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EVALUATION OF NANO AND CONVENTIONAL FORMS OF LAMBDA-CYHALOTHRIN TOXICITY IN RATS

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ABSTRACT

Nano-pesticides have been created to substitute traditional pesticides and enhance agricultural techniques. The utilization of nano-pesticides poses a considerable obstacle due to the insufficiency of information regarding their prospective ecotoxicity, especially animal, human, and other non-target organism toxicity. This study was conducted to evaluate the toxicity of the nano-emulsion Lambda-cyhalothrin compared with the conventional one, depending on investigating the organ weight index and histopathological examination of vital organs in treated rats. There was no significant difference between the effects of conventional and nanoform of Lambda cyhalothrin on body weight index. Still, there was a significant elevation in the relative weight of the liver and kidney in conventional Lambda cyhalothrin and nano-treated rats. Histopathological results of conventional Lambda cyhalothrin-treated rats indicated marked vacuolar degenerated hepatocytes, hypercellularity in the glomeruli, vascular hemorrhage in the cerebral cortex, and vacuolization of Leydig cells. While nano Lambda cyhalothrin-treated rats showed hepatocytes with dilated and congested blood vessels and Kupffer cell activation, necrosis of some renal tubular epithelium, and intratubular mononuclear cell infiltration. The cerebral cortex showed some pyknotic Purkinje cells, and spermatogenic cells were decreased.

Keywords: Nano pesticides, Pesticides, Lambda cyhalothrin, Toxicity.

INTRODUCTION

Nano-pesticides have garnered growing interest in contemporary agricultural practices. Prior to widespread application, assessing the environmental hazards and safety of nano-pesticides is imperative. Various pesticides are encapsulated within diverse nano-materials, resulting in varied effects on the growth and metabolism of distinct non-target crops. (Hu *et al.*, 2023). Prior to commercialization and adoption, a thorough assessment of nano-pesticides (including their environmental fate, toxicity, efficacy, modes of action, and interactions within agro ecosystems) is essential. Standardization of protocols for evaluating regulatory aspects of nano-formulations is crucial to ensure environmental safety. (Takeshita *et al.*, 2023). Worries have arisen regarding the toxicity of nano-agrochemicals. In this study, we examined the toxicity of nano-formulated Lambda-Cyhalothrin in rats,

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using them as a non-target animal model. Additionally, we compared the toxicity of the nano-formulation with that of the conventional form of Lambda-Cyhalothrin in rats.

MATERIALS AND METHOD

Chemicals

Lamdada λ-cyhalothrin technical grade (LC) 97.8% purity (CAS number 91465-08-6) was purchased from Kafr El Zayat Pesticides and Chemicals Co. (Kafr El-Zayat, Gharbia, Egypt).

Lambda-cyhalothrin Nanoparticles (LCN) were prepared by a specific nano-technical unit at the Faculty of Pharmacy, Al-Azhar University, Assiut. The primary particle size is from 70.3-77.53 nm.

Animal model

A total of 72 healthy adult Sprague–Dawley male rats, aged 8–10 weeks with an average body weight of 150–200 g, were used in this study. Rats were purchased from the Experimental Animal Center, Faculty of Medicine Assiut University, Egypt. Rats were fed on standard food pellets, and tap water was supplied *ad libitum*. The study was in agreement with the ethical rules prescribed by the ethics committee of the Faculty of Veterinary Medicine, Assiut University identification code 06/2024/0200.

Experimental animal design

Animals were divided randomly into four groups of eighteen animals each. The conventional form of LC was dissolved in corn oil, while the LCN was dissolved in distilled water. **Conventional-treated rats:** 18 rats were given 1/20 of LD_{50} (79 mg/kg b.w) dissolved in 1 ml of corn oil as a vehicle, orally twice weekly. The LD_{50} for Lambda-cyhalothrin has been determined as 79 mg/kg for male rats (Kidd and James, 1991).

Lambda-cyhalothrin Nano- treated rats: 18 rats were given Nano λ cyhalothrin which equals 1/80 of LD50 (Kidd and James, 1991), orally, twice weekly**.**

Control group [1,2]: 36 rats were kept as control and divided into two sup- group have18 rat each. First and second sup-groups administered 1 ml of corn oil and 1 ml of distilled water, respectively, orally, twice weekly.

Adopted method

Preparation and Characterization of Lambda-cyhalothrin Nano-emulsion (LCN) according to (Elsharkawy *et al.*, 2022).

Body weight and absolute and relative organ weight

The total body weight and the absolute organ weight of rats from different experimental groups were obtained by a digital weight scale. Also, the relative weight of the liver, kidney, brain and testes was calculated by the equation: relative organ weight = [organ weight/body weight] × 100.

Histopathological investigation

Fresh specimens from the liver, kidneys, brain, and testes of rats from all experimental groups were collected and fixed in 10% neutral buffered formalin. The tissues were dehydrated in a graded alcohol series, cleared with methyl benzoate, and embedded in paraffin wax, sectioned at 4 µ thickness, and stained with hematoxylin and eosin, histopathological examination by light microscopy (Bancroft and Gamble, 2008).

Statistical analysis:

The values are expressed as mean ± standard error (S.E) for three animals at each time. Differences between groups were assessed by one-way analysis of variance (ANOVA).

RESULTS

Results of body weight (Fig.1) revealed that both conventional and Nano ʎ cyhalothrin treated rats, showed a non-significant change in comparison with the control group. Also, the comparison between the results of two groups, conventional and Nano ʎ cyhalothrin treated rats, showed a

non-significant change during the whole period of the experiment. The relative weight of the brain (Fig.2), in both conventional and Nano ʎ cyhalothrin treated rats showed a non-significant change during the whole period of experiment in comparison with the control group and with each other. The relative weight of liver (Fig.3) of conventional ʎ cyhalothrin treated rats showed a significant increase at 4^{th} and 6^{th} week of the experiment in comparison with control group. Nano ʎ cyhalothrin-treated rats, showed a significant increase at 4^{th} and 10^{th} week of the experiment in comparison with control group. The comparison between conventional ʎ cyhalothrin treated rats and Nano ʎ cyhalothrin-treated rats showed a non-significant change. The relative weight of kidneys (Fig.4) of conventional ʎ cyhalothrin-treated rats showed a significant increase during the 4^{th}, 6^{th}, 10^{th} and 12^{th} weeks of the experiment in comparison with the control group. In the group of Nano ʎ ccyhalothrin-treated rats showed a significant increase during the 10^{th} week of the experiment in comparison with the control group. The comparison between, conventional ʎ cyhalothrin treated rats and Nano ʎ cyhalothrin treated rats, showed no significant change during the whole period of experiment. The relative weight of testis (Fig.5) of conventional ʎ cyhalothrin and Nano ʎ cyhalothrin treated rats showed an increase in comparison with control group but this increase was non- significant. The comparison between, conventional and Nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of the experiment.

Histopathological examination

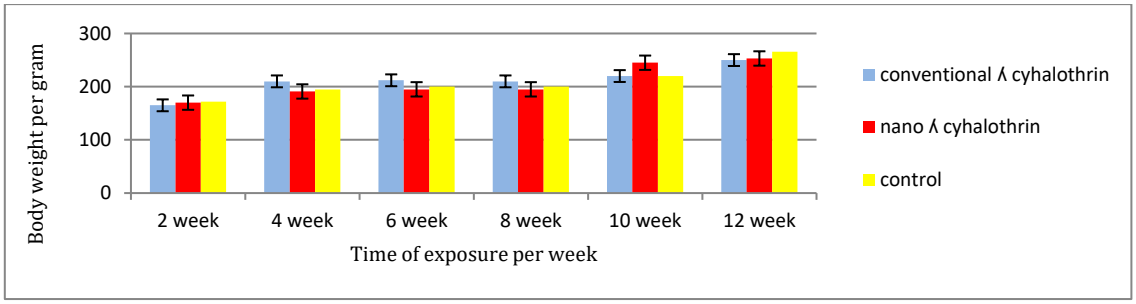
The liver of conventional ʎ cyhalothrin treated rats (Photo. 1, b1, b2) showed marked vacuolar degenerated hepatocytes (Photo. 1, b1), inflammatory cell infiltration with mononuclear cells, marked hydropic degenerated hepatocytes, inflammatory cell infiltration in the portal triad region, and vascular degeneration (Photo. 1, b2). Liver of Nano ʎ cyhalothrin treated rats (Photo. 1, c1, c2) showed marked hydropic degenerated hepatocytes with vacuolation, inflammatory cell infiltration in portal triad region, dilated and congested blood vessels (Photo. 1, c1), Kupffer cell activation, and focal area of necrosis with mononuclear cell infiltration (Photo. 1, c2).

The kidneys of conventional ʎ cyhalothrin treated rats (Photo. 2, b1, b2) showed renal tubular cast (Photo. 2, b1); showing severe degeneration and necrosis of renal tubular epithelium (Photo. 2, b2). Kidneys of Nano ʎ cyhalothrin treated rats (Photo. 2, c1, c2) showed swollen and congested glomerular tuft (Photo. 2, c1); and renal medulla with vacuolar degeneration and necrosis in renal tubular epithelium (Photo. 2, c2).

The brain of conventional ʎ cyhalothrin treated rats (Photo. 3, b1, b2) they also showed pyknotic neurons and congestion of blood vessels (Photo. 3, b1); showed degenerative and pyknotic pyramidal cells (Photo. 3, b2). The brain of Nano ʎ cyhalothrin treated rats (Photo. 3, c1, c2) showed severe perivascular hemorrhage in the cerebral cortex (Photo. 3, c1) and degenerated and pyknotic pyramidal cells (Photo. 3, c2).

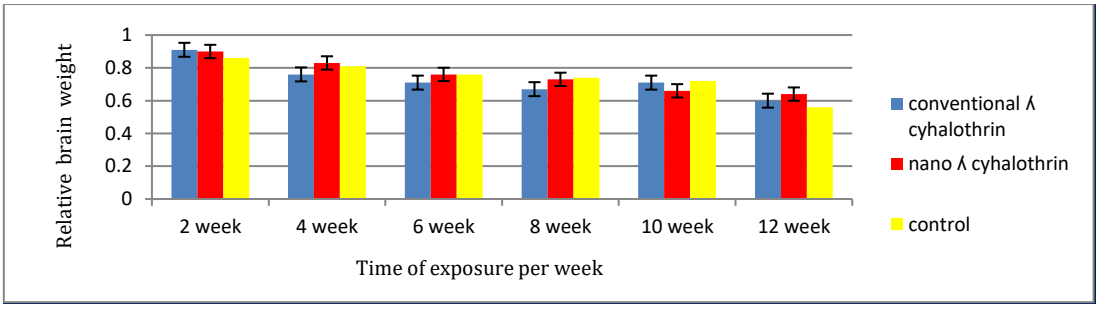
Testis of conventional ʎ cyhalothrin treated rats (Photo. 4, b1, b2) showing sever vacuolation and disorganization of normal successive stages of spermatogenic cells, spermatogonia, and spermatocytes (Photo. 4, b1) showing severe degeneration and absence of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes (Photo. 4, b2). Testis of Nano ʎ cyhalothrin-treated rats (Photo. 4, c1, c2) showed degeneration and disorganization of normal successive stages of spermatogenesis, and Leydig cells degeneration (Photo.4, c1); showed pyknosis and disorganization of spermatogonia and spermatocytes (Photo. 4, c2).

Figure 1: The effect of conventional and Nano ʎ cyhalothrin on body weight (g) in male rats



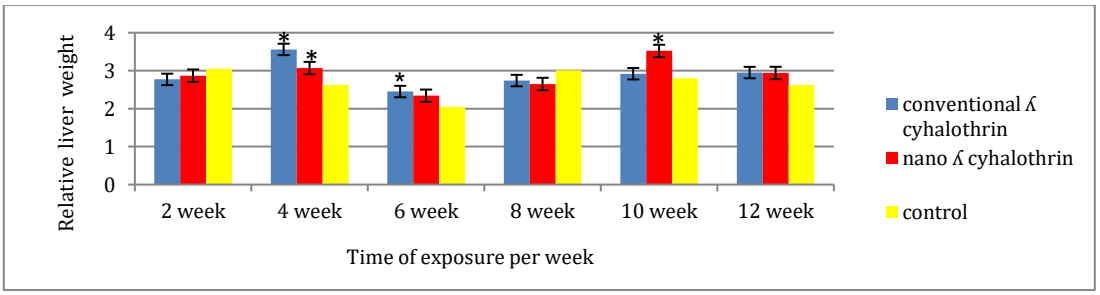
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a. Indicate significant at p≤0.05 compared to the control group, b. Indicate significance at p≤0.05 compared to Conventional ʎcyhalothrin-treated group, c. Indicate significance at p≤0.05 compared to Nano- ʎcyhalothrin-treated group.

Figure 2: The effect of Conventional and Nano ʎ cyhalothrin on brain relative weight (%) in male rats



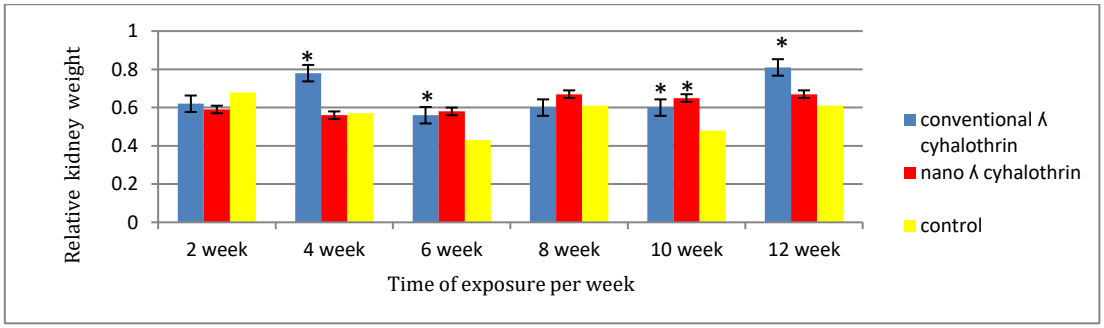
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a. Indicate significant at p≤0.05 compared to the control group, b. Indicate significance at p≤0.05 compared to Conventional ʎcyhalothrin-treated group, c. Indicate significance at p≤0.05 compared to Nano- ʎcyhalothrin-treated group.

Figure 3: The effect of Conventional and Nano ʎ cyhalothrin on liver relative weight (%) in male rats



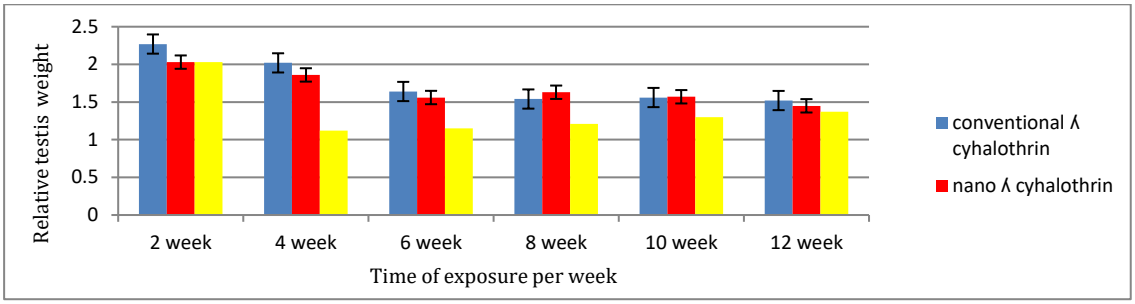
Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a. Indicate significant at p≤0.05 compared to the control group, b. Indicate significance at p≤0.05 compared to the Conventional ʎcyhalothrin-treated group, c. Indicate significance at p≤0.05 compared to Nano- ʎcyhalothrin-treated group.

Figure 4: The effect of Conventional and Nano ʎ cyhalothrin on kidney relative weight (%) in male rats

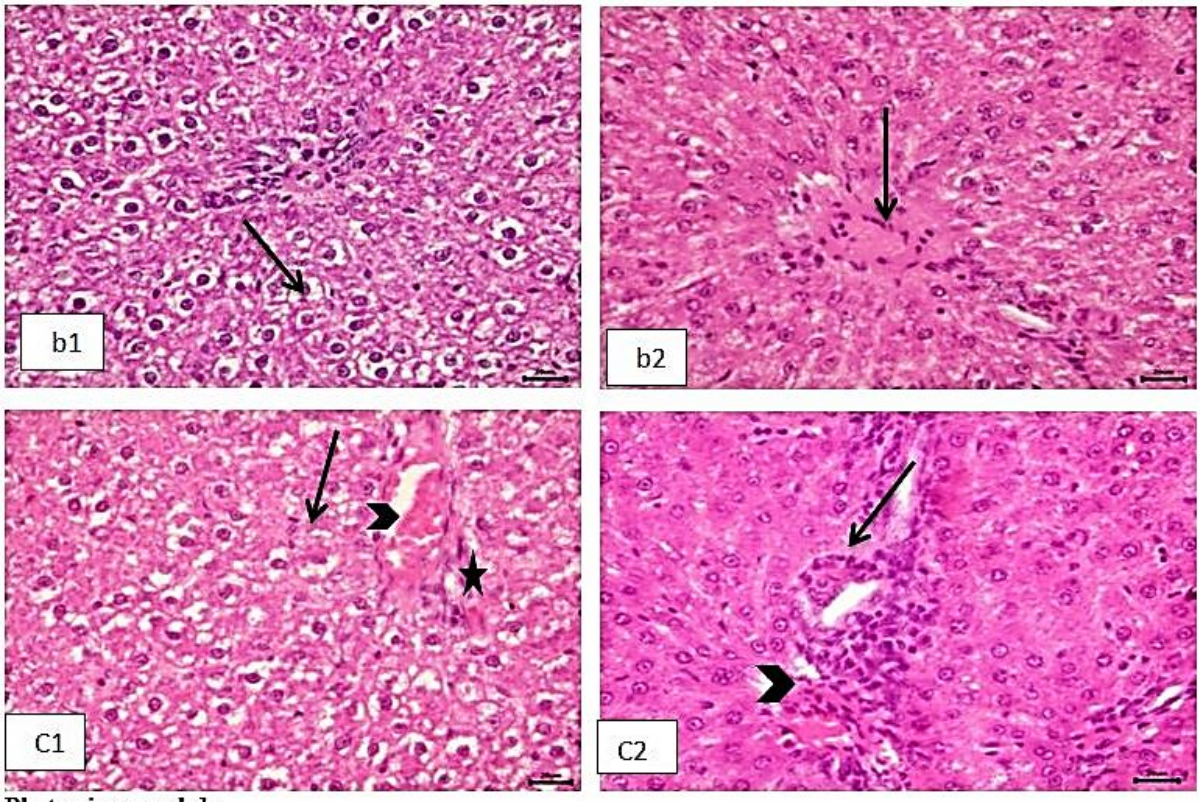


Values are expressed as means ± SE (n=3). * indicate significance at p≤0.05 ** indicate high significance at p≤0.01, a. Indicate significance at p≤0.05 compared to the control group, indicate significance at p≤0.05 compared to Conventional ʎcyhalothrin-treated group, c. Indicate significance at p≤0.05 compared to Nano- ʎcyhalothrin-treated group.

Figure 5: The effect of Conventional and Nano ʎ cyhalothrin on testis relative weight (%) in male rat

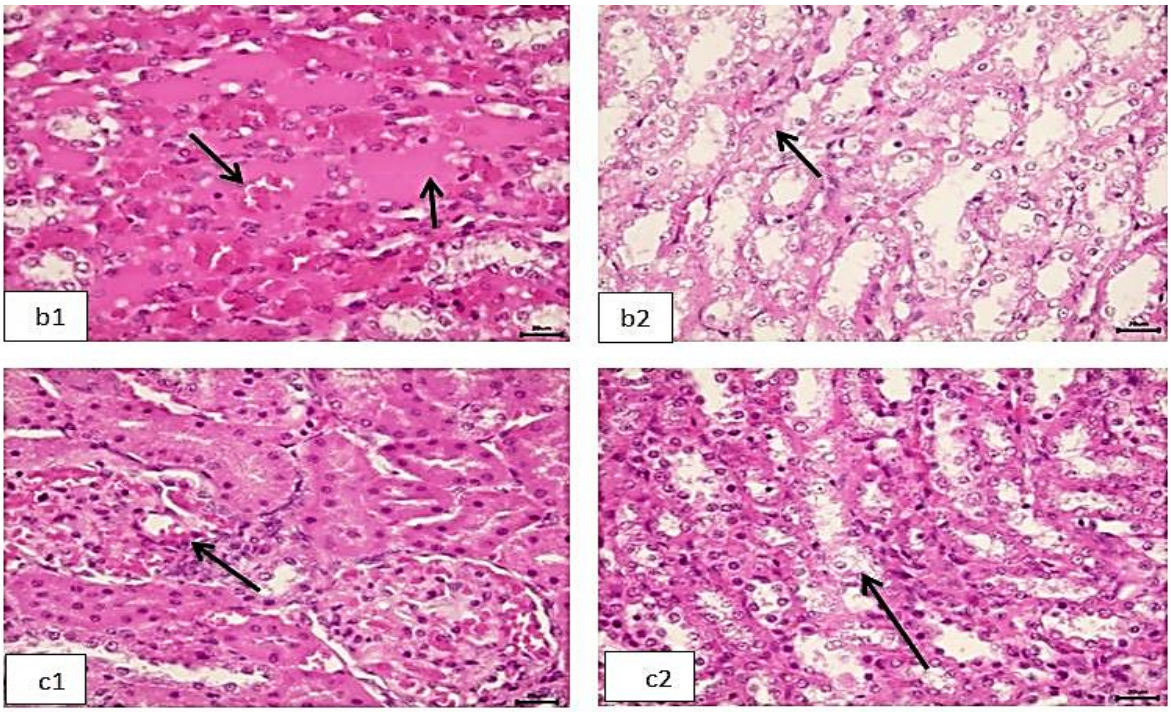


Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a. Indicate significant at p≤0.05 compared to the control group, b. Indicate significance at p≤0.05 compared to Conventional ʎcyhalothrin-treated group, c. Indicate significance at p≤0.05 compared to Nano- ʎcyhalothrin-treated group.



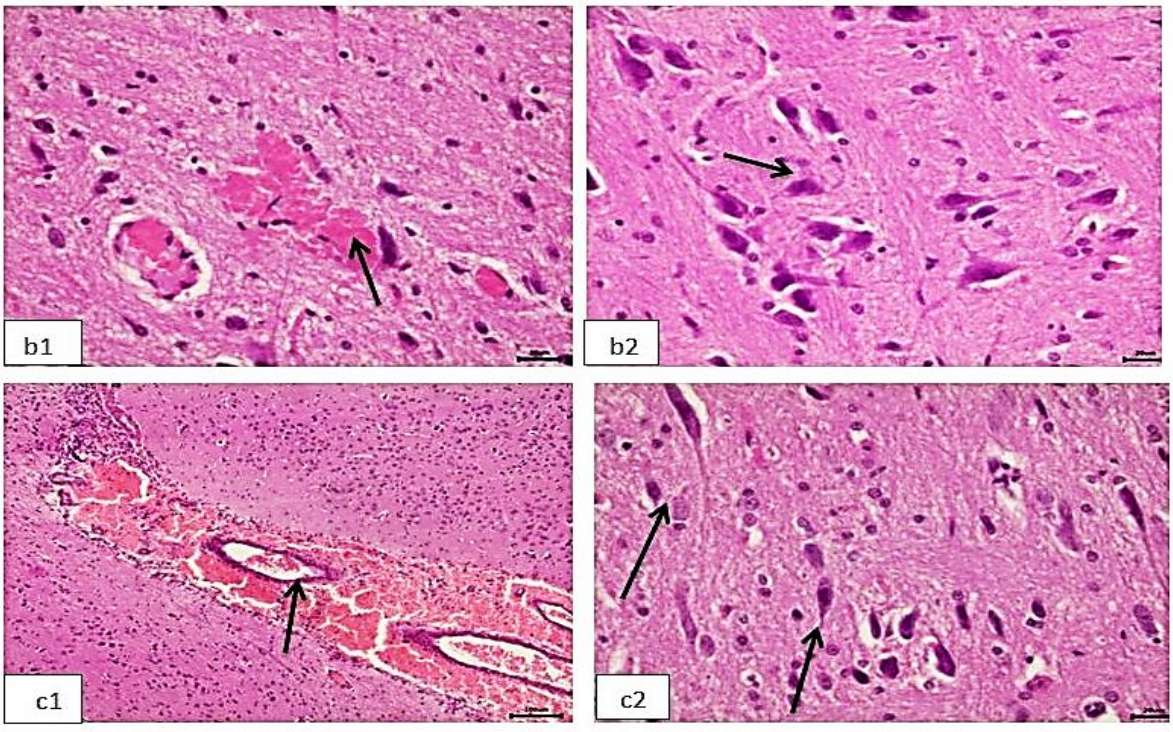
Photomicrograph 1:

Photomicrograph 1: Liver of **conventional ʎ cyhalothrin treated rats (b1, b2)**; (b1) showing marked vacuolar degenerated hepatocytes (arrowhead), inflammatory cell infiltration with mononuclear cells (arrow) (b2) inflammatory cell infiltration in portal triad region (arrow) and vascular degeneration (arrowhead); **liver of Nano ʎ cyhalothrin treated rats (c1, c2)** ; (c1) showing focal area of necrosis (arrowhead); infiltrated with mononuclear cells (arrow) and hepatocytes with vacuolation (star) (c2) showing marked hydropic degenerated hepatocytes with vacuolation (arrow), inflammatory cell infiltration in portal triad region, dilated and congested blood vessel (arrowhead) (H & E stain, bar = 20µ).



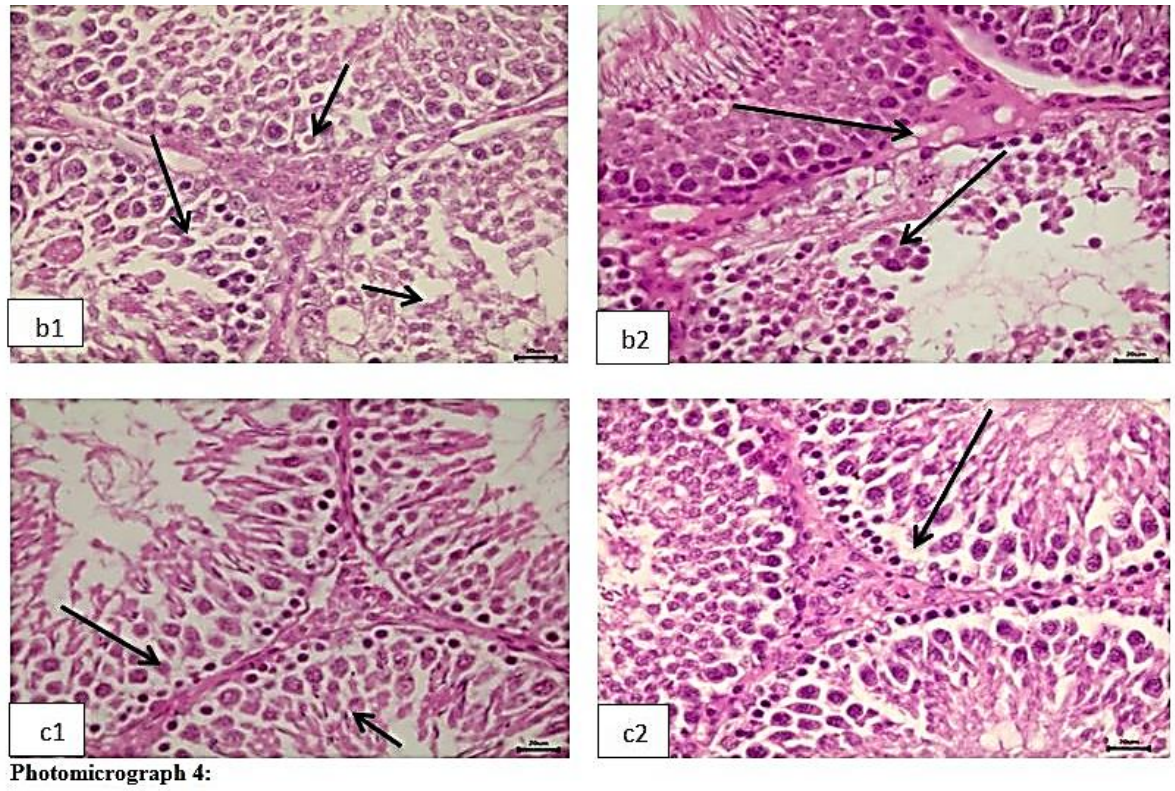
Photomicrograph 2

Photomicrograph 2: Kidney of **conventional ʎ cyhalothrin treated rats (b1, b2,)**; (b1) showing renal tubular cast (arrow); (b2) showing severe degeneration and necrosis of renal tubular epithelium (arrow); **kidney of Nano ʎ cyhalothrin treated rats (c1, c2**) (c1) showing swollen and congested glomerular tuft (arrow); (c2) showing renal medulla with vacuolar degeneration and necrosis in renal tubular epithelium (arrow) (H & E stain, bar = 20µ).



Photomicrograph 3

Photomicrograph 3: Brain of **conventional ʎ cyhalothrin treated rats (b1, b2)** (b1) showing Pyknotic neurons and congestion of blood vessels (arrow); (b2) showing degenerative and pyknotic pyramidal cells (arrow); **brain of Nano ʎ cyhalothrin treated rats (c1, c2)** (c1) showing severe perivascular hemorrhage in the cerebral cortex (arrow); (c2) showing degenerated and pyknotic pyramidal cells (arrow) (H & E stain, bar = 20µ).



Photomicrograph 4:

Photomicrograph 4: Testis of **conventional ʎ cyhalothrin treated rats (b1, b2,**) showing severe interstitial edema (arrow); (b1), showing severe vacuolation and disorganization of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes; (b2) showing severe degeneration and absence of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes; **testis of Nano ʎ cyhalothrin treated rats (c1, c2)** (c1) showing degeneration and disorganization of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes (arrow) and Leydig cells degeneration; (c2) showing pyknosis (arrow) and disorganization of spermatogonia, spermatocytes (H & E stain, bar = 20µ).

DISCUSSION

In the present study, the findings regarding body weight revealed that in both groups, conventional and Nano ʎ cyhalothrin treated rats, showed no significant change during the whole period of the experiment in comparison with the control group. Also, the comparison between the results obtained by two groups, the conventional group and the Nano ʎ cyhalothrin treated rats, showed a non-significant change during the whole period of the experiment. In the same line, Orlu and Obulor (2021) declared that the body weight of male mice receiving 10 mg/kg/bw/day of Lambda-Cyhalothrin for thirty-five days showed no significant change in the body weight in control group compared with the treatment groups. In contrast, Kumar and Yadav (2021) reported that the body weight was significantly decreased in the lambda cyhalothrin treated group. The non-significant differences observed in body weight in this study could be attributed to the impact of insecticide exposure on body weight appears to be inconsistent, likely contingent upon various factors such as dosage and method of administration, species, gender, and duration of treatment (Haratym-Maj, 2003). The non-significant differences observed in the body weight of the experimental animals show that ʎ cyhalothrin is not a systemic toxin. However, it can silently destroy the vital organs of the body without conspicuous changes in the body weight (Orlu and Obulor, 2021). In the present study, the results of brain relative weight revealed that in both groups, conventional and Nano

ʎ cyhalothrin-treated rats showed no significant change during the whole period of the experiment in comparison with the control group. In contrast, Boumezrag *et al*. (2021) declared that the brain weight of rabbits orally administrated 20 mg/kg b.w. /48h of ʎ cyhalothrin for 25 days, was significantly increased compared to the control group. The results of liver relative weight in the present study revealed a significant increase in conventional ʎ cyhalothrin treated rats in comparison with the control group. In Nano ʎ cyhalothrin-treated rats, there was a significant increase at 10 weeks of the experiment in comparison with the control group, and, in comparison, with the group of conventional ʎ cyhalothrin-treated rats. In agreement with Adam *et al.* (2020), who declared that the effects of ʎ cyhalothrin on hepatic-toxicity indicators, resulted in a dose-dependent significant increase in liver weight. In contrast, Boumezrag *et al.* (2021) declared that liver weight showed no significant change compared to the control group. The increased liver weight recorded in this study might be because of increased circulation due to raised requirements for the detoxifying compounds (Vemo *et al.,* 2017). Results of kidney relative weight revealed a significant increase in conventional ʎ cyhalothrin treated rats in comparison with the control group. Also, there was a significant increase in Nano ʎ cyhalothrin treated rats during the 10 weeks of the experiment in comparison with the control group, in agreement with Adam *et al.* (2020). In contrast, Nieradko-Iwanicka and Rutkowski (2022) stated that there were no significant changes in kidney mass after receiving 2 mg/kg cyhalothrin orally for 8 successive days. The kidneys contribute to the maintenance of the body's homeostasis thanks to the excretion of unnecessary metabolic products. Concentrating urine in the tubular fluid also increases the concentration of xenobiotics in it. Renal transport and the accumulation and biotransformation of pyrethroid metabolites contribute to the susceptibility of the kidneys to damage by this group of xenobiotics (Nieradko-Iwanicka and Rutkowski, 2022). In the present study, the results of testis relative weight in both groups of conventional and Nano ʎ cyhalothrin treated rats showed a non-significant change along the time of the experiment in comparison with the control group and in comparison, with each other. In agreement with Titus *et al.* (2019), who stated that the gonadosomatic indicators of Sprague-Dawley rats exposed to Cyalothrin at a dose of 30 mg/kg/bw/day for 35 days, showed a non-significant difference between the control and treatment groups. In contrast, Oshoke *et al.* (2016) reported that male Wistar rats receiving 25, 50, 75, and 100 mg/kg body weight of ʎ cyhalothrin, for five weeks resulted in a significant decrease in the absolute weight of testes and seminal vesicles. Our results could suggest that cyhalothrin may not have an obvious effect on the gonadosomatic indices, but silently destroys target cells of the body over time.

The result of histopathological examination revealed that the liver of conventional ʎ cyhalothrin-treated rats showed marked vacuolar degenerated hepatocytes, inflammatory cell infiltration with mononuclear cells, Liver of Nano ʎ cyhalothrin treated rats showed marked hydropic degenerated hepatocytes with vacuolation, inflammatory cell infiltration in the portal triad region, dilated and congested blood vessels, hyperplasia in the bile duct, and Kupffer cell activation. The brain of conventional ʎ cyhalothrin-treated rats showed pre-vascular hemorrhage in the cerebral cortex, some pyknotic Purkinje cells and granular cell layers, congestion of blood vessels, and degenerative neurocytes. The brain of Nano ʎ cyhalothrin-treated rats showed degeneration in Purkinje cells and few pyknotic nuclei, severe pre-vascular hemorrhage in the cerebral cortex, degenerated pyramidal cells and pyknotic

neurocytes. The liver and brain findings in the present study are in accordance with Alrawe and ALzubaidy (2022), who reported that lambda-cyhalothrin administration revealed liver and brain congestion, focal infiltration of mononuclear cells, hemorrhage, coagulative necrosis, and vasogenic edema. In the brain, the lesion was represented by shrunk Purkinje, demyelination of the axon, and hypertrophy of the astrocyte. The lesion was more severe in both organs when exposed to a high concentration and for longer periods. The liver is a crucial organ in the detoxification processes. However, the morphological frame cannot be interpreted as a specific response to pyrethroid intoxication; instead, it can be attributed to xenobiotics in general. One of the most frequent hepatic alterations observed after exposure to xenobiotics is an increase in vascularization. This physiologic response leads to an increase in blood flux and increases in catabolite excretion from pyrethroid metabolism, which occurs mainly through bile (Bradbury and Coats, 1989; Kolo *et al.*, 2010). Local lesions seem to be the most plausible hypothesis and might be due to exacerbated ROS production, owing to ʎ cyhalothrin action on cell mitochondria. The influence of synthetic pyrethroids in the electron transport chain, could increase ROS generation, and obviously enhance local cellular lesions. There was a clear cytoplasmic vacuolization in hepatocytes linked to ʎ cyhalothrin intoxication. The disorganization of typical hepatocyte cords suggests that the organelle distribution was altered, affecting organ functions (Marinho *et al.*, 2014). In the kidneys of conventional ʎ cyhalothrin treated rats, hyper-cellularity in the glomeruli, necrosis of some renal tubular epithelium, mild intratubular mononuclear cell infiltration, intratubular hemorrhage, pre vascular fibrosis, renal tubular cast, severe degeneration, and necrosis of renal epithelium in the renal medulla. Kidneys of Nano ʎ cyhalothrin-treated rats showed mild intratubular mononuclear cell infiltration, a swollen, congested, and hypercellularity glomerular tuft, degenerated renal tubular epithelium in the renal medulla, and mild intratubular hemorrhage. In accordance with our findings, Pawar *et al.* (2017) reported that characteristic pathological findings were noted in the kidney, especially degenerative changes in the convoluted tubules and hypercellularity of the glomerulus. Degenerative changes in convoluted tubules resulted in occlusion of the lumen and reduced Bowman's space. Tubular degeneration with cystic dilatation of tubules and the presence of a homogenous pinkish mass was evident in the kidneys which strongly corroborated the biochemical perturbations observed after ʎ cyhalothrin exposure. Testis of conventional ʎ cyhalothrin treated rats showed degeneration of Leydig cells, disorganization of spermatogonia, spermatocytes, and nuclear pyknosis of germ cells, and degeneration of spermatogenic cells with massive vacuolation, nuclear pyknosis of germ cells, marked interstitial edema. Testis of Nano ʎ cyhalothrin treated rats showed degeneration of Leydig cell, spermatogonia, spermatocytes, mild vacuolation, degeneration and disorganization of normal successive stages of spermatogenesis, spermatogonia, and spermatocytes. Testis pathological lesions are in agreement with Hussein *et al.* (2012), who declared that ʎ cyhalothrin exposure showed a significant decrease in the numbers of germinal, Leydig and spermatocyte (primary and secondary) cells when compared to the controls. The structures of the seminiferous tubules in cyhalothrin-treatment groups were pathologically damaged. The number of germinal cells was greatly decreased by a disturbance in their diameter. Reactive oxygen species (ROS) caused by insecticide treatment may be involved in the toxicity of different pesticides (Walsh

et al., 2000). Increased ROS may decrease the effective concentration of antioxidants, increasing the harmful effects of ROS on reproductive tissue (Agarwal and Prabakaran, 2005).

CONCLUSION

The outcome of this study confirmed that the administration of the conventional and Nano form of the insecticide Lambda cyhalothrin had a non-significant change in rat body weight while causing an increase in liver and kidney weight. Where they play an essential role in the metabolism, detoxification, and excretion of toxic metabolites. Histopathological results indicated marked vacuolar degeneration in hepatocytes, hypercellularity in the glomeruli, cerebral cortex with vascular hemorrhage, and vacuolization of Leydig cells.

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تقييم سمية المبيد النانوي والتقليدي لامبادا سيهالوثرين على الفئران

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إن تطور مبيدات الآفات النانوية لتحل محل مبيدات الآفات التقليدية أصبح ضرورة ملحة في ظل الثورة التقنية الهائلة في المجال الزراعي، ونظراً لنقص المعلومات حول السمية البيئية المحتملة للمبيدات النانوية وتأثيرها علي البشر والحيوان وجميع الكائنات الحية فإن استخدام مبيدات الآفات النانوية يعد تحديًا كبيرًا. مما دفعنا لإجراء هذه الدراسة لتقييم سمية مستحلب النانو لامباداسيهالوثرين كأحد المبيدات النانوية والمقارنة بينه وبين نظيره المبيد التقليدي، وللتحقق من تأثيرهما على أوزان الجسم وأوزان الأعضاء الحيوية للجسم وكذلك فحص الأنسجة للأعضاء الحيوية.

أشارت النتائج إلي أنه لايوجد فرق معنوي بين تأثير الشكل التقليدي والنانوي من لامبادا سيهالوثرين في مؤشر وزن الجسم ولا الوزن النسبي للأعضاء الحيوية بين المجموعتين على الرغم من الزيادة المعنوية في الوزن النسبي للكبد والكلى في مجموعتي الفئران التي تعرضت للمبيد التقليدي والمبيد النانوي بالمقارنة بالمجموعة الضابطة، كما أن نتيجة فحص الأنسجة في مجموعة الفئران التي تعرضت للمبيد التقليدي تشير إلى ظهور تلف وفقدان التركيب المنتظم للخلايا المكونة للكبد بالاضافة للتورم والارتشاح بالأنسجة المكونة لخلايا الكلى وأنزفة خارج الاوردة في أنسجة المخ وفقدان التركيب المنتظم للخلايا المكونة للحيوانات المنوية داخل نسيج الخصية. أما في مجموعة الفئران التي تعرضت للمبيد النانوي فلقد أظهر الفحص احتقان وتورم في أنسجة الكبد وتخلل الخلايا المناعية للأنسجة، كما حدث تلف وتآكل وتخلل الخلايا المناعية في أنسجة الكلى، بالاضافة إلى التلف وزيادة التصبغ في الخلايا العصبية، كما أظهر الفحص الميكرسكوبي لأنسجة الخصية وجود احتقان وتورم وتآكل في الخلايا المكونة للحيوانات المنوية، والخلاصة أنه لا يوجد فروق معنوية كبيرة بين تأثير كليها على ماتم بالتجربة وينصح باستخدام المبيد النانوي لأسباب اقتصادية ولأنه أكثر فاعلية.