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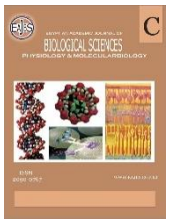

Spirulina Platensis Attenuates Chlorpyrifos-Induced Hepatotoxicity in Rats via Antioxidant, Anti-Inflammation, and Anti-Apoptotic Mechanisms

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ABSTRACT

Background and Aim: The current study was designed to estimate spirulina's (SP) potential role against chlorpyrifos-triggered hepatotoxicity in adult male albino rats. **Experimental Approach:** 28 Wistar male albino rats were categorized to 4 groups (n=7) as following: a control group; a chlorpyrifos group that received chlorpyrifos (5 mg/kg; daily oral administration for 21 consecutive days); a spirulina group that received SP (500 mg/kg; daily oral administration for 21 consecutive days); and a fourth group that received SP 1 h after CPF administration for 21 consecutive days. **Findings and Conclusions:** CPF intoxication increased the levels of serum liver function markers alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as compared to control group. Also, there was an elevation in the content of oxidative stress biomarkers malondialdehyde and nitric oxide (MDA and NO) contaminants with a significant reduction in superoxide dismutase (SOD), reduced glutathione (GSH) levels and catalase (CAT) concentration as antioxidant indicators. Furthermore, animals' livers intoxicated with CPF recorded a significant elevation of interleukin-1beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-κB) as indicators for inflammation as well as apoptosis biomarkers Bcl-2-associated X protein (Bax) and cysteine-aspartic acid protease-3 (Caspase-3). At the same time, there was a marked decrease in anti-apoptotic markers B-cell lymphoma-2 (Bcl-2). However, the post-treatment of SP showed that, there was an improvement in ALT, AST and ALP levels. The levels of these enzymes were reduced. Furthermore, increasing of GSH levels, SOD and CAT activities in liver tissue homogenate and significantly decreasing the levels of MDA and NO. administration of SP (500mg/kg) after one hour of CPF (5mg/kg) administration for 21 consecutive days, significantly reduced the increased levels of proinflammatory cytokines in liver tissue homogenate. Also, significantly alleviate the level of apoptotic markers BAX and caspase-3, but increase the level of antiapoptotic markers Bcl-2 in the liver tissue. In conclusion, the present study showed that SP has free radical scavenging, inflammation-suppressing, and apoptosis-inhibiting properties in the hepatic tissue of CPF-intoxicated wistar male albino rats.

INTRODUCTION

Pesticides are a group of compounds that include substances used as insecticides, herbicides, fungicides, molluscicides, rodenticides and nematicides (Budzinski & Couderchet, 2018, and Chio and Li, 2022). World Health Organization (WHO) announced that, around1,000,000 individuals were influenced via severe poisoning through pesticide exposure. Every year, a mortality range ranging from 0.4 to 1.9% has been demonstrated (Eddleston, 2020, Jia *et al.,* 2020). Almost all organophosphates categorized within two groups: diethyl (e.g., chlorpyrifos, parathion, diazinon, dichlorfenthion and phorate) and dimethyl (e.g., dimethoate, fenitrothion, dichlorvos, fenthion and malathion) (Wu *et al.,* 2024).

Chlorpyrifos (CPF; O, O-diethyl-O(3,5,6-trichloro-2-pyridyl) phosphorothionate) considered as a widely used chlorinated insecticide which easily uptakes through both the respiratory and digestive system in experimental animals and humans. it is assumed to be mainly converted within the liver via several cytochrome P450 multifunction oxidases via various reaction mechanisms. The main toxic biotransformation product is CPF oxon (Farhadi *et al.,* 2024, Eaton *et al.,* 2008). CPF oxon functions to inhibit acetylcholinesterase function in the intended tissues, resulting in the endogenous acetylcholine accumulation, which leads to the overstimulation and disruption in the function of the nervous system activity (Rawat and Singh, 2024, Timchalk *et al.,* 2002). CPF prompts a number of other toxic impacts, such as reproductive (chlorpyrifos remarkably reduced the total sperm count, gonadotropin levels, and serum testosterone, and the enzyme activity contributed to sperm production, resulting in testis oxidative stress), DNA impairment, and genotoxicity (Eaton *et al.,* 2008). A specific molecular pathway of CPF-induced toxicity includes the production of reactive oxygen species (ROS), causing oxidative damage in intended tissues (Bai *et al.,* 2023). Oxidative stress may occur due to increased exposure to oxidants, reduced antioxidant defense, or both of them (Jelic *et al.,* 2021). ROS can destroy almost all components of the cell, such as lipids, proteins and DNA, which can be utilized as markers to indicate the level of oxidative damages. OP insecticides have been demonstrated to produce free radicals within biological systems (Hazarika *et al.,* 2003). Due to its lipophilic nature, CPF can simply permeate the cell membrane and trigger extensive cellular damage (Ncibi *et al.,* 2008).

Over several decades, natural products have been considered as one of the most significant things for life because of their unlimited advantages. So, in the present study, one of these natural products was used, which is spirulina, considered a blue-green algae. it belongs to the phylum of Cyanobacteria. SP is exclusive to lakes that show a high pH level (Sahil *et al.,* 2024, AlFadhly *et al.,* 2022). Spirulina contains high levels of vitamins, proteins, minerals, required amino acids and fatty acids like gamma-linolenic acid (GLA). It is introduced commercially and promoted as a food supplement in health shops all over the world. Until very recently, the concern with SP was primarily due to its nutritive value (Spinola *et al.,* 2024). Moreover, SP comprises 60–70% protein, including all required amino acids. Also, SP is abundant in antioxidants and essential fatty acids. The abundant nutrients in SP offer various health benefits such as anti-inflammatory, antioxidant, insulin-sensitizing properties and immune modulation, as well as beneficial impacts on different diseases, which could also be valuable for athletes (Chaouachi *et al.,* 2024). Furthermore, SP is reported to provide protection against xenobiotic-triggered hepatotoxicity, cardiotoxicity and nephrotoxicity (Abdel-Daim *et al.,* 2013).

While CPF-induced hepatotoxicity

is known to be mediated by oxidative stress, effective natural interventions are still being explored. Spirulina, a potent antioxidant, has shown protective effects against various toxins, but its specific role against CPF-induced liver damage through modulation of inflammation and apoptosis pathways remains unclear. For this reason, this study was designed to estimate the potential role of SP against CPF-induced hepatotoxicity in Wistar male albino rats.

Impact Statement:

Pesticides are a group of compounds that include substances used as insecticides, fungicides, herbicides, rodenticides, molluscicides and nematicides (Chio and Li, 2022). Pesticides can be classified according to various categories such as chemical classes, modes of action, functional groups and toxicity (Garcia *et al.,* 2012). Chlorpyrifos (CPF) is considered as an organophosphorus pesticide that is a neutral, organic and non-volatile poison (Rathod and Garg, 2017). The chlorine group existing in CPF increases not only the compound's half-life in the body, but also lipid solubility, leading to a slower and sustained reduction in cholinesterase (ChE) levels compared to other organophosphorus pesticides (Rathod *et al.,* 2017; Raj and Kumar, 2022). The liver is the primary organ that processes ingested nutrients, drugs and chemicals such as pesticides that access the hepatic portal vein from the digestive system, and liver function can be negatively affected by injury due to chronic or acute exposure to toxicants. The liver is primarily responsible for the activation and detoxification of CPF. It is confirmed that OP pesticides like CPF causes hepatotoxicity by altering the profile of liver marker enzymes (Abdel-Naim *et al.,* 2023). The lipophilic CPF simply invades the blood–brain barrier, therefore, CPF destroy brain integrity, stimulates permeability and free-radical generation and eventually results in CNS morphology and function changes (López-Granero *et al.,* 2016). Alternations impact the CPF-induced oxidant/antioxidant irregularity in several processes, i.e., the breakdown of antioxidant enzyme is accelerated by elevated ROS production, reduction of antioxidants, damage to protein and DNA and peroxidation of lipid (Chiapella *et al.,* 2013).

MATERIALS AND METHODS

The Egyptian Company for Pesticides and Chemicals (EPIC), located in Cairo, Egypt, supplied the CPF Prior to administration, distilled water was used to dilute CPF. Egyptian Herbal Store supplied SP, it was diluted was distilled water to a concentration of 200 mg. A dose of 500 mg/kg b.wt. was formulated.

Methodology:

In this work, Wistar adult male albino rats (150-200g) were utilized, bought from The Holding Company for Biological products and Vaccines (VACSERA), Giza, Egypt. They were acclimatized for a week before the experiment. They were kept under the control of the environmental conditions of humidity and temperature. rats were maintained under a standard light-dark cycle, with standard feeding and watering. The work was accepted, as well as all steps of the experiment were conducted as stated by the instructions of the Committee for Ethical Review of Laboratory Animal Use, Department of Zoology and Entomology, Faculty of Science, Helwan University.

Randomly, rats were separated into four groups as following: group 1, considered as control group, given 5 ml of saline solution (0.9% NaCl); group 2, considered as the SP-administered group, given 500 mg/kg of SP solution; group 3, considered as the CPF-treated group, given 5 mg/kg of CPF; and group 4 given a CPF dose (5 mg/kg), followed by a dose of SP (500 mg/kg) one hour later daily for 21 consecutive days. Oral intake of both CPF and/or SP was repeated for 21 sequential days in all groups. All of the rats were humanely euthanized 24 h after the final dose under anesthesia, as well as blood was collected for more examination. The dissection of livers occurred, livers were

quickly excised under an ice plate, and the liver was divided into half's, one used for biochemical analysis and the other one used for histopathological examination.

Biochemical Investigations:

Preparation of Tissue Homogenate:

Homogenization of liver tissue occurred in chilled phosphate buffer (0.1 M, pH 7.4) with a yellow line disperser (IKA, USA). Homogenized tissue was subjected to centrifugation at 3000 x g for 10 minutes in a refrigerated centrifuge at 4°C. The supernatant was then kept at -80°C for further examination of different parameters.

Estimation of Liver Function Markers:

Alkaline phosphatase (ALP) was assayed using colorimetric determination according to Belfield and Goldberg (1971). The phenol that was liberated is determined at 510 nm calorimetrically with $C_{13}H_{17}N_3O$ and $C_6FeK_3N_6$. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were assayed using colorimetric determination as stated by Reitman and Frankel (1957).

Estimation of Oxidative Stress Markers:

Malondialdehyde (MDA), an indicator for peroxidation of lipid, was analyzed through the utilization of thiobarbituric acid, and the thiobarbituric acid-reactive components produced were examined at 535 nm. The data were presented based on the concentration of MDA formed (Ohkawa *et al.,* 1979). For evaluation the content of nitric oxide (NO), Griess's reagent (sulfanilic acid and N-(1-naphthyl) ethylenediamine) was utilized, and the produced azo dye was examined at 540 nm (Green *et al.,* 1982).

Estimation of Antioxidant Enzyme Activities:

Glutathione (GSH) content was examined using the mechanism of Ellman (1959). It relies on protein precipitation with tungstate-sulfuric acid solution and yellow color was formed after interaction with 5,5`-dithiobis-25-nitrobenzoic acid (DNTB) and reading at a wavelength of 412 nm. Catalase (CAT) activity was analyzed according to Aebi (1984). The superoxide dismutase (SOD) levels was assayed according to the procedure of Niskikimi *et al.* (1972).

Estimation of Inflammatory Markers:

IL-1β, IL-6, and TNF-α, cytokines that promote inflammation, were examined by enzyme-linked immunosorbent analysis (ELISA) kits. IL-1β purchased from Abcam Company (Catalog number ELR-IL-1β), IL-6 purchased from Abcam Company (Catalog number MBS269892), and TNF-α purchased from Abcam Company (Catalog number ab213899). NF-κB was measured using an ELISA kit that utilizes the sandwich-ELISA concept (Catalog number E-EL-R0674, Elabscience, USA).

Determination of Apoptotic/Anti-Apoptotic Markers:

BAX, purchased from Novus Biologicals Company, Colorado, United States, was assayed using the quantitative sandwich enzyme immunoassay technique (Catalog number CSB-EL002573RA). The Bcl-2, purchased from Novus Biologicals Company, Colorado, United States, assay relies on the sandwich ELISA concept. (Catalog number, LS-F4135). Caspase-3, purchased from Novus Biologicals Company, Colorado, United States, was determined by the sandwich ELISA concept. (Catalog Number, E-EL-R0160).

Histopathological Examination:

Histopathological analysis, liver tissue sample were preserved in 10% neutral phosphate-buffered formalin, and the tissue sections were rehydrated, with a thickness of 50 µm. Hematoxylin and eosin stain were utilized. Examination of the sections was performed under a light microscope.

Statistical Analysis:

SPSS 21.0 software (Version X, IBM, Armonk, NY, USA) was used for the statistic process. The analysis results were reported as means ± standard error (SE). To evaluate the difference among all groups, one-way ANOVA with Tukey's multiple comparison test was applied. Histograms

were drawn using Microsoft Excel.

RESULTS

The current study designed to discover the potential role of SP as antioxidant and anti-inflammatory natural product on chlorpyrifos (CPF)-induced hepatotoxicity in adult male albino rats.

Liver function markers were determined in terms of ALT, AST, and ALP. CPF-induced hepatotoxicity as proved by a notable ($p < 0.05$) raise in ALT, AST, and ALP concentrations (Fig. 1**).** These alterations were noted in the liver homogenate of rats treated with CPF versus to control group. In addition, the daily oral administration of SP led to a non-significant alteration in ALT, AST, and ALP in liver tissue homogenate relative to the control group. In contrast, the treated rats by SP after being treated with CPF for 21 consecutive days indicated a remarkable rise in the levels of the three enzymes as opposed to the control group, but a marked decrease in AST level was noticed in serum when compared to CPF group. Furthermore, a significant elevation in the levels of the three enzymes was observed when compared to SP group.

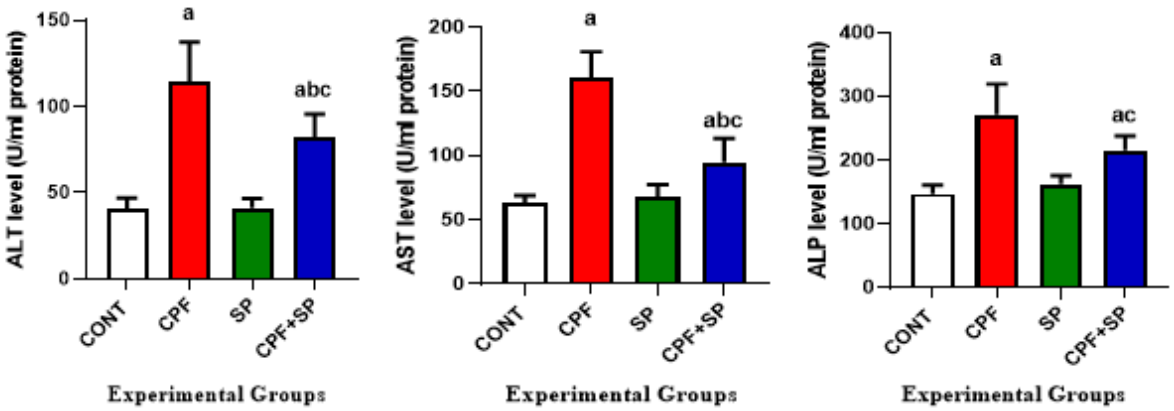

Fig. 1: Impact of daily oral intake of CPF (5mg/kg b.wt) and/or oral intake of SP (500mg/kg b.wt) for 21 consecutive days on alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (ALT, AST, and ALP) levels in serum of adult male albino rats. Data stated as mean ± SE (7 rats/group). [a] Varies from the control group ($p < 0.05$). [b] Varies from the CPF-intoxicated rats ($p<0.05$). [c] Varies from the SP-treated rats ($p<0.05$).

Oxidative damage in the liver tissue homogenate was evaluated based on MDA (outcome of lipid peroxidation), NO, and enzymatic (SOD and CAT) and nonenzymatic (GSH) antioxidants. A significant elevation in MDA and NO levels as well as a noteworthy reduction in the levels of SOD, CAT, and GSH (Figs. 2 and 3) in the CPF-treated rats were detected versus to control. But in case of the SP group, a non-significant change in levels of both MDA and NO as oxidative stress markers and SOD and GSH as antioxidant markers, while the CAT level showed a slight increase in comparison to the control group. The liver tissue of the CPF+SP group displayed an increment in both MDA and NO levels and a decrement in both GSH and CAT levels, but a non-significant alteration is recognized in SOD levels against control group. Moreover, a significant decline in MDA and NO levels in liver tissue homogenate was observed but, it was obtained a remarkable significant increase in the level of GSH, SOD and CAT as compared to CPF group. A significant elevation in hepatic MDA level was noticed when compared to SP group. In contrast, a non-significant change in NO level was noticed as compared to SP group. At variance a reduction in GSH, SOD and CAT levels was decreased if compared to the rats treated with SP.

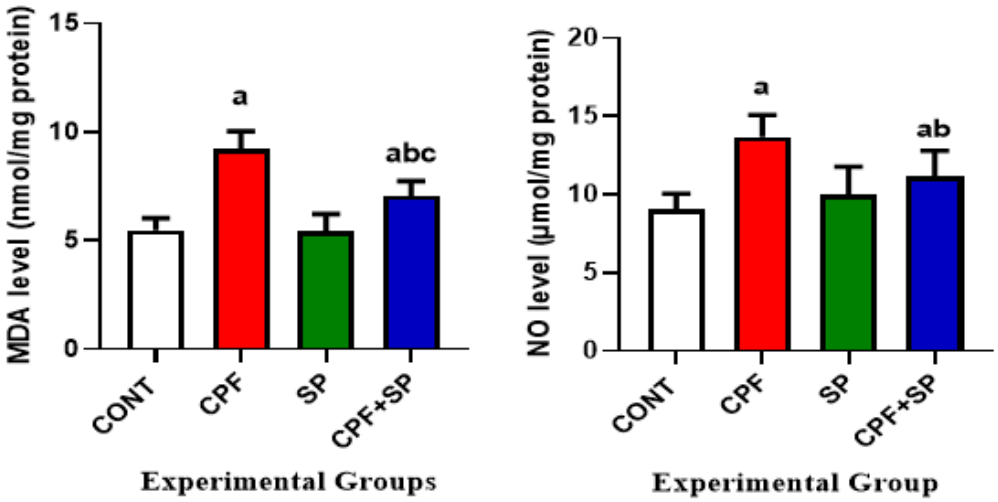

Fig. 2: Impact of oral intake of CPF (5mg/kg b.wt) and/or oral intake of SP (500mg/kg b.wt) for 21 consecutive days on malondialdehyde and nitric oxide (MDA and NO) levels in liver tissue. Data stated as mean ± SE (7 rats/group). [a] Varies from the control group ($p<0.05$). [b] Varies from the CPF- intoxicated rats ($p<0.05$). [c] Varies from the SP-treated rats ($p<0.05$).

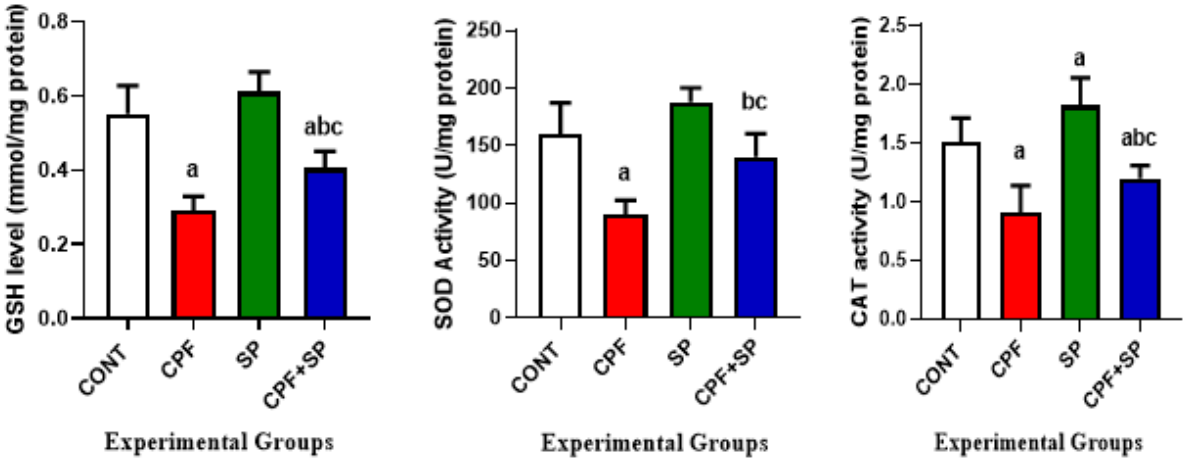

Fig. 3: Impact of oral intake of CPF (5mg/kg b.wt) and/or oral intake of SP (500mg/kg b.wt) for 21 consecutive days on glutathione, superoxide dismutase and catalase (GSH, SOD, and CAT) levels in liver tissue. Data stated as mean ± SE (7 rats/group). [a] Varies from the control group ($p < 0.05$). [b] Varies from the CPF-intoxicated rats ($p<0.05$). [c] Varies from the SP-treated rats ($p<0.05$).

The data in the present work (Fig. 4), which concerned IL-1β, IL-6, TNF-α and NF-κB levels in liver homogenate in the control group, CPF, SP, as well as CPF+SP groups, revealed a noteworthy and remarkable increase in the levels of all of them if compared with the control one. Otherwise, a non-notable change was observed in their concentrations in the group of SP versus the control. Moreover, the oral treatment of SP one hour after the daily oral administration of CPF led to a remarkable elevation in IL-1β, IL-6, TNF-α and NF-κB levels compared to the control values. Moreover, a significant decline in hepatic IL-6, IL-1β and TNF-α levels but, a non-significant decrease in hepatic NF-κB level if compared to CPF group. Furthermore, a significant elevation in IL-1β, IL-6, TNF-α and NF-κB levels was observed as compared to SP group.

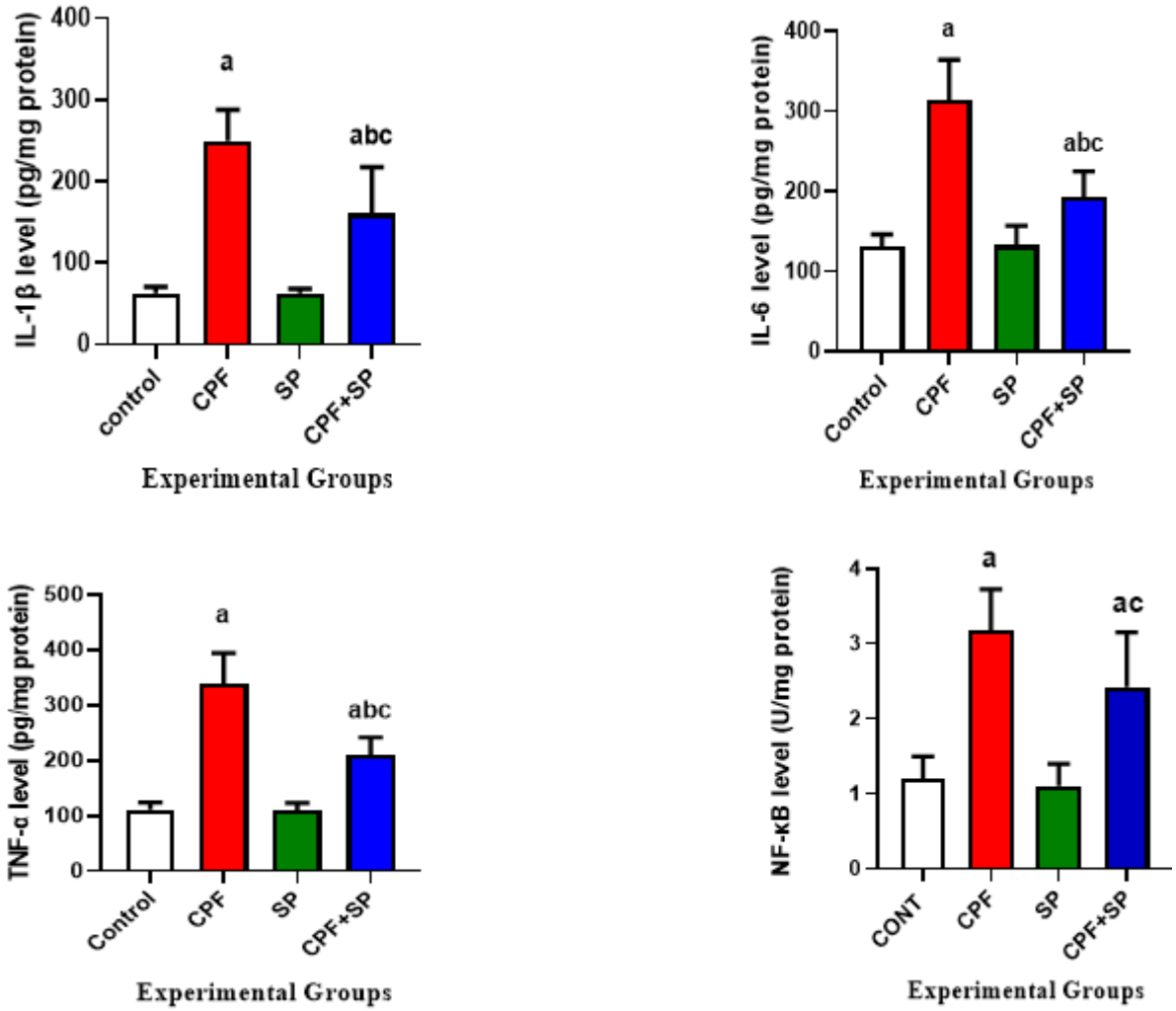

Fig. 4: Impact of oral intake of CPF (5mg/kg b.wt) and/or oral intake of SP (500mg/kg b.wt) for 21 consecutive days on interleukin-1β, interleukin-6, tumor necrosis factor-α and nuclear factor κappa B (IL-1β, IL-6, TNF-α and NF-Kb) levels in liver tissue. Data stated as mean ± SE (7 rats/group). [a] Varies from the control group ($p < 0.05$). [b] Varies from the CPF-intoxicated rats ($p<0.05$). [c] Varies from the SP-treated rats ($p<0.05$).

According to Figure 5, the oral administration of CPF for 21 consecutive days showed a rise in both Bax and caspase-3 levels as well as a reduction in Bcl-2 level compared to the control group. SP group, Bax, caspase-3, and Bcl-2 levels recorded a non-notable alteration versus control group. However, the data obtained from the daily oral administration of SP one hour after the administration of CPF showed that there was a non-significant change in BAX and caspase-3 levels, but Bcl-2 decreased compared to the control group. Moreover, the comparison between this group and the CPF-treated rats' group showed a significant decline in BAX and caspase-3 levels, while a significant increment in hepatic Bcl-2 level. Furthermore, a significant increase in hepatic BAX level was noticed when compared to SP group. A significant reduction in caspase-3 activity and Bcl-2 level as compared to SP group.

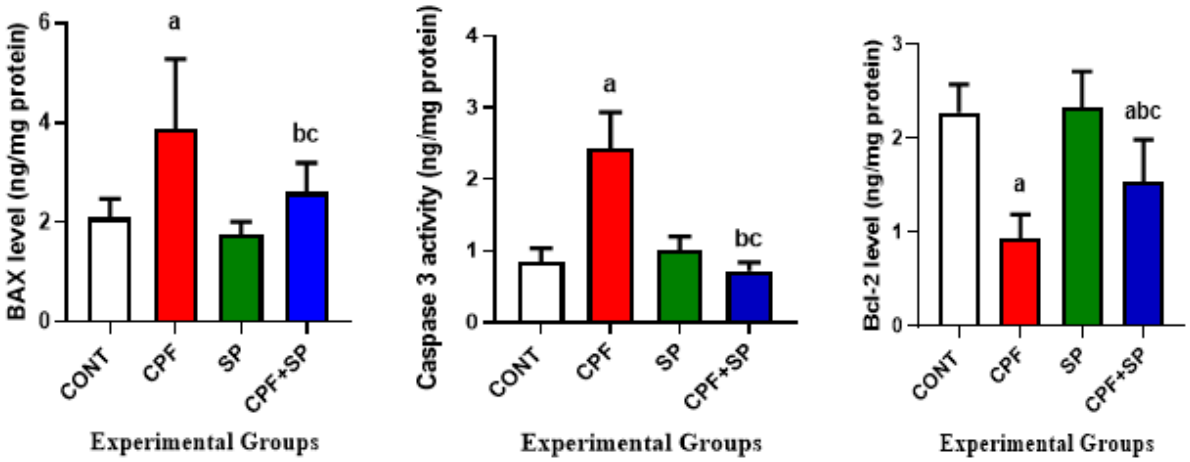

Fig. 5: Impact of oral intake of CPF (5mg/kg b.wt) and/or oral intake of SP (500mg/kg b.wt) for 21 consecutive days on Bcl-2 associated x, caspase-3 and B- Cell Lymphoma-2 **(**Bax, caspase-3, and Bcl-2) levels in liver tissue. Data stated as mean ± SE (7 rats/group). [a] Varies from the control group ($p < 0.05$). [b] Varies from the CPF-intoxicated rats ($p<0.05$). [c] Varies from the SP-treated rats ($p<0.05$).

Hepatic tissues from different treated groups were histopathologically screened and showed in Figure 6. The results revealed that, control and SP-treated rats have normal liver histology featuring a central vein and neatly organized liver cells (Figs. 6A and 6B, respectively). Additionally, earlier biochemical findings are confirmed by a histopathological examination of liver sections from intoxicated rats with CPF, which revealed that the hepatic capsule thickening, hepatocyte cytoplasmic vacuolization, inflammatory cell infiltration, pyknotic nuclei, and hepatocyte apoptosis were all visible in liver slices (Fig. 6C). Interestingly, the hepatic lesions in the CPF-treated rats with SP were only greatly improved, indicating the protective role of SP against CPF-induced hepatotoxicity (Fig. 6D).

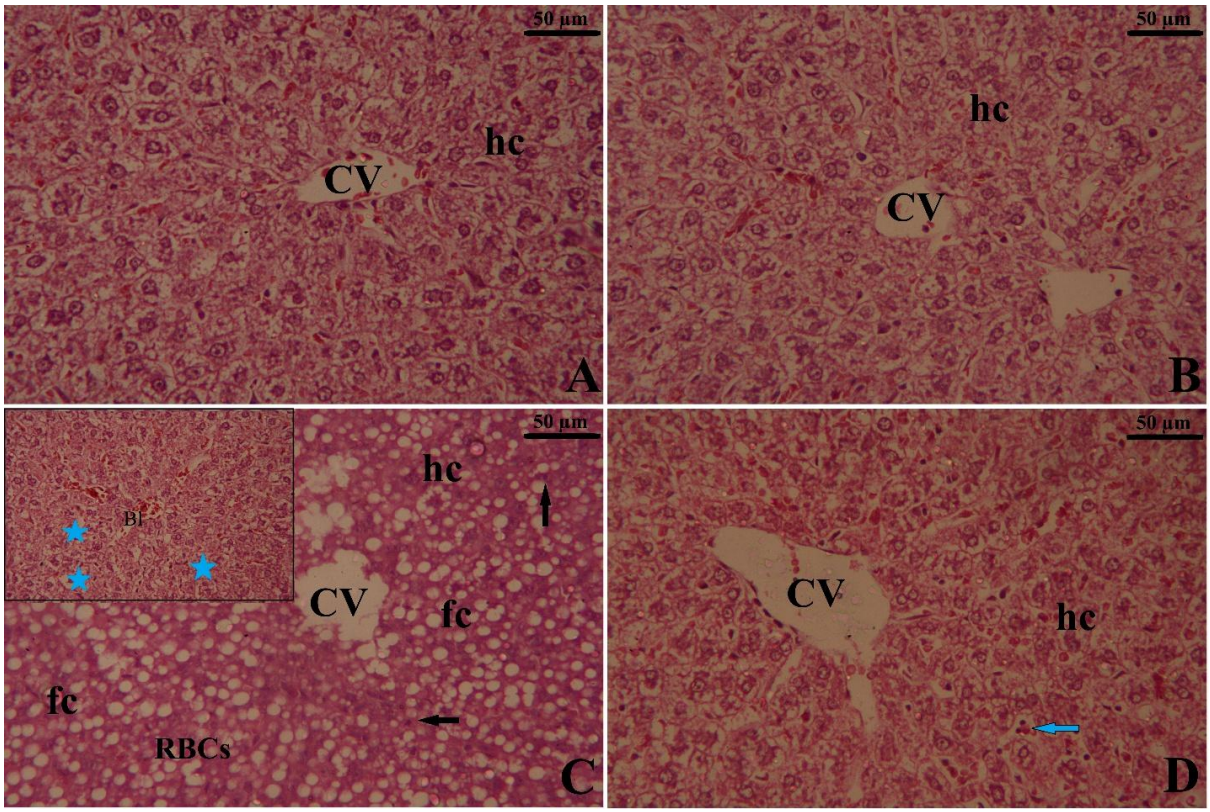

Fig. 6: Effect of oral administration of spirulina (SP; 500 mg/kg body weight) and/or oral administration of chlorpyrifos (CPF; 5 mg/kg body weight) for 21 consecutive days on histopathological deformation of adult male albino rats' liver. A: Control, B: SP, C: CPF and D: CPF+SP. Magnification = x400. hc: hepatocyte, fc: fat cell, CV: central vein, blue arrows indicate inflammatory cells, black arrows indicate apoplectic cells.

DISCUSSION

The current work designed to estimate the role of SP on CPF-triggered hepatotoxicity in the liver of adult male albino rats, through examining oxidant/antioxidant biomarkers, inflammatory indicators, and apoptotic/ antiapoptotic indicators. Pesticides may lead to hepatic injury and trigger liver dysfunction, involving in changes of ALT, serum bilirubin, AST, total protein, ALP and albumin, usually utilized as markers in tests of liver activities (Mohafrash *et al.,* 2021, Morsi *et al.,* 2024). Insecticides are able to interrupt the regular toxin clearance and metabolic regulation in liver, causing inflammation, oxidative damage, fibrosis and eventually cellular death (Gangemi *et al.,* 2016, Xin *et al.,* 2024). In the current work, the oral intake of CPF (5 mg/kg) for 21 sequential days leads to increases in serum levels of ALT, AST, as well as ALP. These observations align with Abdel-Karim *et al.* (2024), who identified the increase in ALP, AST, and ALT could be attributed to liver dysfunction after CPF administration which also leads to disruption in production of such enzymes, along with changes in liver membrane integrity. According to Tanvir *et al.* (2015), CPF exposure can cause a notable increase in liver damage markers. Therefore, the increase that was observed is likely due to the liver enzymes leaking into blood after liver tissue damage. The leakage of such enzymes could cause liver necrosis and inflammatory actions (Xun *et al.,* 2020, Abdel Moneim, 2016).

The data observed from the treatment by SP (500 mg/kg) for 21 consecutive days demonstrated that there was improvement in ALP, ALT, and AST levels. The levels of these enzymes were reduced and almost returned to their normal

value. The outcomes are aligned with Ismail *et al.* (2009), confirming the hepatoprotective impact of *Spirulina platensis*, in addition, the authors assumed that, the observed hepatoprotective actions of algal extracts resulted from their antioxidant activities and their effectiveness in minimizing lipid peroxidation. In this regard, Gad *et al.* (2011), showed the hepatoprotective ability of spirulina in carbon tetrachloride-triggered hepatotoxicity in mice and were linked to antioxidant properties of spirulina and to its role in enhancing the antioxidant defense system. Furthermore, spirulina supplementation was showed to decrease lipid oxidation and enhance antioxidant system activity in rat's liver subjected to cadmium-intoxicated *Xenopus laevis,* as reported by Pérez-Alvarez *et al.* (2021).

Xenobiotics such as pesticides, are widely recognized for enhancing ROS production, subsequently lead to oxidative damage in various tissues (Abdel Moneim, 2016, Morsi *et al.,* 2024). Chlorpyrifos exerts different impacts on cells, including the production of ROS and the stimulation of intracellular oxidative stress, which in turn disrupts normal processes of cellular differentiation and development (Kaur and Sandhu, 2008). Damage induced by ROS includes deleterious alternations to key cellular macromolecules such as proteins, membrane lipids, or DNA. Such damage can impair cellular function by disrupting intracellular pH or intracellular calcium and finally leading to cell death (Uzun and Kalender, 2013, Hajam *et al.,* 2022). CPF may exert its toxicity through a non-cholinergic mechanism, particularly by promoting oxidative stress (Cupic Miladinovic *et al.,* 2021, Ozturk Kurt and Ozdemir, 2023).

The levels of both NO and MDA were elevated due to the oral administration of CPF for 21 consecutive days. While SOD, CAT, and GSH levels in this study were reduced after oral administration of CPF. MDA is a compound that is formed from polyunsaturated fatty acids peroxidation. It has been utilized as an indicator to evaluate oxidative stress in different biological samples in patients who are influenced by a wide array of diseases. MDA is the major and most broadly studied compound formed from lipid peroxidation, known to own toxic and mutagenic effects (Abdel Moneim, 2016). Other studies Mahmoud *et al.* (2019), and Albasher *et al.* (2020), displayed increased MDA levels in various tissues of rats due to the exposure to CPF, including the liver, brain and testis. The increased MDA levels caused oxidative injury of the hepatocytes in rats treated by CPF (Mahmoud *et al.,* 2019, Taychaworaditsakul *et al.,* 2024).

Nitric oxide, a key RNS, can be synthesized enzymatically by nitric oxide synthase (NOS) from arginine or through nonenzymatic pathways. NO can metabolize into NO_2, NO_3, NO^-_3, and other RNS (Abu-Soud *et al.,* 2025). CPF chronic exposure improved NO production in the hepatic tissue, which was linked to the upregulation of inducible nitric oxide synthase (iNOS).

The body's system to combat oxidative stress operates through two key systems. The first involves enzymatic clearance of free radicals and ROS via enzymes like SOD, CAT, GPx and the second system removes free radicals using electron-donating molecules like GSH, ascorbic acid, tocopherols, thioredoxin, etc. (Tumilaar *et al.,* 2024). Pesticides trigger oxidative damage through elevating the generation of ROS or through reducing the abundance of antioxidants in living systems. CPF exposure caused a notable oxidative damage in male rat's liver as evidenced by reduced SOD and CAT enzymes activity in the liver (Omar *et al.,* 2023). The result of EL-Sharkawy *et al.* (2014), supports the proposition and demonstrates that triggering of oxidative stress is maybe the key pathway through which OP pesticides affect cells. Oxidative damage mainly occurs by the generation of ROS, like hydrogen peroxide and hydroxyl radicals, which target biomolecules and

disrupt cellular membrane (Abdel Moneim, 2016).

It has been demonstrated that spirulina owns a powerful antioxidants and free radical scavenging activities (Calella *et al.,* 2022) besides, its powerful chelating ability (Chen and Pan, 2005). These properties are likely due to the abundance of antioxidants like vitamins B_1 and B_2, phycocyanin and carotenoids in spirulina (Mazo *et al.,* 2004). The antioxidant potential of phycocyanin, a major water-soluble antioxidant compound in spirulina, is estimated to be 20 times potent than vitamin C (Grover *et al.,* 2021). The bioactive compound in SP is believed to exert synergistic action, enhancing its overall antioxidant capacity. C-phycocyanin possesses strong antioxidant activities, demonstrated by its potential to act as free radical scavenger involving hydroxyl and superoxide radicals, as well as its inhibitory effect on lipid peroxidation process (Fernandes *et al.,* 2023). The antioxidant role of SP was confirmed by a decreased MDA level, increased GSH contents and improved SOD activity in liver in several experiments (Wu *et al.,* 2016).

The results observed from current work showed that the oral intake of CPF for 21 consecutive days resulted in significant elevation in the levels of IL-1β, IL-6, TNF-α and NF-κB, and these data are aligned with a several study (Albasher *et al.,* 2020, Albasher *et al.,* 2019). Exposure to xenobiotics has been reported to triggers toxicity via several mechanisms such as promoting of nitrosative and oxidative stress, inducing the leakage of pro-inflammatory cytokines like TNF-α and IL-1βas well as initiating apoptosis (Wu *et al.,* 2016). Organophosphorus pesticides exposure has been linked to overproduction of IL-1β and TNF-α via glial cells activation (Mahmoud *et al.,* 2019). Organophosphorus compounds have been shown to enhance the expression of genes associated with cytokine signaling pathways (Tigges *et al.,* 2022).

It was reported that, SP owes antioxidant, antiapoptotic, anti-inflammatory, lipid- and glucose-reducing activities (El-Boghdady *et al.,* 2020) and these results are compatible with the current work, which exhibited that the levels of IL-1β, IL-6, TNF-α and NF-κB have been improved after administration of SP for 21 consecutive days. In previous research by Okuyama *et al.* (2017), SP possesses both anti-inflammatory properties and the ability to modulate the immune respnse (Wu *et al.,* 2016). Abdel-Daim *et al.* (2015) demonstrated that the rats treated by spirulina recorded a notable decrease in TNF-α activity.

Albasher *et al.* (2020) reported that CPF administration caused an elevation in the contents of Bax and caspase-3, but on the other side, the content of Bcl-2 has been decreased. All of these results are consistent with the current work, which proved that the oral intake of CPF for 21 sequential days led to a reduce in Bcl-2 level and a raise in the contents of both Bax and caspase-3. Several studies have confirmed that oxidative stress triggers apoptosis (Sharma *et al.,* 2023, An *et al.,* 2021). Apoptosis refers to a programmed, gene-controlled mechanism of cell death that is fundamental for proper development and homeostasis balance in eukaryotes (Singh *et al.,* 2019).

Spirulina pretreatment decreased Bax generation and improved Bcl-2 expression. Spirulina's effect on apoptosis differs according to the cell type used in the test (Mahmoud *et al.,* 2021). The outcomes of Jadaun *et al.* (2018) further demonstrated that spirulina extract owes a protective impact against cell apoptosis. The extract could apply its impact through scavenging the free radicals, resulting in reduction of the apoptotic pathway activation. Phycocyanin, that exists in Spirulina decreases apoptosis in beta cell of pancreas through inhibiting excess production of ROS and improving the enzymes' function including GSH and SOD (Liu *et al.,* 2022). Also, Jadaun *et al.* (2018), demonstrated

that, co-administration of spirulina attenuates apoptosis by suppressing caspase-3 activity.

Conclusion

In conclusion, oral administration of SP exhibits a partial or incomplete protection and incomplete amelioration effect against CPF-induced hepatotoxicity in adult male albino rats. This partial effect may be attributed to its properties, including antioxidants, antiapoptotic and anti-inflammatory, due to it's rich in chlorophyll, carotenoids, amino acids, GLA, vitamins B_1, B_2, and trace elements including iodine, Fe, Zn and Se, and the major valuable constituent is C-phycocyanin, because of its antioxidant activities. All of these advantages permit the SP to be able to enhance the survival of hepatic cells from CPF-induced hepatotoxicity. Finally, SP could be considered a supportive but not sufficient natural source for mitigating liver toxicity caused by certain toxic agents.

Declarations:

Ethical Approval and Consent to Participate: Our experimental procedures were carried out in accordance with the Institutional Animal Care and Use Committee (IACUC) Guidelines, as approved by the Zoology Department, Faculty of Science, Helwan University in Cairo, Egypt (Approval no: HU-IACUC/Z/MMH1122-01).

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Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Authors' Contributions: Conceived and designed the experiments: NAE, AEA, FHS, and HME; Performed the experiments: MM; Analyzed and interpreted the data: MM and HME; Contributed reagents, materials, analysis tools, or data: MM; Wrote the paper: MM, FHS, and HME; All authors have read and agreed to the published version of the manuscript.

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