student's "t-test" at a 5% level of significance. SPSS IBM® version 17 (2016) was used for the analysis (Gravether & Wallnau, 2004).

Results

Mean serum concentration of diminazene aceturate of 2.43 ± 0.95 µg/ml was obtained in goats administered diminazene aceturate alone while 1.73 ± 0.44 µg/ml of diminazene aceturate was obtained in goats given a combination of diminazene aceturate and oxytetracycline at 0.17 h. These serum concentrations increased until a peak concentration of 6.91 ± 0.59 µg/ml and 7.55 ± 1.20 µg/ml were reached at 2.0 h in goats treated with diminazene aceturate alone and those treated with diminazene aceturate plus tetracycline respectively. The peak concentrations subsequently decreased and at 72 h post diminazene aceturate administration, serum concentrations were 0.00 ± 0.00 and 0.32 ± 0.28 µg/ml respectively in Sahel goats given diminazene aceturate alone and those given a combination of diminazene and tetracycline as shown in Table 1.

The pharmacokinetic parameters of diminazene aceturate in Sahel goats administered with diminazene aceturate alone and diminazene aceturate in combination with oxytetracycline are shown in Table 2. The pharmacokinetic evaluation of diminazene aceturate used in this study indicated that the data should fit a two-compartment open model. The values of time maximum (T_{max}),

Table 1: Mean serum diminazene aceturate concentrations and its combination with oxytetracycline in Sahel goats

Time (h)	DA (µg/ml)	DA+OTC (µg/ml)
	Mean ± SD	Mean ± SD
0.0	0.00 ± 0.00	0.00 ± 0.00
0.17	2.43 ± 0.95	1.73 ± 0.44
0.33	4.06 ± 1.28	3.44 ± 0.69
0.5	5.27 ± 0.49	4.43 ± 1.07
1.0	6.42 ± 0.69	5.62 ± 1.41
2.0	6.91 ± 0.59	7.55 ± 1.20
3.0	5.69 ± 1.33	6.34 ± 1.16
6.0	4.96 ± 0.91	5.54 ± 0.77
9.0	3.94 ± 0.41	4.59 ± 0.80
12.0	3.36 ± 0.65	3.58 ± 1.24
24.0	2.12 ± 0.67	2.62 ± 0.77
36.0	1.12 ± 0.34	1.62 ± 0.75
48.0	0.58 ± 0.34	1.13 ± 0.74
60.0	0.25 ± 0.20	0.67 ± 0.32
72.0	0.00 ± 0.00	0.32 ± 0.28

Diminazene aceturate was intramuscularly administered at a dose rate of 3.5 mg/kg and tetracycline at 20 mg/kg
DA: Diminazene aceturate
OTC: Oxytetracycline

absorption half-life ($T½_{\alpha}$), and mean absorption time (MAT) in the goats administered with diminazene aceturate alone were significantly high in Sahel goats given diminazene plus oxytetracycline. The concentration maximum (C_{max} = 7.55 ± 0.26 µg/ml), elimination half-life ($T½\beta$ = 17.56 ± 1.63 h), absorption rate constant (α = 4.35 ± 0.16), mean resident time (MRT = 25.11 ± 1.81 h), volume of distribution (Vd = 7.09 ± 0.31 L/kg) and area under the concentration-time curve from zero to 72 h (AUC_{0-72} = 157.81 ± 29.35 µg/ml : h) were significantly ($p < 0.05$) higher in diminazene aceturate plus oxytetracycline combination group in comparison with the C_{max} (6.91 ± 0.34 µg/ml), $T½\beta$ (13.72 ± 1.82 h), α (3.37 ± 10.40 /h), MRT (19.70 ± 2.53 /h), Vd (6.11 ± 0.67 L/kg) and AUC_{0-72} (124.25 ± 15.61 µg/ml . h) of the group treated with diminazene aceturate alone. The rate constant of elimination (β = 0.03 ± 0.01 L/h) and total body clearance (CL = 0.29 ± 0.06 L/kg/h) of the group administered diminazene aceturate plus oxytetracycline were significantly lower ($p < 0.05$) than the values of β (0.05 ± 0.01 L/h) and CL (0.35 ± 0.04 L/kg/h) of the group treated with diminazene aceturate alone.

Discussion

The pharmacokinetic profile of diminazene aceturate administered alone in Sahel goats and its combination with oxytetracycline shows that the drug undergoes a two-compartment open model of elimination whether given alone or in combination with oxytetracycline. This agrees with the report of Gilbert (1983) in rabbits, Kellner *et al*. (1985) in calves, Onyeyili & Anika (1989) in dogs and Mamman *et al*. (1993) in cattle. However, this result does not agree with the report of Klatt & Hajdu, (1976) in which a single-phase elimination occurred following diminazene aceturate and rolitetracycline treatment. The result of this study indicates that diminazene aceturate is absorbed from the intramuscular route and this is supported by the presence of the peak serum concentration of diminazene aceturate of 6.91 ± 0.59 and 7.55 ± 1.20 µg/ml in goats administered with diminazene aceturate alone and those pre-treated with oxytetracycline respectively. A previous study in dogs showed that a peak serum concentration of 1.4 ± 0.2 µg/ml was obtained at 0.5 h when diminazene aceturate was administered alone intramuscularly (Eke, 2016). This peak serum concentration in the dog was lower than the diminazene aceturate peak concentrations obtained in the present study, this could be due to drug pharmacokinetics, pharmacodynamics, and specie variations. The peak concentrations in the present study appear to be like those obtained in American goats administered with diminazene aceturate

Table 2: Pharmacokinetic parameters of diminazene aceturate and its combination with oxytetracycline in Sahel goats

Parameters	DA	DA+OTC
	Mean ± SD	Mean ± SD
C_{max} (µg/ml)	6.91 ± 0.34	7.55 ± 0.26[a]
T_{max} (h)	2.00 ± 0.02	2.00 ± 0.03
α (per h)	3.37 ± 0.40	4.35 ± 0.16[a]
t½α (h)	0.21 ± 0.02	0.20 ± 0.07
MAT (h)	0.31 ± 0.03	0.28 ± 0.10
β (per h)	0.05 ± 0.01	0.03 ± 0.01[a]
t½β (h)	13.72 ± 1.82	23.56 ± 2.63[a]
MRT (h)	19.70 ± 2.53	25.11 ± 1.81[a]
CL (L/kg/h)	0.35 ± 0.04	0.29 ± 0.06[a]
Vd (L/kg)	6.11 ± 0.67	7.09 ± 0.31[a]
AUC_{0-t} (µg /ml.h)	124.25 ± 15.61	157.81 ± 29.35[a]
$AUC_{0-\infty}$ (µg.ml.h)	132.85 ± 17.73	169.71 ± 32.83
AUMC (µg /ml.h^2)	2692.32 ± 700.95	4445.94 ± 1255.17[a]

Results are presented as Mean ± SD and mean with superscripts [a] indicate significant ($p < 0.05$) differences between DA and TCN values along the same row.
DA: Diminazene aceturate
OTC: Oxytetracycline

intravenously and intramuscularly at 3.5 mg/kg, whose peak plasma concentrations were 7.2 ± 0.25 and 6.0 ± 0.20 µg/ml respectively (Onyeyili, 1982). The fact that serum diminazene aceturate concentration in goats administered with diminazene aceturate alone was significantly ($p < 0.05$) lower than in goats administered with the combination of diminazene aceturate and oxytetracycline in this study, especially from the time of peak concentration could be due to lower rate constant of absorption in the goats administered with diminazene aceturate alone. The absorption rate constant is the fractional rate of drug movement from the site of administration into the systemic circulation (Smith *et al.,* 2015). The absorption rate constant of 3.37 ± 0.40 / h was calculated for goats administered with diminazene aceturate alone as against 4.35 ± 0.16 /h for goats given diminazene plus oxytetracycline. An estimate of the absorption rate of a drug in a particular dosage form is given by the time at which the peak concentration is reached on the serum concentration versus the time curve. However, absorption continues after peak serum concentration has been reached in goats administered with the combination of diminazene aceturate and oxytetracycline. The continuous absorption after peak serum concentration has been reached was reported by Baggot (1978). The decrease in absorption rate constant in goats administered diminazene aceturate alone compared to those administered with diminazene aceturate plus oxytetracycline shows that the rate of entry of the drug into the systemic circulation is slow in the group given diminazene aceturate alone.

The volume of distribution in goats administered with diminazene aceturate alone (6.11 ± 0.67 L/kg) was significantly ($p < 0.05$) lower than that of goats administered with diminazene aceturate and oxytetracycline (7.09 ± 0.31 L/kg). This is an indication of the less extensive distribution of the drug in goats that were administered diminazene aceturate alone. The less extensive distribution in goats given diminazene aceturate alone may be suggestive of faster elimination of the drug in goats treated with diminazene aceturate alone. It is a fact that the greater the volume of distribution, the longer the half-life of elimination ($T_{½β}$) and the drug will be eliminated slowly from the body (Baggot, 1977). The values of the volume of the distribution obtained in this study were at variance with the findings in American goats administered diminazene aceturate via intramuscular and intravenous routes whose volume of distributions were 0.47 L/kg and 0.41 L/kg respectively (Onyeyili, 1982). A lower volume of distribution was recorded in dogs administered diminazene aceturate alone (3.25 ± 0.07 L/kg) than its combination with secnidazole (3.03 ± 0.12 L/kg) (Eke, 2016). The high volume of distribution in both groups in the present study could be because diminazene aceturate is well distributed in the body fluids and tissues of the body. However, pre-treatment with oxytetracycline increased the volume of distribution

and increased the serum concentration of diminazene aceturate as seen in Table 2. The clinical implication of this result is that higher diminazene aceturate concentrations are present in the serum of goats treated with diminazene aceturate and oxytetracycline combination which may translate to higher trypanocidal activity.

The elimination half-life ($T_{½\beta}$ = 13.72 ± 1.82 h and 23.56 ± 2.63 h) of Sahel goats administered with diminazene aceturate alone and that administered diminazene aceturate plus oxytetracycline respectively were lower than that of cattle and calves administered with diminazene aceturate via the intramuscular route (65 to 188 h) (Klatt & Hadju, 1976; Kellner *et al*., 1985).

The lower elimination half-life in the group administered diminazene aceturate compared to with diminazene aceturate plus oxytetracycline is suggestive of a higher level of clearance and decreased level of distribution of the drug to the body fluids and tissues. The value of the elimination rate constant in the group administered diminazene aceturate alone (0.05 ± 0.01/h) was higher than that of the group administered diminazene aceturate and oxytetracycline combination (0.03 ± 0.01/h). The lower the elimination rate half-life, the higher the elimination rate constant (Baggot, 1977). This is observed in goats administered with the combination of diminazene and oxytetracycline.

The total body clearance reflects the elimination of drugs from the body (Bourne, 2010). The higher the drug concentration in the serum, the more the drug is presented for elimination. Therefore, clearance is the coefficient of proportionality between serum drug concentration and elimination (Bourne, 2010). Total body clearance of diminazene aceturate was significantly ($p < 0.05$) higher in goats administered diminazene aceturate alone (0.35 ± 0.04 L/kg/h) compared with that of goats administered with diminazene aceturate and tetracycline combination (0.29 ± 0.06 L / kg / h). These suggest that more drug is presented to the metabolic and excretory organs at any point in the goats treated with diminazene aceturate alone.

The area under the concentration-time curve (AUC) is the integral of the serum drug concentration-time curve (Bourne, 2010). It indicates the actual exposure of the body to the drug after administration of a drug dose (Bourne, 2010). AUC is dependent on the elimination rate and the administered dose and is inversely proportional to the total drug clearance (Bourne, 2010). In the present study, diminazene aceturate co-administration with oxytetracycline increased the AUC (157.87 ± 29.35 µg/ml/h) compared to the value obtained with diminazene aceturate alone (124.25 ± 15.61 µg/ml/h). This may indicate that the body exposure to the drug is increased in the group administered diminazene aceturate and oxytetracycline because of lower clearance and a slower decline in the serum drug concentration. Clinically, this implies an increased risk of diminazene aceturate toxicity.

In conclusion, there were marked differences in the pharmacokinetic behaviour of diminazene aceturate in Sahel goats administered with diminazene alone and its combination with oxytetracycline. The significantly high volume of distribution, elimination half-life and low elimination rate constant observed in goats administered with the combination of diminazene aceturate and oxytetracycline as compared with those administered with diminazene aceturate alone is indicative of a slower clearance rate of the drug from the body of goats administered with the combination therapy. Effect of oxytetracycline on the serum of diminazene aceturate in trypanosome-infected Sahel goats should be undertaken to know the extent of the drug penetration into the various tissues following infection and treatment with the combination of diminazene aceturate and oxytetracycline.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Aliu YO, Odegaard S & Sognen E (1984). Diminazene/Berenil: bioavailability and disposition in dairy goats. *Acta Veterinaria Scandinavia,* **25**(4): 593-596.

Aliu YO, Mamman M & Peregrine AS (1993). Pharmacokinetics of diminazene in female Boran (*Bos indicus*) cattle. *Journal of Veterinary Pharmacology and Therapeutics*, **16**(3): 291-300.

Baggot JD (1977). Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary

Clinical Pharmacology, first edition. W. B. Sounders Company, Philadelphia. Pp 1-185.

Baggot JD (1978). Some aspects of clinical pharmacokinetics in Veterinary medicine I. *Journal of Veterinary Pharmacology and Therapeutics,* **1**(1): 5-18.

Bauer LA (2006). Clinical Pharmacokinetics Equations and Calculations. In: *Applied Clinical Pharmacokinetics,* third edition. The McGrawhill Companies Inc. Pp 3-8.

Boothe DM (2016). Tetracyclines. In: *The Merck Veterinary Manual.* Merck, Sharp and Dohme, Whitehouse station, New York. Pp 14-19.

Bourne DWA (2010). Method of residuals; pharmacokinetics. http://www.boomer.org/c/p4/c25/c2504.html), retrieved 12-06-2021.

Chopra I & Roberts M (2001). Tetracycline antibiotics: Mode of action, application, molecular biology, and epidemiology of bacterial resistance. *Microbiological Molecular Biology Review,* **65**(2): 232-260.

CIOMS (Council for International Organisations of Medical Sciences) (1985). International guiding principles for biomedical research involving animals. Retrieved July 2021, from https://www.ncbi.nlm.nih.gov/books/NBK25438/, retrieved 27-07-2021.

Eke IG (2016). Pharmacologic and Pharmacokinetic Effects of the Combination Therapy of Secnidazole and Diminazene Aceturate in Rats and Dogs Experimentally Infected with *Trypanosoma brucei*. PhD Thesis. Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria Nsukka. Pp 77-121.

Gibaldi M & Perrier D (1975). Drugs and Pharmaceutical Sciences: Pharmacokinetics, second edition. Marul Dekker inc., New York. Pp 45-463.

Gilbert RJ & Newton BA (1982). Pharmacokinetics and efficacy of the trypanocide diminazene aceturate (Berenil) in rabbits. *Veterinary Record*, **111**(17): 377-397.

Gilbert RJ (1983). Studies in rabbits on the disposition of trypanocidal activity of the anti-trypanosomal drug, diminazene aceturate (Berenil). *British Journal of Pharmacology*, **80**(1): 133-139.

Gravether FJ & Wallnau LB (2004). Statistics for the Behavioural Sciences, sixth edition. Thomson Wadsworth Belmonth, USA. Pp 746.

Kellner HM, Eckert HG & Volz MH (1985). Studies in cattle on the disposition of the antitrypanosomal drug diminazene diaceturate (Berenil). *Tropical Medicine and Parasitology,* **36**(4): 199-204.

Klatt P & Hajdu P (1976). Pharmacokinetic investigation on diminazene and rolitetracycline in comparison to a combination of both. *Veterinary Record*, **99**(19): 372–374.

Mamman M, Aliu YO & Peregrine AS (1993). Comparative pharmacokinetics of diminazene in non-infected boran *(Bos indicus*) cattle and Boran cattle infected with *Trypanosoma congolense*. *Antimicrobial Agents and Chemotherapy*, **37**(5): 1050-1055.

Milne AH, Robson J & Lwebandiza T (1955). The efficacy of berenil against *Trypanosoma congolense* in zebu cattle. *Veterinary Record*, **67**: 280-281.

Odika IE, Asuzu IU & Anika SM (1995). The effects of hyperosmolar agents lithium chloride and sucrose on the brain concentration of diminazene aceturate in rats. *Acta Tropica,* **60**(2): 119-125.

Onyeyili PA & Anika SM (1989). The influence of *Trypanosoma congolense* infection on the disposition kinetics of diminazene aceturate in the dog. *Veterinary Research Communications*, doi.10.1007/BF00142049.

Onyeyili PA & Anika SM (1991). Diminazene aceturate residues in the tissues of healthy, *Trypanosoma congolense* and *Trypanosoma brucei brucei* infected dogs. *British Veterinary Journal,* **147**(2): 155-162.

Onyeyili PA & Onwualu JE (1991). Efficacy of combination of DMFO and diminazene aceturate in the treatment of late stage *Trypanosoma brucei brucei* infection in rats. *Tropical Medicine and Parasitology*, **42**(2): 143-145.

Onyeyili PA (1982). The Absorption, Distribution, and Excretion of Diazoaminobenzene Diaceturate (Diminazene INN) (ganaseg) in Goats. M.Sc. Thesis, Tuskegee Institute, Alabama, USA. Pp 1-50.

Reather W, Hajdu P, Seidenath H & Damm D (1972). Pharmacokinetic and chemoprophylactic studies on berenil in Wistar rats infected

with *Trypanosoma rhodensiense*. Z *Tropenmed Parasitol*, **23**(4): 418-427.

Roberts MC (2003). Tetracycline therapy: Update. *Clinical Infectious Diseases*, **36**(4): 462-467. doi: 10.1086/367622.

Smith DA, Beaumont K, Maurer TS & Di L (2015). Volume of distribution in drug design. *Journal of Medicinal Chemistry*, **58**(15): 5691-5698.

Van Miert ASJPAM, Van Duin CTh, Busser FJM, Periea N, Van Den Ingh, TSGAM & De Neys-Backer MHH (1978). The effect of flurbiprofen, a potent non-steroidal anti-inflammatory agent, upon *Trypanosoma vivax* infection in goats. *Journal of Veterinary Pharmacology and Therapeutics,* **1**(1): 69-76.

Verma BB, Gautam OP & Malik PD (1973). Diminazene aceturate in the treatment of experimental *Trypanosoma evansi* infection in buffalo calves. *Veterinary Record*, **93**(17): 465-467.

Watson ADJ, Van Gogh H, Van Duurzaam EJM, Van Dam CTM, Van Miert AS & Pam J (1987). Pharmacokinetics of three sulphonamides in ruminant and pre-ruminant kids. *Research in Veterinary Science*, **43**(2): 208 – 216.