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INFLUENCE OF IVERMECTIN ON THE PHARMACOKINETICS OF DOXYCYCLINE IN RABBITS

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SUMMARY

The pharmacokinetic aspects of doxycycline were studied in normal and ivermectin-medicated rabbits after a single intravenous and oral administrations of 20 mg kg^{-1} b.wt. Plasma concentrations of doxycycline were determined by microbiological assay . After intravenous injection, the plasma concentration-time curve of doxycycline was characteristic of a two-compartment open model with a rapid distribution phase and slower elimination phase .

The elimination half-lives (t1/2ß) were 7.98 and 6.19 hours and the mean residence times (MRT) were 11.15 and 8.61 hours in normal and ivermectin-medicated rabbits , respectively . The volume of distribution and the total clearance values were quite similar in both groups . Following oral administration , doxycycline was rapidly absorbed in normal and ivermectin-medicated rabbits with absorption half-lives ($t^1/_{2ab}$) of 33.85 and 28.81 minutes, respectively . The peak plasma concentrations (Cmax) were 15.60 and 18.93 µg ml^{-1}, and were attained at (Tmax) 2.30 and 1.87 hours , respectively . The elimination half-life ($t^1/_{2el}$) of doxycycline after oral administration was shorter in pre-medicated rabbits than in normal ones . The systemic bioavailability percentages (F%) of doxycycline after oral administration were 88.63 % and 90.64 % in normal and ivermectin medicated rabbits , respectively . The present study showed that if ivermectin is used concomitantly, altered doxycycline pharmacokinetics should be anticipated.

INTRODUCTION

Antibiotics and ivermectin are commonly used concurrently in rabbit practice . Doxycycline is a broad spectrum antibiotic closely related to oxytetracycline , currently the most widely used member of the tetracyclines in food animal medicine (Xia et al.,1983 and Riond and Riviere

,1988). The major advantage of doxycycline lies in its greater lipid solubility relative to other tetracyclines . This character probably accounts for its enhanced antimicrobial effectiveness for some organisms , more efficient absorption after oral administration , and enhanced distribution in the body (Aronson ,1980) . Doxycycline has been used in the treatment of chronic respiratory diseases (Migaki and Babcock ,1977) and systemic colibacillosis (George et al.,1977) of poultry . It is generally believed that tetracyclines inhibit protein synthesis in susceptible bacteria by blocking the binding of aminoacyl-transfer RNA to the messenger RNA-ribosome complex (Suzuka et al.,1966) . The binding is reversible and most of the binding is to the 30 S ribosomal subunits.

The efficacy of ivermectin against many endoparasitic and ectoparasitic species has led to its extensive use in domestic animals (Campbell and Benz , 1984) . In rabbits , it has been used principally for the treatment of ectoparasitic conditions (Mc Kellar et al.,1992) . The objectives of the study reported here was to elucidate the influence of ivermectin on the pharmacokinetic behaviour of doxycycline after single intravenous and oral administration in rabbits .

MATERIAL AND METHODS

Animals :

Twenty adult male New Zealand white rabbits each weighing 3-3.5 kg were allowed a conditioning period of approximately 6 weeks before beginning the experiment to insure complete clearance from any drugs . All rabbits were kept under observation and were fed on barseem and a concentrated ration in a pellet form and watered ad-libitum .

Drugs :

1- Doxycycline hydrochloride: was supplied in the form of standard pure powder , highly soluble in water from the Arab Veterinary Industrial Co. (AVICO) Amman – Jordan .
2-Ivermectin : was supplied in the form of injectable solution (Ivomec®, MSD, AGVET Division of Merk Sharp & Dohme Ltd, Holland).

Experimental design :

The animals were allotted to four groups of 5 rabbits each . Rabbits were individually weighed before drug administration and doses were calculated precisely . Rabbits of groups I and III were received 400 µg kg^{-1} ivermectin subcutaneously in the scruff , using graduated 1 ml syringes . After 24 hours , animals of groups I and II were given doxycycline at a dose of 20 mg kg^{-1} intravenously through the left ear vein , while rabbits of groups III and IV were orally received the same dose of doxycycline via stomach tube . Food but not water , was withheld for 12 hours before oral dosing until 6 hours after drug administration .

Blood samples (1ml) were taken by cutaneous venesection of the marginal right ear vein of each rabbit and collected in heparinized tubes at 5 , 10 ,

15 , 30 min., 1 ,2 , 4 , 6 , 8 , 12 , 24 , 48 and 72 h after doxycycline administration . Plasma was separated after centrifugation at 1500 rpm for 20 min. and was stored at –20 °C until assayed .

Analytical procedure :

Concentrations of doxycycline were determined by an agar well diffusion microbiological assay method described by Arret et al .,(1971) using Bacillus cereus var.mycoids (ATCC 11778) strain as the assay organism. Standard curves of doxycycline for both normal and ivermectin-medicated rabbits were prepared in pooled antibacterial free plasma . Each sample or standard was assayed in triplicate by adding 100 µl to each well in each plate . The plates were incubated at 37 °C for 24 hours . The diameters of the inhibition zones were then measured , registered and expressed in (mm) . The mean correlation coefficient (r^2) of the standard curves was 0.999. The limit of detection of doxycycline assay was 0.020 µg ml $^{-1}$.

Pharmacokinetic analysis :

A computerized curve-stripping program (Rstrip, Micromath Scientific Software, Salt Lake City, UT, and USA) was used for data analysis for each rabbit after administration of doxycycline. Following intravenous injection the disposition curve of doxycycline which expresses the decline in plasma drug concentration as a function of time was described by a biexponential expression :

$Cp0 = Ae^{-at} + Be^{-ßt}$

Cp0 = The concentration of drug in plasma at time t

A = Intercept of the distribution line with the concentration axis expressed in (µg ml^{-1})

B = Intercept of the elimination line with the concentration axis expressed in (µg ml^{-1})

α = Distribution rate constant expressed in units of reciprocal time (h^{-1}) .

ß = Elimination rate constant expressed in units of reciprocal time (h^{-1}) .

e = Base of natural logarithm .

Following oral administration , each individual curve of doxycycline vs time was analyzed to determine peak concentration (Cmax), time to peak concentration (Tmax) . This program also calculated compartmental analysis by statistical moment theory . Values calculated included elimination half-life ($t^1/_{2el}$), area under the curve (AUC) from zero to infinity by the trapezoidal integral , (AUMC) area under the first moment curve and mean residence time (MRT) in plasma . The results obtained were statistically analyzed by using student "t" test , according to Snedecor and Cochran (1976)

RESULTS

Mean plasma doxycycline concentrations following single intravenous and oral administration of 20 mg kg^{-1} b.wt. are presented in Figures (1&2). Values of pharmacokinetic constants for doxycycline both alone and following ivermectin treatment are shown in Tables (1&2). After intravenous injection , disposition of doxycycline conformed to a two-compartment model (Fig.1). A rapid distribution phase and slower elimination phase were observed after intravenous injection of

Table 1 : Kinetic parameters of doxycycline following intravenous injection (20 mg kg-1 b.wt.) to normal and ivermectin-medicated rabbits . (n=5) .

Parameter	UNIT	Doxycycline	Doxycycline+ Ivermectin
A	μgml^{-1}	36.94 ± 1.75	33.28 ± 1.30**
α	h^{-1}	5.05 ± 0.24	3.64 ± 0.18**
T1/2α	min	8.31 ± 0.29	11.54 ± 0.44***
B	μgml^{-1}	20.90 ± 0.89	23.09 ± 0.91
ß	h^{-1}	0.087 ± 0.004	0.111 ± 0.009*
T1/2ß	h	7.98 ± 0.37	6.19 ± 0.24**
MRT	h	11.15 ± 0.49	8.61 ± 0.27**
Cp^0	μgml^{-1}	57.87 ± 3.23	56.33 ± 3.06
V_c	L/kg	0.33 ± 0.010	0.35 ± 0.022
K_{21}	h^{-1}	1.90 ± 0.09	1.56 ± 0.06*
K_{el}	h^{-1}	0.22 ± 0.017	0.26 ± 0.018
K_{12}	h^{-1}	3.01 ± 0.20	1.94 ± 0.09**
$V_{d(area)}$	L/kg	0.99 ± 0.04	0.81 ± 0.03**
$V_{d(ss)}$	L/kg	0.89 ± 0.03	0.80 ± 0.02*
ClB	L/h/kg	0.08 ± 0.005	0.09 ± 0.006
AUC	µg/ml/h	245.47 ± 8.63	216.88 ± 7.22*
AUMC	µg/ml/h	2754.03 ± 191.87	1884.73 ± 126.39*

* Significant at p 0.05
** Significant at p 0.01
*** Significant at p 0.001

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Table 2 : Kinetic parameters of doxycycline following oral administration (20 mg kg-1 b.wt.) to normal and ivermectin-medicated rabbits . (n=5) .

Parameter	UNIT	Doxycycline	Doxycycline+ Ivermectin
A	μgml^{-1}	23.40 ± 1.85	30.47 ± 1.39*
K_{ab}	h^{-1}	1.24 ± 0.06	1.45 ± 0.04*
T1/2ab	min	33.85 ± 1.76	28.81 ± 1.19*
MAT	min	89.41 ± 5.30	30.62 ± 1.92***
B	μgml^{-1}	20.80 ± 0.91	26.46 ± 1.13**
K_{el}	h^{-1}	0.087 ± 0.005	0.122 ± 0.010*
T1/2el	h	7.94 ± 0.43	5.77 ± 0.30**
MRT	h	12.67 ± 0.68	9.10 ± 0.41**
Cmax	μgml^{-1}	15.60 ± 0.65	18.93 ± 0.78*
Tmax	h	2.30 ± 0.11	1.87 ± 0.09*
AUC	µg/ml/h	219.49 ± 7.72	196.05 ± 6.19*
AUMC	µg/ml/h	2756.82 ± 192.46	1798.06 ± 155.28**
F	%	88.63 ± 4.99	90.64 ± 5.05

*** Significant at p 0.05**

**** Significant at p 0.01**

***** Significant at p 0.001**

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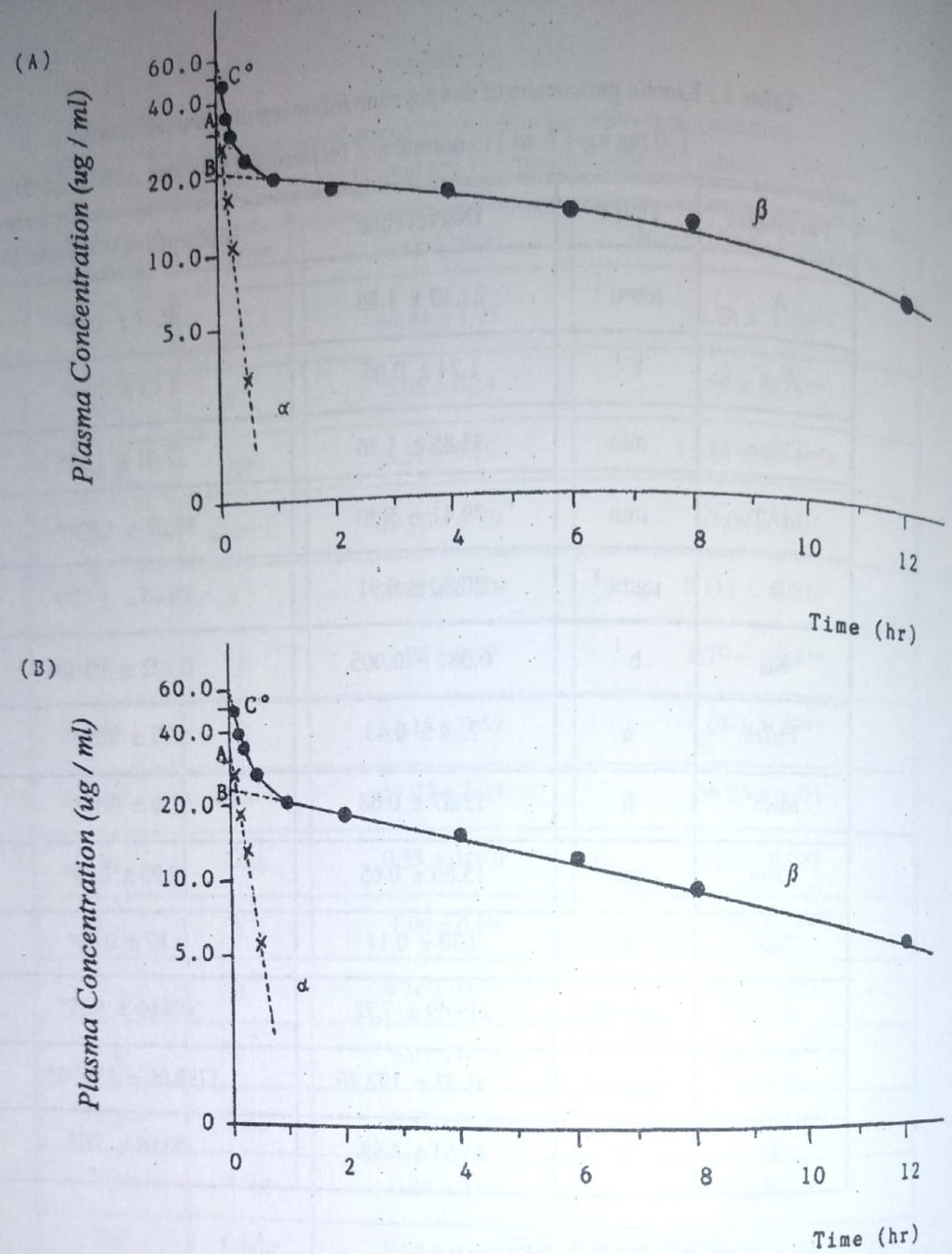

Fig.(1): Semilogarithmic graph depicting the time concentration course of doxycline in plasma of normal (A) and ivermectin-medicated (B) rabbits after a single intravenous injection of 20 mg Kg^{-1} b.wt (n=5).

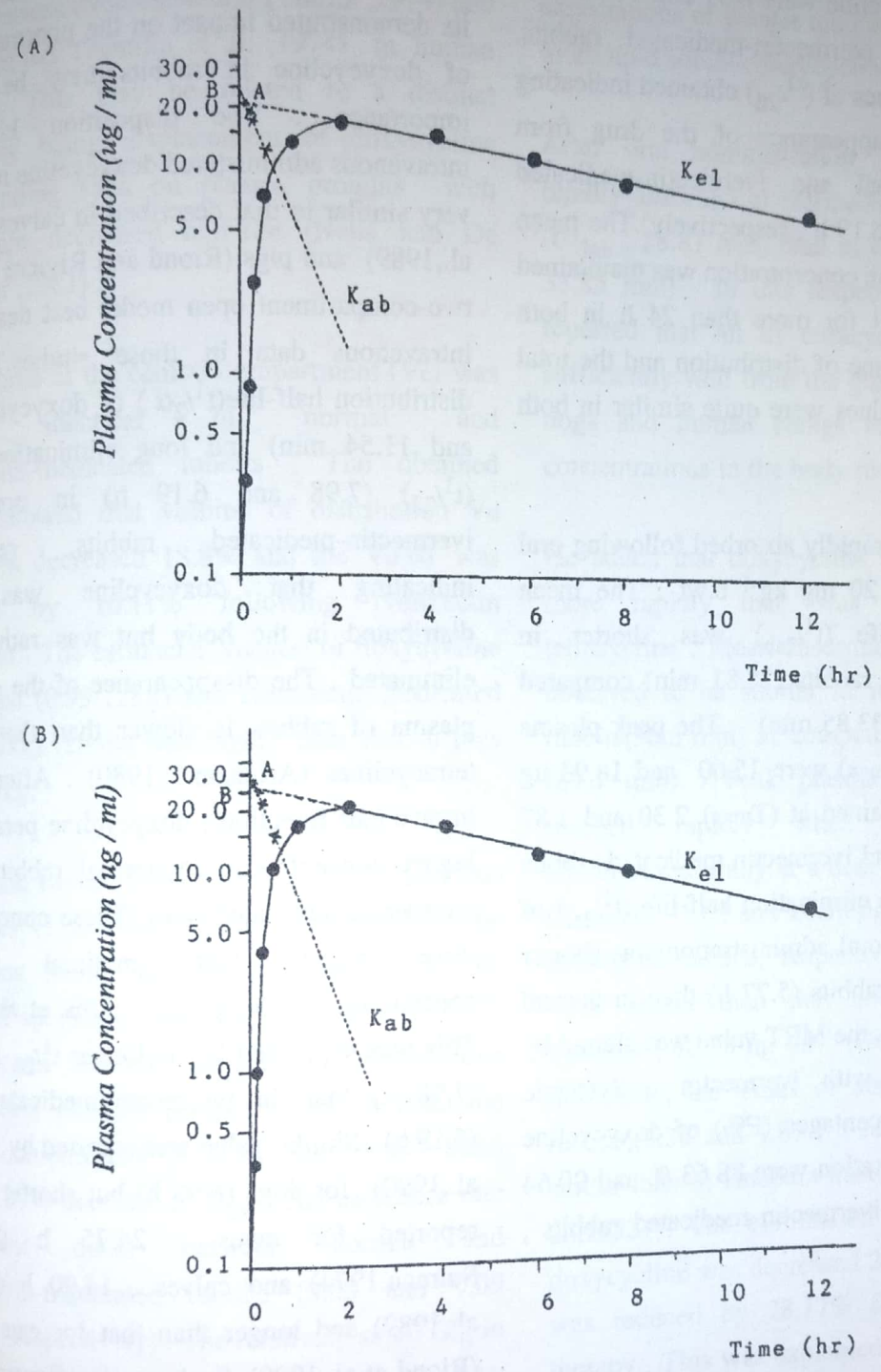

Fig.(2): Semilogarithmic graph depicting the time concentration course of doxycline in plasma of normal (A) and ivermectin-medicated (B) rabbits after a single oral administrationn of 20 mg Kg^{-1} b.wt (n=5).

doxycycline . The mean distribution half-lives ($t^1/_2\alpha$) of doxycycline were 8.31 and 11.54 min. in normal and ivermectin-medicated rabbits, respectively . Values of ($t^1/_{2\beta}$) obtained indicating a slow final disappearance of the drug from plasma of normal and ivermectin-medicated rabbits (7.98 and 6.19 h , respectively).The mean plasma doxycycline concentration was maintained above 0.5 µg ml-1 for more than 24 h in both groups . The volume of distribution and the total body clearance values were quite similar in both groups.

Doxycycline was rapidly absorbed following oral administration of 20 mg kg^{-1} b.wt . The mean absorption half-life ($t^1/_{2ab}$) was shorter in ivermectin-treated rabbits (28.81 min) compared to normal ones (33.85 min) . The peak plasma concentrations (Cmax) were 15.60 and 18.93 ug ml^{-1} and were attained at (Tmax) 2.30 and 1.87 hours in normal and ivermectin medicated rabbits , respectively. The elimination half-life ($t^1/_{2el}$) of doxycycline after oral administration was shorter in pre-medicated rabbits (5.77 h) than in normal ones(7.94 h). Also, the MRT value was altered by co-administration with ivermectin . Systemic bioavailability percentages (F%) of doxycycline after oral administration were 88.63 % and 90.64 % in normal and ivermectin medicated rabbits , respectively .

DISCUSSION

Doxycycline is a broad-spectrum antibiotic that is useful for treatment of infectious diseases in poultry and animals (Migaki and Babcock ,1977 and Riond and Riviere ,1988). Ivermectin is likely to be used in the treatment of ectoparasitic conditions (Mc Kellar et al.,1992) and therefore, its demonstrated impact on the pharmacokinetics of doxycycline in rabbits may be of great importance . The disposition kinetics of intravenous administered doxycycline in rabbits is very similar to that described in calves (Riond et al.,1989) and pigs (Riond and Riviere ,1990) . A two-compartment open model best described the intravenous data in those studies.The short distribution half-life($t^1/_2\alpha$) of doxycycline (8.31 and 11.54 min) and long elimination half-life ($t^1/_{2\beta}$) (7.98 and 6.19 h) in normal and ivermectin-medicated rabbits, respectively indicating that doxycycline was rapidly distributed in the body but was rather slowly eliminated . The disappearance of the drug from plasma of rabbits is slower than that of other tetracyclines (Aronson , 1980) . After 24 h of intravenous injection , doxycycline persisted in a higher concentration in normal rabbits than in ivermectin-medicated ones .These concentrations were higher than minimal inhibitory concentration (0.5 µg ml^{-1} ; Jha et al., 1989) . This was supported by its longer $t^1/_{2\beta}$ in normal (7.98 h) than in ivermectin-medicated rabbits (6.19 h) . Similar value was reported by (Riond et al.,1990) for dogs (6.99 h) but shorter than that reported for ewes , 24.75 h (Ziv and Sulman,1974) and calves , 14.90 h (Riond et al.,1989) and longer than that for cats , 4.56 h (Riond et al.,1990). Such species discrepancies in the estimation of $t^1/_{2\beta}$ are commonly encountered in the literature and are related to various pharmacokinetic curve-fitting routines and analytical techniques used (Dix et al., 1985). Some investigators noted a dramatic decrease in

$t^1/_{2\beta}$ of doxycycline following co-administration of barbiturates (Neuvonen and Pentilla ,1974) and anti-epileptics (Pentilla et al. ,1974) in human beings . This may be related to a distinct possibility being displacement of doxycycline from binding sites on plasma proteins with subsequent decreased half-life (Nelis and De Leenheer ,1981) .

The volume of the central compartment (Vc) was almost identical in normal and ivermectin-medicated rabbits . The obtained results showed that volume of distribution $V_{d(area)}$ was decreased 18.8% and the $V_{d(ss)}$ was reduced by 10.11% following ivermectin treatment . The estimated $V_{d(area)}$ of doxycycline in normal (0.99 L/kg) and ivermectin-medicated (0.81 L/kg) rabbits was higher than that in pigs (0.57 L/kg, Riond and Riviere ,1990) but was lower than that in calves (1.38 L/kg, Riond et al.,1990) . The variations found may be due mainly to the different species . Ivermectin induced a 35.54% decrease in the rate constant of doxycycline distribution into the peripheral tissues from the central compartment (K_{12}) and there was a trend to a 17.89% decrease in K_{21} . The clearance rate did not differ between normal and ivermectin-medicated rabbits (0.08 and 0.09 L/h/kg , respectively) . The relatively small Cl_B in rabbits of both groups may have been caused by extensive binding of doxycycline to plasma proteins and its reabsorption from renal tubules (Aronson ,1980). In human beings, the renal clearance of doxycycline was shown to be considerably less than was of tetracycline (Jaffe et al.,1973) . This observation is consistent with expectations of greater tubular reabsorption of the more lipid soluble doxycycline .

After oral administration , doxycycline was rapidly absorbed in ivermectin-medicated rabbits ($t^1/_{2ab}$; 28.81 min) than in normal ones ($t^1/_{2ab}$; 33.85 min) . In this respect , Aronson (1980) reported that all of tetracyclines are absorbed sufficiently well from the gastrointestinal tract of dogs and human beings to produce effective concentrations in the body for systemic infections

He added that doxycycline was absorbed much more rapidly than was oxytetracycline or tetracycline . Mean absorption time (MAT) was observed to be shorter in ivermectin-medicated rabbits(30.6 min) as compared with normal ones (89.4 min) . Peak plasma concentration was reached rapidly when doxycycline was administered orally at a dose of 20 mg kg^{-1} with (C_{max}) of 15.60 and 18.93 µg ml^{-1} in normal and medicated rabbits, respectively . These values were higher than that observed in Houbara bustards ; 10.25 µg ml^{-1} (Greth et al.,1993) . In this study, the Tmax of normal and medicated rabbits,(2.30 and 1.87 h , respectively) occurred earlier than in Houbara bustards ; 12 h (Greth et al.,1993) . The elimination half-life ($t^1/_{2el}$) of doxycycline was decreased 27.32% and the MRT was reduced by 28.17% following ivermectin therapy . This was explained by Mc Kellar et al., (1992) who mentioned that high concentrations of ivermectin are sustained in the body for 13 days as it binds to plasma proteins in a high proportion . Consequently , it decreases the binding of doxycycline to plasma proteins and elevates its

free portion and thus the rate of doxycycline excretion is increased as compared to normal animals .

In conclusion , the present study showed that if ivermectin is used concomitantly, altered doxycycline pharmacokinetics should be anticipated

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