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Potential Role of Leporacarus Gibbus (Commensal Mite) in the Pathogenesis of Iodide-induced Toxicosis in New Zealand Rabbits

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Abstract: The goal of this study is to report clinical and histopathological manifestations in New Zealand rabbits naturally infested with Leporacarus gibbus (L. gibbus) and experimentally induced to develop iodidetoxicosis by oral administration of iodide. Iodine was used as potassium iodide (KI) in female New Zealand rabbits at a concentration of 200 mg/L in drinking water administered over a 4-week period. Leporacarus gibbus is considered a commensal rabbit fur mite. Infestations for L. gibbus usually are asymptomatic, even if heavy infestations are present. However, the immunological system dysfunctions may lead to the occurrence of clinical signs. In this report, rabbits receiving KI developed a multisystem syndrome that affected not only the skin, causing moist dermatitis and intense pruritus, but also affected the liver, thymus, spleen, and ovaries. Iodine-induced cytotoxicity was observed in several organs. Immunotoxicity was detected in the thymus and the spleen as a depletion of parenchymal compartments, lymphopenia, and neutrophilia. We believe that immunological disruption caused by KI toxicosis may lead to L. gibbus pathogenicity. Further observation at the macroscopic and microscopic level detected polycystic ovaries. Our preliminary results indicate skin mite L.gibbus may have a role in host immune responses. Fur mites in laboratory rabbits represent an underestimated variable that may impact not only research outcomes but research findings reproducibility.

Keywords: Cytotoxicity, rabbits, Leporacarus gibbus, potassium iodine

I. Introduction

Leporacarus gibbus (L. gibbus), formerly Listrophorus gibbus, is a common rabbit fur acarid[1]. Its role as a commensal parasite is unclear since infestations accompanied by dermatitis in rabbits that were naturally infected has been reported [2]. However, it is believed that clinical manifestations attributed to L. gibbus are related to its association with other ectoparasites, suchas Cheyletiella parasitivorax (mite) [3]and Spilopsyllus cuniculi (flea) [4]. Although L. gibbus is an obligate parasite that has completed all of its life stages (including adult and two nymphal stages) on the host, the life cycle of this mite has not yet been fully described [5]. L.gibbus mites usually affect pets and laboratory rabbits [2]. The mites do not locate directly on the epidermis; instead, they adhere to the hair and feed on sebaceous secretions, squamous epithelial cells, and hair debris [6]. Therefore, these mites are nonpathogenic[5]. However, it has been reported that rabbits infested with L. gibbus may develop hypersensibility that is manifested as pruritus, moist dermatitis, and alopecia [2].

Iodide is an essential micro-nutrient required for the proper function of all organ systems. The main source of iodide in the diet is through the intake of salt, dairy products, bread, and, seafood [7]. Toxicological studies in pregnant rabbits indicate that the administration of iodine at 250 ppm (250 mg/L) over a 2-day period prior to parturition lead to an increased mortality rate among new-born rabbits [8]. In male rabbits, iodine supplementation in drinking water at levels of 400 ppm (400 mg/L) as potassium iodide (KI) induces drastic hypothyroidism [9]. In mice, iodine overload is associated with thyroid dysfunction and cytotoxicity on spleen white cells (lymphocytes sub-populations and natural killer (NK) cells) [10]. Although the role of iodide in the normal thyroid hormone synthesis and reproduction is well-established, its direct cytotoxic effects on the immune system compartments remainrelatively unexplored [11]. The goals of the present work are: 1) To report histopathological damage caused by an overdose of iodide (iodide toxicosis) administered to rabbits naturally

infested with L.gibbus, to describe clinical manifestations associated with this ectoparasite and 2) To document the potential cytotoxic effects on immune system compartments and target tissues.

II. Material and Methods

2.1. Iodide toxicosis

Iodide toxicosis was induced according to the protocol of Soliman et al. [9], with some modifications. Briefly, Potassium Iodide (KI) (Sigma-Aldrich catalog Nº 221945) was administrated *ad libitum* in the drinking water at a concentration of 200 mg/L (approximately 9.37 mg/day/ rabbit) over a 4-week period. A KI-supplemented water taste reactivity test was conducted by the principal investigator (PI) using a single-blind test to evaluate palatability and determinate whether rabbits will accept or reject it. Daily water intake and body weight were also recorded.

2.2 Experimental design, animal housing and management

This project was conductedby implementing the principles of the 3Rs: Replacement, Reduction and Refinement, which are the guiding principlesfordriving ethical research in toxicological studies. They were established by the Laboratory Animal Welfare Act (AWA), in the United States (1966) [12]. This policy was applied in an effort to reduce the number of animals used for toxicological studies *in vivo.* Five adult New Zealand rabbits (5 female), age 3 months, identified as R1 to R5, weighing 2.168 ± 442 g, were enrolled in a preliminary study to develop iodide toxicosis. Four rabbits received KI in their drinking water at a concentration of 200 mg/L for 4 weeks. The untreated control animal received filtered tap water *ad libitum* without KI, and was used for histopathological, organ weight and clinical evaluation pattern comparisons.Animals were obtained from the Universidad de Carabobo Animal Resources Facility. Animals came from a colony of rabbits naturally infested with *L. gibbus.* Rabbits from this colony were otherwise healthy. To determine the level of infestation, skin exfoliative cytology was performed on all the animals. All procedures were performed in accordance with the published guidelines of the Institutional Animal Care and Use Committee (IACUC) regarding the use of rabbits for research. Preliminary study protocol number CBDCS-01-2015 was reviewed and approved by the Medicine School Bioethics Committee of the Universidad Centroccidental "Lisandro Alvarado". Rabbits were housed in an animal facility in a stable environment at a temperature of 25-26 °C, with 50-55 % relative humidity. Rabbits were exposed to a 12:12 light/dark cycle. Animals were housed in sanitized cages within a sanitized room. The general overall health condition of the rabbits was monitored daily during 24 days of pre-experiment acclimation period. Water intake, body weight and general animal behavior were observed and recorded. At day zero (0), before treatment began (pre-treatment period), and at day 32 (the last day of treatment),the general health of the rabbits was determined by taking samples of peripheral blood, feces, and skin. Skin samples (scraping and tape test) were collected from the dorsal neck and internal ear canal skin. To perform blood collection, rabbits were safely restrained. The back of the hind leg was shaved using an electric trimmer and then disinfected with 70% ethanol. The saphenous vein was punctured with a *23*-gauge needle. Blood smears were prepared and stained withHemacolor® rapid staining kit (Merck Millipore®)to manually determinate leukocyte differentials and study cells morphology.

2.3 Animal Husbandry and Housing

The students and staff responsible for the routine management of the animals were trained in appropriate methods to manipulate animal of experimentation. Rabbits were housed in an enriched environment. Cages had separate nesting and bathroom areas, as well as toys, and enough opportunities to express their specie-specific natural behaviors (socialize, exploring, jumping, foraging, scratching, grooming). In each cage, 1/3 of the total floor was made up of a solid wood resting area. Rabbits were housed individually in special cages that allow them to maintain physical, olfactory and visual contact with each other. To establish a positive interaction with humans to facilitate stress-free sampling and handling, animals were exposed to positive reinforcement when exposed to human contact. Animals were removed from their cages daily to partake in petting sessions. Rabbits were rubbed on their forehead, nose, ears and dorsal area. After that, animals were rewarded with fresh vegetables (carrots, cauliflower, kale, and broccoli). Potable tap water was administrated *ab libitum* (800 ml per day) and was changed daily. Diet consisted of 100 g of balanced commercial food containing 0.02 PPM (0.02 mg/kg) of iodide, hay (also working as a nesting material), and fresh vegetables (carrots, celery, chard).

2.4 Clinical and behavioral symptoms assessment

The welfare of the animalswas monitored daily using the following criteria: Posture, body weight, water/food intake, response to sound stimuli and position of ears.

2.5 Clinical experimental endpoint

The primary clinical end-point was defined as the time of occurrence of possible adverse effects including ≥20% body weight lost infection, pain, distress, not reacting to stimuli.

2.6 Gross and histopathological evaluation

Necropsy to assess injuries will be performed either at the clinical endpoint or during euthanasia of animals showing an advanced degree of disease, distress or persistent clinical signs. We used a semi-quantitative clinical and body-condition score to determinate when to euthanize. Terminal body weight was recorded, and target organs (spleen, liver, kidneys) weighed. Samples were collected from target tissues, including skin and tissues showing signs of injury. Tissue sections were processed using a hematoxylin and eosin (H&E) staining routine for general morphological diagnostic analyses.Euthanasia was performed at the end of the study (day 32) to facilitate sample collection and postmortem examination. Premedication was performed with Fentanyl at 0.03 mg/Kg/IV (Innovar-Vet®); after 5 minutes, the level of sedation was measured by stimuli response to a gentle ear punch, followed by an overdose of pentobarbital 100 mg/Kg/IV. Death was confirmed by auscultation of heart sounds. Cardiac arrest was confirmed by auscultation, and terminal body weight was recorded. To perform manual transcardial perfusion, 1 ml of heparin was injected into the heart followed by perfusion with a Dulbecco's Phosphate Buffered Saline (DPBS, Invitrogen, cat # 14190-250) and then 10% neutral buffered formalin using a 50 ml syringe and a 15 gauge 1 inch blunt end needle. During postmortem macroscopic observation, prominent ovary cystic follicles were observed in treated rabbits; samples were collected for further histopathological analysis. One cm^2 samples were collected and processed by using the paraffin technique processed at the Universidad Centroccidental "Lisandro Alvarado" Veterinary School Histopathology Laboratory. Tissue samples were fixed via immersion in 10% neutral buffered formalin for 72 hours. Each tissue sample was sectioned at 4 micron thickness and stained with hematoxylin and eosin (H&E) and evaluated microscopically. The histopathological parameters evaluated include inflammatory cell infiltration, epithelial cell degeneration, atrophy, hyperplasia, necrosis, and fibrosis.

2.7 Chemical induced toxicity in organ weight

Iodide toxicosis by administration of KI was estimated in target tissues by measuring organ weight following the Society for Toxicologic Pathology (STP) recommendations [13]. Spleen, liver, and the kidneys were carefully collected and weighed. Quantitative variables were summarized using descriptive statistics.

III. Results

Although the goal of this study was to assess the sub-chronic effects of oral administration of KI in a 90-day study, the animals started to show severe clinical signs and distress at week 4. The KI administration was immediately suspended. The general health status of the rabbits was monitored for 4 days after the treatment interruption.

3.1 Water palatability test

The addition of KI at 200 mg/L did not change the water taste and should not affect water uptake by rabbits.

3.2 Skin scrapes and tape test evaluation

Microscopic examination of dorsal skin samples revealed a slight infestation with *L. gibbus* on arrival (day 0). Microscopic observation of fur samples revealed between 2-3 adults and 0 -1 eggs per sample. The ear canal samples were negative for mites. No macroscopic skin damage was observed. At day 28, the follow-up fur sample evaluation revealed that the number of adult mites were the same as day 0 of the pre-treatment period (2-3 adult mites per sample and 1-2 eggs per sample).

3.3 General health condition and daily body weight

Fecal flotation test revealed no eggs or adult forms of gastrointestinal parasites in rabbits during the pretreatment period or at day 28 day of the treatment period. The study of peripheral blood smears revealed no numeric or morphological variations during day 0 of the pretreatment period when compared to reference values [14].However, at day 28 of the treatment period, morphological and numeric alterations in leucocytes were detected in blood smears of rabbits receiving KI at a concentration of 200 mg/L. Reactive lymphocytes were observed in peripheral blood from treated rabbits. A slight increase in the number of circulating monocytes (from 11.5±2.07 to 19±4.43). A slight lymphopenia (55±6.01 to 51.67±5) and mild neutropenia (33.5±5.32 to 23.33±10) with a shift to the left were noticed. No numeric or morphological modifications in peripheral leucocytes were observed in the untreated control rabbit. During the 4th week (day 28) treated animals displayed a hunched posture, down-turned ears, and an intense skin pruritus. Clinical examination revealed small roundedalopecia areas and skin wounds (Fig. 1). In order to identify the nature of the skin rash, imprint exfoliative citology and fur samples studies were performed on all rabbits. Fur samples were placed in a drop of mineral oil on a microscopic slide. A coverslip was placed on the sample, and it was evaluated under a microscope at 4X and 10X. Between 3-4 adults and 1-2 eggs per sample were manually counted. Imprinting citology (impression smear) and scrape citology samples were fixed with methanol and stained with Giemsa. The evaluation of imprinting citology revealed the presence of a purulent exudade containing various inflammatory cells (macrophages, lymphocytes and neutrophils) (data not shown). No adult mites, eggs or nymphal stages of *L. gibbus* were observed in imprinted or exfoliatived skin samples. However, adult mites and eggs were present in fur samples (Fig. 2). Animals presenting skin rash were shaved and injuries were treated topically twice per day (every 12 hours) for a period of 7 days with a mixture of antiseptic solution containing 50:50 of Gerdex® and Lociotan® (LaboratoriosAsociados CALA). All rabbits were treated with two doses of ivermectine (Ivomec® 1% Merial Caribbean) at 0.25 ml/Kg via sub-cutaneous. Five days after initial ivermectine injection, *L. gibbus* was almost undetected and by day 7, a contraction in the wound borders was observed. However, purulent multiple infection foci were still present under the wound scabs. While four female rabbits receiving KI treatment developed different degrees of skin damage, the untreated control female rabbit did not develop clinical signals of skin injuries, even though they were previously diagnosed as having the parasite *L. gibbus*.

3.4 KI induced toxicity

Changes in the absolute body weight and relative organ weight of the liver and spleen oftreated female rabbits in comparison to specie and gender specific reference values. All treated female rabbits showed a reduced liver weight. The untreated female (80 g) did not display any reduce liver weight. It should be noticed, that one female treated rabbit (R3) showed a drastic reduction in absolute and relative spleen, liver and kidneys weight (Table 1). A reduction in liver color and size comapred with reference values [15] were macroscopically observed in all 4 female rabbits exposed to KI treatment. Histopathological evaluation of liver tissue samples revealed moderate periportal mononuclear cell infiltration in rabbits treated with KI at 200 mg/L over a 4 week period (Fig.3). An increased cytoplasmic eosinophilia was detected in periportal and midzonal hepatocytes, together with sinusoidal dilation in center-lobular areas. These histopathological patterns indicate hepatotoxicity. Evaluation of parenchymal splenic compartments revealed a decrease in cellularity of the white pulp formed by B cells of the lymph node. The T cells of periarteriolar lymphoid sheaths (PALS) surrounding the nodule arterioles were still preserved (Fig.4). The mantle zone which normally surrounds the lymph node appears to be thinner in comparison with untreated control tissue samples. In the red pulp, which is the spleen compartment for hemocateresis and hematopoiesis, constituted by venous sinus and splenic cords, histopathological damage was not observed. No microscopic damage was observed in the kidneys.

3.5 Skin toxicity

Histopathological evaluation was performed at the clinical end point (4 weeks). The main injuries detected were dermis atrophy (Fig. 5), subdermal vascular rete atrophy, dermo-epidermal separation with vacuolar modification of basal epidermal cells, and loss of focal epidermal cells. In all 4 female rabbits exposed to KI at 200 mg/L in drinking water over the 4 week period, there was a focal sclerotic atrophy of the skin. The sclerotic and dermal appendage atrophy was observed in a dispersed focal pattern. The cutaneous muscle injuries were characterized by an endomysial mononuclear cell infiltration (endomyositis), myofiber degeneration and necrosis (Fig. 5 panels D, E and F) and focal endothelial cells proliferation. Thickening of

small vasculature walls, dermo-epidermic separation, and reduction in papillary dermis thickness were also observed.

3.6 Macroscopic and Histopathological finding of polycystic ovary syndrome (PCOS):

Macroscopic observation of ovaries dissected from 4 rabbits exposed to KI at a concentration of 200 mg/L compared with 1 untreated (control) rabbit revealed gross characteristics of polycystic ovary syndrome (PCOS) (Fig.6); prominent cystic follicles were observed on the ovary surface. The histopathological study confirmed the existence of atretic cystic follicles which showed disaggregation and detachment of granulosa cells, the absence of corpus luteum, and the presence of small lutein cells (Fig. 7).

IV. Discussion

The present study addresses clinical manifestations and histopathological changes in target tissues caused by an excess of potassium iodide in New Zealand rabbits. In our pilot study a mean dose of iodide 9.37 mg/day/ animal, under the form of KI induced a multisystem toxicity syndrome. In our study, we also found gross (Fig.6) and histological evidence (Fig.7) of PCOS together with dermatomyositis (Fig. 5), a cutaneous myositis diagnosed by a skin rash along with perifascicular skeletal muscle atrophy [16]. In our preliminary study, female rabbits induced to develop toxicity by overdose of KI, also presented dermatomyositis and scleroderma with atrophy of dermal appendages (Fig. 5). Since it has been reported *L. gibbus* populating the distal 1/3 dorsal lumbar area causes moist dermatitis and pruritus [1], we believe that similar symptoms observed in the test rabbits were due to *L. gibbus.* However, although all the 5 rabbits had a similar level of parasites before treatment(day 0) and at day 28 of the treatment period, only the 4 females induced to develop toxicity by an overload of KI at 200mg/L in drinking water developed alopecia, moist dermatitis and intense pruritus. In concordance with a previous report [1] dermatitis caused by *L. gibbus* began in the lower dorsal lumbar region (see Fig. 1B) and soon extended to all dorsal, facial and leg areas. Since the number of adult mites was virtually the same at day 0 of the pre-treatment and day 28 of treatment periods, the histopathological damages observed in the skin may not be attributed to their direct damage, but to immune system impairment. Degenerative changes in skin and subcutaneous muscle (dermatomyositis) were observed in rabbits treated with KI during 28 days.This damage may be exacerbated by the underlying dermatomyositis. Rabbit fur mites *L.gibbus* (formerly Listrophorus gibbus), are reported affecting laboratory animals and pet rabbits [2] Infestations for this ectoparasite are frequently asymptomatic [17]. The finding of neutropenia, combined with the presence of band cells, as well as a slight reduction in circulating lymphocytes, may be connected with skin-associated infection foci observed in rabbits treated with KI over a 4 week period (Table 1, direct effect of KI on immune cells, or indirect (via effects on other systems, such as endocrine or cardiovascular). We believe that the clinical signs observed in this study may be associated with down-regulation of immunological responses due to dysregulation of T lymphopoiesis in the thymus. In rats, toxicological studies have demonstrated that oral administration of periodate is conducive to a substantial reduction in leucocyte population in both the spleen's white pulp and thymus compartments [18]. Here we found cytotoxicity in the spleen and thymus. These hematopoietic organs exhibited an apparent reduction in the population of parenchymalcells ofthe spleen (Fig. 4) and thymus lobule cortex (Fig. 4D). Since cells from endoderm-derived organs such as thymus are able to uptake iodide[19, 20], we hypothesize that iodide may affect the functionality of endoderm- derived epithelial reticular cells (ERCs), which provide the structural support for T lymphocytes. Additionally, ERCs form an integral part of the thymic hematopoietic barrier that prevents the circulating antigens to reach the proliferating T lymphocytes. They also secrete trophic factors that promote proliferation and maturation of lymphocytes [21]. We conjecture that excessive iodide uptake by ERCs may affect the cortex lymphocytes subpopulation proliferation rate and, as a consequence, their trafficking to thymodependent zone in secondary lymphoid organs such a T-cell dependent periarteriolar lymphoid sheath (PALS) in the spleen leading to immune insufficiency.

In mice, excess of iodine may be correlated with hepatotoxicity [22].In the present study, a reduction in the absolute liver weight was observed (Table I). In toxicological studies, organ weight is accepted as an indicator of chemically-induced toxicity [23]. Therefore, we propose that the reduction in the absolute liver weight may be due to KI-induced cytotoxicity. Liver damage was confirmed by histopathological study, which revealed periportal necrosis with mononuclear cell infiltration (Fig. 3).

Although a characterization of skin microbiotawas not performed, it would be interesting to study the existence of a potential association or symbiotic mechanism between skin microbial flora and *L.gibbus*that may control or modulate cutaneous immune responses.

V. Conclusion

After the mouse and rat, therabbit is the most common species used in research [24]; thus, studying factors that influence research outcomes in this species is relevant. The preliminary data for the present study suggest *L. gibbus*,a putative commensalmite may become a pathogen if a disruption of immunological tolerance occurs as a consequence of KI toxicosis. Exogenous dietary factors, such as iodide, may lead to immune system dysregulations that ultimately alter the cutaneous microbiota equilibrium. Fur mites in laboratory rabbits may represent an underestimate variable affecting research outcomes.

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Figures and Tables

Table I

28 days Oral toxicity study in Rabbit experimentally induced to develop toxicity by administration of potassium iodide (KI) in drinking water. Absolute and Relative Organ Weight

		Organ weight					
	Terminal body	Spleen		Liver		Kidneys	
Rabbit ID	weight (g)	mg	%	g	%	g	%
R1	2555	1.400	0.055	64.55	2.53	12.4	0.485
R2	2085	1.240	0.059	66.00	3.17	12.5	0.599
R3	2615	0.700	0.027	45.06	1.72	9.60	0.367
R4	2605	1.330	0.051	63.81	2.45	12.9	0.495
R5	3105	1.180	0.038	80.00	2.58	12.5	0.402
Ref. values *	2541	0.940	0.037	83.21	3.28	12.96	0.510

***** Female New Zealand Rabbits (n=21) reference organ weight values per 100 grams of body weight [15]. Reference values are expressed as absolute values.

FIGURE 1

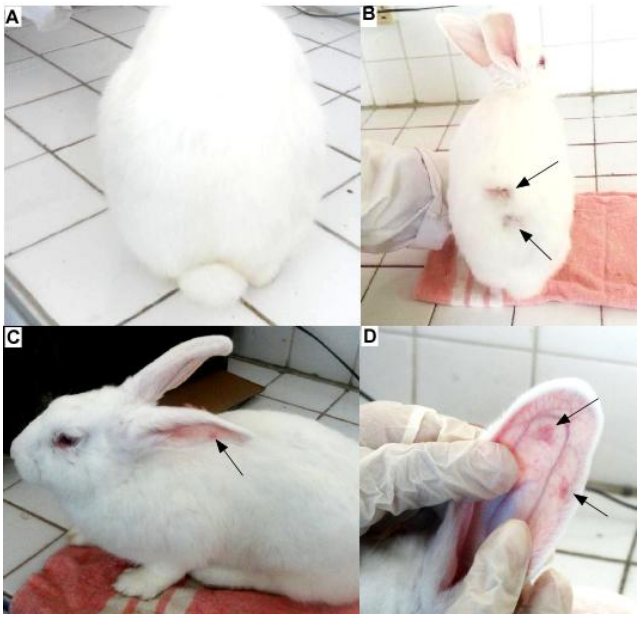

Figure 1.Clinical manifestations of potassium iodide (KI) toxicity in New Zealand rabbits in 4 weeks of treatment. A) Control (untreated) female rabbit showing skin integrity and a good body condition. B) Treated female rabbit at week 4 receiving KI at 200 mg/L in drinking water. During week 4 after initial treatment, the animal showed pruritus, intense skin alopecia together with moist dermatitis located in back, ears (panel D) and nose, face and periocular skin. C) Some signals of animal distress such as unusual posture, hair rough coat, ear, and back were observed, an arrow indicates rash in the inner skin of the ear. D) At higher amplification, rounded skin lesion was detailed.

FIGURE 2

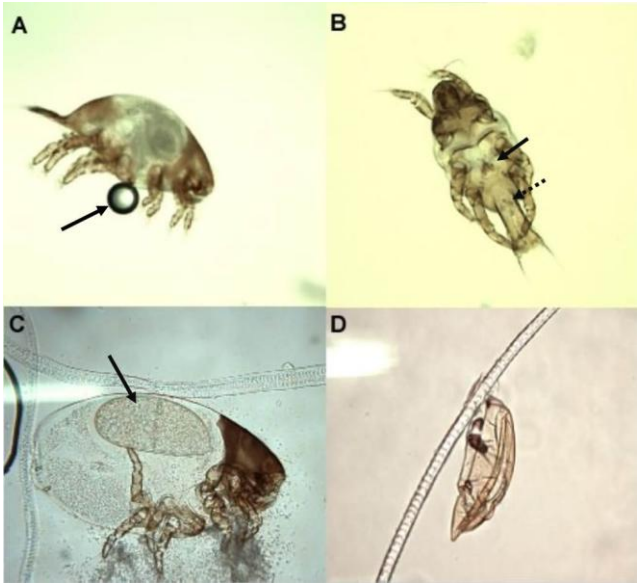

Figure 2: Dark field microscopy pictures of rabbits commensal mite Leporacarus gibbus (L. gibbus). All the evolutionary stages of L. gibbus were present in all rabbits. A) Lateral side of an adult female, the arrow indicates a sample preparation artifact, 10 X ocular magnification. B) Ventral view of an adult male, the arrow indicates the ventral hook, dashed arrow indicates male reproductive organs (10X ocular magnification). C)

Lateral view female L. gibbus mite, the arrow indicates the reproductive system with eggs. D) Larvae stage adhered to hair (20X ocular magnification).

FIGURE 3

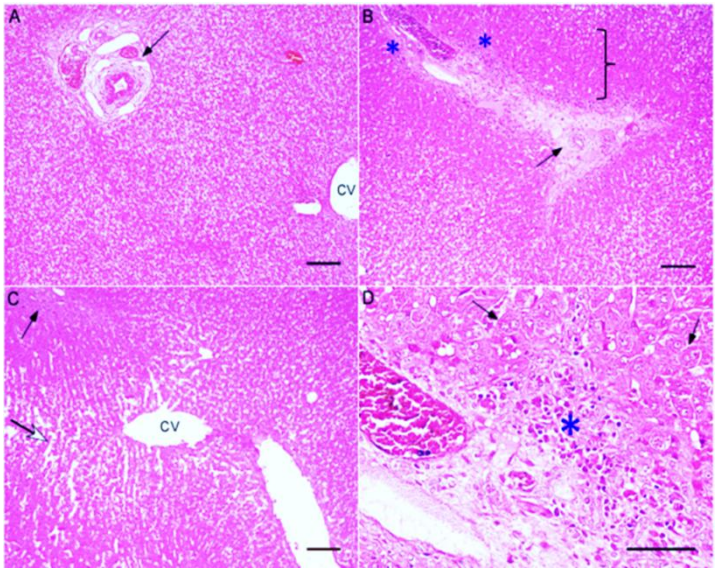

Figure 3: Hepatotoxity of KI at 200 mg/L concentration in rabbits. A) Photomicrographs of sections liver of untreated control rabbit, arrow indicates portal area, central vein of parenchymal lobule is signed as CV. B) Tissue section from KI at 200 mg/L concentration during 4 weeks in drinking water, portal area (arrow) present inflammatory infiltrations (asterisk), the bracket indicates the periportal zone showing an increment in acidophilia. C) Portal area is indicated by black head arrow, sinusoidal dilation can be observed (white head arrow) in hepatocytes from parenchymal mid-zone. D) At higher magnification of panel B portal area shows hepatocytes ballooning (arrows) and inflammatory infiltrate (asterisk). H&E stain, panels A, B and C, ocular magnification 10X, bar scale= 100µm.Panel C, ocular magnification 40X, bar scale=50 µm.

FIGURE 4

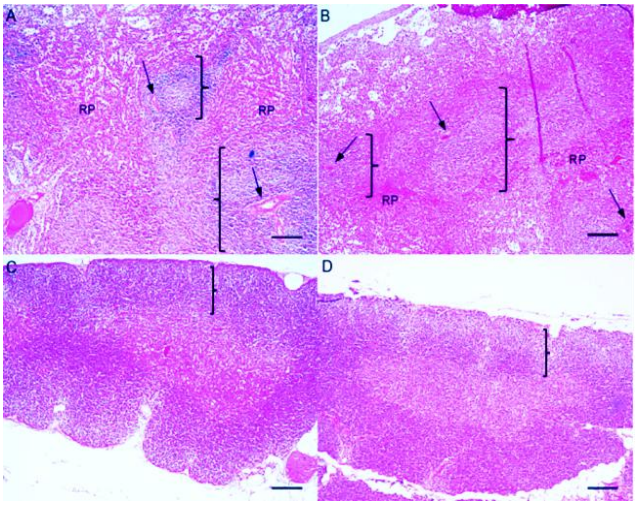

Figure 4: Photomicrographs of lymphoid organs spleen and thymus. A) Spleen tissue section of untreated control rabbits showing lymphatic nodules (white pulp) surrounding by the red pulp (RP), the arrows indicate the nodular arteriole and brackets show the estimated nodule size. Notice that each nodule has a germinal center (central paler area) surrounded by a more dense packed cap of lymphocytes. B) Depletion of white pulp components is observed, brackets indicate the area occupied by a lymphocyte depleted nodule, and

the nodular arteriole is indicated by arrows. C) A tissular section of a thymic lobule, bracket indicates the outer cortex fill by lymphocytes. In the panel D, a reduction in basophilic stain in the cortex is observed, which is associated with a reduction in the number of lymphocytes (bracket). H&E stain, ocular magnification 10x, bar scale= 100µm.

FIGURE 5

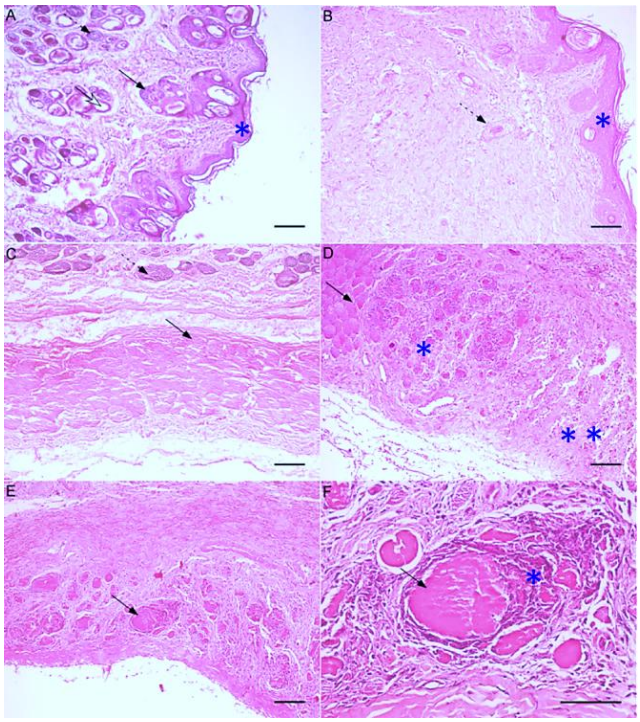

Figure 5: Photomicrographs of comparatives sections from back skin female rabbits exposed and no exposed to KI at 200 mg/L concentration in drinking water for 4 weeks. A) Skin section from normal control healthy rabbit, epithelial lining of epidermis is indicated by an asterisk, the dermis is occupied by cutaneous appendages sebaceous gland (black straight head arrow), pilose follicle (dashed blackhead arrow) and sweet gland (white head arrow). B) Skin section from a rabbit treated with KI, epidermis is indicated by an asterisk, notice that only a remaining atrophic pilose follicle (arrow) is observed in dermis, an increase in collagen deposition was also observed. C) Skin section from control healthy rabbit, part of deep dermis (dashed arrow) is indicated, the cutaneous muscle (straight arrow), bundled of skeletal fibers (straight arrow) are surrounded for smooth connective tissue (endomysium), all the fascicle are surrounded by perimysium. D) Cutaneous muscle from KI treated rabbit showing inflammatory infiltration in endomysium (*) together with muscle degeneration (dermatomyositis) (**), the arrow indicates areas with apparently normal skeletal muscle cell morphology. E) Cutaneous muscle from KI treated rabbit; the arrow indicates a hypertrophic skeletal fiber, surrounded by inflammatory cells. a closer image of panel E (F), reveals an endomysial mononuclear inflammation (*) together with variability in myofiber size, H&E paraffin sections, panels A-E ocular magnification 10x, bar scale=100; panel F 40x ocular magnification, bar scale=50µm

FIGURE 6

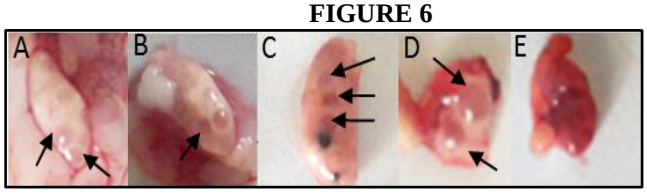

Figure 6.Macroscopic characteristics of ovaries from rabbit exposed to KI at 200 mg/L during 4 weeks. Photographs from plates A to D, correspond to rabbit treated with KI, notice the pale colors of ovaries, the

arrows indicate follicular cysts. In contrast, panel e shows ovary from untreated (control) rabbit characterizes from a smooth surface and higher irrigation.

FIGURE 7

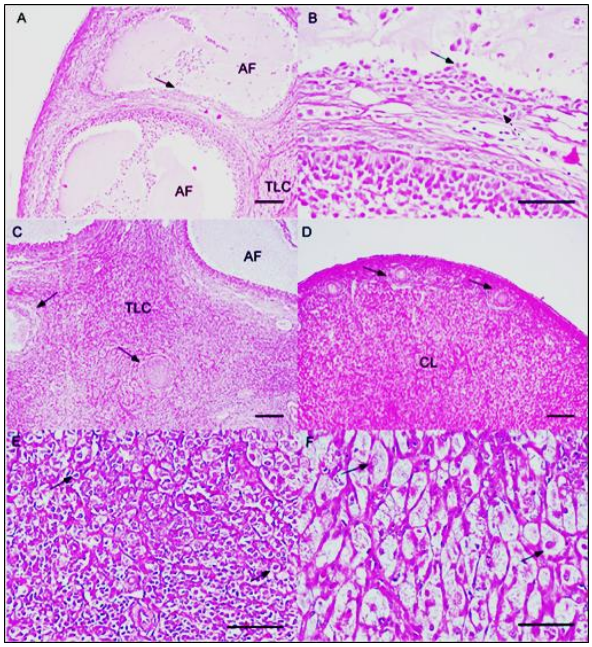

Figure7.Photomicrographs of ovaries dissected from rabbit treated with KI in drinking water at dose of 200 200 mg/L during weeks (panels A, B, C ,E) and untreated control rabbit (panels D and F). A) Notice the ovarian cortex with two prominent atretic follicles (AF) and theca lutein cells (TLC). B) At higher can be observed disaggregation and detachment of granulosa cells (arrow), small lutein cells (TLC) (ocular magnification 40x, bar scale 50µm). C) A section of ovarian medulla showing atretic follicles in several stages of regression (arrows). D) A tissue section from a normal (untreated control) rabbit ovary notice the presence of immature follicles (pre-antral) localized in the cortex (arrows), large lutein cells are conforming a corpus luteum (CL). E) Higher magnification of the panel C, the TLC can be detailed (arrows). F) Higher magnification of the panel D showing ovarian corpus luteum conformed by large lutein cells. H&E stain. Panels A, C and D ocular magnification 10x, bar scale 100µm; panels B, E and F ocular magnification 40x, bar scale= 100µm

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