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Toxicity of cyhalothrin lambda on erythrogram and renal parameters attenuated by Lavendula stoechas essential oil in rabbit

Toxicidad de la cihalotrina lambda sobre el hemograma los parámetros renales atenuados por el aceite esencial de Lavendula stoechas en conejo

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ABSTRACT

Among the pyrethroid insecticides used in Algeria against a wide range of agricultural pests is lambda-cyhalothrin (LCT). The aim of this study was to investigate the beneficial effects of Lavendula stoechas essential oil on the hematotoxicity and nephrotoxicity of the insecticide lambda cyhalothrin in rabbits (Cuniculus Lepus). Twenty-seven (27) healthy local rabbits, with an average weight of 1.8 kg, were divided into three groups of nine each: the first group considered as control, the second group treated with (100 mg/kg/BW) (LCT) and the third group treated with a mixture of (100 mg/kg/BW) (LCT) and (500 mg/kg/BW) Lavandula s. essential oil (LS.EO). The doses tested occurred daily in the diet over a 4-week period. Blood analysis revealed significant variations in the haemogram between the treated and control groups. A highly significant increase was recorded in the white blood cell, lymphocyte count in the LCT-treated group compared with the control group ($P<0.001$), highly and very highly significant decreases were observed in Red Blood Cell (RED), Hematocrit (HCT) and Hemoglobin(HGB) levels in the LCT-treated group compared with the control ($P<0.001$; $P<0.0001$), with the same result obtained for erythrocyte indices . Biochemical analysis of renal parameters showed a highly and very highly significant increase in urea and creatinine concentrations respectively in the LCT-treated group versus the control ($P<0.001$; $P<0.0001$). In contrast, the addition of lavendula essential oil improved the previous hematological and biochemical alterations.

Key words: Rabbit; lambda cyhalothrin; Lavendula s essential oil; biological parameter

RESUMEN

Entre los insecticidas piretroides utilizados en Argelia contra una amplia gama de plagas agrícolas se encuentra la lambda-cialotrina (LCT). El objetivo de este estudio fue investigar los efectos beneficiosos del aceite esencial de Lavendula stoechas sobre la hematotoxicidad y nefrotoxicidad del insecticida lambda cihalotrina en conejos (Cuniculus Lepus). Veintisiete (27) conejos locales sanos, con un peso medio de 1,8 kg, fueron divididos en tres grupos de nueve cada uno: el primer grupo considerado como control, el segundo grupo tratado con (100t mg/kg/BW) (LCT) y el tercer grupo tratado con una mezcla de (100 mg/kg/BW) (LCT) y (500 mg/kg/BW) aceite esencial de Lavandula s (LS.EO). Las dosis probadas se suministraron diariamente en la dieta durante un periodo de 4 semanas. Los análisis de sangre revelaron variaciones significativas en el hemograma entre los grupos tratados y de control. Se registró un aumento altamente significativo en el recuento de glóbulos blancos y linfocitos en el grupo tratado con LCT en comparación con el grupo de control ($P<0,001$), se observaron descensos altamente y muy altamente significativos en los niveles de RBC, HCT y HGB en el grupo tratado con LCT en comparación con el control ($P<0,001$; $P<0,0001$), obteniéndose el mismo resultado para los índices eritrocitarios. El análisis bioquímico de los parámetros renales mostró un aumento altamente y muy altamente significativo de las concentraciones de urea y creatinina respectivamente en el grupo tratado con LCT frente al control ($P<0,001$; $P<0,0001$). Por el contrario, la adición de aceite esencial de lavándulas mejoró las alteraciones hematológicas y bioquímicas anteriores.

Palabras clave: Conejo; lambda cihalotrina; aceite esencial de Lavándula s.; parámetros biológicos

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INTRODUCTION

Pesticides are becoming increasingly important as a result of various epidemics in various countries around the world [1]. The main purpose of pesticides is to control harmful organisms (animals, plants, fungi) but they can also regulate plant growth, have defoliating properties, or improve the storage or transport of crop products**.** The use of these products is constantly increasing throughout the world. According to world experts, the demand for pesticides is such that their production practically doubles every ten years. Around 2 million tons of pesticides are used internationally, of which 47.5% are herbicides, 29.5% insecticides, 17.5% fungicides and 5.5% other pesticides [2 , 3]. Pesticides pose a health risk because the majority of the human population remains actively or passively exposed to them. The exposure pattern is also a matter of concern as it determines the exposure dose [4]. The abundance of these products and the small quantities applied have two consequences: the need to study these molecules in order to understand what happens to them in the environment and the difficulty of detecting and measuring the active substances which are found in low concentrations [5]. As a result, the extensive use of these products and the presence of their metabolites in the body can develop pathologies and environmental disturbances.

The main pathologies suspected to result from exposure to pesticides are cancers, reproductive and developmental problems, weakening of the immune system, hormonal and neurological disorders [6 , 7]. Natural products are a formidable alternative for combating the toxicity of pesticides in order to reduce the negative impact on human health and the environment. The move towards green chemistry processes and the ongoing need for new tools with original modes of action represent an attractive area of research that deserves particular attention. Unlike a chemical, remedy whose mode of action the synthetic molecule on a specific receptor site determines.The most studied natural products are ascorbic acid (vitamin C) and α-tocopherol (vitamin E) together or with other natural products such as quercetin [8] as well as, the therapeutic qualities of a medicinal plant, which are based on the interactions of all its different components. Aromatic plants have played a vital role in all times and places, particularly in tropical countries. First used in their raw state, then infused, digested, in the form of ointment, perfume or alcoholic extracts, they have always played a major role in the culture of many peoples throughout the ages, whether religiously or medicinally. Aromatic plants have healing and curative effects [9]. The first elements of photosynthesis are primordial metabolites: sugars, fatty acids and amino acids. They produce specific metabolites called essential oils, which have therapeutic properties. Essential oils are molecules with an aromatic core and a volatile character, giving the plant a characteristic odor. They are found in secretory organs such as leaves (peppermint, basil), flowers (lavender, ylang ylang), wood (Atlas cedar, white sandalwood), roots (ginger, valerian, vetiver) and seeds (coriander, green anise, carrot) [10]. They are chemical messengers used by aromatic plants to interact with their environment and play a role in protecting against excess light and attracting pollinating insects). Oils have great potential in terms of their biological activity: antioxidant, anti-inflammatory, antiseptic, antimicrobial, antiviral, antifungal, bactericidal, antitoxic, insecticidal and insect repellent, invigorating, stimulating, calming, etc [11]. They are free radical scavengers and metal chelators. The use of essential oils has been the subject of much research over the last decade and has aroused considerable scientific interest, reflected in the number of studies into the effectiveness of essential oils against the harmful effects of various xenobiotics. The purpose of this study was to determine the relationship between the use of *Lavendula steochas* essential oils and their antioxidative effects in the treatment of hematological and renal issues brought on by long-term exposure to a pesticide.

MATERIALS AND METHODS

Characteristics of the Ouled Bechih study area

The Ouled Bechih study area is located in north-eastern Algeria, in the Machroha locality (36° 21’24’’ N, 7° 50’83’’ E) and the Bouhadjar locality (36° 30’12’’ N, 8° 06’20’’ E) (FIG.1). The Ouled Bechih forests cover an area of 6,582 hectares and provide a rich diversity of species [12]. Climatic factors have a major impact on a region’s biodiversity. The climate of the Souk Ahras region is conducive to the development of extensive forest areas on very hilly terrain, where slopes can exceed 20%, known as the agri-forest zone and covering a vast area.

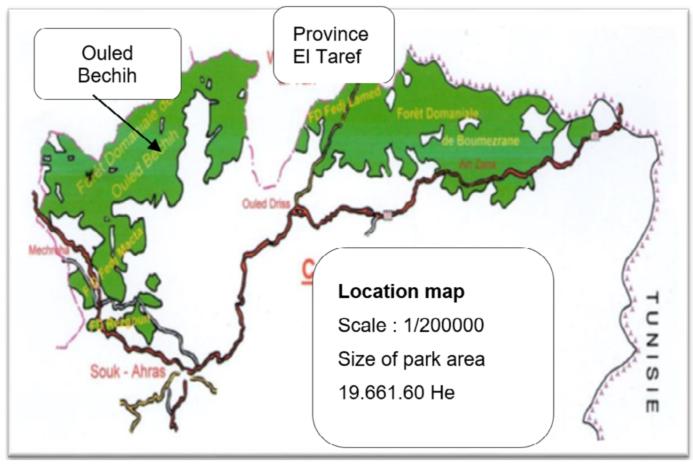

FIGURE 1. Geographical location of the study area (Ouled Bechih in Machroha forest-Souk Ahras. Northeast of Algeria)

Compound tested

KARATEKA® is a commercial name, multi-crop foliar insecticide formulated from the active ingredient lambda cyhalothrin (LCT), which belongs to the family of synthetic pyrethroids. This combination provides a very broad spectrum of efficacy against aphids and many other pests. It came from the Souk Ahras Agricultural Directorate (Algeria).

Lavendula steochas used

Lavender harvesting takes place during flowering, between March and May. The aerial parts of *Lavendula stoechas* were collected in March 2024 in the Ouled Bechih region of the El-machroura Mountains, Souk Ahras, Algeria. The identification was carried out by Doctor Ketfi Louisa, botanist at the botany laboratory of the Faculty of Natural and Life Sciences at Mohammed Cherif Messaadia University, and then deposited in the botany laboratory herbarium. Aerial parts underwent drying at room temperature (20-25°C) for 14 d.

Extraction of Lavandula stoechas essential oils by hydrodistillation

The plant is air-dried, away from light, to preserve the integrity of the molecules and prevent alteration and reproduction by microorganisms. Next, 100 g of the dried aerial part of the plant got into a double 1000 mL flask saturated with 530 mL distilled water, which is brought to the boil for 3 h, adding distilled water from time to time to prevent the mixture from drying out. The essential oil-filled vapor passing through the condenser is condensed and collected in a clean bottle. The process of hydrodistillation, which includes the use of a Clevenger, F77, JOANLAB apparatus, is a way to extract essential plant oils using water vapor. This method releases the volatile components of plant cells, which are then condensed and dissipated. The goal is to increase yield by utilizing various hydromodulary ratios (plant/water) and distillation times while examining the effects of various parameters on the quality and chemical makeup of the obtained essential oils.

Animals

A total of 27 local male rabbits (*Cuniculus Lepus*) aged 5 to 6 months and weighing 1.8 kg (Olba, WS-20 digital scale, French), were housed individually in galvanised batteries at room temperature (22 - 26 °C) and relative humidity (60 ± 5%). Food and water served ad libitum. The experimental protocol was in accordance with the National Guidelines for the Appropriate Care and Use of Animals in Laboratory Research established by Mohammed Cherif Messaadia University (Algeria).

Experimental design

The rabbits entered three groups of 9 animals each.

Group I: nine rabbits kept as controls

Group II: nine rabbits exposed to(100mg/kg/d)lambda cyhalothrin (LCT) [13]

Group III: nine rabbits received both (100 mg/kg/d) lambda cyhalothrin (LCT) and (500 mg/kg/d) *Lavandula stoechas* [14] essential oil (LS.EO). The doses tested took place daily in the diet over a 4-week period.

Blood Specimens

Hematological parameters

Complete blood counts (CBC) performed on anticoagulated samples using Ethylene diamine tetra acetic acid (EDTA) tubes. Data recorded in the following come from the use of automatic hematology analyzer (BK-6310.Biobase, China): white blood cells (WBC); red blood cells (RBC); hemoglobin (HB); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Renal biomarkers

The blood sample then underwent centrifugation at 10000 g for 10 minutes (min) by using centrifuge (Rotina 380, 380R and 380RHK, HETTICH®, French) to obtain plasma, to measure urea and creatinine by using an automatic biochemistry analyzer (ARCHITECT ci4100) supplied with commercial kits (Spinreact, Spain).

Statistical analysis

Statistical significance was assessed using ANOVA analysis with Tukey's multiple comparison test after detection of a normal distribution of the data and an appropriate P. $P<0.0001$ considered significant.

RESULTS AND DISCUSSION

Hematological parameters

According to TABLE I, the results show a highly significant increase in the WBC rate, the percentage of lymphocytes and the platelet rate in the group treated with LCT compared with the control and the group treated with LCT+L.S.EO. Concerning the parameters following red blood cells, hemoglobin, hematocrit, MCH, MCHC and MCV the values indicate a very highly significant decrease in the group treated with lambda- cyhalothrin compared to the control group and the group treated with the mixture of lambda-cyhalothrin and *Lavandula s* essential oil. On the other hand, the group receiving the mixture of insecticide and essential oil recorded significant, highly significant and non-significant differences for the following parameters: red blood cells, MCV and MCH; white blood cells, lymphocytes and hematocrit as well as platelets and MCHC respectively compared to the group treated with lambda cyhalothrin.

TABLE I.
Variation in the levels of some hematological parameters in rabbits in the different experimental batches (X ± SD) compared with the control.

Markers \ Groups	control	LCT	LCT+LS.EO	Observation
WBC (10^3/µL)	6,79±0,97[a]	11,11±1,45[b]	10,87±1,73[a]	a** b=NS c=**
Lymphocytes (%)	33,60±4,56[a]	47,64±3,99[b]	45,62±4,46[a]	a** b=NS c= **
RBC (10^6/µl)	4,94±0,61[a]	3,22±0,30[b]	4,68±0,51[a]	a*** b=NS c=*
HGB (g/dl)	10,64±1,40[a]	7,37±0,53[b]	8,94±0,63[a]	a*** b=NS c=*
HCT (%)	34,57±4,96[a]	24,22±2,67[b]	35,02±4,09[a]	a** b=NS c=**
PLT (10^3/µl)	699±42,35[a]	966,66±93,0[b]	755,7±39,60[ab]	a*** b=NS c =NS
MCH (pg)	25,10±1,55[a]	20,11±1,44[b]	23,76±1,32[a]	a** b=NS c= *
MCHC (g/dl)	36,08±1,46[a]	25,35±2,67[b]	27,01±2,00[ab]	a** b=NS c=NS
MCV (fl)	75,70±4,65[a]	60,41±2,55[b]	73,37±7,36[a]	a** b=NS c= *

LCT: 100mg/kg/d of lambda-cyhalothrin ; LCT+LS.EO: 100mg/kg/bw of lambda-cyhalothrin+500 mg/kg/bw of Lavandula stoechas essential oil
NS: No significant P<0.05; *: Significant P<0.01; **: Highly significant, P<0.001; ***: Very highly significant p<0.0001.
a= Control x LCT; b= Control x LCT+LS.EO; c= LCT x LCT+LS.EO

Cyhalothrin lambda is a broad-spectrum pyrethroid insecticide that induces physiological, biochemical and behavioural disturbances in laboratory animals, particularly with regard to body growth rate, the haematopoietic system, the kidneys and the liver. A complete blood count is an essential test for detecting changes in the various components of the blood. Blood count parameters provide important information about health and can help in the diagnosis and monitoring of various diseases and disorders. The results of the present study show a significant increase in white blood cell count, lymphocyte and platelet counts in the LCT and LCT+LS.EO treatment groups, compared with the control group. These results are consistent with the work of indicated reported a significant increase in these parameters when rabbits were exposed to lambda-cyhalothrin [15]. Other authors concluded that the increase in the value of these parameters could be due to a pathological response, indicating that the body's immune system was attempting to overcome and eliminate the toxic substances. With regard to the following parameters: red blood cells, hemoglobin and hematocrit, the data showed highly significant reductions in the LCT-treated rabbit group compared with the control. This result is in agreement with that others study who highlighted a decrease in these parameters [16], which could be due to reduced erythropoiesis or altered heme biosynthesis in the bone marrow [17].

In parallel was reported that hemoglobin concentration and hematocrit significantly reduced after LCT injection in rabbits and those pyrethroids generally [18], induce hemolysis, which ultimately leads to a reduction of Red Blood Cells in the circulation [19]. Furthermore, these results are not consistent with those obtained by Azafiarimanga *et al.* [13] who found no significant variation in hemoglobin and hematocrit concentrations and red blood cell counts in the LCT-treated groups compared with the control group.

The importance of erythrocyte indices (MCH, MCHC, and MCV) on the hemogram is to characterize the type of anemia. In general, their decrease indicates the presence of microcytic anemia (reduced globule size) and hypochromic anemia due to a lack of iron, heme and globin [20]. In the present study, the results of the erythrocyte indices (MCV, MCH, MCHC) showed highly significant decreases in the LCT-treated group compared with the control group. Our results are comparable with those reported, who found that the increase or decrease in these indices could be due to the increased activity of the bone marrow and the deficiency of certain hemopoietic factors [15 , 18]. Contrary to the conclusion the other study who noted no change in MCV, MCHC and MCH in rabbits treated orally with LCT [21].

Additional treatment with *Lavendula* essential oil significantly improved the disruption of erythrocyte indices caused by the pesticide. According to Batiha *et al***.** [22] *Lavandula* essential oil offers prospects for various biological applications, in particular for its antioxidant activity.in vitro and in vivo studies on the synergistic effect of lavender's bioactive components with other molecules could prove an effective alternative for treating pathologies linked to oxidative stress. According to the results obtained by Iretiola *et al.* [23], treatment with *Lavandula* essential oil in rabbits exposed to insecticides brought erythrocyte index levels closer to normal values.

Biochemical parameters

The results of the renal function parameters shown in TABLE II, where urea levels showed a highly significant and a very highly significant increase in the LCT and LCT+LS.EO groups compared with the control. Creatinemia showed a very highly significant and another significant increase in the LCT and LCT+LS.EO treated groups respectively compared to the control group.

TABLE II.
Evaluation of some kidney function parameters in rabbits in the different experimental batches (X ± SD) compared with the control.

Groups / Markers	control	LCT	LCT+LS.EO	Observation
Urea (g/L)	0,25±0,03[a]	0,44±0,15[b]	0,22±0,05[a]	a* b=NS c=**
Creatinine (U/L)	5,89±0,02[a]	8,58±0,79[b]	7,52±0,80[b]	a*** b= * c= NS

LCT: 100mg/kg/bw of lambda-cyhalothrin; LCT+LS.EO:100mg/kg/bw oflambda-cyhalothrin+500mg/kg/bw of Lavandula stoechas essential oil
NS: No significant<0.05; *: Significant p<0.01; **: Highly significant, p<0.001; ***: Very highly significant p<0.0001.
a= Control x LCT; b= Control x LCT+LS.EO; c= LCT x LCT+LS.EO

The toxicity of lambda cyhalothrin on renal parameters appears in table 2. Increased blood urea, creatinine concentrations are a specific and sensitive indicator of impaired renal function due to oxidative stress. Nadia *et al.* [24] and Aramjoo *et al.* [25] reported results similar to those obtained in this study. According to Eraslan *et al.* [26], the increase in urinary parameters is linked either to significant protein degradation, or to the toxic effect of pesticides on the kidneys, whose blood filtration capacity has been reduced. However, no significant difference and a significant difference were found in creatinine and urea concentrations respectively between the study groups compared with the control group [27]. Debabsa *et al*. [28] demonstrated that elevated plasma urea levels could indicate a decrease in glomerular filtration. Furthermore, the combined treatment with LCT+LS.EO in the present study showed renal function values almost similar to those of the control group. Essential oil of *Lavandula stoechas* used for its potential to reduce the toxicity of cyhalothrin lambda in male rabbits thus, the antioxidant properties of *Lavendula stoechas* extracts are mainly due to the presence of phenolic constituents, which could have a protective effect against nephrotoxicity induced by LCT administration [22].

CONCLUSION

The present study has demonstrated that exposure to lambda-cyhalothrin, a potent toxic insecticide, induces cytotoxic and hematological disorders, particularly on renal biochemical markers and various hemogram indices reflecting a metabolic disorder. In the light of these observations, further research on the mechanism of organ tissue damage with different doses is required to better assess the dose-effect relationship. In parallel, preventive supplementation with lavender, essential oil known for its antioxidant activity due to the synergistic effect of its bioactive components may prove a promising solution against insecticide toxicity.

Conflict of Interests

The authors declare no conflict of interest regarding the publication of this manuscript.

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