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Ameliorative effect of date palm pollen against carbendazim toxicity in lymph node of rats

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Abstract: Carbendazim (CBZ) is a widely used fungicide that controls a variety of fungal diseases affecting crops. The widespread use of CBZ has raised concerns regarding its environmental persistence and its potential toxicological effects on non-target organisms, including humans. To the best of our knowledge, the CBZ toxicity on lymph node structure, that plays a critical role in immune response, was not reported. Therefore, we examine the chronic exposure of sub-lethal dose of CBZ on the histological structure of lymph node in rats. Moreover, we examine the therapeutic potential of date palm pollen (DPP) in counteracting CBZ toxicity. 32 of male albino rats (*Rattus rattus*) were divided into 4 groups: control, CBZ (10 mg/Kg bw), DPP (100 mg/Kg bw), and CBZ with DPP; and the exposure experiment was lasted for 2 months. CBZ caused severe histopathological changes in the lymph node structure such as atrophic lymphoid follicle, thick capsular, hemorrhage, and congested lymphoid sinus. Treatment with DPP alone showed normal structure of lymph node as control group; while it seems that DPP mitigates the negative effects of CBZ when combined together and showed mostly normal structure of lymph node. The capacity of DPP to mitigate the CBZ toxicity might return to its chemical composition that enhance the ability to detoxify harmful substances by neutralizing reactive oxygen species (ROS) that might be generated during CBZ exposure. However, future biochemical and physiological studies are needed to examine CBZ toxicity and the therapeutic potential of DPP.

Keywords: carbendazim, natural product, date palm pollen, lymph node, pesticide, immune system.

1. Introduction

Carbendazim (CBZ) is a fungicide that belongs to the benzimidazole class, primarily used in agriculture to control a variety of fungal diseases affecting crops. It's effective against Ascomycetes, Fungi Imperfecti, and Basidiomycetes, making it suitable for a range of plants [1-3]. Chemically, CBZ contains an aromatic hydrocarbon ring, which is predictable to be oxidized by cytochrome P450 (CYPs) [4-7]. CYPs-mediated oxidation and reduction reactions that could result in either detoxification or activation effects [5,7]. However, there was still little information available on the metabolic enzymes, kinetic behaviors, and exact metabolites involved in CBZ metabolism in various species. CBZ exhibits relatively low acute toxicity in humans and non-target organisms when compared to many other pesticides [8]. Its LD50 values (the dose required to kill 50% of a test population) ranged between 2000 and more than 10000 mg/kg bw for oral and dermal exposures indicates a low level of acute toxicity to mammals [9-10]. However, due to its low acute toxicity, the widespread use of carbendazim has led to growing concerns regarding its environmental persistence, bioaccumulation in soil, water, and food products, and potential toxic effects on non-target organisms, including humans [11-13]. Long-term exposure, particularly at high doses, has been associated with potential reproductive toxicity, hepatotoxicity, and mild immunotoxic effects in laboratory studies. Indeed, carbendazim's toxicity is well-documented, with studies revealing its toxicity to induce oxidative stress, genotoxicity, carcinogenicity, and organ-specific damage in some organs as in liver, kidney and reproductive organs [14-18]. These adverse effects are primarily linked to the compound's ability to generate reactive oxygen species (ROS), disrupt cellular signaling pathways, and impair the normal functioning of various physiological systems [10,16,18]. Despite the lymphatic system, including lymph nodes, might be a susceptible target for CBZ toxicity, to the best of our knowledge, the toxicity of CBZ on lymph node was not reported. Lymph nodes, integral to the immune response, are critical sites for antigen processing and lymphocyte activation [19-20]. Disruption in their structure or function due to toxicants like CBZ could lead to compromised immunity and increased susceptibility to infections and diseases [19-22].

Recently, research has focused on mitigating pesticide-induced toxicity by using natural products due to their safety and multifaceted therapeutic properties [23-25]. Among these natural products, date palm pollen (DPP), a male reproductive cell of palm tree (*Phoenix dactylifera*), has been extensively recognized for its role in mitigating toxin-induced damage [26-28]. DPP is a rich source of bioactive molecules, including flavonoids, phenolic acids, sterols, vitamins, and minerals, which collectively exhibit potent antioxidants, anti-inflammatory, and immunomodulatory properties; hence can neutralize free radicals, enhance cellular repair mechanisms,

and promote the regeneration of damaged tissues [29-30]. Therefore, it can be used as a protective agent against toxicants on the hematological parameters [28], anti-hepatotoxicity [31], anti-nephrotoxicity [32], and anti-inflammatory [33]. To this end, the current study was conducted to investigate the effect of sub-lethal dose of CBZ toxicity on the lymph nodes, focusing on potential histological alterations for the first time. Furthermore, to evaluate the therapeutic potential of DPP in mitigating the possible toxic effects of CBZ on lymph node.

2. Materials and methods

2.1. Animals and experimental design:

Male albino rats (*Rattus rattus*; n = 32; with mean weight 200 ± 20 g) were obtained from animal house of Faculty of Science, Sohag University, Egypt. In the University facilities, rats were maintained in clean stainless-steel cages; in a well-ventilated room with optimum conditions (temperature 25 ± 1 °C; and under 12 h light: 12 h dark photoperiod). Each cage was covered with hardwood bedding that changed weekly. Animals were allowed to acclimate to these conditions for 2 weeks before starting the experiment. During the acclimation, rats were fed daily with standard rat's chow and drank tap water *ad libitum*. All experiments were done with the consent of the institutional ethical committee for animal experimentation (permission CSRE-30-24).

Rats were randomly divided into 4 groups (8 rats / cage), and the exposure experiment lasted for 2 months. First group (G1) acted as a control group that provideder and food only without any exposure; while the second group (G2) and the third group (G3) were orally treated with carbendazim (CBZ; 10 mg/Kg bw; [43]) and date palm pollen grains (DPP; 100 mg/Kg bw; [28]), respectively. Finally, the fourth group (G4) was simultaneously treated with DPP (100 mg/Kg bw) and CBZ (10 mg/Kg bw). No mortality was observed in any groups during the experiment.

2.2. Chemicals:

Carbendazim, methyl benzimidazol-2-ylcarbamate, (CBZ; Loba Chemie company, India) was prepared as a stock solution by dissolving in sunflower oil. Similarly, date palm pollen grains (DPP; obtained from the farm of Faculty of Agriculture, Sohag University, Egypt) were extracted from the date bark, washed, dried, blended, and the powder was kept in the fridge until use. The stock solution of DDP was prepared by dissolving the powder in the distilled water.

2.3. Histological studies:

At the end of the experiment, rats were sacrificed and the (axillary, mediastinal, and mesenteric) lymph nodes were excised and fixed in 10% of neutral buffered formalin (Sigma-Aldrich, St. Louis, MO) after washing with physiological saline (0.9% NaCl) solution. The lymph node was prepared for histological examination and routinely processed for paraffin embedding technique. Embedded tissues were sectioned at 5 µm and stained with hematoxyline and eosin (HE) as previously described in detail [34-35]. Histological sections were examined and captured by using Olympus CX43 light microscope with an Olympus SC52 camera adapted for the microscope (Olympus, Tokyo, Japan).

3. Results:

Histological examination for G1 (control group) revealed normal histological structure of lymph node with normal capsular structure, lymphoid follicular size, lymphocytic cellular density in germinal center, and vascular sinuses (Fig. 1). In G2 (CBZ group), CBZ caused severe histopathological changes in the lymph node structure as atrophic lymphoid follicle filled with necrotic cells admixed with fibrous network, thick capsular structure (Fig. 2A, B), subcapsular hemorrhage, dispersed parenchymal cells (depletion), and dilated and congested lymphoid sinus (Fig. 2C-F). Treatment with DPP (G3) alone showed normal structure of lymph node as control group (G1) (Fig. 3A, B); while it seems that DPP soothed the negative effect of CBZ when combined together (G4), and showed mostly normal structure of lymph node (Fig. 3C-F) with exception of presence some necrotic cells (Fig. 3D).

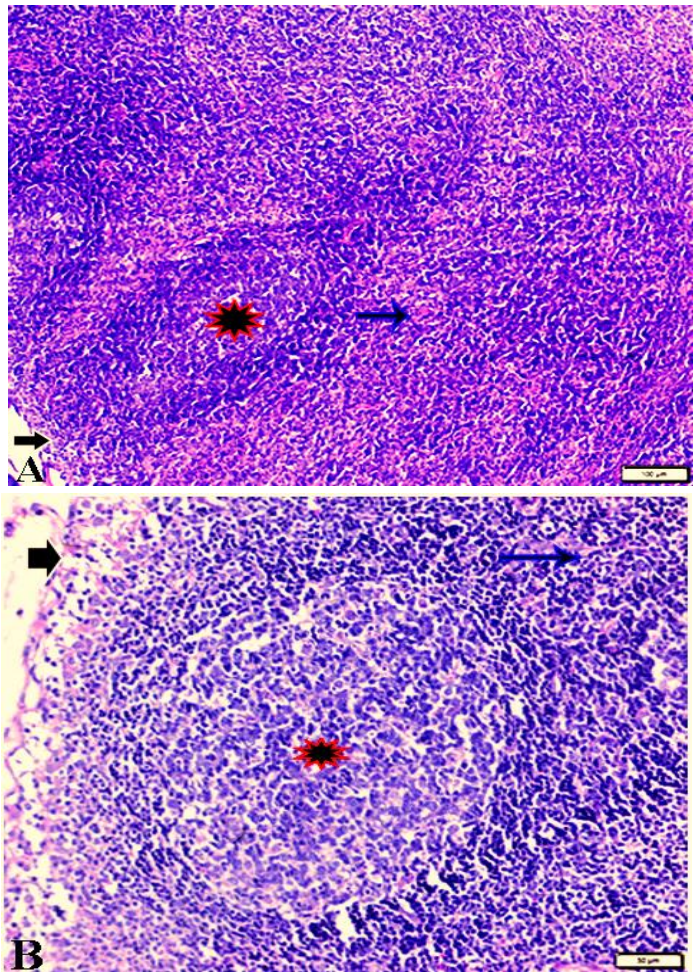

Fig. 1. Photomicrograph of lymph node section of control group (G1) (**A and B**) showing normal capsular structure (thick arrows), normal lymphoid follicle size with normal lymphocytic cellular density in germinal center (stars), and vascular sinuses (thin arrows). **A** magnified **B**. H and E stain. Scale bar equal 20 µm.

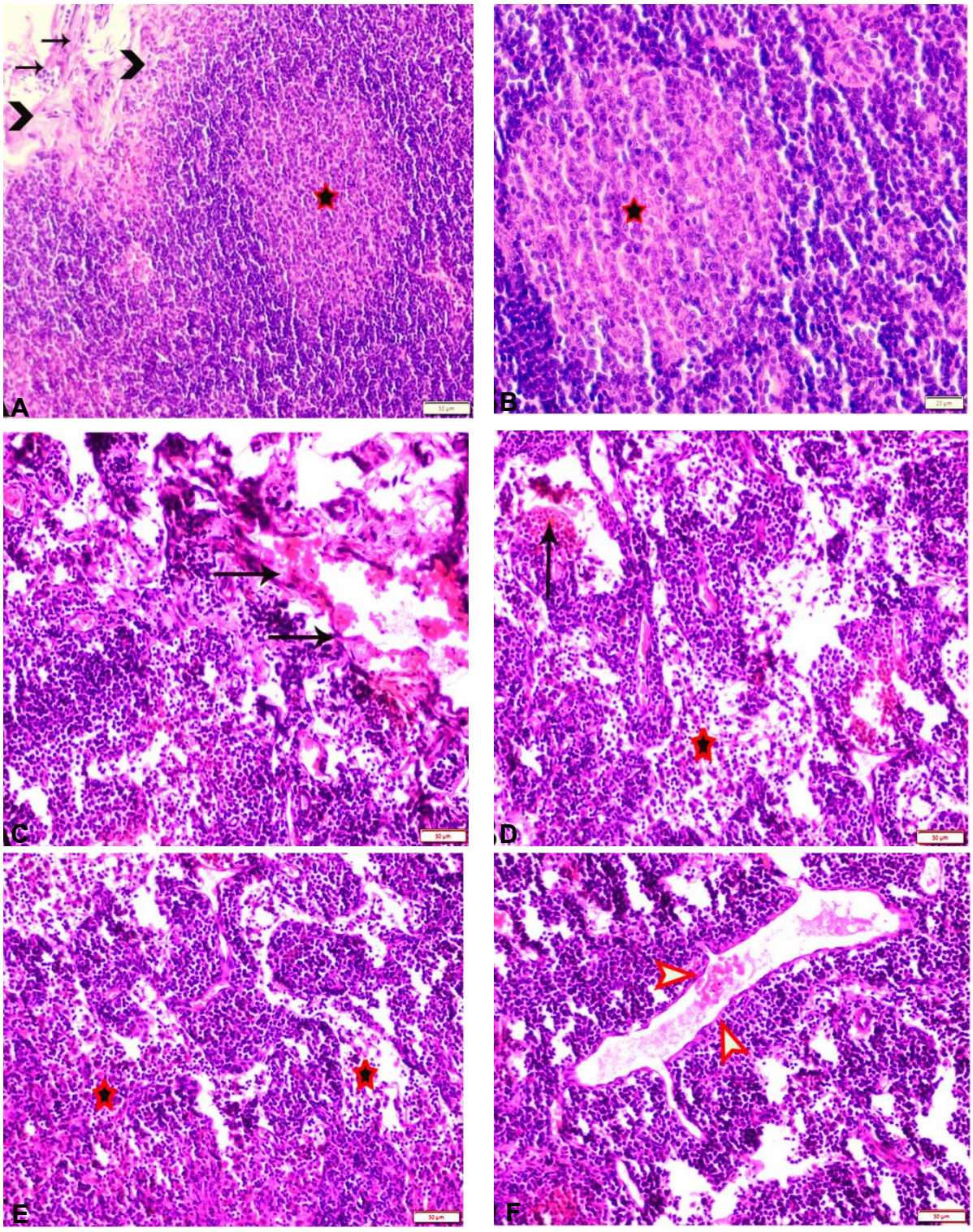

Fig. 2. Photomicrograph of lymph node sections from carbendazim group (CBZ; G2) showing thick capsule (arrow heads) (**A**); atrophic lymphoid follicle filled with necrotic cells admixed with fibrous network (star) (**A,B**); subcapsular hemorrhage (arrows) (**C,D**); dispersed parenchymal cells (depletion) (stars) (**D,E**); and dilated and congested lymphoid sinus (red arrow heads) (**F**). H and E stain. Scale bar equal 20 µm (**B**) and 50 µm (**A, C-F**).

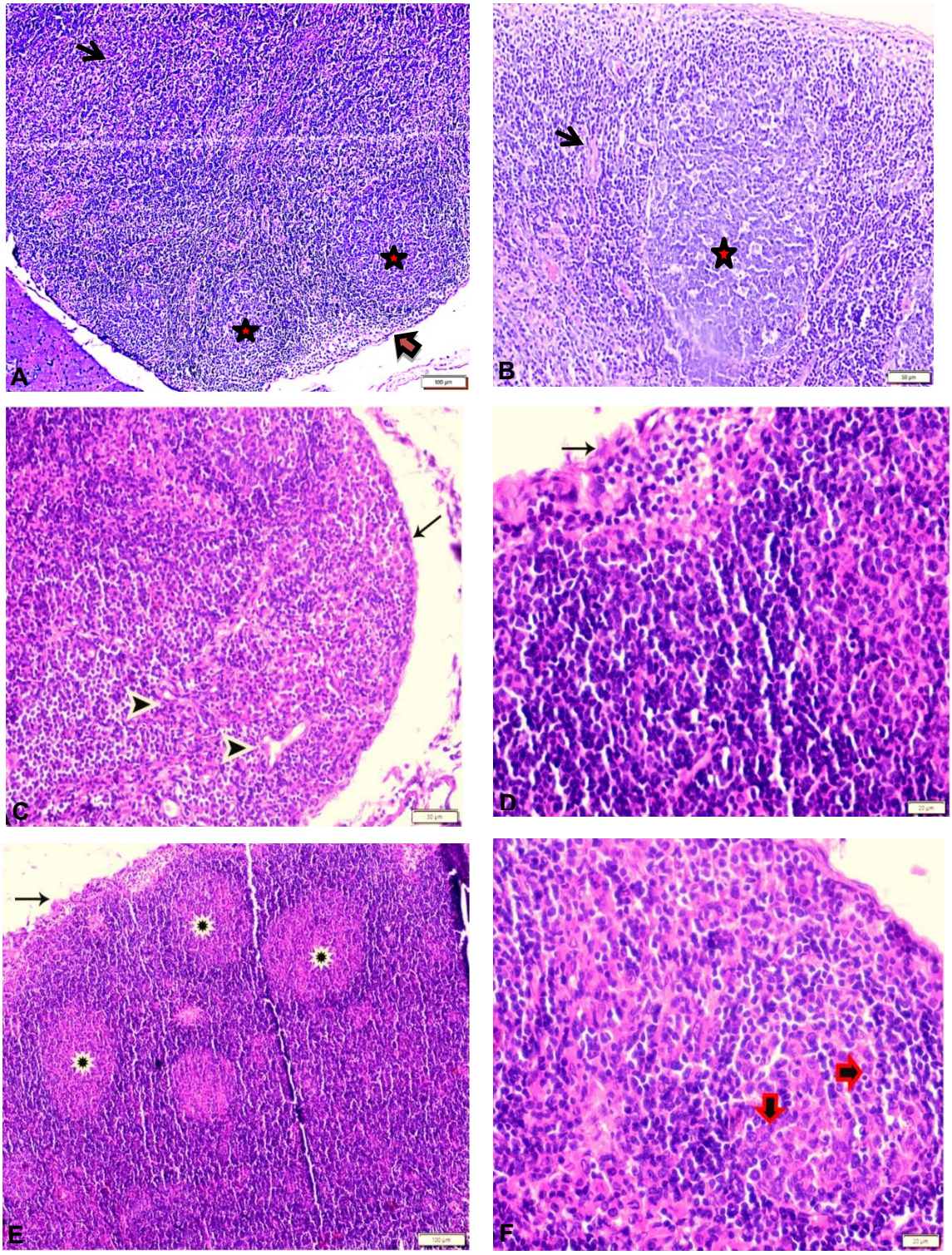

Fig. 3. Photomicrograph of lymph node section of date palm pollen group (DPP; G3; **A-B**); and combination of CBZ with DPP group (G4; **C-F**). (G3; **A-B**) Showing normal capsule (red arrow) (**A**); normal lymphoid follicular structure (stars); and normal sinuses (black arrows) (**A-B**). (G4; **C-F**) Showing normal capsule (thin arrows) (**C-E**); normal lymphatic sinuses (arrow heads) (**C**). Lymphoid follicles restored its normal structure (stars) (**E**), except for the presence of some necrotic cells (red arrows) (**F**). H and E stain. Scale bar equal 100 µm (**A,E**), 50 µm (**B,C**), and 20 µm (**E,F**).

4. Discussion:

Carbendazim (CBZ) is a widely used fungicide in agriculture, but its toxicological effects have raised concerns, particularly regarding its impact on the immune system and lymphatic tissues such as lymph nodes. CBZ, a teratogenic, mutagenic and neurogenic agent that exerts its toxicity via generation of reactive oxygen species (ROS) [10,18,36]. CBZ increases the oxidation of thiols, proteins, lipids, and reduces the antioxidant enzymes' activities [37,38]. Moreover, it causes hematological abnormalities, hepatic and renal dysfunction and structure, neurodegenerative disorders, immunological disorders, disrupting the antioxidant defense mechanism [14-18,37]. Although many studies have reported the side effects of using CBZ as pesticide for controlling fungal diseases in agriculture, the CBZ toxicity on lymph nodes was not reported.

Lymph nodes play a vital role in the immune response by filtering lymph fluid, housing immune cells, as T and B lymphocytes, and responding to infections and foreign substances [20, 39-40]. Recently, great attention has been paid to explore the potential of natural substances, such as date palm pollen, to counteract the toxic effects of xenobiotics and synthetic products such as pesticides [26,28]. To the best of our knowledge, the current study reveals the toxicity of carbendazim on lymph node for the first time; and the role of DPP as a natural therapeutic agent to counteract the carbendazim toxicity.

Carbendazim exhibits low acute toxicity in mammals, with high lethal dose for 50% of the population (LD50) values ranged between 2000 to 10000 mg/kg bw in CBZ orally administration studies for rats, based on specific experimental conditions and methodologies, indicating that substantial amounts would be required to reach lethal levels [9-10]. However, chronic exposure can lead to significant long-term health effects, including hepatotoxicity, nephrotoxicity, and reproductive toxicity [14-18]. Previous histopathological examinations have shown significant kidney damage characterized by mononuclear cell infiltration and degeneration and fibrosis of renal tubules [14,41-42]; and liver damageacterized by congestion, enlargement of sinusoids, necrosis, and infiltration of mononuclear cells in the liver tissue [14,41,43]. Similarly, in the current study, oral administration of a few milligrams of CBZ (10 mg/Kg bw) for 2 months caused thick lymphatic capsule, infiltrated with mononuclear inflammatory cells, atrophic lymphatic nodule filled with necrotic cells admixed with fibrous network, subcapsular hemorrhage dispersed parenchymal lymphatic cells (depletion), and dilated and congested lymphatic sinus. Accordingly, previous studies have reported that lymphoid necrosis depending on the inciting factor accompanied by inflammatory cells including neutrophils and phagocytic macrophages with intracytoplasmic cellular debris and can lead to lymphatic sinus ectasia with few lymphocytes, plasma cells and macrophages admixed with fibrous network [39]. Moreover, lymphoid depletion is a sign of chronic necrosis or apoptosis which is characterized by a decrease in the number and size of follicles and germinal centers [39].

On the other hand, date palm pollen (DPP) is gaining attention for its various applications and health benefits. DPP contains significant amounts of antioxidants, which may help mitigate oxidative stress caused by carbendazim toxicity. The presence of phenolic compounds and flavonoids in date palm pollen can enhance the ability to detoxify harmful substances by neutralizing reactive oxygen species (ROS) including that might be generated during carbendazim exposure [10,29-31]. By reducing oxidative stress, DPP may protect tissues from damage associated with pesticide toxicity. Previous studies have demonstrated that DPP can inhibit lipid peroxidation, a process that contributes to cellular damage during toxic exposure, which in turn is vital for maintaining cellular integrity and function in tissues affected by carbendazim [44-45]. Research indicates that DPP treatment can ameliorate liver damage caused by various toxins, including those like carbendazim [14,28,46]. It has been shown to reduce histological lesions and inflammatory changes in liver tissue, suggesting its potential to protect against carbendazim-induced hepatotoxicity [14,18,41,46]. Moreover, similar protective effects have been observed in kidney tissues. DPP has been reported to influence renal function parameters positively and mitigate histological damage in the kidneys, which may also be relevant for recovery from carbendazim toxicity [14,18,41-42,46]. Consistently, the current study revealed that the administration of DPP before CBZ restored completely the lymph node structure. Therefore, DPP, as an antioxidant, could prevent the generation of free radicals, and hence protect the lymph node structure against the toxicity of CBZ.

5. Conclusion

Chronic exposure of few milligrams of carbendazim was enough to cause severe histopathological changes in lymph node structure and thereby might impair the immune system. Indeed, orally doses of carbendazim (600 mg/Kg/day) caused a decrease in white blood cell and lymphocyte counts in male albino rats [14]. Alternatively, the incorporation of date palm pollen may serve as a natural therapeutic agent to counteract the toxic effects of carbendazim on lymph node. Its antioxidant properties and ability to reduce oxidative stress make it a promising candidate for further research into its protective roles against pesticides toxicity. Future biochemical and physiological studies are needed to examine the toxicity of carbendazim and the protective effect of DPP on the biological system.

CRediT authorship contribution statement:

Author Contributions: Ahmed Badr, Mohamed F. El-Sayed, Sary Kh. Abd El-Ghaffar, Basma H.H. Nasser; methodology, Ahmed Badr, Mohamed F. El-Sayed, Basma H.H. Nasser; validation, Ahmed Badr, Basma H.H. Nasser; formal analysis, Ahmed Badr, Basma H.H. Nasser; investigation, Ahmed Badr, Mohamed F. El-Sayed, Sary Kh. Abd El-Ghaffar, Basma H.H. Nasser; resources, Ahmed Badr, Mohamed F. El-Sayed, Basma H.H. Nasser; data curation, Ahmed Badr, Basma H.H. Nasser, writing—original draft preparation, Ahmed Badr, Mohamed F. El-Sayed, Sary Kh. Abd El-Ghaffar, Basma H.H. Nasser; writing—review and editing, Ahmed Badr, Basma H.H. Nasser; visualization, Ahmed Badr, Mohamed F. El-Sayed, Sary Kh. Abd El-Ghaffar, supervision.

All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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