

## Pharmacological Studies on Tetracycline and Tetracycline Nanoemulsion Formulas.

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THE study was done to compare the pharmacokinetic and pharmacodynamics of 50 mg/kg b.wt tetracycline hydrochloride (TC-hcl) and tetracycline nanoemulsion (TC-nm) formulas in rabbits and detection of their effect on standard and field bacterial strains. After oral TC concentration in plasma started to be detected at 0.25 h, reached the maximum at (0.5 h TC-hcl) and 1 h (TC-nm) and declined at 12 hours. Following a single i.v. administration a volume of distribution  $V_2$  ( $0.292 \pm 0.111$  L/kg) in TC-nm than for TC-hcl ( $0.216 \pm 0.183$  L/kg) and was slowly cleared ( $0.393 \pm 0.183$  L.h/kg) in TC-nm than in TC-hcl ( $0.415 \pm 0.311$  L.h/kg). After oral administration a rapidly absorbed with significant slow absorption half-life  $t_{1/2\alpha}$  ( $0.550 \pm 0.090$  h and  $0.176 \pm 0.058$  h.) and elimination half-life  $t_{1/2\beta}$  ( $4.215 \pm 1.661$  h. and  $1.58 \pm 1.447$  h.) with higher calculated  $C_{max}$  of ( $4.215 \pm 1.661$   $\mu$ g/ml and  $1.58 \pm 1.447$   $\mu$ g/ml) achieved at prolonged calculated  $t_{max}$  ( $0.759 \pm 0.149$  h. and  $0.356 \pm 0.305$  h.) in TC-nm than in TC-hcl, respectively.

The value of TC-hcl and TC-nm MIC was the same for *Staph. Aureus* 6538, *Staph. Epidermidis* 12228, *E. coli* 8739 were 0.14, 0.8 and 0.12  $\mu$ g, respectively, and interpreted as sensitive. Field sensitive *Corynebacterium*, *E. coli*, *S. typhimurium*, *S. enteritidis* and *Staph. leutus* isolates MIC value was 1.4, 8, 2, 1.6 and 2  $\mu$ g for, respectively. Tetracycline resistant *Staph. scuri* (18 and 16  $\mu$ g) and *Staph. xylois* (18 and 6  $\mu$ g).

**Keywords:** Pharmacokinetics, pharmacodynamics, Tetracycline, nanoemulsions, MIC, Rabbits.

### Introduction

Tetracyclines were discovered in the 1940s and exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. They are inexpensive antibiotics used extensively in the prophylaxis and therapy of human and animal infections and also as growth promoters [1,2]. Tetracyclines are widely used in veterinary medicine mainly for the treatment of gastrointestinal, respiratory and skin bacterial infections [3]. Dissemination of tolerance and resistance determinants has limited their tetracycline usage [4]. Tetracycline resistance

in pathogenic, opportunistic, and commensal bacteria is often due to the acquisition of new genes, which code for energy-dependent efflux of tetracycline or for a protein that protects bacterial ribosomes from the action of tetracycline [1].

Nanoemulsions are colloidal dispersion systems that are thermodynamically stable, composed of two immiscible liquids mixed along with emulsifying agents (surfactants and co-surfactants) to form a single phase and have been investigated as drug delivery systems [5]. Nanoemulsions formulation improve drug delivery system [6-8], entrapment efficiency (EE) and loading efficiency (LE) of the drug [9], pharmacokinetic and biodistribution, target

selectivity, enhanced activity against intracellular pathogens, protection of antibiotic drugs against hydrolytic activity of enzymes, decreased toxicity, enhanced penetrability, and thereby increased residence time of the drug in macrophages [10,11].

Tetracycline hydrochloride-loaded particles was reported to be effereent against *H. pylori* [12], *P. aeruginosa* [13], *E. coli* strains in pigs [14], *Salmonella* spp. *E. coli* 0157:H7 (VT-), *P.aeruginosa*, *Staph.aureus* and *L.monocytogenes* [15].

The synthesized tetracycline-loaded calcium phosphate nanoparticle (Tet-CPNP) bactericidal activity of nano-particulate tetracycline was investigated by agar plating, spectrophotometry, and phase contrast-fluorescence-atomic force microscopy and flow cytometry techniques. Efficiency of tetracycline loading in CPNP was about 20% and the minimum inhibitory concentration (MIC) was in the range of 20–40 µg/ml on multiple antibiotic resistant bacteria like *E. coli*, *S.kentuckey* and *Shigella*flexneri, whereas MIC of free tetracycline was in the range of 150–180 µg/ml [16].

The integration of PK (bioavailability and clearance) and PD (MIC) indices allows predicting efficacy and potency of a drug in the early phase of drug development and supports post-marketing surveillance [17, 18].

Therefore this study was planned to evaluate the Pharmacokinetics and antibacterial activity of prepared tetracycline nanoemulsion formulas as compared with tetracycline and in vitro.

## **Materials and Methods**

### *Tetracycline*

#### *Tetracycline-loaded nanoemulsion (TC-nm)*

Prepared and characterized TC-nm was supplied by Amer et al. [30].

#### *Tetracycline hydrochloride (TC-hcl)*

TC-hcl was obtained as pure powder 100% from El-Nasr pharmaceutical chemicals Co. (Abu Zaabal, Egypt).

### *Pharmacokinetics*

#### *Rabbits*

Male New Zealand white rabbits, weighing 3.25-3.75 kg were obtained from animal house Faculty of Veterinary Medicine Cairo University. Rabbits were allowed for acclimatization for 15 days before being used. Animals were housed singly in stainless steel cages in a separate animal room at an environmental temperature of 20-24°C and will ventilation and a 12 hour light/

dark cycle. Rabbits were fed on antibacterial free balanced commercial pelleted ration free from antibacterial drugs. Rabbits were given ration and drinking water ad libitum.

### *Groups and administration*

Sixteen (16), white male New Zealand rabbits were randomly divided into 2 groups, 8 animals/group. Animals of group 1 given TC-nm and animals of group 2 given Tc-hcl. Single dose of 50 mg/kg body weight (BW) from each preparation will be given for each rabbit using oral and intravenous (i.v) route, with 14 day interval to insure complete drug clearance from rabbits body [30,32,33]. Blood samples were collected at different time intervals at 0.083 (5 min), 0.15 (0.15 min), 0.5 (30 min), 1, 2, 4, 6, 8, 10, 12 and 24 hours after each dose administration. Individual non-coagulated blood samples were collected from ear vein through i.v catheter for separation of plasma [34, 35]. The collected plasma was stored at -80 °C till determination of tetracycline using microbiological technique.

### *Antibiotic assay*

Blood samples were centrifuged at proximately 1500 rpm. The plasma was collected and either tested immediately or stored frozen at -80°C in individual vials until assayed. Each sample was induplicate assayed for the presence of tetracycline using the plate disk method as previously described [36]. Cultures of *Bacillus cereus varmycoides* ATCC 1177815 (Difco Laboratories, Detroit, Michigan) freshly prepared were used as the test organism in antibiotic assays. All tests were done in duplicate, including standard controls. The minimum level of sensitivity of the assay was 0.02 µg/ mL of serum and compared with standard curve. Samples with drug levels lower than 0.02 µg/ mL were recorded as undetected.

### *Pharmacokinetic modeling*

Compartmental analysis is a widely used technique to quantitatively evaluate and predict the in vivo fate of a drug by modeling the concentration–time data with a suitable. PK compartment model. Pharmacokinetic values were calculated using PKsolver program [37] and values were expressed as mean ± SD. The actual maximum concentration in plasma (Cmax) and time to maximum concentration (tmax) were determined from the concentration–time relationship for each rabbit. The duration of time that the plasma concentration of tetracycline exceeded 0.12µg/mL was determined for each rabbit. This concentration cut point was selected

based on data from rabbit where the in vitro MIC for *Bacillus cereus varmycoides* ATCC 1177815 0.07µg/ [38].

A two compartment open model was applied to data obtained following IV injection and pharmacokinetic values calculated using standard equations [39]. A two compartment model provided the best model fit based on residual analysis when compared to 1 or 3 compartment models. Bioavailability (F) of tetracycline after oral injection was calculated as a percentage using a standard equation [40] as:  $F = \text{AUC oral} \times 100 / \text{AUC IV}$ .

#### *Pharmacodynamics*

Antibacterial activity and MIC determination

#### *Bacterial strains*

Field and stander bacterial isolates were obtained and used for testing their susceptibility to tetracyclines. Standard strains *Staph. Aureus* 6538, *Staph. Epidermidis* 12228, *Ecoli* 8739. Field bacterial strains including tetracycline sensitive strains including *Corynebacterium*, *E coli*, *S. typhimurium* *Staph Leutus* and *S. enteritidis* [41,42]. Coagulase negative staphylococci tetracycline resistant include 2 *Staph. xylois* and 2 *Staph. scuiiri* [43]. Field resistant strains *Corynebacteriumcervicis*, *E.coli* and *S. typhimurium* were supplied by Dr. M.M. Amer, poultry clinic lab. Fac. Vet. Med. Cairo University).

#### *Culture and preparation of bacterial inoculum*

Overnight Mueller Hinton broth cultures of all bacterial strains at 37°C were prepared. Bacterial inoculum density for preparation of inoculum suspension was adjusted to be equal that of the 0.5 MacFarland standards was done by picking up of 4:5 colonies from 24 hour culture in 2 ml of Mueller-Hinton broth. To aid comparison compare the test and standard against a white background with a contrasting black line. Suspension contain between  $10^7 - 10^8$ cfu/ml according to the genera and the inoculum was adjusted to  $10^4$ cfu/ml by dilution with Mueller-Hinton broth and dispensed on the surface of the agar.

#### *Minimum inhibitory concentration (MIC)*

Stock solutions of both TC-hcl powder and TC-nm was prepared in concentration of 1000 mg/L in sterile saline. Working solutions of each tested formulas were freshly prepared in concentrations 0.06 - 128 µg/ml. MIC for all bacterial strains was performed using Mueller Hinton agar (Oxoid) plates in Petri dish 9 cm in diameter. for control without antibiotic 2 mL of sterile distilled water in a Petri dish and 2 mL of each dilution antibiotic

from the lowest to the highest concentration were added to a series of Petri dishes followed by 18 mL of Mueller-Hinton agar medium, Mixed well and allowed to dry at 35 to 37°C for 30 min, a 1ml of suspension was delivered on to the surface of the agar and allowed the inoculum to be absorbed into the agar before incubation. Inoculated dishes were incubated 37°C for 18 h. All tests were done in triplicate. *Bacillus cereus varmycoides* ATCC 1177815 was used as MIC control positive control strain for 0.16µg/ml. MIC endpoint as the lowest concentration of antibiotic in in mg/L at which there is no visible growth and interpreted [38, 44].

#### *Statistical analysis*

Data was presented as mean ± SD. Selected pharmacokinetic values were compared for IV and oral administration of tetracycline using mixed models analysis of variance and a compound symmetry covariance matrix (PROC MIXED, SAS 9.2, SAS Inc, Cary, NC). A P value <0.05 or P < 0.001 was considered significant.

### **Results and Discussion**

Since the discovery of Tetracyclines in the 1940s, it used extensively in the prophylaxis and therapy in human and animal infections. Tetracyclines still widely used in veterinary medicine for the treatment of gastrointestinal, respiratory and skin bacterial infections [1-3].

#### *Pharmacokinetics*

Tetracycline concentration in rabbit plasma was determined following TC-hcl powder and TC-nm administration in a single dose of 50 mg/kg b.wt via oral and IV. Micro-biological assays for determination of tetracyclines were previously used [45-47].

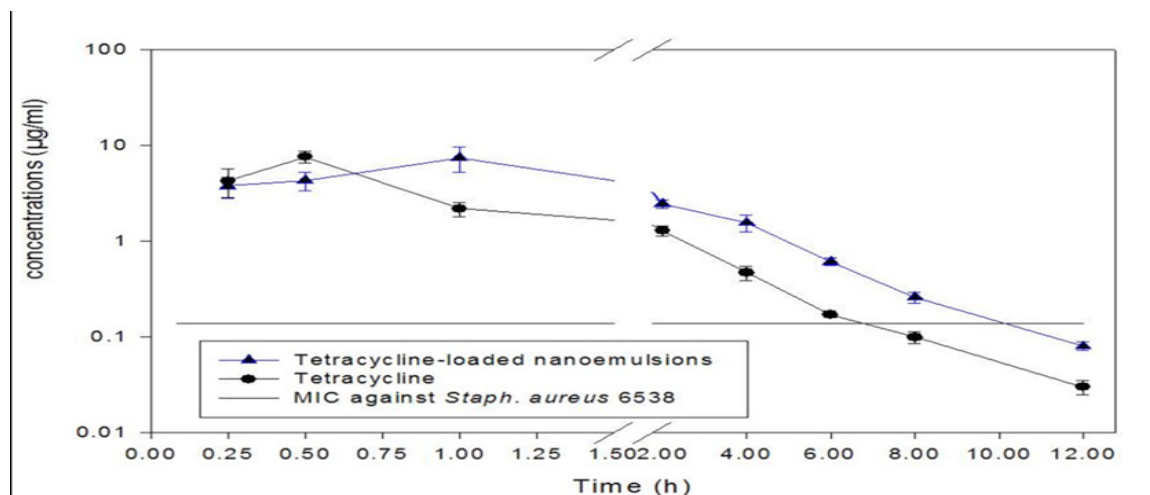
The mean plasma tetracycline concentration-time relationship following a single oral administration of 50 mg/kg of BW (Table 1) Tc-hcl concentration in plasma started to be detected at 0.25 h, reach the maximum at 0.5 h followed by decline at 12 hours as  $4.26 \pm 1.458$ µg/ml,  $7.60 \pm 1.102$ µg/ml and  $0.03 \pm 0.005$ µg/ml, respectively. While, TC-nm was determined at 0.25 h and reach the maximum at 1 h and decline to the minimum value at 12 h as  $3.77 \pm 0.923$ µg/ml,  $7.41 \pm 2.184$ µg/ml and  $0.08 \pm 0.008$ µg/ml, respectively. Similar results were detected in rat [48], in dogs [49], in man [50], in rabbit [30]. The drug had a rapid distribution phase [31, 51]. The non-detected concentration at 24 was reported in dog [27]. µNanoemulsion showed higher concentrations persisted higher than MIC for longer time (more

**TABLE1. Plasma concentration of TC- hcland TC-nm after oral or i.v administration (50 mg/kg b.wt) in rabbits (N =8, Mean ± SD)**

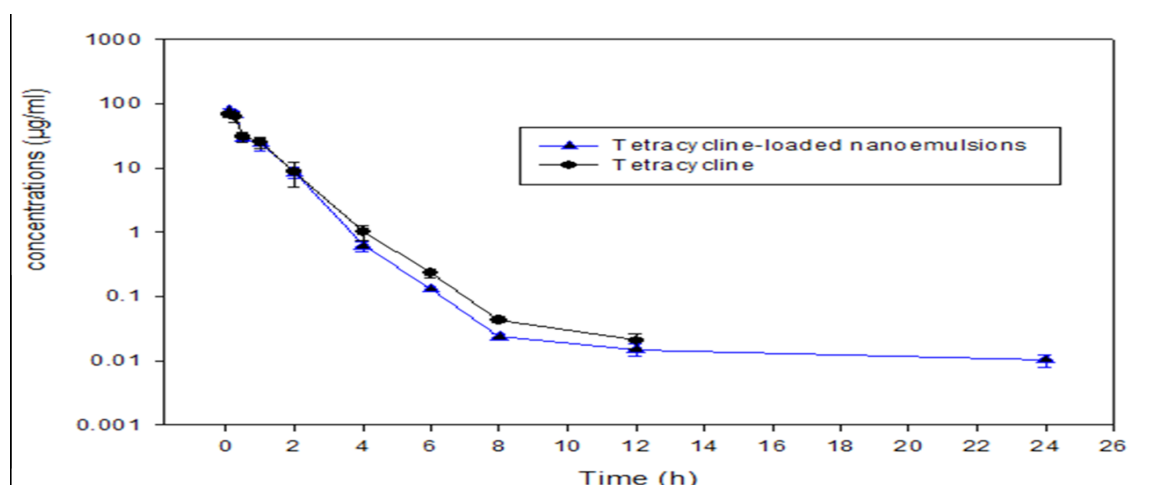
Time (h)	Tetracycline concentration µg/ml (mean ± SD)			
	Oral administration		IV administration	
	TC-hcl	TC-nm	TC-hcl	TC-nm
0.083	ND	ND	67.879 ± 6.555	78.566 ± 5.295**
0.25	4.26 ± 1.458	3.77 ± 0.923	63.051 ± 11.280	68.547 ± 7.284**
0.5	7.60 ± 1.102	4.32 ± 0.969	30.664 ± 5.717	28.455 ± 3.377
1	2.18 ± 0.368	7.41 ± 2.184*	25.319 ± 4.922	24.318 ± 5.595
2	1.29 ± 0.152	2.45 ± 0.270	8.754 ± 3.772	8.527 ± 1.736
4	0.47 ± 0.079	1.56 ± 0.322**	1.013 ± 0.260	0.613 ± 0.111*
6	0.17 ± 0.011	0.61 ± 0.056**	0.232 ± 0.039	0.130 ± 0.010**
8	0.10 ± 0.014	0.26 ± 0.034**	0.043 ± 0.004	0.024 ± 0.002*
12	0.03 ± 0.005	0.08 ± 0.008**	0.021 ± 0.005	0.015 ± 0.003
24	ND	ND	ND	0.010 ± 0.002**

ND: Non-detected.

\*Significant <0.005 \*\*Significant P < 0.001.



**Fig. 1. Semilogarithmic graph depicting the time concentration relationship of TC-hcl or TC-nm after oral administration (50 mg/kg b.wt) in rabbits. (N =8, Mean ± SD)**



**Fig. 2. Semilogarithmic graph depicting the time concentration relationship of TC-hcl or TC-nm after i.v administration (50 mg/kg b.wt) in rabbits. (N =8, Mean ± SD)**

than 10 hours) than that for powder form 6 hours (Table 1, Fig 1) [30].

The pharmacokinetic variables that describe the disposition of tetracycline following a single i.v administration presented in (Table 2). Tetracycline had higher volume of distribution V<sub>2</sub> (0.292 ± 0.111 L/kg) in TC-nmand was slowly cleared (0.393 ± 0.183 L.h/kg) than for TC-hcl(0.216 ± 0.183L/kg) and (0.415 ± 0.311 L.h/

kg) after i.v administration, respectively. Also, higher k<sub>12</sub> 0.765 ± 0.361 1/h and slow k<sub>21</sub> 1.431 ± 0.75 1/h were recorded in TC- nm as compared with those of TC-hcl k<sub>12</sub> 0.683 ± 0.511 1/h and k<sub>21</sub> 2.1 ± 1.8 1/h., respectively. These results are in accordance with those reported previously by many animal species in rats [48], female rats and male guinea-pigs [52], adult white Californian rabbits [30,54] and dogs [49]. These findings represented by higher and prolonged tetracycline

**TABLE 2. Pharmacokinetic parameters of TC-hcl and TC-nm after oral or i.v administration (50 mg/kg b.wt) in rabbits (N =8, Mean ± SD)**

Parameter	Unit	Oral administration		IV administration	
		TC-hcl	TC-nm	TC-hcl	TC-nm
k <sub>10</sub>	1/h	1.917 ± 4.266	0.701 ± 0.102**	1.199 ± 0.218	1.407 ± 0.198
k <sub>12</sub>	1/h	1.566 ± 6.784	0.379 ± 0.105**	0.683 ± 0.511	0.765 ± 0.361
k <sub>21</sub>	1/h	0.903 ± 1.338	0.406 ± 0.335**	2.1 ± 1.8	1.431 ± 0.75
t <sub>1/2</sub> Alpha	h	0.176 ± 0.058	0.550 ± 0.090**	0.324 ± 0.263	0.25 ± 0.074
t <sub>1/2</sub> Beta	h	1.58 ± 1.447	4.215 ± 1.661**	1.041 ± 0.277	1.12 ± 0.184
t <sub>1/2</sub> ka	h	0.05 ± 0.027	0.519 ± 0.091**		
V	L/kg	1.26 ± 0.0570	0.216 ± 0.0208**	0.626 ± 0.09	0.515 ± 0.042*
CL	L.h/kg	3.77 ± 0.0335	0.301 ± 0.0235**	0.736 ± 0.071	0.720 ± 0.082
V <sub>2</sub>	L/kg	3.98 ± 0.0548	0.122 ± 0.0463**	0.216 ± 0.183	0.292 ± 0.111
CL <sub>2</sub>	L.h/kg	4.58 ± 0.1459	0.167 ± 0.0811**	0.415 ± 0.311	0.393 ± 0.183
T <sub>max</sub>	h	0.356 ± 0.305	0.759 ± 0.149**		
C <sub>max</sub>	µg/ml	10.689 ± 21.491	6.326 ± 1.173**		
AUC 0-t	µg/ml.h	8.768 ± 9.397	16.679 ± 1.246*	68.51 ± 7.16	70.1 ± 7.2
AUC 0-inf	µg/ml.h	8.8 ± 9.42	18.67 ± 3.07*	68.54 ± 7.16	70.1 ± 7.2
AUMC	µg/ml.h <sup>2</sup>	13.17 ± 13.77	97.67 ± 77.24**	79.32 ± 20.06	79.04 ± 13.03
MRT	h	1.277 ± 1.215	4.796 ± 2.781**	1.151 ± 0.238	1.125 ± 0.128
F	%	13.7 ± 15.03	23.79 ± 17.31**		
C <sub>0</sub>	µg/ml	0.356 ± 0.305	0.759 ± 0.149	81.27 ± 11.25	97.62 ± 8.08
V <sub>ss</sub>	L/kg	10.689 ± 21.491	6.326 ± 1.173	0.842 ± 0.168	0.807 ± 0.103

\*Significant <0.005 \*\*Significant P < 0.001

plasma concentration after TC-nm administration than TC-hcl powder [30].

After oral administration tetracycline was rapidly absorbed with significant slowly absorption half-life t<sub>1/2</sub><sup>alpha</sup> (0.550 ± 0.090 h and 0.176 ± 0.058 h.) and elimination half-life t<sub>1/2</sub><sup>beta</sup> (4.215 ± 1.661 h. and 1.58 ± 1.447 h.) with higher calculated C<sub>max</sub> of (4.215 ± 1.661 µg/ml

and 1.58 ± 1.447 µg/ml) achieved at prolonged calculated t<sub>max</sub> (0.759 ± 0.149h. and 0.356 ± 0.305 h.) in TC-nm than in TC-hcl powder treated rabbits, respectively. A significant higher AUC<sub>0-inf</sub>. (18.67 ± 3.07 and 8.80 ± 9.42 µg/ml.h.) at prolonged MRT (4.796 ± 2.781 and 1.277 ± 1.215 h.) in TC-nm than in TC-hcl powder treated rabbits, respectively. Tetracycline pharmacokinetic variables indicated higher bioavailability in nanoemulsion 23.79 ± 17.31 %



than TC-hcl  $13.7 \pm 15.03\%$  treated rabbits. TC-nm showed lower volume of distribution VSS  $6.326 \pm 1.173$  L/kg than that for tetracycline  $10.689 \pm 21.491$  l/kg. The recorded serum pharmacokinetic parameters after oral administration of single dose was studied in rabbit [29, 30], in sheep [23]. While tetracycline was detected for 30 hours after oral dose of 40 mg/kg in pigs [55]. These findings were recorded after oral administration represented by higher and prolonged tetracycline plasma concentration for TC-nm administration than TC-hcl. This can be attributed to the nanoemulsion increases drug solubility and bioavailability, reduced patient variability, controlled drug release, and protection from enzymatic degradation [56]. The effect of nanoemulsions clarified by Mishra *et al.* [57] stated that nanoemulsions exhibited sufficiently high level of stability for them to be proposed as vehicle for drug delivery as it eliminates the side effects in the transdermal route, increases patient compliance, avoids first-pass metabolism, enhance bioavailability and maintains the plasma drug level for a longer period of time. It was reported that the pharmacokinetic parameters of tetracycline are dose dependent where its parameters in man after single oral doses 250 mg resulted in  $C_{max}$  (2 mg/L),  $t_{max}$  2-4 h and  $t_{1/2}$  6-11 h [58], doses 300 mg the  $C_{max}$  (2.5 mg/L),  $t_{max}$  3 h and  $t_{1/2}$  7.8 h [59] as well as in an oral doses of 500 mg  $C_{max}$  (3-5 mg/L),  $t_{max}$  2 h and  $t_{1/2}$  8.5 h [60].

#### Pharmacodynamics

To evaluate the efficiency of antibiotic there are two factors, the 1st is the measure of potency of the antibiotic for the pathogen in question

MIC and MBC, the 2<sup>nd</sup> is relationship between the concentration time profile and potency of the antibiotic [61-63]. Results were interpreted according to CLISI [44] where, MIC  $\mu$ G/ML value interpretive standards for *Staphylococcus* spp and *Enterobacteriaceae* are  $\leq 4$ : sensitive, 8: intermediate and  $\geq 16$   $\mu$ G/ML: resistant.

The result of MIC to determined and compare the antibacterial activity of TC-hcl powder and TC-nm on different Gram positive and Gram negative bacterial strains are presented in (Table 3). MIC for both TC-hcl and TC-nm are the same for *Staph. Aureus* 6538, *Staph. Epidermidis* 12228 and *E. coli* 8739 (Stander strains) were the similar 0.14, 0.8 and 0.12  $\mu$ g, respectively, and interpreted as sensitive [44]. This result agree with MIC for reference *E. coli* and *S. aureus* strains were 1-2 mg/L and 0.06 - 0.5 mg/L, respectively [38].

Field sensitive isolates had MIC values of 1.4, 8, 2, 1.6 and 2  $\mu$ g for *Corynebacterium*, *E. coli*, *S. typhimurium*, *S. enteritidis* and *Staph. leutus*, respectively, interpretation showed all were sensitive except *E. coli* was intermediate. *S. Enteritidis* was sensitive to oxytetracycline [63]. *E. coli* resistance tetracycline was reported in vitro and confirmed genetically by detection of gens tet(A) and tet(B) [41]. Tetracycline resistant *Staph. scuiri-1*, *Staph. xyloxis-1*, *Staph. scuiri-2* and *Staph. xyloxis-2* showed equal values of MIC 18, 18, 16 and 6  $\mu$ g, respectively, all still resistant except *Staph. xyloxis-2* that interpreted as intermediate. This result was previously reported in vitro and resistance tetK was also detected [41,42]. Tetracycline bacterial resistance was previously determined [1,4]. Our results still in the suggested MIC of tetracycline

**TABLE 3. MIC values of tested Gram –ve and Gram +ve bacterial strains to both TC-hcl and TC-nm formula.**

Source	Bacterial strain	TC-nm		TC-hcl	
		$\mu$ g/ml	Interpretation	$\mu$ g/ml	Interpretation
Standard strains	<i>Staph. Aureus</i> 6538	0.14	S	0.14	S
	<i>Staph. Epidermidis</i> 12228	0.8	S	0.8	S
	<i>E. coli</i> 8739	0.12	S	0.14	S
Field Sensitive isolates	<i>Corynebacterium</i>	1.4	S	1.6	S
	<i>E. coli</i>	8	I	8	I
	<i>S. typhimurium</i>	2	S	2	S
Field multidrug resistant strains	<i>S. enteritidis</i>	1.6	S	1.4	S
	<i>Staph. Leutus</i>	2	S	2.2	S
	<i>Staph. Scuiri-1</i>	18	R	18	R
	<i>Staph. Xyloxis-1</i>	18	R	18	R
	<i>staph. scuiri-2</i>	16	R	16	R
	<i>staph. Xyloxis-2</i>	6	I	6	I

S: Sensitive

I: Intermediate.

R: Resistant.

rangeto Enterobacteriaceae, Staphylococci are 0.25- 128 and 0.06 - 128 mg/L [38].

MIC value of tetracycline against *Staph. aureus*, *Shigella* spp. and *E. coli* were found to be 0.5, 1.0 and >64.0 mg/ml, respectively [62]. The obtained results showed no difference in MIC values of the bacterial strain between the activity of TC-hcl and TC-nm. This indicated that the nanoemulsion formulation reported to be efferent against many bacterial strains [12-16]. The active tetracycline in oil phase of oil-in-water nano-emulsion is protected from hydrolysis and oxidation [64,65]. While Vatsraj et al. [66] reported the solubility and the bioavailability of clarithromycin has increased in the formulated nanoemulsion system. Nanoemulsion as a drug delivery system improve bioavailability and pharmacokinetic activity of tetracycline [5-11,30, 67].

In conclusion: the obtained results indicated that the nanoemulsion formulation of tetracycline hydrochloride improves pharmacokinetic parameters than usual formula and not affect the antibacterial efficacy. Therefore, the pharmacokinetic/pharmacodynamics pattern of nanoemulsion formulation must be applied intensively as system for drug delivery in veterinary medicine.

#### Ethical approval

The research plan was approved from Cairo University institutional animal care and use committee (CU-IACUC) with approval number CU-II-F-99-18.

#### Conflict of Interest

The authors have no conflict of interests to declare regarding the publication of this paper. Also, the authors declare that the work was self-funded.

#### Authors' Contributions

A.M.A, S.A E. and M.M.A designed and planned this study. M.S.S, S.A E. and M.M.A performs experimental work, collects samples and all laboratory tests. All authors shared samples collection, performing the tests, manuscript writing, drafted, revised the manuscript and approved the final manuscript.

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TC-nm was prepared in Dept. of Pharmaceutical, faculty of Pharmacy, Cairo University. The experimental work of the research was facilitated and completed in department of

pharmacology faculty of veterinary medicine, Cairo University.

#### References

1. Chopra, I. and Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiol. Mol. Biol. Rev.*, **65** (2), 232- 260(2001). doi: [10.1128/MMBR.65.2.232-260.2001]
2. Eliopoulos, G.M., Eliopoulos, G. M. and Roberts, M. C. Tetracycline Therapy: Update. *Clinical Infect. Dis.*, **36** (4), 462-467(2003). <https://doi.org/10.1086/367622>.
3. Prescott, J.F., Baggot, J.D. and Walker, R.D. *Antimicrobial Therapy in Veterinary Medicine*. Iowa State University Press, Ames, 277 p. (2000).
4. Granados-Chinchilla, F. and Rodríguez, C. Review Article: Tetracyclines in Food and Feeding stuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. *Journal of Analytical Methods in Chemistry*. Article ID 1315497, 24 ps(2017). <https://doi.org/10.1155/2017/1315497>
5. Gurpreet, K. and Singh, S.K. Review of Nanoemulsion Formulation and Characterization Techniques. *Indian J. of Pharm. Sci.*, **80**(5)781-789(2018).
6. Severino, P., Andreani, T., Macedo, A.S., Figueiro, J.F., Santana, M.H.A., Silva, A.M., and Eliana, B. Review Article: Current State-of-Art and New Trends on Lipid Nanoparticles (SLN and NLC) for Oral Drug Delivery. *Souto J. Drug Deliv.*, Article ID 750891, 10 ps(2012). doi:10.1155/2012/750891.
7. Vijayan, V., Aafreen, S., Sakthivel, S. and Reddy, K.R. Formulation and characterization of solid lipid nanoparticles loaded Neem oil for topical treatment of acne. *J. Acute Dis.*, **2**: 282- 286(2013).
8. Kalhapure, R.S., Suleman, N., Mocktar, C., Seedat, N. and Govender, T. Nanoengineered drug delivery systems for enhancing antibiotic therapy. *J Pharm Sci.*, **104** (3), 872-905(2015). doi: 10.1002/jps.24298.
9. Singh, K.K. and Vingkar, S.K. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. *Int. J. Pharm.*, **347**. 136-143(2008).
10. Drulis-Kawa, Z. and Jach, A.D. Liposomes as delivery system for antibiotics. *Int. J. Pharm.*, **387**, 187-198 (2009).

11. Sharma, A. K., Arya, D., Dua, M., Chhatwal, G.S. and Johri, A.K. Nanotechnology for targeted drug delivery to combat antibiotic resistance. *Expert Opin Drug Deliv*, **9**, 1325-32 (2012).
12. Hejazi, R. and Amiji, M. Stomach-specific anti-H. Pylori therapy. I: Preparation and characterization of tetracycline-loaded chitosan microspheres. *Int. J. Pharm.*, **235**, 87-94. (2002).
13. Mahmoud, H.A., Melake, N.A. and El-Semary, M.T. Bactericidal Activity of Various Antibiotics versus Tetracycline-loaded Chitosan Microspheres against *Pseudomonas aeruginosa* Biofilms. *Pharmaceut. Anal. Acta.*, **S15**, (2012). doi:10.4172/2153- 2435.S15-007.
14. Ahmad, A., Græsbøll, K., Christiansen, L. E., Toft, N., Matthews, L. and Nielsen, S. S. Pharmacokinetic-Pharmacodynamic Model To Evaluate Intramuscular Tetracycline Treatment Protocols To Prevent Antimicrobial Resistance in Pigs. *Antimicrob. Agents Chemother*, **59** (3), 1634–1642(2015). doi: [10.1128/AAC.03919-14]
15. Teixeira, P.C., Leite, G.M., Domingues, R.J., Silva, J., Gibbs, P.A. and Ferreira, J.P. Antimicrobial effects of a microemulsion and a nanoemulsion on enteric and other pathogens and biofilms. *Int. J. Food Microbiol.*, **118** (1), 15-19 (2007).
16. Mukherjee, R., Patra, M., Dutta, D., Banik, M. and Basu, T. Tetracycline-loaded calcium phosphate nanoparticle (Tet-CPNP): Rejuvenation of an obsolete antibiotic to further action. *Biochimica et Biophysica, Acta*, **1860**, 1929–1941 (2016).
17. Toutain, P.L., Lefebvre, H.P. and King, J.N. Benazeprilat disposition and effect in dogs revisited with a pharmacokinetic / pharmacodynamics modeling approach. *J. Pharmacol. Experimental Therapeutics*, **292**, 1087–1093 (2000).
18. Toutain, P.L. Pharmacokinetics/pharmacodynamics integration in drug development and dosage regimen optimization for veterinary medicine. *AAPS Pharm Sci.*, **4**, article 38 (2002) (<http://www.aapspharmsci.org/scientificjournals/pharmsci/journal/ps040438.htm>)
19. Nouws, J.F.M., Smulders, A. and Rappalini, M. Comparative pharmacokinetics and bioavailability of eight parenteral oxytetracycline-10% formulations in dairy cows. *Vet. Quart.*, **7**, 306-314, (1985).
20. Riond, J.L., Tyczkowska, K. and Riviere, J.E. Pharmacokinetics and metabolic inertness of doxycycline in calves with mature or immature rumen function. *Am. J. Vet. Res.*, **50**, 1329-1333(1989).
21. Meijer, L.A., Ceyskens, K.G.F., Dejong, W.T.H. and Greve, B.I.J.A.C. Three phase elimination of oxytetracycline in veal calves: the presence of an extended terminal elimination phase. *J. Vet. Pharmacol. Ther.*, **16**, 214-222.(1993).
22. Wilson, R.C. and Green, N.K. Pharmacokinetics of minocycline hydrochloride in clinically normal and hypoproteinemic sheep. *Am. J. Vet. Res.*, **47**, 650-652.(1986).
23. Rajaian, H.1 and , E. M. Pharmacokinetics of tetracycline hydrochloride in fat-tailed sheep. *Iranian J. Vet. Res., Shiraz University*, **8**, (2), Ser. No. 19, 138-143 (2007).
24. Escudero, E., Carceles, C. M. and Serrano, J.M. Pharmacokinetics of oxytetracycline in goats: modifications induced by a longacting formulation. *Vet. Rec.*, **135**, 548-552 (1994).
25. Hall, W.F., Kniffen, T.S., Bane, D.P., Bevill, R. F. and Koritz, G.D. Plasma concentrations of oxytetracycline in swine after administration of the drug intramuscularly and orally in feed. *J. Am. Vet. Med. Assoc.*, **194**, 1265-1268 (1989).
26. Wilson, R.C., Kitzman, J.V., Kemp, D.T. and Goetsch, D.D. Compartmental and non-compartmental pharmacokinetic analyses of minocycline hydrochloride in the dog. *Am. J. Vet. Res.*, **46**, 1316-1318 (1985).
27. Rajaian, H., Fazeli, M. and Jalaei, J. Pharmacokinetics of tetracycline hydrochloride after single intravenous injection in dogs. *Iranian J. of Vet. Res., Shiraz University*, **9** (3) Ser. No. 24, 266-270. (2008).
29. Percy, DH and Black, WD. Pharmacokinetics of tetracycline in the domestic rabbit following intravenous or oral administration. *Can. J. Vet. Res.*, **52**, 5-11.(1988).
30. Amer, M. A. , El Badawy A. S., Saber, S.M, Ahmed-Farid , A.O., Abd-Elsalam, H. W. and Amer, M.M. Comparative pharmacokinetics of tetracycline and tetracycline nanoemulsion formula in rabbits. *accepted in RJPBCS*, **9** (1) (2019).
31. Kniffen, T.S., Bane, D.P., Hall, W.F., Koritz, G.D. and Bevill, R.F. Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine. *Am. J. Vet. Res.*, **50**, 518-521(1989).
32. Abdel Aziz, E.A., Khairy, M.H., El-Nabity, S.M., Hamed, E.E. and Saffaf, B.A. Pharmacodynamic and Pharmacokinetic Studies on Tetracycline Hydrochloride in Rabbits. *Zagazig Vet. J.*, **45** (3), 218- 227(2017).



33. Saber, S.M., Amer, M.A., ElBadawy, A.S., Othman, M.A., Ahmed-Farid, A.O and Amer, M.M. Comparative pharmacodynamic and histopathological studies on tetracycline loaded nanoemulsion and tetracycline in rabbits. *RJPR*, **10** (1), (2018).
34. Parasuramanm, S., Raveendran,R.andKesavan, R. Blood sample collection in small laboratory animals. *J. Pharmacol. Pharmacother*, **1** (2), 87-93. (2010).
35. Baby, P.M., Jacob,S.S.,Kumar, R.and Kumar, P. An innovative approach for serial injection in marginal vein and blood collection from auricular artery in New Zealand white Rabbit. *Methods X*,**4**, 457-460(2017).
36. Arret, B., Johnson, D.P.andKirshbaum, A. Outline of details for microbiological assays of antibiotics: Second revision. *J. Pharm. Sci.*,**60**, 1689-1694(1971).
37. Zhang, Y., Huo,M.andXie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine*, **99**, 306-314 (2010).
38. Andrews, J. M. Determination of minimum inhibitory concentrations. *J. Antimicrobial. Chemotherapy*, **48** (6), 5-16(2001).
39. Baggot, J.D.Some aspects of clinical pharmacokinetics in veterinary medicine.*Int. J. Vet. Pharmacol. Ther.*,**1**, 5- 18 (1978).
40. Toutain, P.L.andBousquet-Melou, A. Bioavailability and its assessment. *J. Vet. Pharmacol. Ther.*,**27**,455-466(2004).
41. Abd-El Mawgoud, A.I. Studies on yolk sac infection in baby chicks in El-Fayoum and Beni-suef governorates.M.V.Sc. Thesis(poultry diseases),Fac. Vet. Med.Beni-suef University(2016).
42. Amer, M.M., Mekky, H.M., Amer, M.A.and Fedawy, H.S. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. *Vet. World*, **11**(8), 1082-1088(2018).
43. Mariam-Shokery, H.M., Redwan, I.A.H., Abd El-Ghany, A.W. and Amer, M. M.Molecular detection of antibiotic resistance genes in identified of Coagulase Negative Staphylococci from chickens flocks and hatcheries in Egypt. *Egypt. J. Vet. Sci.*, **49** (1), 58-70 (2018).
44. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26<sup>th</sup> ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute. , 950 West Valley Roadn Suite 2500, Wayne, Pennsylvania 19087, USA (2016).
45. Grove, D. C., and Randall, W. A. Assay Methods of Antibiotics: A Laboratory Manual. New York, Medical Encyclopedia, Inc., 50p. (1955).
46. Andrews, J. M. Microbiological assays. In: Reeves DS, Wise R, Andrews JM, White LO, eds. Clinical Antimicrobial Assays. Oxford: Oxford University Press, pp. 35-44 (1999).
47. White, L.O.andLovering, A. M. Non-microbiological assays. In: Reeves D.S., Wise R., Andrews J.M., White L.O. (Ed.) Clinical Antimicrobial Assays. Oxford: Oxford University Press, 45- 64 (1999).
48. Berté, F. and Vandoni, G. On the intestinal absorption and organotropism of some tetracyclines. *Chemotherapy*, **5**, 219-230 (1962).
49. Kanegis, L. The comarative pharmacology of tetracyclines: initial studies on serum levels and urinary excretion of antibiotic A-VIII following single oral and intravenous doses in the dog. *Report P.R.*, **4**, 884-921(1958).
50. Martindale. The Extra pharmacopoeia, 29<sup>th</sup> ed., Reynolds, J.E.F. (Ed.), London, The Pharmaceutical Press, (1989).
51. Agwuh, K. N. and MacGowan, A.Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylyclines. *J. Antimicrob. Chemother.*, **58**, 256-265 (2006).
52. Faghihi, M.The disposition of tetracycline in sheep. *MS Thesis, The University of Illinois, USA* (1980).
53. Eisner, H.J., Stirn, F.E., Dornbush, A.C.andOleson, J.J. The enhancement of serum levels of Aureomycin experimental animals. *J. Pharmacol. Exp. Ther.*, **108**, 442- 449 (1953).
54. Neuschl, J. Comparison of some pharmacokinetic parameters of tetracyclines which are most frequently used in veterinary medicine. *Arch. Exp. Veterinarmed.*, **45**, 105-112 (1991)
55. Nielsen,P.andGyrd-Hansen, N. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. *Vet. Pharma.and Therap.*, **19** (4), 305-311 (1996).

56. He, W., Tan, Y., Tian, Z., Chen, L., Hu, F. and Wu, W. Food protein-stabilized nanoemulsions as potential delivery systems for poorly water-soluble drugs: preparation, in vitro characterization, and pharmacokinetics in rats. *Inter. J. of Nanomed.*, **6**, 521–533 (2011).
57. Mishra, R.K., Soni, G. C. and Mishra, R. Nanoemulsion: A Novel Drug Delivery Tool. *Inter. J. of Pharma.Res. & Review*, **3** (7), 32-43 (2014).
58. Steigbigel, N.H., Reed, C.W. and Finland, M. Absorption and excretion of five tetracycline analogues in normal young men. *Am. J. Med. Sci.*, **255**, 296- 312 (1968).
59. Sjolín-Forsberg, G. and Hermansson, J. Comparative bioavailability of tetracycline and lymecycline. *Br. J. Clin. Pharmacol.*, **18**, 529- 533 (1984).
60. Kunin, C. M., Dornbush, A.C. and Finland, M. Distribution and excretion of four tetracycline analogues in normal young men. *J. Clin. Invest.*, **38**, 1950- 1959 (1959).
61. NCCLS. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing -11<sup>th</sup> Informational Supplement. *NCCLS Document M100-S11*, 2001, **21** (1), 156-163 (2001).
62. Coyle, M.B. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. Washington DC., (2005).
63. Kowser, M. M., Hoque, M. M. and Fatema, N. Determination of MIC and MBC of selected tetracycline capsule commercially available in Bangladesh. *The ORION Medical J.*, **32** (3), 684-686 (2009).
64. Amer, M.M., ELbayoumi, Kh. M., Zeinab, M.S. Amin Girh, Hoda M. Mekky and Nagwa S. Rabie. A Study on Bacterial Contamination of Dead in Shell Chicken Embryos and Culled One Day Old Chicks. *Inter. J. of Pharmac. and Phytopharma. Res. (EIJPPR)*, **7** (2), 5- 11 (2017).
65. Devarajan, V. and Ravichandran, V. Nanoemulsion: as Modified Drug Delivery Tool. *Pharmacie Globale: Int. J. of Comprehensive Pharmacy*, **4** (1), 1-6 (2011).
66. Fadli, A., Nizam, A. G., Ibrahim, A., Nurulnadiyah, A.K., Jiyuddin, K., Samer, A.D., Budiasih, S., Kaleemullah, M., Jawad, A., Rasha S., Todo, H., Sugibayashi K. and Eddy, Y. Formulation of tetracycline nano-emulsion. *World J. of Pharmac. Res.*, **4** (4), 134-144. (2015).
67. Vatsraj, S., Chauhan, K. and Pathak, H. Formulation of a Novel Nanoemulsion System for Enhanced Solubility of a Sparingly Water Soluble Antibiotic, Clarithromycin. *J. of Nanoscience*, 7 pages (2014). <http://dx.doi.org/10.1155/2014/268293>
68. Sharma, S., Sahni, J. K., Ali, J. and Baboota, S. Effect of high-pressure homogenization on formulation of TPGS loaded nanoemulsion of rutin-pharmacodynamic and antioxidant studies. *Drug Deliv.*, **22**, 541–551 (2014).
69. Yen, C. C., Chen, Y.C., Wu, M.T., Wang, C.C. and Wu, Y.T. Nanoemulsion as a strategy for improving the oral bioavailability and anti-inflammatory activity of andrographolide. *Int. J. Nanomed.*, **13**, 669 - 680 (2018).

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## دراسات دوائية على صيغ التتراسيكلين والمستحلب النانومتري للتيتراسيكلين

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أجريت هذه الدراسة لمقارنة المسار الحركي والديناميكيه الدوائية لىصيغتي للتتراسيكلين و المستحلب النانومتري للتيتراسيكلين بعد اعطاؤه بجرعة واحده مقدارها ٥٠ ميليجرام / كيلوجرام من وزن الجسم عن طريق الفم والحقن الوريدي في الارانب ودراسة تأثيرهما علي عترات بكتيرييه عياريه و حقيقيه، بدأ تركيز الدواء في البلازما عند ٠,٢٥ ساعة ووصل إلى الحد الأقصى عند نصف ساعه في التتراسيكلين وساعه في المستحلب النانومتري ليصل للحد الأدنى عند ١٢ ساعه من اعطاء جرعه واحده. اتبع الحقن الوريدي للتتراسيكلين اعلى معدلات الانتشار ( V2 )  $0.292 \pm 0.111$  لتر / كجم في المستحلب النانومتري للتيتراسيكلين ( TC-nm ) من  $0.216 \pm 0.183$  لتر / كجم في التيتراسيكلين ( TC-hcl ) وتم تنقيه البلازما من المستحلب ببطء  $0.393 \pm 0.183$  (لتر ساعة / كجم ) عن التيتراسيكلين  $(0.415 \pm 0.311)$  (لتر ساعة / كجم ) في التتراسيكلين.

اتبع اعطاء الدواء بالفم امتصاصا سريعا و امتصاص معنوي بطئ نصف عمر  $t_{1/2}^{\alpha}$   $0.550 \pm 0.090$  ساعة و  $0.176 \pm 0.058$  ساعة ) و استخلاص نصف عمر  $t_{1/2}^{\beta}$   $(4.215 \pm 1.661)$  ساعة و  $1.58 \pm 1.447$  ساعة ) مع ارتفاع  $C_{max}$   $(1.661 \pm 1.447)$  ميكرو جرام / مللي و  $1.58 \pm 1.447$  ميكرو جرام / مللي ( منعكسا على قيمه  $t_{max}$  المحسوبة  $(0.759 \pm 0.149)$  ساعة و  $0.356 \pm 0.305$  ساعة ) في المستحلب عن الصيغة العادية في الارانب المعالجة، على التوالي.

كانت قيم الحد الأدنى للتركيز المثبط للبكتيريا متساويه في كل من التتراسيكلين العادي و المستحلب النانومتري للمكور العنقودي البرتقالي ٦٥٣٨ و المكور العنقودي البشراوي ١٢٢٢٨ و المكروب القولوني ٨٧٣٩ هي 0.14 و 0.8 و 0.12 ميكروجرام ، على التوالي وتم تقييمها على انها حساسه. اما العترات الحقيقيه الحساسه من انواع الوتديتو الميكروب القولوني و السلمونيلا التيفودييه و السلمونيلا المعويه و المكور العنقودي لينتس فكانت قيمها ١ و ٤ و ٨ و ٢ و ٦ و ٢ ، على التوالي. اما القيم في عتراتي المكور العنقودي سيريس (١٨ و ١٦ مللجرام) وعترتي الزيلبيوس (١٨ و ٦ مللجرام) فتأكد مقاومتهم للصيغتي الدواء. تشير النتائج ان صياغه التتراسيكلين في مستحلب نانومتري لم لا تؤثر على فعالية كمضاده للجراثيم البكتيرييه.

في الختام: أشارت النتائج المتحصل عليها إلى أن صيغة مستحلب من هيدروكلوريد التتراسيكلين يحسن قياسات الحراك الدوائي عن الصيغة العادية ولا يؤثر على الفعالية المضادة للبكتيريا. ولذلك ، من الممكن تطبيق هذا النمط من صياغة مستحلبات النانو كنظام لإيصال الدواء بشكل في الطب البيطري.