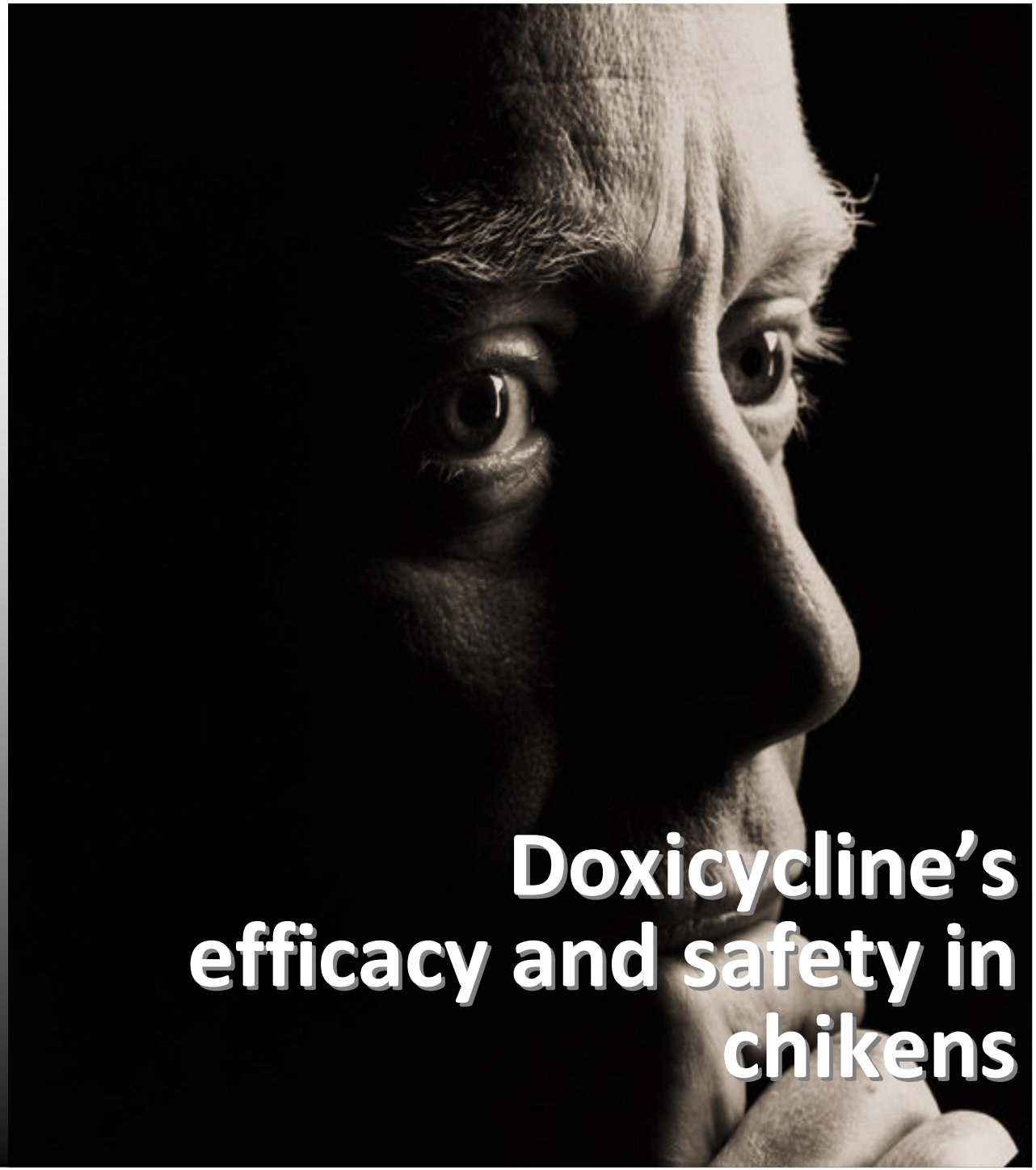

Doxicycline's efficacy and safety in chikens

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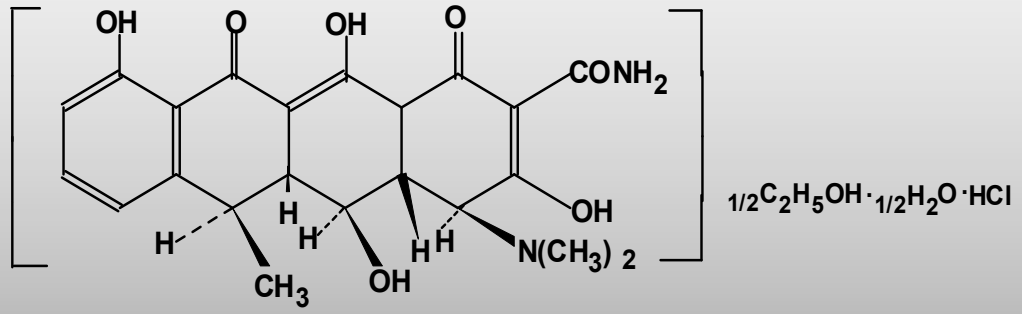
Doxicycline hyclate



A structural isomer, who meets the:

- ►EU's reference standards (Ph.Eur. III 1997: 0272) and respects:
- ►C.E.E. Regulations **(No. 2377/90),** for MRL (Maximum Residue Limits) and also:
- ►latest MRL-regulations: Commission Regulation (EU) No **37/2010** of 22/12/2009, (OJ L15/1 of 20.1.2010).
- ► Molecular formula: **$C_{22}H_{25}ClN_2O_8$, ½ C_2H_6O, ½ H_2O**
- ► Molecular mass: **M: 512,9**

Aim of the study

- Assessment of doxicycline's hyclate efficacy in broilers in a colisepticemia outbreak.
- Safety evaluation for an a.u.v. doxicycline, destined for broiler chickens in water administrations.
- Ascertaining the clinical effects and tolerance consecutive to risen doses administration, as part of safety study for this antibiotic in poultry.

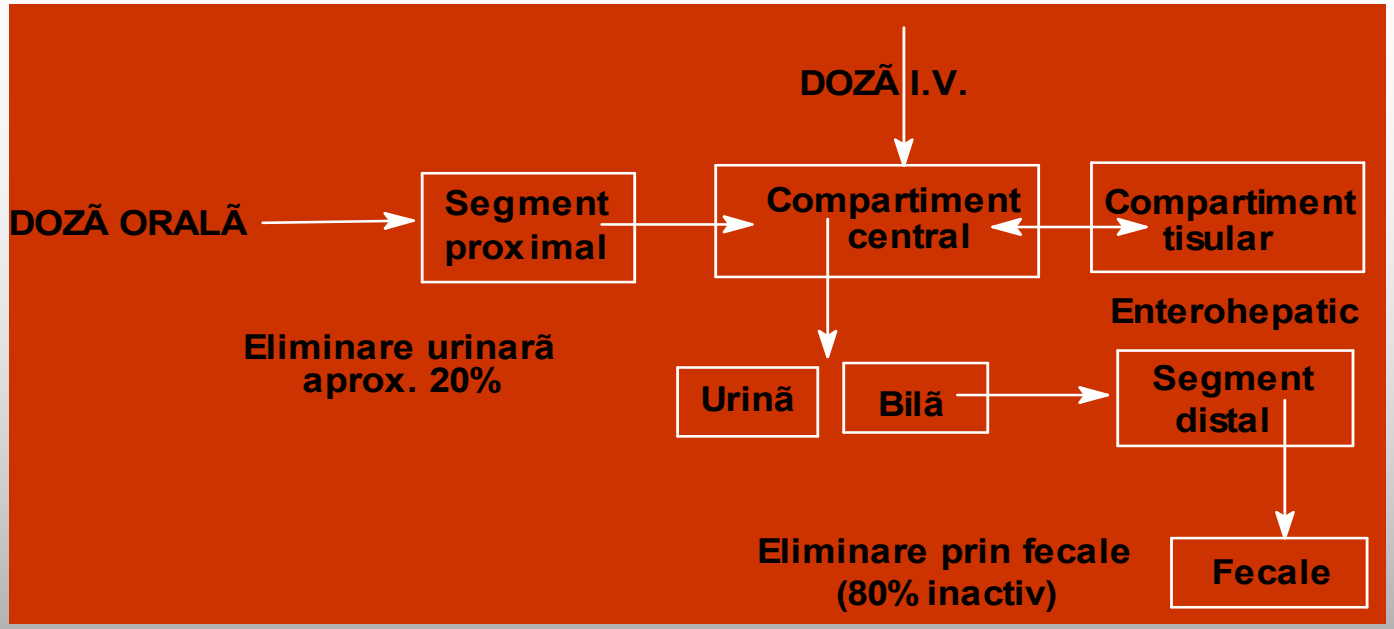
Pharmacologic activity

- ►Doxicycline is an inhibitor of protein synthesis in sensible organisms and it traverse directly through the bilipidic layer of bacterial wall.
- ►Once reached in the bacterial cell, doxicycline is coupling to the 30S ribosomal unit inhibiting the bacterial complex named *tARN-aminoacyl-mARN-ribosomal*, implied in the translation phase.
- ►Coupling to ribosomes assure the transport in the bacterial cell's interior, being considered a biphasic process:

  I: plasmatic absorption

  II: active transport process.
- ► Additionally, effects on leukocytes (chemotaxis and phagocytary inhibition) are also registered.

Doxicycline's pharmacokinetic model



Tetraciclyne's digestive absorbtion rate

Compound	Absorbed rate
Doxicycline	93 %
Tetracycline	77 – 80 %
Dimethylchlortetracycline	66 %
Metacycline	58 %
Oxitetracyclina	58 %
Chlortetracyclina	25 – 30 %

Therapeutic indications

Spectrum and sensibility of microorganisms to doxicycline hyclate

Microorganism	Great sensitive (CMI_{90}<4µg/ml)	Medium sensitive (CMI_{90}<16µg/ml)	Resistant species (CMI_{90}>16µg/ml)
Gram negative	*Haemophilus* *Neisseria* *Brucella* *Campilobacter* *Moraxella* *Yersinia* *Pasteurella* *Vibrio*	*Klebsiella* *Escherichia* *Salmonella* *Enterobacter* *Serratia* *Shigella* *Bordetella*	*Proteus* *Pseudomonas*
Gram positive	*Staphylococcus* *Corynebacterium* *Bacillus* *Listeria*	*Streptococcus*	-
Anaerobic bacterias	*Fusobacterium* *Actinomyces* *Propionibacterium*	*Bacterioides* *Clostridium* *Lactobacillus*	-
Other microorganisms	*Spirocheta* *Mycoplasma* *Rickettsia* *Chlamidia* *Ureaplasma* *Erlichia* *Anaplasma*	-	-

Sensibility to doxicycline of poultry isolates

In vitro	Sensibility to doxicycline	Source
Chicken isolates : *E.coli, Staphylococcus, Streptococcus, Pasteurella, Enterobacter, Pseudomonas, Bacillus, Klebsiella*	To numbered genus: 25% sensibility	Ramasastry, 1991 Bousquet, 1994
Campylobacter coli	100%	EMEA
Campilobacter jejuni	98%	
E. coli 65	Low sensibility	Prasad, 1997
Salmonella gallinarum	43%	Ramasastry, 1991
Salmonella enteritidis	98%	
Tukey isolates: *Chlamidia psittaci*	100%	EMEA

General description

- ►Doxicycline a.u.v. is a soluble powder suitable for administrations in drinking water to poultry.
- ►Before treatments chickens will be deprived by water, introducing the fluid diet.
- ► Doxicycline is diluted in a water quantity required for 3 hours and in the necessary of water amount calculation ambient and body temperature of animals must be considered.

Experiment I.

Research methodology:

- Disease cases identifying and lots' setting
- Etiologic diagnosis
- Antibiogram (difusometric method)
- Product administration after recommendations
- Clinical monitoring after treatments
- Weight gain evolution monitoring

The animals

- ►Three week old Cobb hybrids in a 3,000 heads farm, where broilers are grown on the ground
- ► Epidemiological investigation = favorising combined zoo-hygiene deficiencies, moisture, agglomeration, temperature variations and thermal discomfort
- ►Disease started at 20 days broiler's age and mortality rate reached at 32.5%
- ►Clinical signs: lack of appetite and polydipsia
- ►Colisepticemia confirmed by morphopathologic & bacteriologic exams

The experiment's setting

- In the hall was built a 4 x 5 m pen, where 120 chickens, all with clinical colisepticemia signs, were randomly placed for the experiment.
- 12 chickens constituted the Control lot who do not received any treatment, only clinic evolution was monitorized.
- Chickens were fed *ad libitum* with a balanced mixed fodder: CC 1115 *(Vivabio)*.
- Watering was done at the discretion.

- Bacteriological examination revealed numerous *E. coli* strains (11 strains) presence
- The behaviour to antibiotics of isolated bacterial strains and antibiogram (Sanofi) was accomplished
- Used micro tablets were:

  - Colistin sulphate,
  - Neomycin,
  - Amoxiklav,
  - Enrofloxacin,
  - Norfloxacin,
  - Erythromycin,
  - Spectinomycin and
  - Doxicycline

- *E. coli* have proven to be highly sensitive to:

  - Colistin sulphate & doxicycline

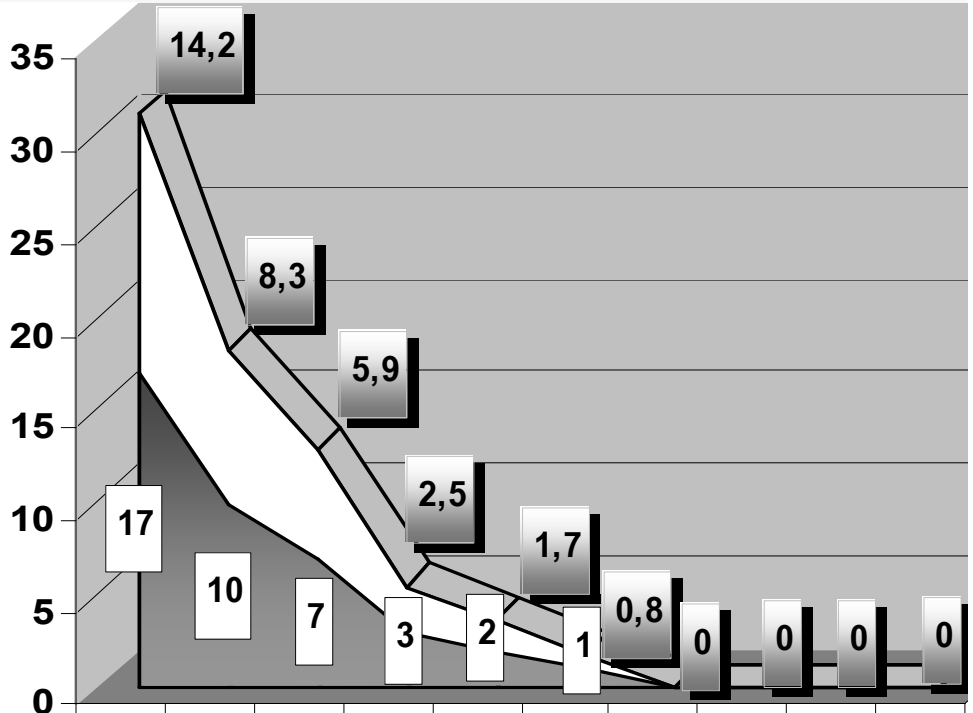
- Treatments duration: five continuous days
- Dosage: 10 mg x kg.bw.-1, p.o. way in water
- Monitoring period: ten days after treatments' starting

Results and discussion

Favorable clinical evolution was recorded after the third day of treatment, (in the seventh day mortality being totally stopped), at the end of experiment from 120 remaining live 80 chickens, (representing 66.7%).

After 5 days of treatment when the clinical signs have entirely disappeared

In the untreated Control group, all chickens have died until fourth day.



	I	II	III	IV	V	VI	VII	VIII	IX	X
□ %	14,2	8,3	5,9	2,5	1,7	0,8	0	0	0	0
■ Pui morți	17	10	7	3	2	1	0	0	0	0

Losses' evolution in the studied group ■ = dead chicken

Average daily weight gain, fodder and water daily consumption/studied period

Lot in day	Total weight/ Lot (g)	Average Weight (g)	Average daily body gain /period (g)	Average daily fodder consumption (g)	Average daily watter consumption (ml)
Day 20 120 heads	73800	655	30,75	56 (≅ 6,5 kg)	≅ 200
Day 30 40 losses	75280	941	24,60	110 (8,80 kg)	300
Day 40 80 remained	119600	1495	51,40	170 (13,60 kg)	370
Day 50 Slaughtering	174400	2180	68,50	190 (15,20 kg)	450

Conclusions

- Doxicycline in dose of 10 mg x kg.b.w.-1 administered for five days resulted in a complete clinical and bacteriological healing of the broilers with colisepticemia and recovered in day ten.
- In all cases, therapy is preferable, even if the economic parameters are significantly lower than disease-free situations.
- After treatments, the clinical and biological recovery was evidenced also by the evolution of weight gain in the remaining chickens.

Experiment II.

The studied compound:

Doxicycline hyclate	0,30 g.
Excipients q.s. ad	1,00 g.

Animals:

Tolerance and effects estimation consecutive to:
x2, x3 and x5 times therapeutic doses as follows:

- ► **Experimental lot 1 (E1):** therapeutic dose = 10mg x kgbw-1 /day
- ► **Experimental lot 2 (E2):** two times the therapeutic dose
- ► **Experimental lot 3 (E3):** three times the therapeutic dose
- ► **Experimental lot 4 (E4):** five times the therapeutic dose
- ► **Control lot (C):** no drug administration, only water

► **Administrations:** individually, p.o.,

Health status established after:

- ► **Clinic examination:** general and local tolerance, appetite
- ► **Paraclinic examination:** haemoleucograme, erythrocytes, leucocytes, haemoglobin, haematocrit, leucograme;
- ► **Sanguine biochemical exam:** total proteins, albumins, globulins, creatinine, uric acid and enzyme values for: ASAT, ALAT and ALP.
- ► **Histologic and morphopathologic examinations**

Methods:

- ► **Haematologic examination** (haemoleucograme) was accomplished with **MS-9-VET automatic analyzer**
- ► **Biochemical examination** with **VET SCREEN** semi automatic analyzer.
- ► **Tissues** (liver, kidney and spleen) were fixed in 80c alcohol, included in paraffin, sectioned to 5µ and H.E., respectively Mallory tri-chromic coloration.

Results and discussions

Daily b.w. medium gain, daily fodder consumption and daily water consumption in the studied lots			
Lot	Daily b.w. medium gain (g)	Daily fodder consumption (g)	Daily water consumption (ml)
E_1	17,06	34,5	1,6
E_2	13,98	33,78	67,6
E_3	13,66	33,78	52,51
E_4	13,42	29,58	51,9
C	17,7	34,5	72,96

Note: Accepted reference values are:

Daily body weight gain (for Cobb, hybrid Hubbard broilers): 23,0 g, 16,7 g

Daily fodder consumption: 21 g, 24-40 g, 32,2 g.

Daily water consumption: 76-100 l/1000 chicken.

Haemoleucograme for the broilers treated with doxicycline

Specification	Arithmetic mean ± Average mean error				
	E_1	E_2	E_3	E_4	C
Erythrocytes (x 10^{12}/l)	2,33 ± 0,09	2,28 ± 0,063	2,24 ± 0,06	2,06 ± 0,076	2,52 ± 0,082
Haematocrit (%)	31,75 ± 0,77	30,8 ± 1,23	29,7 ± 1,10	28,75 ± 0,92	32,3 ± 1,42
Haemoglobin (g/100 ml)	10,75 ± 0,33	10,4 ± 0,24	10,7 ± 0,27	10,9 ± 0,36	10,15 ± 0,17
Leucocytes (x 10^9/l)	19,03 ± 1,6	18,4 ± 1,71	18,58 ± 1,43	19,68 ± 1,22	18,69 ± 0,92
Lymphocytes (%)	92,10 ± 2,42	93,05 ± 3,13	92,85 ± 2,32	92,2 ± 2,84	91,5 ± 1,92
Granulocytes (%)	4,35 ± 0,51	3,45 ± 0,36	3,7 ± 0,45	4,35 ± 0,43	4,6 ± 0,66
Monocites (%)	3,55 ± 0,62	3,5 ± 0,73	3,5 ± 0,48	3,55 ± 0,53	3,55 ± 0,7

Note: accepted reference values (x ± DS) for broilers are:

- erythrocytes 2,35 ±0,25;
- haematocrit 26 ±4;
- haemoglobin 7,3 ±1,3;
- leucocytes 26 ±4;
- lymphocytes 63 ±10;
- granulocytes 35,5 ±7,8;
- monocites 6,1.

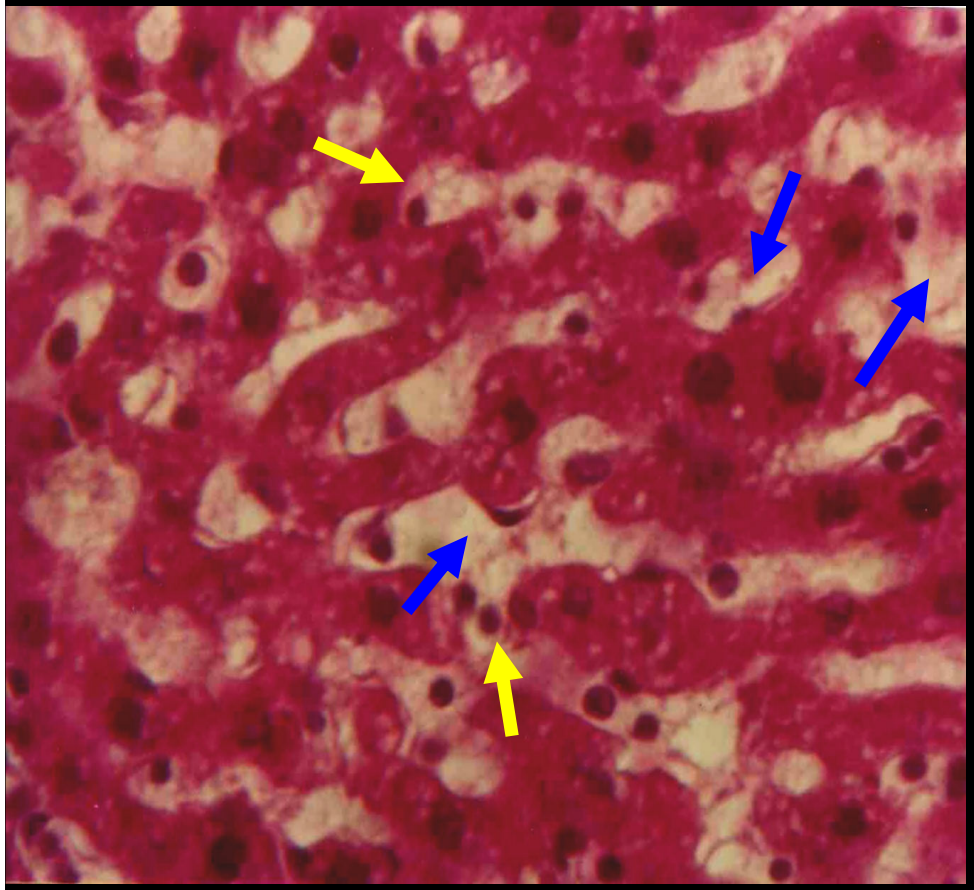
Morpho-histopathological investigation

Registered morphopathological modifications consisted in:

E2
- light hepatomegaly
- growth in volume of the caecal sacs

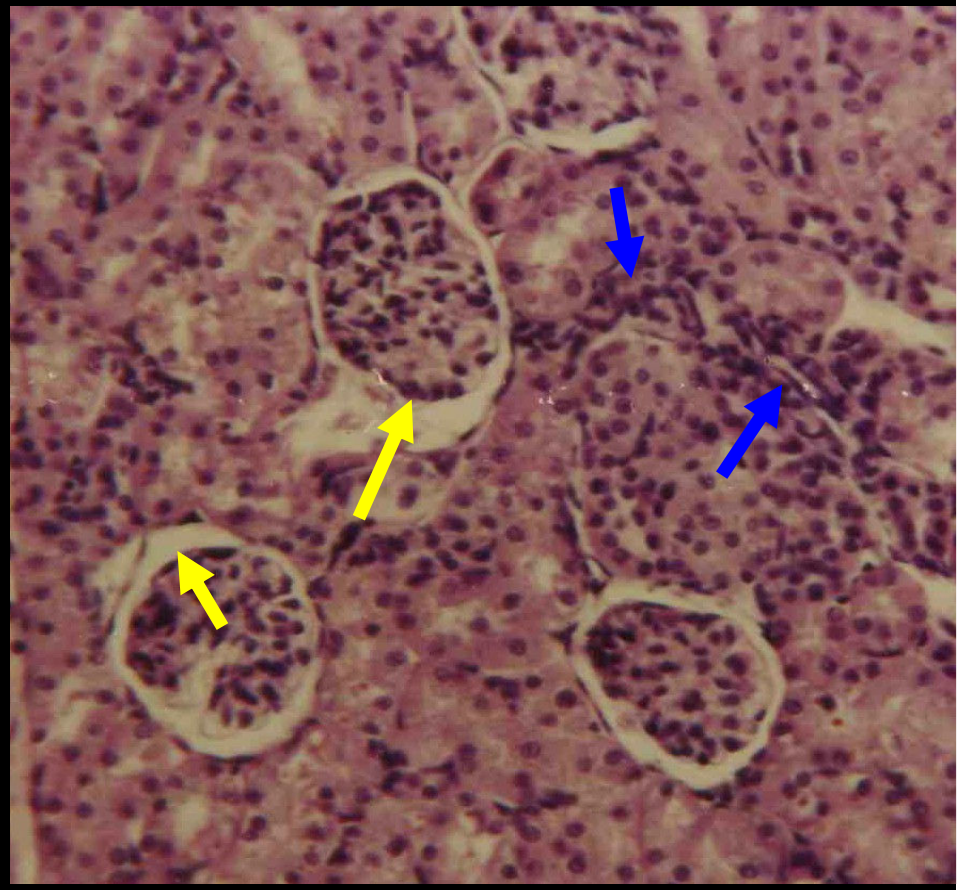
E3
- light hepatomegaly
- low intensity and diffuse zones of hepatic degenerescence
- light splenomegaly
- intestinal vascular ectasia
- caecal sacs distension and gaseous content

E4
- hepatomegaly
- extended but diffuse zones of hepatic degenerescence
- light splenomegaly
- distension of caecal sacs; brown-yellowish gaseous content.



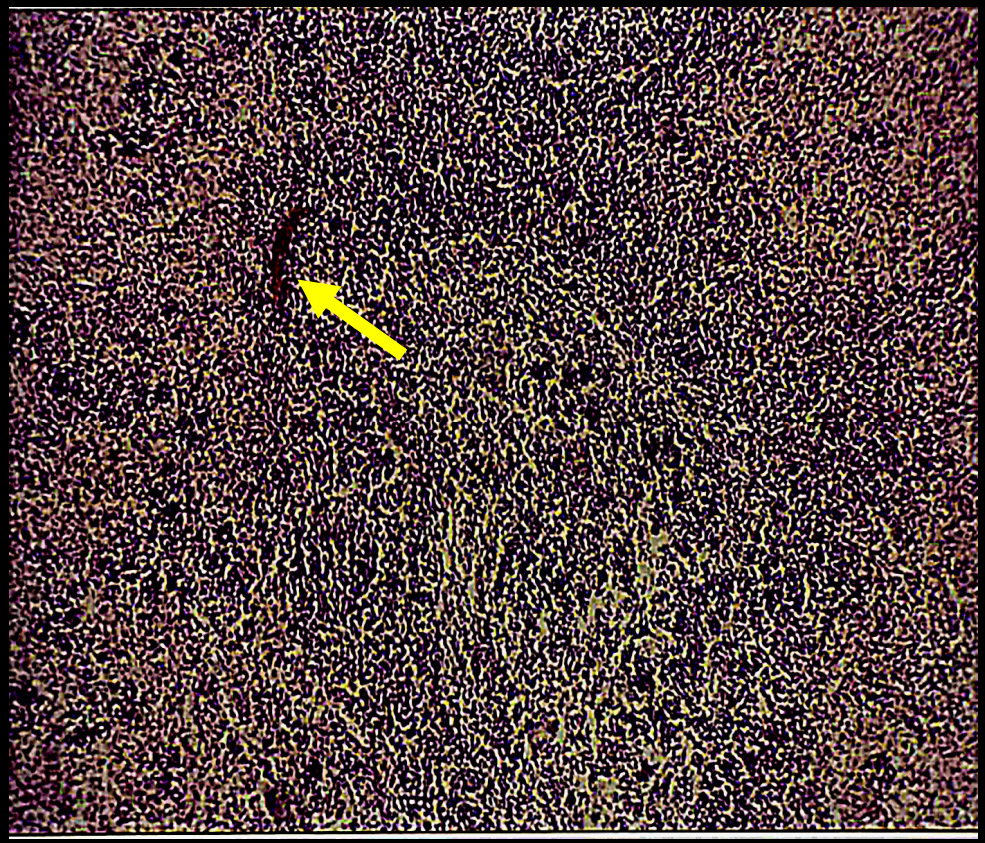
Histologic section through hepatic tissue from E4

Nuclear heterochromatinization (picnotic nucleous - necrobiosys (yellow)
Hepatocite's vacuolar degenerescence (blue)



Histologic section through kidney tissue from E4

Renal corpuscles degenerescence, with Bowman's capsule altering (yellow)
Red marrow increasing with erythrocytes' abundance (blue)



Histologic section through spleen in E4 lot:
Spleen's vascular ectasia

Conclusions

- Good local and general tolerance to therapeutic (10 mg x kgb.w.-1) and to the x2 doses
- Diarrhoea presence to lots which received x 3 and x 5 times than therapeutic dose
- Haemoleucogram does not suffer evident changes comparatively with C lot, values being between the reference limits (exception- leucocitary formula)
- Evident increase comparatively to the control lot of creatinine's concentration consecutively to x3 and x5 greater doses administration without passing the reference values limits;
- Marked increasing of ASAT level and limited for ALAT, comparatively to control lot in the case of E4 lot, but light to reference values;
- Macro and microscopic changes especially for liver and renal samples to the individuals from the x3 and x5 therapeutic doses lots.


CHEERS!