

# **PROCEEDINGS OF THE 12<sup>th</sup> WORLD RABBIT CONGRESS**

Nantes (France) - November 3-5, 2021 ISSN 2308-1910

## Session REPRODUCTION

#### Khaldoun Oularbi H.. Makhlouf C., Bokreta S., Settar A., Tarzali D., Zitouni G., Hamadou D., Kais S., Daoudi-Zerrouki N.

AMPLIGO® INSECTICIDE INDUCES INJURIES ON THE TESTES OF RABBIT ORYCTOLAGUS CUNICULUS: ALLEVIATING EFFECTS OF VITAMINS C AND E (ASCORBATE/α-TOCOPHEROL)

> Full text of the communication + Slides of the oral presentation

How to cite this paper

Khaldoun Oularbi H.. Makhlouf C., Bokreta S., Settar A., Tarzali D., Zitouni G., Hamadou D., Kais S., Daoudi-Zerrouki N., 2021. Ampligo® insecticide induces injuries on the testes of rabbit *Oryctolagus cuniculus* : alleviating effects of vitamins C and E (ascorbate/α-tocopherol). Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication R-13, 5 pp.

### AMPLIGO® INSECTICIDE INDUCES INJURIES ON THE TESTES OF RABBIT ORYCTOLAGUS CUNICULUS: ALLEVIATING EFFECTS OF VITAMINS C AND E (ASCORBATE/α-TOCOPHEROL)

# Khaldoun Oularbi H<sup>1. 2\*</sup>. Makhlouf C<sup>1,2</sup>., Bokreta S<sup>1,2</sup>., Settar A<sup>1</sup>., Tarzali D<sup>1</sup>., Zitouni G<sup>3</sup>., Hamadou D<sup>2</sup>., Kais S<sup>2</sup>., Daoudi-Zerrouki N.<sup>2</sup>

<sup>1</sup>Department of Biology, Faculty of Nature and Life Sciences, University Blida 1, BP 270. Soumaa. Blida. Algeria <sup>2</sup>Natural Resources Laboratory, University Mouloud Mammeri, BP 15017. Tizi-Ouzou. Algeria <sup>3</sup>Technical Breeding Institute (Institut Technique d'Elevage (ITELV), 16111 Baba Ali, Alger, Algeria <sup>\*</sup> Correspondance Khaldoun Oularbi Hassina : e-mail : khaldounhassina@hotmail.fr

#### ABSTRACT

This study was conducted to evaluate the potential reproductive toxicity induced by "Ampligo® 150 ZC" (AP) (Chlorantraniliprole 9.3% + Lambda cyhalothrin 4.6% ZC) in male rabbits and to examine the protective effect of vitamins C and E (CE) against AP hormonal and histological toxicity. Twenty male rabbits "*Oryctolagus cuniculus*" were divided into four groups: Control, vitamins C and E (CE), ampligo (AP) and AP plus CE (AP+CE) groups. Testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured from the serum, while the testes tissue samples were used for histopathological examinations. Ampligo exposition induced decreased body weight gain and reproductive organs testes and epididymis weights. This study revealed no changes in serum hormonal LH and FSH concentrations, while, testosterone concentration were found significantly reduced in AP treated group than the other groups. Our observations also showed that treatment with AP significantly decreased the morphometrycal parameters of seminiferous tubules. Histomorphometrycal examination revealed a decrease in germinal layer thickness, disorganization of seminiferous tubules, degeneration of the epithelium and congestion. Vitamins C and E supplementation with AP significantly reversed the above mentioned damages. The present results indicate that vitamins C and E combination exerts curative effects against Ampligo®-induced male reproductive toxicity.

Key words: Ampligo 150ZC. Vitamins C and E, Hormonal Parameters, Testicle Histology, Rabbits

#### **INTRODUCTION**

The causal factors of male infertility consist of internal and external factors able to disrupt the spermatogenesis; principally exposure to environmental chemicals such as pesticides (insecticides) (Fang et al., 2013), heavy metals (Yamaguchia et al.2009), high temperature, smoking, stress, alcohol and obesity (Shrem et al., 2019). Synthetic pyrethroids have been considered potentially toxic to male reproductive system (Kilian et al., 2007), they have ability to disrupt estrogen signaling and affect male reproductive organs and semen quality. Exposure to pyrethroids causes decreased sperm counts, impairment of sperm motility, reduced fertilization ability, producing abnormal sperm in the rodents following repeated exposure (Wang et al., 2010). Ampligo® 150 ZC" (AP) is a new insecticide formulation containing chlorantraniliprole 9.3% and lambda cyhalothrin 4.6%. Chlorantraniliprole is an anthranilic diamide insecticide while lambda cyhalothrin (LCT) is a type II pyrethroid insecticide widely used in pest management. During pyrethroid metabolism, reactive oxygen species (ROS) were generated and caused oxidative stress in intoxicated animals (Yousef et al., 2006). Antioxidants can protect against the damaging effect of oxygen species on male infertility. Numerous vitamins have proven beneficial in treating male reproduction, such as vitamin C (khaldoun oularbi et al., 2014; khaldoun oularbi et al., 2017), vitamin E, folic acid (Yousef et al., 2006) vitamin E (Mohamed et al., 2014). Ascorbic acid is a water-soluble antioxidant which has potential protective effects on oxidative stress and environmental toxicities (Guo et al., 2016). Vitamin E is a potent lipid soluble antioxidant in biological systems that play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors (Yousef *et al.*, 2010). The main aim of this study was (1) to assess the toxic effect of a new insecticide formulation Ampligo  $\mathbb{B}$  on rabbit's testicle histology and endocrine testicular functions and (2) to investigate the Protective effect of vitamins C and E combination against ampligo  $\mathbb{B}$  reproductive toxicity in male rabbit.

#### MATERIALS AND METHODS

#### Animals and experimental design

Twenty male adults rabbits "*oryctolagus cunuculus*" (2.5 - 2.9 Kg) were obtained from the Technical Breeding Institute (ITELV, Baba-Ali) and kept for experimentation in the CRD Saidal Algeria. The rabbits were acclimated for a period of 3 weeks and had free access to food and water *ad libitum*. Animals were randomly distributed into four groups : (1) control group; (2) CE group, rabbits were given Vit E 200 mg/kg + Vitamin C 200 mg/kg; (3) Ampligo ® group (AP), rabbits were administered ampligo (at dose that containing 12.24 mg/kg per day LCT and 24.48 mg/kg per day); (4) in group AP+CE, rabbits were given the same doses of vitamins C and E as in group CE 12h after the administration of ampligo treatment of rabbits.

#### **Chemical Analyses**

On the day 21 after treatment, rabbits were sacrificed by decapitation and blood samples were collected. Plasma was obtained by centrifugating 5 mL of blood per rabbit at 2.000 rpm for 15 minutes. Samples were then frozen and stored at -20 degrees Celsius until processing. Plasma concentrations measured included: LH (ref: 11775863122), FSH (ref: 11732234122) and testosterone (ref: 05200067190), by electrochemiluminescence immunoassay (ECLIA). This assay is based on the principle of competitive union using a monoclonal antibody with reagents for the Cobas e-411 immunology analyzer (Roche). The whole testes and epididymis were weighed than testes were dissected out, collected, fixed in 10% neutral buffered formaldehyde solution for histological examination, processed, sectioned (2-3 $\mu$ m) and stained with hematoxylin and eosin (HE). For morphometrycal analysis, digital images of testicular parenchyma were obtained by photomicroscope in order to determine the diameter of the area of seminiferous tubules, the epithelium height and the luminal diameter of the seminiferous tubule using the Axio Vision Ziess image analysis software.

#### **Statistical Analysis**

All statistical analyses were performed using Statistica version 10.0 (Stat Soft Inc., Tulsa, Oklahoma, USA). Data were calculated using one-way analysis of variance followed by the Duncan's post hoc tests. Data were expressed as the mean  $\pm$  SD. A p-value < 0.05 was considered as the level of significance.

<b>Table 1</b> : Body weightcontrol, CE, AP and AP	gain (% BWG P+CE-treated gro	), average feed oups, 21 days af	l and ter t	d water intake i reatment.	in rabbits from	
Groups / Period	CONTROL	CE		AP	AP+CE	
Initial BW (Kg)	$2.70\ \pm 0.02$	$2.65 \ \pm 0.03$		$2.61\pm0.06$	$2.83\pm0.02$	
Final BW (Kg)	$3.04 \pm 0.02$	$2.99 \ \pm 0.28$		$2.81\pm0.05*$	$3.16\pm0.34$	
% BWG	3,38	3,45		1,99*	3,29	
Absolute : Testis + epididymis Weight (g)	$5.32\pm0.03$	$4.99\pm0.41$		$4.32 \pm 0.16^{*}$	6.1 ± 0.2	
Relative : Testis + epididymis Weight	0.18 ± 0.01	$0.17\pm0.02$		0.13 ± 0.01*	0.19 ±0.02	
Average feed intake (g / rabbit)						
Acclimatation	$98.2\pm5.1$	$97.4\pm7.7$	97.4 ± 7.7		$98.7\pm6.3$	
Experimentation	$164.0 \pm 4.6$	$185.5 \pm 10.1$	l	$159.9\pm13.0^*$	$176.4 \pm 6.5$	
Average water consumption (ml / rabbit)						
Acclimatation	$31.2\pm0.7$	30.8 ± 2	2.6	31.6 ± 1.5	34.3 ± 1.6	
Experimentation	$138.2 \pm 5.1$	117.2 ± 6	5.9	100.08±8.08*	106.8±7.33	
Results are given as a mean $\pm$ SD for five rabbits in each group. * p < 0.05.						

#### **RESULTS AND DISCUSSION**

Effects of treatments on food intake, water consumption, body and Testes and epididymis weights: In ampligo-treated rabbits food intaka

rabbits, feed intake and water consumption were reduced when compared with controls. The decrease in the body weight of treated rabbits with ampligo appeared as a result of lesser intake of feed. Significant

<b>Table 1</b> : Body weigfrom control, CE, Al	ht gain (% BV P and AP+CE	VG), average f -treated groups	eed and water in s, 21 days after tr	take in rabbits eatment.		
Groups / Period	CONTROL	CE	AP	AP+CE		
Initial BW (Kg)	$2.70 \pm 0.02$	$2.65 \pm 0.03$	$2.61\pm0.06$	$2.83\pm0.02$		
Final BW (Kg)	3.04 ± 0.02	2.99 ± 0.28	$2.81\pm0.05*$	$3.16\pm0.34$		
% BWG	3,38	3,45	1,99*	3,29		
Absolute : Testis + epididymis Weight (g)	$5.32\pm0.03$	$4.99 \pm 0.41$	$4.32\pm0.16^{\ast}$	$6.1\pm0.2$		
Relative : Testis + epididymis Weight	$0.18\pm0.01$	$0.17\pm0.02$	$0.13\pm0.01\ast$	0.19 ±0.02		
Average feed intake (g / rabbit)						
Acclimatation	$98.2\pm5.1$	$97.4\pm7.7$	86.3 ± 4.0	$98.7\pm6.3$		
Experimentation	$164.0\pm4.6$	$185.5\pm10.1$	159.9 ± 13.0*	$176.4\pm6.5$		
Average water consumption (ml / rabbit)						
Acclimatation	$31.2\pm0.7$	30.8 ± 2	.6 31.6 ± 1.5	34.3 ± 1.6		
Experimentation	$138.2\pm5.1$	$117.2 \pm 6$	.9 100.08±8.08*	106.8±7.33		
Results are given as a mean $\pm$ SD for five rabbits in each group. * p < 0.05.						

**CE:** vitamins C and E group **AP:** Ampligo group; AP+CE: ampligo + vitamins C and E - treated groups.

differences in testis epididymis plus weights were observed in ampligo treated group. Those results corroborated with the findings of previous studies (Yousef et al., 2010: Khaldoun Oularbi et al., 2014) showing that exposure to pyrethroids causes decreased body and organs weights in the rodents following repeated exposure.

Effects of treatments on plasma hormonal parameters and morphometrycal

#### parameters

The synthetic pyrethroids are expected to cause deleterious effects on most of the organs and especially on the male reproductive system. Lambda-cyhalothrin caused sexual dysfunction in male rats and caused alterations in reproduction (Yousef *et al.*, 2010; Ratnasooriya *et al.*, 2002). The effects of ampligo® treatment on testosterone, FSH and LH in plasma were analyzed. This study revealed no changes in the hormone plasma levels of LH and FSH. The concentrations of testosterone in plasma reduced significantly in the AP group. Similarly to Yousef *et al.* (2010) treatment with lambda cyhalothrin insecticide decreased testosterone levels. Li *et al.* (2013) established that decrease in serum levels of testosterone in the adult rats exposed to cypermethrin could result from decreases in the number of Leydig cells and/or the damage of their structure. Morphometric study demonstrated a decrease in the total area, diameter of the epithelium of seminiferous tubules of AP rabbits. Vitamins C E supplementation with AP significantly reversed the above mentioned damages.

Table 2: Hormone concentrations in rabbit's	control	and treated-groups	(CE, AP, AP+CE) and
histomorphometric parameters of seminiferous	tubules	at the end of the e	xperimental period. EH:
Epithelium height, LD: Luminal diameter			

Groups	Contro	ol CE	AP	AP + CE	
		(1)	Hormonal Parameters		
FSH (ng/ml)	0.33±0.	00 0.35±0.01	0.31±0.00	0.29±0.86	
LH (ng/ml)	0.21+0.0	0.23+0.03	0.24 + 0.07	0.21 + 0.00	
Testosterone (ng/n	<b>nl</b> ) 2.91+0.	8 13.36+0.23	1.84+ 0.25**	5.66+5.42*	
	(2) Histomorphometric Parameters of seminiferous tubules				
Total area (µm²)	$79.85.x10^3 \pm 15.2x1$	$0^2$ 63.9x10 <sup>3</sup> .±17.4 x10 <sup>2</sup>	$45.91 \times 10^3 \pm 16.5 \times 10^{2*}$	$52.73 \text{ x}10^3 \pm 21.1 \text{ x}10^2$	
<b>ΕΗ (μm)</b>	$77.5 \pm 12.4$	$79.0 \pm 11.2$	49.2 ± 7.91*	51.4 ± 6.93	
LD (µm)	$1129\pm24.4$	$1018\pm19.3$	990 ± 18.7	956 ± 17.5	
EH / LD	$0.68\pm0.2$	$0.77\pm0.1$	$0.49 \pm 0.1^{*}$	$0.53 \pm 0.1$	
LD/ EH	$1.45\pm0.3$	$1.28\pm0.2$	2.01 ± 0.4*	$1.85 \pm 0.4$	

Results are given as a mean  $\pm$  SD for five rabbits in each group. \* p < 0.05. CE: vitamins C and E group AP: Ampligo group; AP+CE: ampligo + vitamins C and E -treated groups.

**Figure 1:** Histology of Testis: (A and B) the histoarchitecture of the testis is intact in control and CE rabbits. (C) Rabbits treated with AP show disorganization of seminiferous tubules and degeneration of the epithelium and congestion. C and D) Rabbits treated AP+ CE (Haematoxylin Eosin stain, X 400).



# Effect of treatments on testis histological structure

The testis section of control and CEtreated rabbits appeared normal. It is formed of many somniferous tubules with a normal spermatogenic lineage. The testicular parenchyma showed structural changes after ampligo treatment, the arrangement of germ cells was in disorder, the interspaces between seminiferous tubules enlarged and the interstitial Leydig cells were smaller than their control. The number of cell layers of the seminiferous tubules was significantly reduced, the damage involving both germinal and interstitial (Leydig) cells. Histopathological changes were more intense in rabbits from the APtreated group than in those from the AP+CE-treated group.

Similar results were reported in male rats exposed to other synthetic pyrethroids such as fenvalerate, permethrin and Beta-cypermethrin and lambda cyhalothrin (Elbetieha *et al.*, 2001; Mani *et al.*, 2002; LI *et al.*, 2013; khaldoun oularbi, 2014). Authors demonstrated that pyrethroid insecticide induces impairment of the structure of seminiferous tubules and spermatogenesis in the male adult rats. The said impairment may be attributed to the imbalance of circulating testosterone (Li *et al.*, 2013). Vitamins C E supplementation with AP significantly reversed the histological damages.



#### CONCLUSIONS

The findings of the present study clearly indicate a significant protective effect of vitamins C and E combination on testicular dysfunctions against ampligo® reprotoxicity and give explanation for the use of the antioxydant as fertility enhancer in the management of pesticide-derived male infertility.

REFERENCES

- Elbetieha A., Da'as S.I., Khamas W., et al. 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol, 41, 522-8.
- Fang L., Chen P., Xia H., Chun X. 2013. Effects of cypermethrin on male reproductive system in adult rats. Biomed Environ Sci; 26(3):201–8
- Guo M., Liu J.H., Ma X., Luo D.X., Gong Z.H., & Lu M.H. 2016. The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. *Frontiers in plant science*, 7, 114
- Khaldoun Oularbi H. 2014. Biochemical and histopathological changes in the kidney and adrenal gland of rats following repeated exposure to lambda-cyhalothrin. *Journal of Xenobiotics*, *4*(1)
- Khaldoun Oularbi H., Richeval C., Lebaili N., Zerrouki-Daoudi N., Baha M., Djennas N., & Allorge D. 2017. Ameliorative effect of vitamin C against hepatotoxicity induced by emamectin benzoate in rats. *Human & experimental toxicology*, 36(7), 709-717.

Kili multaneous exposure to low concentrations of oestrogens has negative effects on the reproductive parameters in male Spraque-Dawley rats. *Andrologia*, *39*(4), 128-135

- Li Y.F., Pan C., Hu J.X., Li J., Xu L.C. 2013. Effects of cypermethrin on male reproductive system in adult rats. Biomed. Environ. Sci. 26, 201-208.
- Mani U., Islam F., Prasad A.K. et al. 2002. Steroidogenic alterations in testes and sera of rats exposed to formulated Fenvalerate by inhalation. Hum Exp Toxicol, 21, 593-97.
- Mohamed D., Saber A., Omar A., Solima A. 2014. Effect of cadmium on the testes of adult albino rats and the ameliorating effect of zinc and vitamin E. *British Journal of Science*. 11(1):72-95.
- Ratnasooriya W.D., Ratnayake S.S.K., Jayatunga Y.N.A. 2002. Effects of pyrethroid insecticide ICON (lambda-cyhalothrin) on reproductive competence of male rats. Asian J. Androl. 4, 35–41.
- Shrem G., Brudner Y., Atzmon Y., Michaeli M., Ellenbogen A., & Shalom-Paz E. 2019. The influence of obesity, smoking, and serum follicular stimulating hormone in azoospermic patients on testicular sperm extraction-intra cytoplasmic sperm injection outcomes: A retrospective cohort study. *Medicine*, *98*(4).
- Wang H., Wang Q., Zhao X.F., Liu P., Meng X.H., Yu T., Ji Y.L., Zhang H., Zhang C., Zhang Y., Xu D.X. 2010. Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Arch. Toxicol. 84, 53–61.
- Yamaguchia S., Chiemi M.C., Kikuchi K., Celino F.T., Agusa T., Tanabe S., Takeshi M.T. 2009. Zinc is essential trace element for spermatogenesis. PNAS.,; 106: 10859- 10864.
- Yousef M. I., El-Demerdash F. M., Kamil K. I., & Elaswad F. A. 2006. Ameliorating effect of folic acid on chromium (VI)induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. *Reproductive Toxicology*, 21(3), 322-328
- Yousef M.I. 2010. Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. Food and Chemical Toxicology 48; 1152–1159







# AMPLIGO® INSECTICIDE INDUCES INJURIES ON THE TESTES OF RABBIT ORYCTOLAGUS CUNICULUS: ALLEVIATING EFFECTS OF VITAMINS C AND E (ASCORBATE/α-TOCOPHEROL)

Khaldoun Oularbi H1. 2\*. Makhlouf C1,2., Bokreta S1,2., Settar A1., Tarzali D1., Oularbi Y3., Zitouni G4., Hamadou D2., Kais S2., Zerrouki N.2

1Department of Biology, Faculty of Nature and Life Sciences, University Blida 1, BP 270. Soumaa. Blida. Algeria
2Natural Resources Laboratory, University Mouloud Mammeri, BP 15017. Tizi-Ouzou. Algeria
<sup>3</sup> École Nationale Supérieure Agronomique, EL-Harrach, 16200, Alger, Algérie
4Technical Breeding Institute (Institut Technique d'Elevage (ITELV), 16111 Baba Ali, Alger, Algeria



H. Khaldoun







(1)

(2)

 to assess the toxic effect of a new insecticide formulation Ampligo <sup>®</sup> on rabbit's testicle histology and endocrine testicular functions

E combination against ampligo <sup>®</sup> reproductive

toxicity in male rabbit .

# Hormonal assays



Plama samples/ Electrochemiluminescence immunoassay (ECLIA) : LH ; FSH ; Testosterone





# Histological preparation/ **Morphometrycal analysis**

Hematoxylin and eosin). Diameter of the area of seminiferous tubules,

**Epithelium height** Luminal diameter of the seminiferous tubule





# 20 male adults rabbits

- "Oryctolagus cunuculus" (2.5 2.9 Kg)
- Technical Breeding Institute (ITELV, Baba-Ali
- •CR Saidal Algeria





## **Experimental**



# 4 groups

- (1) control group
- (2) CE group: Vit E 200 mg/kg + Vitamin C 200 mg/kg
- (3) Ampligo B group (AP): (12.24 mg/kg)
- (4) arout AP+CE & vitamins C and E (12h as in the

# • Effects of treatments on food intake, water consumption, body and Testes and epididymis weights

Groups / Period	CONTROL	CE	AP	AP+CE
Initial BW (Kg)	2.70 ± 0.02	2.65 ± 0.03	2.61 ± 0.06	2.83 ± 0.02
Final BW (Kg)	3.04 ± 0.02	2.99 ± 0.28	2.81 ± 0.05*	3.16 ± 0.34
% BWG	3,38	3,45	1,99*	3,29
Absolute				
Testis + epididymis Weight (g)	5.32 ± 0.03	$4.99 \pm 0.41$	4.32 ± 0.16*	$6.1 \pm 0.2$
Relative	$0.18 \pm 0.01$	0.17 ± 0.02	0.13 ± 0.01*	0.19 ±0.02
Testis + epididymis Weight				
Averag	e feed intake (g	g / rabbit)		
Acclimatation	98.2 ± 5.1	97.4 ± 7.7	86.3 ± 4.0	98.7 ± 6.3
Experimentation	164.0 ± 4.6	185.5 ± 10.1	159.9 ± 13.0*	176.4 ± 6.5
Average wa	ter consumptio	n (ml / rabbit)		
Acclimatation	31.2 ± 0.7	30.8 ± 2.6	31.6 ± 1.5	34.3 ± 1.6
Experimentation	138.2 ± 5.1	117.2 ± 6.9	100.08±8.08*	106.8±7.33

12

1.

RESULTS

& DISCUSSION

RESULTS & DISCUSSION	• Eff	•Effects of treatments on plasma hormonal parameter and morphometrycal parameters				
Groups	Contro	1 <b>C</b> H	E AP	AP + CE		
		(1) Hormonal Parameters				
FSH (ng/ml)	$0.33 \pm 0.00$	) 0.35±0	.01 0.31±0.00	0.29±0.86		
LH (ng/ml)	0.21+0.01	0.23+0	.03 0.24+0.07	0.21+0.00		
Testosterone (ng/m	<b>1)</b> 2.91+0.8	13.36+0	0.23 <b>1.84+ 0.25**</b>	5.66+5.42*		
	(1) Histomorph	ometric Parameters	of seminiferous tubules			
Total area (µm²)	$79.85.x10^3 \pm 15.2x10^2$	$63.9 \times 10^3 \pm 17.4 \times 10^2$	$45.91 \times 10^3 \pm 16.5 \times 10^{2*}$	$52.73 \text{ x}10^3 \pm 21.1 \text{ x}10^2$		
EH (μm)	77.5 ± 12.4	$79.0 \pm 11.2$	49.2 ± 7.91*	51.4 ± 6.93		
LD (µm)	1129 ± 24.4	$1018 \pm 19.3$	$990 \pm 18.7$	956 ± 17.5		
EH / LD	$0.68 \pm 0.2$	$0.77 \pm 0.1$	$0.49 \pm 0.1*$	$0.53 \pm 0.1$		
LD/ EH	$1.45 \pm 0.3$	$1.28 \pm 0.2$	$2.01 \pm 0.4*$	$1.85 \pm 0.4$		

.

12

1-1





•Effect of treatments on testis histological structure

Histology of Testis (Hematoxylin Eosin stain, X 400)

(A and B) the histoarchitecture of the testis is intact in control and CE rabbits.

(C) Rabbits treated with AP show disorganization of seminiferous tubules and degeneration of the epithelium and congestion.

C and D) Rabbits treated AP+ CE



04/11/2021

