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Hepatoprotective Impact of *Chlorella vulgaris* Powder on Deltamethrin Intoxicated Rats

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Abstract

This study was conducted to evaluate the possible protective impact of *Chlorella vulgaris* (*C. vulgaris*) on liver tissues of adult male albino rats intoxicated with deltamethrin (DLM). Thirty-two adult rats were divided into four equal groups. The control group was orally administered 0.5 mL normal saline once daily for eight weeks. The second (*Chlorella* group (CV)) and third (Deltamethrin group (DLM)) groups, were orally received 50 mg *C. vulgaris* powder/kg BW and deltamethrin 3 mg/kg BW once daily for eight weeks respectively, while the fourth group was orally received CV and DLM together, with the same previous mentioned doses and duration. DLM treated group induced a significant elevation of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities. Liver oxidant/antioxidant status was altered due to DLM intoxication. Meanwhile, *C. vulgaris* powder normalized the changes of ALT and ALP activities. Moreover, it improved oxidant/antioxidant status of the liver related to DLM intoxication. *C. vulgaris* supplementation could overcome the DLM-induced hepatotoxicity through eliminating the oxidative tissue injuries.

Keywords: *Chlorella vulgaris*, Deltamethrin, Liver, Rat.

Introduction

Deltamethrin (DlM) is a type II synthetic pyrethroid, that inhibits humoral and cellular immune responses [1], causes thymic atrophy in mice [2], hepatotoxicity [3], hypertrophy of liver cells, significant increase in koopfer cells, blood circulation disorder and focal necrosis [4,5]. It generates the free radical that may induce oxidative stress [6]**.** The importance of dietary antioxidants and their benefits in the health and disease have been attracted great interest [7]**.** Using synthetic antioxidants decreased because they promote the carcinogenesis and consumer rejection of the synthetic food additives [8]. So, there is a current interest to find out new and safe antioxidants from natural sources such as plant material to decrease the damage of the living cells [9]**.**

Chlorella vulgar (C. vulgaris) is considered as a potential source for healthy food that containing" proteins, essential amino acids, carbohydrates, dietary fibers, fatty acids, nucleic acids, vitamins, growth factors, minerals and chlorophyll" [10]**.** Algae of *Chlorella is* considered one of the oldest organisms on earth due to its sphere form and very stable cell wall. The nutritional value of CV was determined in 1950's - 1960's [11] and its proteins include all essential amino acids required for human growth and health [12]. Moreover, it is considered a functional whole food with lower cholesterol due to it provides large amounts of minerals, vitamins, and dietary fibers [13]. *Chlorella vulgaris* specifically protects against the immunosuppressive effect of stress and gastric ulcer formation [14]. Furthermore, it showed an anti-inflammatory, immune-modulatory, hepatoprotective, antidiabetic, anti-hypertensive and anticancer activities [15-17]. The increasing use of deltamethrin encouraged the researchers to reduce its deleterious effect in the environment. Therefore, the present work was conducted to evaluate the potential protective effect of *Chlorella vulgaris* powder on deltamethrin-induced hepatotoxicity in albino male rats.

Materials and methods

Experimental animals and study protocol

The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt. The present work was conducted on 32 adult male albino rats weighing 140-180 g, collected from the Experimental Research Animal Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. They were kept in

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metal cages and fed on standard pelleted diet. Water was supplied *ad-libitum* allover the study and the rats were left two weeks for adaptation. Rats were randomly divided into four groups. Group I (Control group), was orally administrated 0.5mL normal saline once daily for 8 weeks. Group II (CV), was orally received 50 mg *C. vulgaris* powder/kg BW per day for 8 weeks (dried green powder *(C. vulgaris)* collected from Feed Crop Department, Crop Research Institute, Agricultural Research Center, Giza, Egypt) [18,19]**.** Group III (Deltamethrin) was orally received 3 mg DLM/kg BW once daily for 8 weeks (Deltamethrin (Butox); a product of Bayer company, DLM available by concentration of 50 gm/liter) [20]. Group IV (CV-DLM), was received *C. vulgaris* powder concurrently with deltamethrin at the previous mentioned doses and duration.

Blood and tissue samples collections

At the end of the study (8 weeks), animals were sacrificed and only five blood samples (in anticipation for death and given 5 samples to be suitable for statistical analysis) were collected from each group in test tubes without EDTA then left for 20 min in room temperature and centrifuged at 3000 rpm for 20 min to get serum that preserved at -20˚C until the analysis. The liver was taken from animal after removing the surrounding tissue and then washed by normal saline. It was blotted over a piece of filter paper. Half gram of the liver was homogenized in 5mL cold buffer solution and centrifuged at 3000 rpm for 20 min. The supernatant layer was transferred to Eppendorf tubes and kept at -80°C until the biochemical analysis. Other parts of the examined liver were preserved at 10% neutral buffered formalin solution for histopathological investigations.

Evaluation of hepatic injury biomarkers

The preserved sera were used for evaluating Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP) calorimetrically according to Reitman and Frankel [21] and Tietz *et al*. [22], respectively.

Assessment of oxidant/antioxidant status of liver tissues

The homogenous liver tissue was used for assessing the catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) levels calorimetrically using the method of Aebi [23], Nishikimi *et al*. [24] and Ohkawa *et al*. [25], respectively.

Histopathological preparations

Liver specimens that preserved in 10% neutral buffered formalin solution were dehydrated in ethanol (70-100%) then washed in xylene and putted in paraffin. Prepared sections (5µm thickness), were stained with Hematoxylin and Eosin and then examined microscopically for pathological findings [26].

Statistical analysis

The variation between the animal groups were statistically analyzed using one-way (ANOVA) then Duncan's multiple ranges post hoc test for comparisons among means. Data were considered significant at $p < 0.05$ [27].

Results

Hepatic injury biomarkers and oxidant/antioxidant status

There were significantly ($p<0.05$) increased of ALT and ALP in the DLM group when compared with the control group. Meanwhile, administration of *C. vulgaris* concurrent with DLM induced significantly ($p<0.05$) reduction in both of ALT and ALP level when compared with DLM treated group only (Table 1). The oral daily administration of 50 mg *C. vulgaris* powder/kg BW to adult rats for eight weeks significantly ($p<0.05$) elevated the CAT and SOD levels when compared with the control rat group (Table 2). The oral intoxication in rats with DLM significantly ($p<0.05$) reduced the SOD and CAT meanwhile, significantly ($p<0.05$) elevated the MDA concentration. On the other hand, administration of *C. vulgaris* powder with DLM significantly ($p<0.05$) increased the CAT and SOD with significant reduction in the concentration of MDA than that of DLM intoxicated group.

Table 1: Effects of *C. vulgaris* and deltamethrin on serum ALT and ALP levels of male albino rats, (Mean ±SE, n=5)

Treatments*	Parameters	
	ALT(U/I)[1]	ALP (U/I)[2]
Control group[3]	38.51±1.43[c]	162.68±4.7[c]
Second group (CV)[4]	37.62±2.78[c]	162.27±5.3[c]
Third group (DLM)[5]	66.59±2.58[a]	223.43±5.7[a]
Fourth group (CV-DLM)[6]	51.77±2.59[b]	193.12±3.4[b]

1 ALT: Alanine Aminotransferase; [2]ALP: Alkaline phosphatase.[3]Control rats orally received 0.5mL normal saline once daily for 8 weeks. [4]CV group: rats received *C. vulgaris* (50 mg/ kg BW) orally once daily for 8 weeks. [5]DLM group: rats administered deltamethrin (3 mg/ kg BW) orally once daily for 8 weeks.[6]CV-DLM group: rats received *C. vulgaris* concurrently with DLM with the previous mentioned doses and duration.
Means within the same column carrying different superscripts were significant at ($p < 0.05$).

Histopathological findings of hepatic tissue

Liver tissue sections of the control and *Chlorella* treated groups showed normal morpho- histological structures (Figures 1A and B). But, the DLM intoxicated group showed mild portal and interstitial infiltration of round cells. Moreover, degenerative changes (cloudy swelling and hydropic degeneration) and fatty changes were detected in some hepatocytes (Figures 1C, D, E). The rats treated with both of *Chlorella* and deltamethrin revealed mild congestion of hepatic blood vessels with apparently normal hepatic parenchyma in most of the examined sections and mild degenerative changes in a few number of hepatocytes. Additionally, mild portal, interstitial and sinusoidal infiltration of round cells and mast cells were detected in most of the examined sections (Figure 1F).

Table 2: Effect of *C. vulgaris* and deltamethrin on the hepatic levels of CAT, SOD and MDA in male albino rats (Mean ±SE, n=5)

Treatments*	Parameters		
	CAT (U/mg tissue)[1]	SOD (U/mg tissue)[2]	MDA (nmoL/mg tissue)[3]
Control group[4]	0.31±0.004[b]	0.26±0.016[b]	0.14±0.002[c]
Second group (CV)[5]	0.36±0.010[a]	0.33±0.006[a]	0.13±0.006[d]
Third group (DLM)[6]	0.11±0.006[d]	0.11±0.003[d]	0.31±0.006[a]
Fourth group(CV-DLM)[7]	0.15±0.006[c]	0.17±0.004[c]	0.21±0.003[b]

[1]CAT: Catalase; [2]SOD: Superoxide dismutase; [3]MDA: Malondialdehyde; [4]Control rats orally received 0.5mL normal saline once daily for 8 weeks. [5]CV group: rats received *C. vulgaris* (50 mg/ kg BW) orally once daily for 8 weeks. [6]DLM group: rats administered deltamethrin (3 mg/ kg BW) orally once daily for 8 weeks.[7]CV-DLM group: rats received *C. vulgaris* concurrently with DLM with the previous mentioned doses and duration.
Means within the same column carrying different superscripts were significant at ($p < 0.05$).

Discussion

Deltamethrin is type II pyrethroid insecticide used in variable house, agricultural and landscaping settings; however, it has higher levels of toxicity against insects. At the same time, pyrethroids can affect mammals and ecosystem as a whole, because they remain for a long time in the air, soil, and water. Moreover, it may be used as a vector control for insects [28]. The liver is the vital part in metabolism and detoxification, therefore deltamethrin changes the liver enzymes and function in rats (as a model of mammals). Oxidative stress is an imbalance occurs between free radicals and the body ability to detoxify the harmful effects throughout neutralization by body antioxidants. Also, it is a status which causes cell death, by oxidation of cellular lipids, proteins and DNA [29]. Antioxidant enzymes (SOD and CAT) are the first line of defense against free radical induced oxidative stress. Superoxide dismutase is used for catalytic

dismutation of superoxide radicals to hydrogen peroxide, while catalase is responsible for the catalytic decomposition of hydrogen peroxide to oxygen and H_2O [30]. Our results regarding the oxidative hepatic tissue damage in DLM-induced toxicity which manifested by a significant elevation in the liver tissue MDA concentration with reduction of tissue CAT and SOD when compared to the control group. These alterations may be due to exhaustion of CAT and SOD that caused by an excessive reactive oxygen species production as deltamethrin attacks cellular component inducing lipid peroxidation leading to tissue damage. Similar results were obtained by Manna *et al*. [6], who detected a significant decrease in SOD and CAT in rat liver after deltamethrin administration.

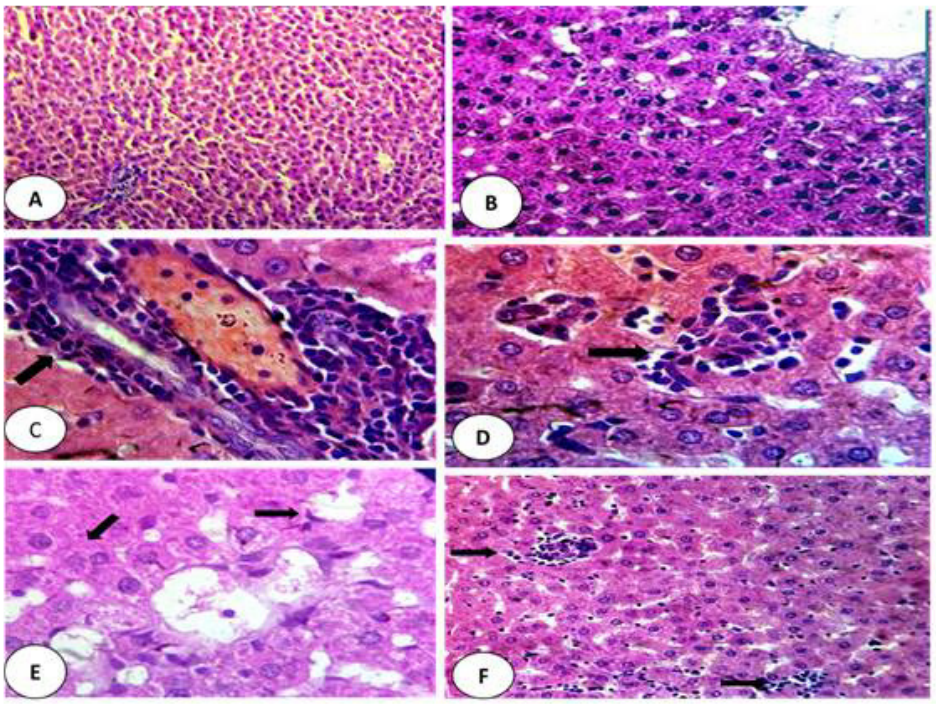

Figure 1: A: liver tissue section of control group, showed the normal morpho-histological structures (H&Ex400). B: liver tissue section of rats treated with *Chlorella*, showed normal hepatic morpho-histological structures (H&Ex1000). C: liver tissue section of rats treated with deltamethrin, showed portal round cells infiltration (arrow) (H&Ex1000). D: liver tissue section of rats treated with deltamethrin showed interstitial round cells infiltration (arrow) (H&Ex1000). E: liver tissue section of rats treated with deltamethrin, showed cloudy swelling and fatty changes (arrows) (H&Ex1000). F: liver tissue section of rats concurrently treated with *Chlorella* and deltamethrin showed interstitial and sinusoidal infiltration of round cells and apparently normal hepatocytes (arrows) (H&Ex400).

Hepatocytes, complex metabolic liver cells, contain a large amount of enzymes such as ALT which permeate into plasma as a result of liver damage. It is evaluated as a marker of liver damage [31]. This enzyme is sensitive to liver toxicity and its histopathological changes compared with other enzymes as ALP and AST that can be evaluated in a shorter period of time [32]. Another enzyme which is assessed to diagnose liver damage is ALP which exists in most tissues and its level is altered due to liver diseases [33]. ALT and ALP levels were increased significantly ($p<0.05$) in the current study after deltamethrin intoxication when compared with the control group. The leakage of these enzymes from liver cytosol into the blood indicates destruction of hepatic cells [34]. Our findings were supported by Evans and Halliwell [5] and Ghassemi *et al*. [35], who found that deltamethrin intoxication, induced a significant increase ALT and ALP levels in female rats.

Chlorella vulgaris is unicellular green algae, broadly used as a food supplement and it has high antioxidant and therapeutic uses such as lutein, α, β-carotene, ascorbic acid and α-tocopherol, which are active against free radicals and may be responsible for *Chlorella* functional activities [36,37]. Furthermore, α and β-carotene in *C. vulgaris* not only react with various ROS but also interfere with the processes of oxidation in the lipid and cellular compartment [38]. *Chlorella vulgar has* many health-promoting benefits as antioxidant and lipid reduction in animals and humans [39] with free radical scavenging action [40,41]. These facts about C. *vulgaris* could explain its antioxidant property present here which manifested by a significant elevation of SOD and CAT levels with significant reduction of MDA concentration in liver tissue of *C. vulgaris* treated groups when compared with the control group. Our results supported by Queiroz *et al*. [19]. *C. vulgaris* treatment before lead, heavy metals, mercury and cadmium, reduces its toxicities and mediate the antioxidant activities so used as a protective effect in rats [19]. Therefore, our results revealed that *C. vulgaris* plays an important role to prevent hepatic toxicity, especially resulted in oxidative stress which manifested by amelioration of oxidant/antioxidant hepatic status at DLM intoxicated animals. The current results may be explained by antioxidant defense mechanisms resulted from *Chlorella* intake against lipid peroxidation of liver tissues of deltamethrin intoxicated rats.

The current results were supported by Aizzat *et al*. [42], who studied the antioxidant effect of *C. vulgaris* in STZ-induced diabetic rats and reported that *C. vulgaris* significantly reduced the blood MDA level in STZ-induced diabetic rats when compared with the control group. Our findings were supported by Vijayavel *et al*. [43], who demonstrated that treatment with *C. vulgaris* significantly decreased the lipid peroxidation with significantly increased the enzymic and non-enzymic antioxidants activation in naphthalene intoxicated rats**.** Similarly, El Makawy *et al*. [44] reported that *C. vulgaris* aqueous extract has hepatoprotective property against MSG and enhanced the activities of liver function that evidenced by the improvement of MDA, antioxidant activities, histological and histochemical changes.

Conclusion

This study demonstrated that deltamethrin intoxication resulted in biochemical changes in the serum parameters and elevated lipid peroxidation with a reduction of the antioxidant enzymes activities. *C. vulgaris* powder could be considered a promising ameliorative agent against DLM-induced hepatotoxicity in rats.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربى

التأثير الوقائى لمسحوق الكلوريلا فولغاريس على كبد الجرذان المسممة بالدلتامثرين

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أجريت هذه الدراسة لتقييم التاثير الوقائى المحتمل للكلوريلا فولغاريس على أنسجة الكبد فى ذكور الجرذان البيضاء البالغة عند معاملتها بمادة الدلتامثرين. تم توزيع عدد إثنين وثلاثون من ذكورالجرذان البيضاء البالغة عشوائيا على أربع مجموعات متساوية كل مجموعة ٨ جرذان : المجموعة الأولى (المجموعة الضابطة)، تلقت ٠.٥ مل من محلول ملى عن طريق الفم مرة واحدة يوميا لمدة ٨ أسابيع. المجموعة الثانية (مجموعة الكلوريلا) وقد تلقت مسحوق (٥٠ ملجم/كجم من وزن الجسم) عن طريق الفم مرة واحدة يوميا لمدة ٨ أسابيع. المجموعة الثالثة (مجموعة الدلتامثرين) وتم إعطاء الجرذان بجرعة (٣ملجم/كجم من وزن الجسم) مرة واحدة يوميا عن طريق الفم لمدة ٨ أسابيع. المجموعة الرابعة (الكلوريلا مع الدلتامثرين) تم إعطاءالجرذان الكلوريلا قبل الدلتامثرين بساعة بنفس الجرعات والمدة سابقة الذكر. وكشفت النتائج أن المجموعات المسممة بمادة الدلتامثرين أسفرت عن زيادة ملحوظة فى مستوى الألنين امينو ترانزفيراز(ALT) والكالين فوسفاتيز(ALP) وزيادة ملحوظة فى الإجهاد التأكسدى لخلايا الكبد. وفى الوقت نفسه فإن مسحوق الكلوريلا ساعد فى الحد من هذا الإجهاد التأكسدى وأثبتت فاعليتها فى تحسين التاثيرات السمية الخا□ة بالكبد .أسفرت النتائج النهائية لهذا البحث عن أهمية مضادات الأكسدة طبيعية المصدر مثل كلوريلا فولغاريس فى التغلب على التسمم الكبدى الناتج عن التسمم بمادة الدلتامثرين.