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Antioxidant and hepatoprotective Potential of *Coriandrum sativum* L. against hepatic injury by Lambda-cyhalothrin insecticide

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ABSTRACT

The objective of this study is to evaluate the antioxidant and hepatoprotective activity of aerial part and seeds of *Coriandrum sativum* plant against Lambda cyhalothrin insecticide. Male Wistar Albinos rats were randomly divided into control, LCT, CsA, CsS, CsS+LCT, CsA+LCT groups, after 90 days of treatments Biochemical, some oxidative stress parameters, and histopathology of liver tissue were evaluated. Total polyphenol content in aerial part and the seed extract estimated at 9.29 and 14.64 mg EAG / mg of extract and IC_{50} for an antioxidant activity equal to 19.38 and 22.62 mg/ml respectively. The obtained results revealed that rats received Lambda cyhalothrin insecticide showed a significant change in enzymes activity (AST, ALT, ALP and c-GT) and Glutathione (GSH) in liver. Meanwhile content of hepatic Malondialdehyde (MDA). Histopathology examination of liver revealed that *Coriandrum sativum* attenuate the incidence of liver lesions triggered by Lambda cyhalothrin intoxication. Therefore, the results of this study show that *Coriandrum sativum* can be proposed to protect the liver against Lambda cyhalothrin induced oxidative damage in rats, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenging effect.

Keywords: hepatoprotective, antioxidant, *Coriandrum sativum* L., Lambda cyhalothrin, Oxidative stress.

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1. INTRODUCTION

The Algerian association of environment protection asserts that Algeria is a major consumer of pesticides; in fact 30,000 tons are spread each year. Half of agriculture production would contain these chemicals, the use of xenobiotic like pesticides induce reactive oxygen formation, reduce antioxidant enzyme and especially oxidative stress [1,2]. Pesticides cause a number of severe health effects and illnesses, but can be cured by conventional and traditional medicine; Furthermore, experimental studies [3] and clinical studies [4] have shown that many medicinal plants have an antioxidant effect through their active ingredient, which exists in plant organs.

The use of natural products from medicinal plant has taken advantage of multiple interests in food, cosmetic and pharmaceutical. For their high antioxidative activity, the natural products are found many kinds of secondary metabolites such as Flavonoids, Alcaloids, Tannins. The natural substances are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress, the latter being considered a cause of ageing and degenerative diseases [5]. Additionally, recent studies have suggested that natural antioxidants in complex mixtures ingested with the diet are more efficacious than pure compounds in preventing oxidative stress-related pathologies due to particular interactions and synergisms [6].

The genus Coriandrum includes a lot of cultivated plants, the most important is coriander (*Coriandrum sativum* L.), and it's native to southern Europe and the western Mediterranean region. This herb widely used and growth in worldwide. It has many therapeutic virtues and use for the treatment of several diseases. The seeds are widely used for the preparation of the powder of spices, their therapeutic properties are due to the presence of active ingredients. In addition, used in the Algerian diet [7].

In this study, we investigated the Antioxidant activity *In vitro* and potential hepatoprotective effects of Coriander: *Coriandrum sativum* areal part and seed powder in the

Lambda-cyhalothrin pesticide induced hepatic damage in Wistar albinos rats.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., USA, and the pesticide Lambda-cyhalothrin was obtained from Nanjing Zonechem Co laboratory.

2.2. Preparation of plant extract

The areal part and seed of *C.sativum* were collected from Kouinine village, Wilaya of El-Oued, Algeria. The areal part was washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and shade dried for 22 days. 30 g of shade-dried powdered plant (areal part and seed) was extracted with 200 ml of water. The resulting extract was filtrated through Whatman N°: 1 filter paper and evaporated under reduce pressure then dried. The percentage yield of two extract was 22.6 and 11.63% for areal part and seed respectively, the two extracts were stored in refrigerator at 4 ±1°C for determination of total phenolic content and DPPH free radical scavenging assay.

2.3. Determination of total phenolic content

Total phenolic content were determined according to the literature [8,9]. Briefly, 200 µl of the diluted sample was added to 1 ml of Folin–Ciocalteu reagent. After 4 min, 800 µl of saturated sodium carbonate solution (about 20%) was added. After 2 h of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm. The same procedure was repeated to all standard gallic acid solutions (0 - 500 µg/ml) and standard curve was obtained.

2.4. DPPH Free Radical Scavenging Assay

The radical scavenging assay was conducted as described by [10]. The DPPH solution was prepared by dissolving 2.4 mg DPPH in 100 ml of methanol. 25 µl of extract or standard antioxidant (Ascorbic acid) were added to 975 µl of DPPH solution. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature and the decreases in the absorbance values were measured at 515 nm. The percentage of DPPH scavenging activity was calculated using the following equation.

$$\%\text{DPPH scavenging activity} = (A_{control} - A_{Sample}/A_{control})\ 100$$

Where $A_{control}$ is the absorbance of the control reaction mixture without the test compounds, and A_{sample} is the absorbance of the test compounds. IC_{50} values, which represented the concentration of the extract that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentages against concentration of the samples [11].

2.5. Experimental animals

Male Wistar albinos rats (146 ±4 g) purchased from Pasteur Institute of Algeria, were used for the study. The animals were housed in large, clean, polypropylene cages and acclimated to our laboratory animal for two week before the start of the experiments. Animals were maintained in a temperature (24 ±3°C), 12 h light/dark cycles with free access to food and tap water.

2.6. Experimental design

The Animals were divided into six groups of six animals per group (n=6) and received their respective treatment for 90 days as follow: group 1: served as untreated control group, Group 2: received Lambda-cyhalothrin (62.5 mg/L in drinking water), group 3 fed on seed of *C.sativum* diet (1% w/w), group 4 fed on areal part of *C.sativum* diet (1% w/w), group 5: received Lambda-cyhalothrin and fed on seed of *C.sativum* diet. Group 6: received Lambda-cyhalothrin and fed areal part of *C.sativum* diet. During experience, body weight was recorded periodically during the experiment weeks.

2.7. Calculation of relative Liver weight

The relative liver weight was calculated according to the formula: (Rats liver weight/rats weight)*100%.

2.8. Blood collection and tissue homogenate

Animals were sacrificed 24 h after the last dose of *C.sativum*. Just before sacrifice, the blood was collected and the serum was separated by centrifugation at 3000 rpm for 5 min and used for biochemical estimations. The liver and was removed, washed with saline solution, weighed and divided into samples. The samples were used for preparation of histological sections and post mitochondrial supernatant (PMS).

2.8.1. Determination of serum enzymes activity

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and Gama-glutamil transferase (γ-GT) levels were determined by using the automatic analyzer (Technicon R.A® lOOO).

2.8.2. Determination of liver homogenate parameters

- Preparation of post mitochondrial supernatant

0.5 g of the liver isolated from sacrificed animals were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (1.17%), the homogenate was centrifuged at 800 rpm for 15 minutes to remove nuclear debris. The supernatant so obtained was further centrifuged at 9600 rpm for 45 minutes at 4°C to get post mitochondrial supernatant (PMS) which was used as a source of enzymes.

- Determination of total protein

The total proteins in liver homogenates were determined by Coomassie Brilliant Blue G-250 methods using bovine serum albumin as the standard [12]. 0.1 ml of liver homogenates was transferred to 5 ml volumetric flask and the volume adjusted with Coomassie Blue and mixed by vortex. Wait 5 minutes and the produced blue color was measured spectrophotometrically at 595 nm.

- Determination of Glutathione (GSH) content

The assay of GSH was estimated by the method of Weckberker and Cory [13]. The liver samples were homogenized in 1 ml of EDTA (0.02 M). The homogenate was then subjected to a deproteinization with sulfosalicylic acid (SSA) 0.25%. Then 0.8 ml homogenate was added to 0.2 ml of the mixture, this was vortexed and left for 15 min in an ice bath before centrifugation (1000 rpm for 5 min). The supernatant (0.5 ml) was supplemented with 1 ml of Tris-EDTA (0.02 M, pH 9.6) and 0.025 ml (5-5'-dithio- bis-2-nitrobenzoic acid (DTNB, 0.01 M) and then left at room temperature for 5 min. The optical density was measured at 412 nm after 5 min.

- Determination of Malondialdehyde (MDA) content

MDA was measured according to the method described by Quintanilha [14]. The MDA reagent (trichloroacetic acid, thiobarbituric acid, and hydrochloric acid) was added to aliquots of the homogenate mixed with 2% (w/v) ethanolic solution of butylated hydroxytoluene. Then, the mixture was heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation, and the absorbance was measured at 532 nm.

2.8.3. Histopathological evaluation

Fresh liver tissues, which were previously trimmed to an approximately 2 mm thickness, were placed in plastic cassettes and fixed in 10% formalin for 24 h. Then the paraffin sections were prepared (Automatic tissue processor, Autotechnique) and cut into 2 µm thick sections in a rotary microtome. The sections were then stained with haematoxylin-eosin (H&E) dye and examined under light microscope for histopathological changes.

2.9. Statistical analysis

Data from each experiment were expressed by the mean ± and respective standard deviation (SD). The data were analyzed by Student test (software Minitab® 13). Values of $P<0.05$ were considered significant.

3. RESULTS

3.1. Total phenol content

The content of phenolic compounds in *C.sativum* extract determined using regression equation of calibration curve (y: 0.0082x + 0.1813, r^2: 0.9524), and expressed in gallic acid equivalents was found to be 9.29 ±0.156 and 14.64 ±0.388 mg GAE/ml of extract for the areal part and seed of *C.sativum* respectively.

3.2. DPPH Free radical scavenging assay

DPPH, is free radicals used to determine the antioxidant activity of plant extracts. When a solution of DPPH (violet color) is mixed with the plant extracts that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of their color (Molyneux, 2004). Table 1 show the results of DPPH scavenging activity.

Table 1: DPPH scavenging activity of seed and areal part of *C.sativum*.

IC_{50} µg/mL	Ascorbic acid	Seed of *C.sativum*	Areal part of *C.sativum*
	0.031	22.62 ±0.04	19.38 ±0.14

Data are expressed as mean±SE (Standard Error), (n=3).

3.3.1. Effect of *C.sativum* on body weight and relative liver weight

Table 2 shows that body weights rats within 90 days of the experimental were not affected by pesticide Lambda-cyhalothrin, areal part and seed of *C.sativum*. However, a significant elevation of relative liver weight was seen in Lambda-cyhalothrin treated group, indicating that Lambda-cyhalothrin induced hypertrophy of these tissues. By contrast, areal part of *C.sativum* in combination with Lambda-cyhalothrin significantly reduced the elevated weight of liver, suggesting the possibility of *C.sativum* areal part to give protection against liver injury upon Lambda-cyhalothrin induction.

Table 2: Effect of *C.sativum* extract on Initial body weight, body weight gain and relative liver.

Group	Initial body weight (g)	Body weight gain (g/d)	Relative Liver weight (%)
Con	164.5 ±3.84	0.793 ±0.045	2.538 ±0.091
LCT	168.1 ±8.16	0.676 ±0.07	2.642 ±0.052*
CsS	164.3 ±3.70	0.778 ±0.03	2.421 ±0.045
CsA	174.6 ±4.75	0.783 ±0.056	2.513 ±0.160
CsS+LCT	154.2 ±8.23	0.77 ±0.05	2.872 ±0.131*
CsA+LCT	174.8 ±11.1	0.98 ±0.195	2.321 ±0.14[a]

Con: Control. LCT: Lambda-cyhalothrin. CsS: Seed of *C.sativum*. CsA: Areal part of *C.sativum.* Results are presented as the mean ± S.E. (Standard Error) (n = 6). *p≤0.05, **p≤0.01 and ***p≤0.001. [a]p < 0.05 as compared with Lambda-cyhalothrin model group. [b]p < 0.05 as compared with normal control group.

3.3.2. Effect of *C.sativum* on serum ALT, AST, ALP and γ-GT

The protective effect of *C.sativum* at dose level (1% w/w) on the Lambda-cyhalothrin-induced modification in serum ALT, AST, ALP and γ-GT levels was shown in Figure 1. A single dose of Lambda-cyhalothrin caused hepatotoxicity in rats as indicated by an increase in serum ALT, AST, activities after Lambda-cyhalothrin administration. Whereas, animals pretreated with *C.sativum* extract exhibited a significant decrease in the activities of the serum marker enzymes.

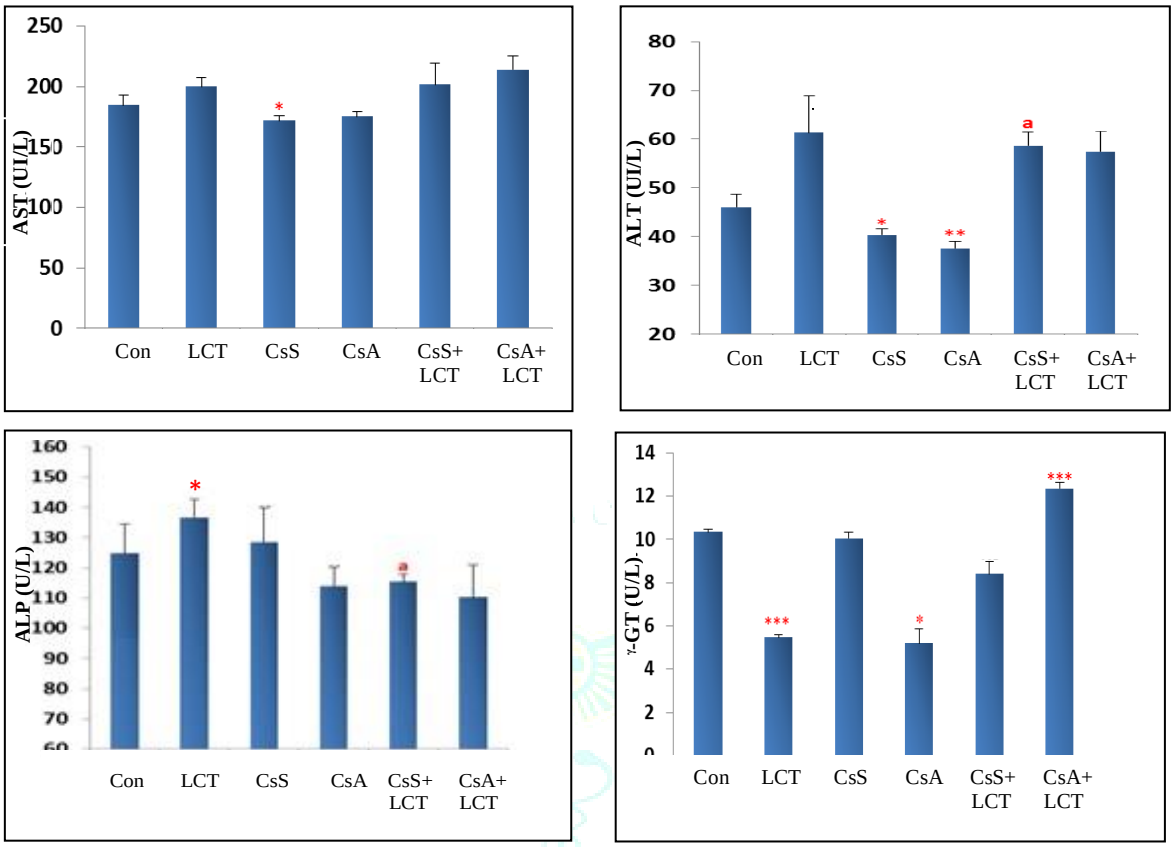

Figure 1: Effect of *C.sativum* extract on serum enzymes activity.

Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), γ-Glutamyl Transpeptidase (γ-GT).Con: Control. LCT: Lambda-cyhalothrin. CsS: Seed of *C.sativum.* CsA: Areal part of *C.sativum.* Results are presented as the mean ± S.E. (Standard Error) (n = 6). *p≤0.05, **p≤0.01 and ***p≤0.001. [a]p < 0.05 as compared with Lambda-cyhalothrin model group. [b]p < 0.05 as compared with normal control group.

3.3.3. Effects of *C.sativum* extract on GSH and MDA

Hepatic injury induced by Lambda-cyhalothrine caused significant increase in MDA and decrease in GSH. Results *C.sativum* effects of on GSH and MDA in Lambda-cyhalothrin induced hepatic injury are shown in Figure 2.

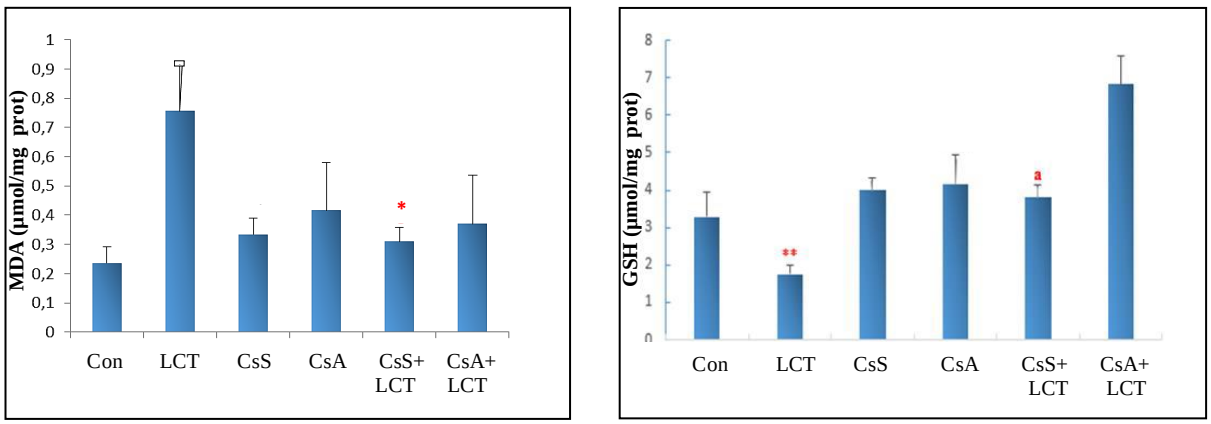

Figure 2: Effect of *C.sativum* extract on GSH and MDA.

Glutathione (GSH). Malondialdehyde (MDA). Con: Control. LCT: Lambda-cyhalothrin. CsS: Seed of *C.sativum.* CsA: Areal part of *C.sativum.* Results are presented as the mean ± S.E. (Standard Error) (n = 6). *p≤0.05, **p≤0.01 and ***p≤0.001. [a]p < 0.05 as compared with Lambda-cyhalothrin model group. [b]p < 0.05 as compared with normal control group.

3.3.4. Histopathological examination of the liver

The histologic examination of the liver section of the normal control group showed normal histologic picture of hepatic central vein and sinusoids (Figure 3). The liver sections of rats treated with Lambda-cyhalothrin alone showed inflammatory cells in the centrolobular zone and the necrosis of hepatocytes. It could also be observed that scattering penetrated inflammatory cells accumulated in liver sinusoids. Pre-administration of *C.sativum* extract (areal part and seed of plant) for 90 days could reduce the hepatic injury score.

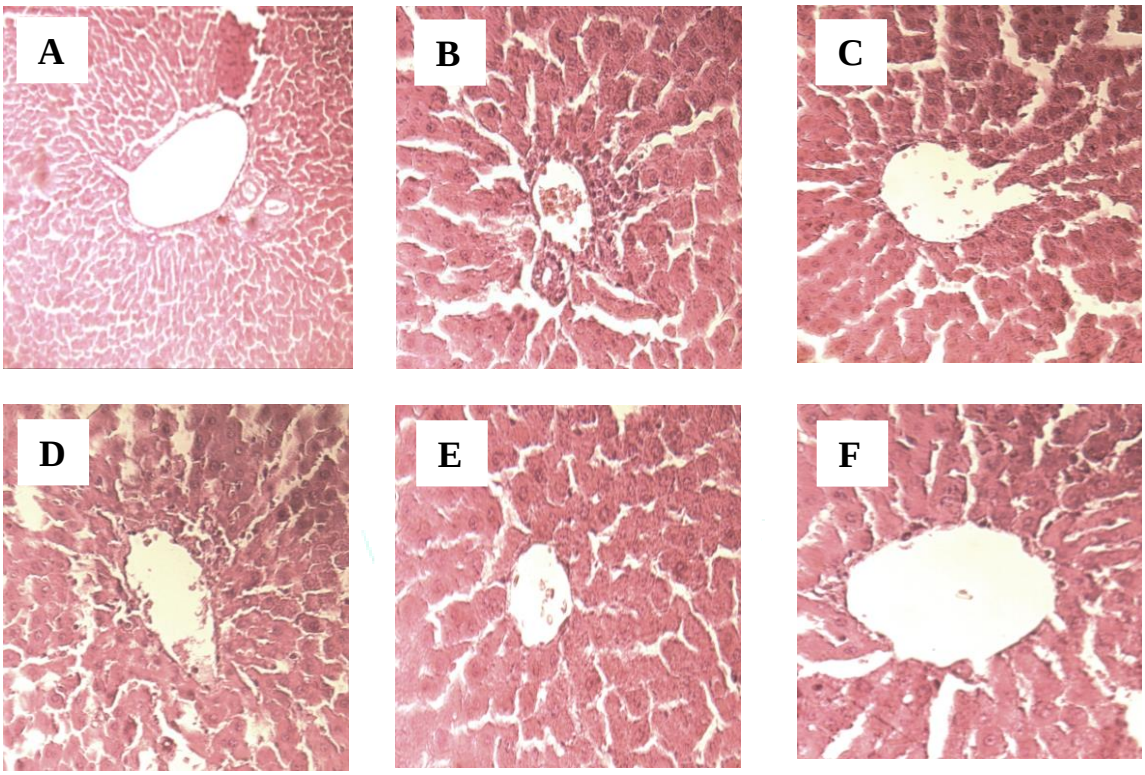

Figure 3: Histological examination of the liver section; normal control and other groups

A: Control. B: Lambda-cyhalothrin. C: Seed of *C.sativum*+Lambda-cyhalothrin. D: Areal part of *C.sativum*+Lambda-cyhalothrin. E: Seed of *C.sativum.* F: Areal part: of *C.sativum* (X40)

4. DISCUSSION

Antioxidant properties make coriander more useful to humans beyond just the use as a seasoning. It can be used as a natural antioxidant to help prolong the shelf-life of foods. Many diseases, such as atherosclerosis, dementia, diabetes, cancer, and inflammation, derive from ageing and an unhealthy modern lifestyle and nearly all of these are related to attack by reactive oxygen species (ROS). Coriander is able to help to reduce the oxygen stress and awaken the body's own antioxidase system, protecting the body from ROS attack. [15]. However, the extracts and essential oils of plant are used on food industry benefits as substitutes for chemical antioxidants [16].

The present study was undertaken to investigate the antioxidant and hepatoprotective effect of areal part and seed of *C.sativum* extract against Lambda-cyhalothrin insecticide in Wistar rats. The IC_{50} value of areal part and seed of *C.sativum* as determined through DPPH (1,1 diphenyl-2-picry hydrazyl) free radical scavenging activity, our results are exhibited better antioxidant potency of areal part extract (IC_{50} 19.38± 0.14 µg/mL) than seed extract (IC_{50} 22.62± 0.04 µg/mL) this results in agreement with research conducted by Sultana et *al* [17] which was demonstrated that both methanolic crude extract of *C.sativum* found to be (IC_{50} 58.36 µg/mL). To our knowledge, the antioxidant activities of this plant due to presence of some phenolics such as linalool, geranyl acetate and α-pinene [18].

Exposing Wistar rats to Lambda-cyhalothrin insecticide for 90 days increased the relative liver weights significantly compared to the control group. The increase in liver relative weight indicates a hepatomegaly caused by Lambda-cyhalothrin, This liver anomaly is a phenomenon result of accumulation by chemicals [19]. The administration of *C.sativum* areal part with Lambda-cyhalothrin caused significant decrease in liver relative weight less than the control, which can indicate the hepatoprotective activity.

Concerning enzymes activity, the present data showed that a significant elevation of all investigated enzymes in both serum. Instead, serum ALT, AST and ALP activities were significantly inhibited after treated with *C.sativum* extracts, indicating predictive of effectiveness of *C.sativum* extracts in liver regeneration after damaged. In addition, the serum enzymes TGO, TGP, PAL and γ-GT are synthesized at cell cytoplasm and discharged into the circulation in case of damaged cells [20,21]. These enzymes considered the best indicators of hepatic parenchyma cytolysis, which source from cell membrane and mitochondrial damages in liver cells. For example, high levels of liver enzymes, such as AST and ALT are frequently attributed to the metabolic of different toxic drugs such as psychotropic drugs [22], alcohols [23], Pollutants [24] notably Lambda-cyhalothrin.

Results from the present study demonstrate that the levels of endogenous antioxidants GSH are significantly decreased in the Lambda-cyhalothrin-treated group compared to the normal control rats, GSH is one of the abundant tripeptides, widely distributed in hepatocytes. Its functions are concerned by the displacement of free radicals such as H_2O_2 and superoxide radicals [25]. Furthermore, GSH is the first line of defence system that protects the cell from the deleterious effects of reactive oxygen species [26]. Previous studies indicated that GSH capable of recuperating superoxide anions and the hydroxyl radical by giving it electrons and becoming oxidized to their radical [27].

There are some effective defense mechanisms to protect against damage induced by free radicals. These mechanisms are ensured by the use of endogenous antioxidant enzymes such as: MDA, GST, CAT, SOD, … etc. against oxygen reactive species [28]. The results obtained indicate increased MDA levels in response to Lambda-cyhalothrin treatment, implying increased oxidative damage to the liver. However, there are some authors reported that the oxidation of fatty acids in the cell membrane results the formation of MDA, which is considered an indicator of lipid peroxidation; can be evaluated by high rates of MDA [29]. Also, Kyle et *al* [30] stated that the increase in enzyme activitie was probable a response towards increase Reactive Oxygen Species (ROS) generation. Generally, Our results demonstrate a very good protective effect of *C.sativum* extract against Lambda-cyhalothrin - induced perturbation in the levels of endogenous antioxidants both enzymatic and non-enzymatic (GSH and MDA) , which is probably due at least partly to its antioxidative properties, scavenging Lambda-cyhalothrin – associated free radicals.

The presence of injuries in livers of Lambda-cyhalothrin treated rats was revealed by histopathological examinations. In case of control group showed normal hepatic central vein, sinusoids and normal architecture of hepatocytes. The liver sections of rats treated with Lambda-cyhalothrin alone showed hepatocytes necrosis, local inflammation around the central vein and destruction of vascular walls. Pretreatment of *C.sativum* extracts reduced the severity of Lambda-cyhalothrin induced liver intoxication. Liver parenchyma was well preserved with radially arranged hepatocytes around the central vein for both groups. Regular sinusoidal structures were reserved. The result of the histologic examination experiment is in concordance with biochemical result, which clearly indicate the hepatoprotective effects provided by plant natural products [21,31].

5. CONCLUSION

The data of the present study suggests that *C.sativum* had a hepatoprotective effect against Lambda-cyhalothrin insecticide-induced hepatic damage in rats. Contributing to the alleviation of Lambda-cyhalothrin triggered typical hepatotoxic characterization. Obtained results show that the *C.sativum* can be used as source of natural antioxidants and as a possible food supplement. The hepatoprotective effect of *C.sativum* is likely due to its ability to scavenge free radicals, suppress the inflammatory responses and improve drug-metabolizing enzyme activity.

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Conflict of interest statement

The authors report no conflict of interest.

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