Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.151

RESEARCH ARTICLE

The Protective Effect of *Chlorella vulgaris* Against Gibberellic Acid (GA3)-Induced Liver Injury in Male Albino Rats

Sahar Jameel Melebary[1]*

[1]Department of Biological Sciences, College of Science, University of Jeddah, P.O. Box 80237, Jeddah 21589, Saudi Arabia
*Corresponding author: Sjmelebary@uj.edu.sa

ARTICLE HISTORY (25-133)

Received: February 15, 2025
Revised: March 24, 2025
Accepted: March 28, 2025
Published online: April 21, 2025

Key words:
Chlorella vulgaris
Gibberellic acid
Liver injury
Oxidative stress
Plant growth regulator
Rats

ABSTRACT

The plant growth regulator, gibberellic acid (GA3), is extensively utilized in agriculture across many countries to hasten the maturation of vegetable and fruit crops. However, there are numerous harmful effects caused by its residues in food. This work aimed to evaluate the possible protection of *Chlorella vulgaris* (CV) extract against gibberellic acid-induced hepatic injury in male albino rats. Forty male rats were allocated randomly into 4 groups (n = 10). The control group received a daily oral gavage of 0.9% normal saline. The CV group was administered an oral dose of CV extract at 70 mg/kg body weight, diluted in 0.9% normal saline, once daily for four weeks. The GA3 group received gibberellic acid (GA3) at a dose of 55 mg/kg body weight via oral gavage daily for four weeks. In the GA3 + CV co-treatment group, rats were orally gavaged with *CV* extract (70 mg/kg BW) one hour prior to GA3 administration (55 mg/kg BW) each day for four weeks. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly elevated in the GA3-treated group, indicating hepatic injury. Additionally, malondialdehyde (MDA) concentrations and caspase-3 expression levels in liver tissue were significantly increased, reflecting enhanced oxidative stress and apoptosis. Conversely, the antioxidant defence system was markedly impaired, as evidenced by reduced activities of superoxide dismutase (SOD) and catalase (CAT). Administration of *CV* extract significantly ($P<0.05$) attenuated GA3-induced hepatic inflammation and oxidative stress, while restoring SOD and CAT activities in liver tissues. Histopathological analysis of liver sections from the GA3 group revealed cytoplasmic vacuolization, cellular degeneration, necrosis, and interstitial fibrosis. These histological alterations were notably ameliorated by *CV* extract treatment, suggesting a protective modulatory effect against GA3-induced hepatotoxicity. In conclusion, *CV* extract exerts hepatoprotective effects by mitigating oxidative damage, reducing inflammation, and preserving liver histoarchitecture in GA3-induced hepatic injury.

To Cite This Article: Melebary SJ, 2025. The protective effect of chlorella vulgaris against gibberellic acid (ga3)-induced liver injury in male albino rats. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.151

INTRODUCTION

Plant growth regulators are naturally occurring endogenous hormones synthesized by plants to regulate growth and development. In agricultural practices, synthetic PGRs are widely employed to manipulate physiological processes and enhance crop yield and productivity (Panda *et al.*, 2022).Gibberellic acid (GA3) is one of the most biologically active gibberellin hormones. Stem elongation, flowering, fruit development, and breaking dormancy are just a few ways it influences plant growth (Abdel-Aty & Masoud, 2016). GA3 has a long half-life in soil and is resistant to natural degradation (Panda *et al.*, 2022). Exposure to gibberellic acid (GA3) residues through the consumption of contaminated fruits and vegetables has been associated with toxic effects on both human and animal health (Abu Amra *et al.*, 2020). The World Health Organization (WHO) classifies GA3 as a pesticide-related compound due to its biological activity and environmental persistence, particularly its ability to remain in soil for extended periods. Consequently, only minimal levels of GA3 residues are permitted. The Environmental Protection Agency (EPA) also recommends its use at low concentrations to minimize potential health risks (Gikas *et al.*, 2022).

Exposure to gibberellic acid (GA3) residues through skin contact, inhalation of powder, or eating of GA3-treated fruits and plants poses potential health risks to both humans and animals.(Iftikhar *et al.,* 2020). Dietary exposure to GA3 residues may occur by consuming various GA3-treated fruits and vegetables unknowingly. Additionally, contaminated drinking water may serve as a secondary source of GA3 exposure. Agricultural workers are especially vulnerable as they can be exposed to GA3 in the workplace, inhaling its powder or coming into direct skin contact with it, increasing the risk of systemic toxicity(Alsemeh *et al.,* 2019).

Exposure to GA3 impairs the cellular reactive oxygen species (ROS) scavenging system, leading to oxidative stress and cell death (Murcia *et al.,* 2024). The free radicals generated by GA3 affect various organs, including the stomach, kidneys, liver, spleen, and heart. This oxidative stress leads to enzyme inactivation, DNA damage (genotoxicity), cell death, and defective cell membranes due to ROS degradation of biomolecules like glutathione, lipids, proteins, and DNA (Abdulrazik *et al.,* 2024; Ma, 2024). It has been suggested that GA3 exhibits both cytotoxic and genotoxic properties, contributing to its overall toxicological profile (Soliman *et al.,* 2022).

Toxicants can accumulate in high concentrations within the body, leading to significant structural and functional damage to the liver. As the primary organ for detoxification, the liver receives the majority of its blood supply from the intestines via the portal vein, which carries concentrated forms of ingested drugs, chemicals, and environmental pollutants. This anatomical and physiological role renders the liver particularly vulnerable to toxic insults. GA3 in particular, has been reported to cause hepatic injury to this soft and metabolically active organ, due to its toxic potential (Tekade *et al.,* 2023).

Given the liver's vulnerability to toxic insults, particularly from compounds GA3, interest has grown in natural agents with hepatoprotective potential. *Chlorella vulgaris* is a type of single-celled green alga that stands out as a "superfood" due to its rich nutritional profile and therapeutic properties. It is considered abundant in bioactive antioxidants, including chlorophyll, carotenoids, lutein, astaxanthin, and phycobiliproteins, which help neutralize reactive oxygen species (ROS) and reduce oxidative stress. *Chlorella* supplementation has shown antihypertensive (Lin *et al.,* 2018), antioxidative, hepatoprotective activities (Wu, 2020), and hypolipidemic effects (Sherafati *et al.,* 2022) and has been approved as a safe alga dietary supplement by the Food and Drug Administration (FDA) (Bauer *et al.,* 2017).

It is hypothesized that *Chlorella vulgaris* extract, due to its rich antioxidant content and nutritional composition, will effectively mitigate GA3-induced hepatic injury in male albino rats by reducing oxidative stress and preserving liver cell integrity. However, to date, not many studies have specifically reported and investigated the mitigative effects of *Chlorella vulgaris* on GA3-induced hepatic toxicity. Therefore, the present study was designed to assess the extent of GA3-induced hepatic injury in male albino rats. The study further aimed to investigate the protective effects of *Chlorella vulgaris* extract on liver function and histological structure in GA3-treated rats and explore the potential mechanisms by which *Chlorella vulgaris* mitigates GA3 toxicity, focusing on its antioxidant properties.

MATERIALS AND METHODS

Chemicals: GA3 identified as 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene1,10-dicarboxylic acid 1,4a-lactone, (≥99% purity), was obtained as white crystalline powder from Sigma-Aldrich Chemical Co., (Germany). It was freshly dissolved in distilled water before administration. *Chlorella vulgaris* powder was outsourced from the Algal Biotechnology Unit, National Research Centre, (Dokki, Cairo, Egypt). The dried extract was reconstituted in normal saline (0.9%) before oral administration and stored at refrigerated temperatures (4°C) until use.

Animals: Forty adult male Albino Wister rats weighing 200- 250 grams (gm) were used in the current study. The rats were obtained from the animal house of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were kept in healthy and clean cages (10 animals each) at a temperature of 25°C and 70% humidity, with a 12h light/ dark cycle, and obtained water and well-adjusted diet *ad libitum* and adjusted to laboratory conditions. All experimental methods were carried out following the national institutional animal care standards and the recommendations of the Committee for Control and Supervision of Animal Experiments regarding the use and care of experimental animals.

Experimental design: The experimental design involved randomly dividing forty male albino rats into four groups (n=10). The control group received 0.5mL/rat of normal saline via oral gavage. The CV group was administered 70mg/kg body weight of CV extract dissolved in 0.9% normal saline via oral gavage for four weeks. GA3 was administered via oral gavage at a dose of 55mg/kg body weight for four consecutive weeks, a dose corresponding to 1/100th of the reported LD_{50}. In the GA3 + CV co-treated group, rats received 70mg/kg of *Chlorella vulgaris* extract orally one hour prior to the GA3 administration. Both treatments were delivered via oral gavage daily for a duration of four weeks.

Liver function assessment: The collected blood samples were centrifuged at 2500 rpm for 10 minutes to separate the serum. The liver's functional capacity was analyzed according to the levels of aspartate (AST) and alanine aminotransferases (ALT) in the blood. Both ALT and AST activities were assessed in the serum according to Bergmeyer & Bernt, (1974) and Tietz *et al.* (1983), respectively.

Assessment of oxidant/antioxidant status of liver tissues: To determine the enzymes that neutralize oxidants and antioxidants, the right side of the liver was cut into small pieces and then mixed with a potassium phosphate buffer solution (50Mm, pH 7.5) using a potter Elvehiem homogenizer until it reached a 10% homogenate. The tissue homogenate was centrifuged at 3000 rpm for 15 minutes at 4 °C, and the supernatant was collected and stored on ice to calculate malondialdehyde

(MDA), superoxide dismutase (SOD), and catalase (CAT). Draper and Hadley's spectrophotometric method was used to determine the MDA level in the liver, which is an indicator of lipid peroxidation (Draper & Hadley, 1990). MDA levels were measured spectrophotometrically at an absorbance of 532 nm, and the results were expressed as nmol of MDA per mg of protein. CAT and SOD activities were determined calorimetrically using the methods described by Aebi (1984) and Nishikimi *et al*. (1972) respectively.

Tissue samples were homogenized in a cold phosphate buffer, and the supernatant was collected after centrifugation. A reaction mixture consisting of phosphate buffer and a defined concentration of hydrogen peroxide (H_2O_2), was prepared. The sample supernatant was added to this mixture, initiating the CAT-catalysed decomposition of H_2O_2. The decomposition rate was monitored spectrophotometrically by measuring the decrease in absorbance at 240nm over time. This decrease, directly proportional to CAT activity, was used to calculate the enzyme's activity based on the molar extinction coefficient of H_2O_2.

SOD activity was determined using a reaction mixture containing phosphate buffer, EDTA, nitroblue tetrazolium (NBT), xanthine, and xanthine oxidase. Briefly, the sample supernatant was added to this mixture, and the reaction was initiated. Xanthine oxidase generated superoxide radicals, which normally reduce NBT to form a blue formazan product. SOD, present in the sample, competed with NBT for these radicals, inhibiting formazan formation. The degree of inhibition was measured spectrophotometrically by monitoring the decrease in absorbance at 560nm. SOD activity was then calculated and expressed as the amount of enzyme that inhibited the NBT reduction by 50%.

Histological analysis: At the end of the experiment (After 28 days), all rats were euthanized by cervical dislocation, and liver samples were collected through dissection. Specimens from the left lobe of each liver were fixed in 10% buffered formalin to preserve tissue structure. Following fixation, the samples were processed for paraffin embedding and sectioned into consecutive 5µM thick slices using a microtome. Liver tissue sections were subjected to Haematoxylin and Eosin (H&E) staining for general histoarchitecture, Masson's trichrome for fibrosis assessment (collagen fibres stained blue), and Periodic Acid-Schiff (PAS) staining to detect glycogen and mucopolysaccharide content (magenta coloration). After staining, the tissue sections were examined and photographed using a light microscope (Leica DM500) with a digital camera (Leica ICC50 W Camera Module).

Immunohistochemical (IHC) examination: For IHC, liver tissue sections were assessed using the avidin-biotin peroxidase complex (ABC) immunohistochemistry method, as described by Bancroft and Gamble (2008). Briefly, sections were incubated with the primary antibody Caspase-3, a class III intermediate filament (Rabbit monoclonal antibody) (1:100, Cat No. ab4051, Abcam, Cambridge, Massachusetts, USA) for 60 minutes at room temperature in a humidified chamber. Immunoreactivity was visualized as cytoplasmic brown staining in caspase-3 positive cells. Rat brain tissue was used as a positive control, while a negative control was prepared by omitting the primary antibody step in one of the liver sections. Slides were then mounted for microscopic examination under light microscopy.

Quantitative histopathological analysis using Image J software: Image J software was utilized for quantitative analysis, wherein ten distinct, non-overlapping fields from five separate regions of liver tissue sections within each experimental group were assessed for each parameter. For H&E-stained sections (200X), liver damage was evaluated using a semi-quantitative grading system based on Gibson-Corley's method, which quantified cellular vacuolation, apoptosis, inflammatory cell infiltrates, and vascular congestion. Scores were assigned as follows: 0 for no observed changes, 1 for changes affecting less than 25% of the field, 2 for changes affecting 26-50%, 3 for 51-75%, and 4 for changes exceeding 75%. Masson trichrome stained sections (400X) were used to determine the percentage area of collagen fibres, representing the extent of fibrosis. Additionally, the mean percentage area of caspase-3 positive cells, indicative of apoptosis, was quantified in immunohistochemically stained sections (400X).

Statistical Analysis: The obtained data was analysed using SPSS version 26 (SPSS Inc., Chicago, IL, USA) statistical package. Data was summarized using mean and standard deviation (Mean ±SD). The group were compared using a One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons test. Histological scores were compared using two-way ANOVA followed by Tukey's post hoc test. The data were recorded as mean+Std. Deviation (S.D) with $P< 0.05$ was considered statistically significant. All the graphs were recorded using GraphPad Prism (Version 10.1.2 for Mac) (GraphPad Software, San Diego, CA, USA).

RESULTS

Liver function assessment: As shown in Figure. 1A and 1B, the GA3 group exhibited a significant increase in AST (123.80U/L) and ALT (93.60U/L) levels the compared to negative control group (80.10 and 58.60 U/L, respectively) ($P<0.0001$). In contrast, CV treatment significantly reduced AST (95.00U/L) and ALT (73.60U/L) levels compared to the GA3 group ($P<0.0001$). No significant differences were observed between the control and CV-only groups.

Oxidant/antioxidant status of liver tissues assessment: As shown in Figure. 1C, GA3 group exhibited a significant increase in MDA (7.592 nmol/g) level in contrast with the negative control group (1.579nmol/g) ($P<0.0001$). In contrast, in CV treatment, the rats exhibited significantly decreased MDA levels (3.083nmol/g) compared to the GA3 group ($P<0.0001$). However, there were no statistically significant differences between the control group and the group treated with *Chlorella vulgaris* alone. Similarly, the GA3 group exhibited a significant increase in SOD (85.88U/g)

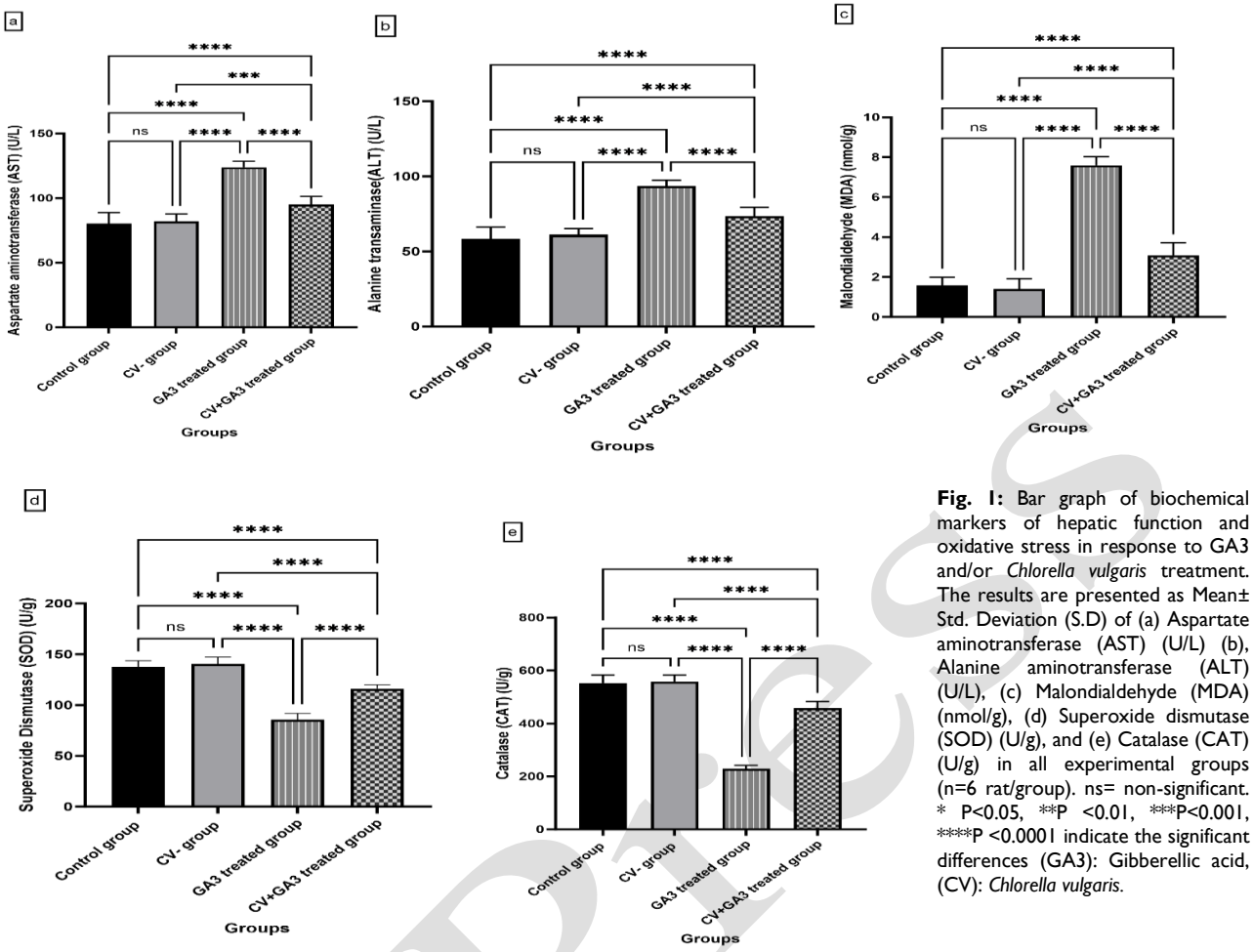


Fig. 1: Bar graph of biochemical markers of hepatic function and oxidative stress in response to GA3 and/or *Chlorella vulgaris* treatment. The results are presented as Mean± Std. Deviation (S.D) of (a) Aspartate aminotransferase (AST) (U/L) (b), Alanine aminotransferase (ALT) (U/L), (c) Malondialdehyde (MDA) (nmol/g), (d) Superoxide dismutase (SOD) (U/g), and (e) Catalase (CAT) (U/g) in all experimental groups (n=6 rat/group). ns= non-significant. * P<0.05, **P <0.01, ***P<0.001, ****P <0.0001 indicate the significant differences (GA3): Gibberellic acid, (CV): *Chlorella vulgaris.*

and CAT (228.6U/g) levels compared to the negative control group (137.8U/g and 552.7U/g, respectively) (P<0.0001). However, after co-administration with CV, the rats demonstrated significantly decrease in SOD (116.3U/g) and CAT (458.0U/g) levels compared to the GA3 group (P<0.0001). No such differences were observed between the control and CV-only groups (Fig. 1D & 1E).

Histological examination: Histological examination using H&E staining revealed normal hepatic architecture in the control and CV-only groups, with well-organized polygonal hepatocytes, intact sinusoids, and normal portal triads (Fig. 2A–D). In contrast, the GA3-treated group exhibited marked hepatic alterations, including cytoplasmic vacuolization, apoptotic nuclei, sinusoidal dilation, periportal inflammatory infiltration, and distorted bile ducts (Fig. 2E–F). Co-treatment with *Chlorella vulgaris* mitigated these changes, preserving liver architecture with only mild vacuolization and minimal inflammatory infiltration (Fig. 2G–H). The histopathological scoring in all experimental groups is presented in Figure. 3. The GA3 group showed significantly higher cellular vacuolation (4.00), apoptosis scores (4.00), vascular congestion (3.70), and inflammatory cell infiltration (3.90) compared to the control (0.30 0.60, 0.20, and 0.70 respectively) and *Chlorella vulgaris* only treated (0.60 0.60, 0.30 and 0.40 respectively) groups (P<0.0001). However, the CV+GA3 group still exhibited significantly higher scores than the control and CV-only groups (P<0.0001). No statistically significant differences were observed between the control and CV-only groups in any of the parameters (P>0.05).

Masson trichrome staining revealed minimal collagen deposition around central veins and portal tracts in both the control and CV-only groups, indicating normal liver architecture (Fig. 4A–B). In contrast, GA3 treatment resulted in a marked increase in collagen fibre accumulation, indicative of hepatic fibrosis (Fig. 4C). Co-treatment with Chlorella vulgaris notably reduced collagen deposition, preserving near-normal architecture with only focal collagen presence (Fig. 4D). Quantitative analysis showed a significant increase in mean collagen area % in the GA3 group (26.12%) compared to the control (1.95%) and CV-only groups (1.96%) (P<0.0001). The GA3+CV group exhibited a significantly lower collagen area % (12.01%) than GA3 alone (P<0.0001), though still higher than control and CV-only groups (P<0.0001). (Fig. 4E).

Periodic acid-Schiff staining revealed abundant glycogen granules (PAS-positive) in the cytoplasm of hepatocytes from both the control and CV-only groups, especially around the central vein and adjacent basement membranes (Fig. 6A & B). In contrast, GA3-treated liver tissues exhibited reduced or absent PAS positivity in vacuolated hepatocytes, indicating glycogen depletion, while enhanced PAS reactivity was noted along the basement membranes of the central and periportal regions

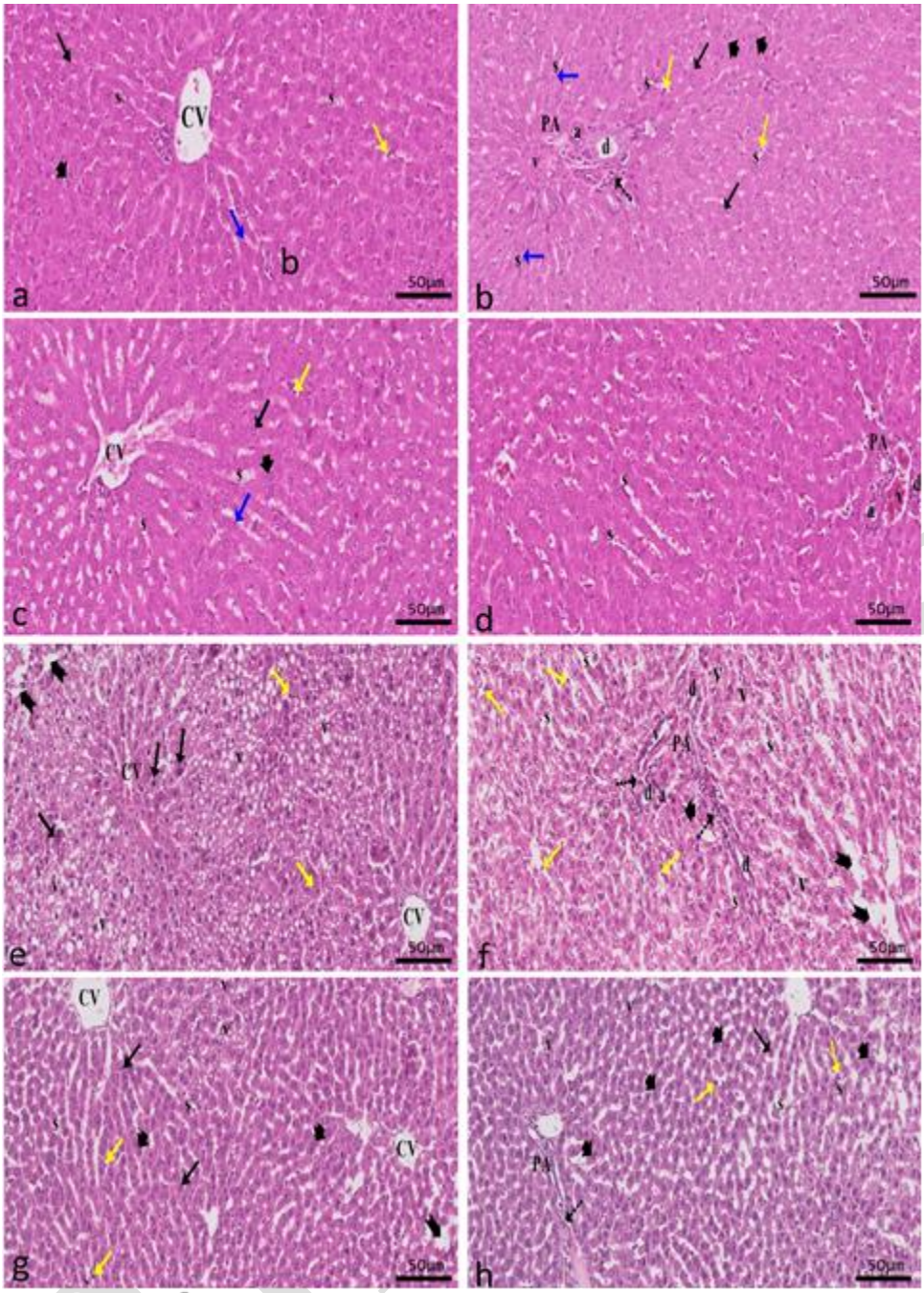


Fig. 2: A photomicrograph of a liver section stained with hematoxylin and eosin stain (H&E 20X) stain showing: (a &b) Control group and (c& d) *Chlorella vulgaris* (CV-group) cords of hepatocytes with large rounded vesicular nuclei (↑) or binucleated (arrowhead) radiating from central vein (CV) and separated by sinusoidal (s) spaces in between. Hepatic blood sinusoids (s) are bordered by flat endothelial cells (blue↑) and Kupffer cells (yellow↑). Portal area (PA) containing branches of hepatic artery (a), vein (v) and bile duct(d) are also detected. Notice extensive cellular infiltration is observed mainly in the portal tracts (dot arrow). (e& f) Gibberellic acid (GA3) lost hepatic architecture. Hepatocytes appear with vacuolated cytoplasm (v) and pyknotic nuclei (↑). Blood sinusoids (S) appear dilated with many Kupffer cells (yellow↑). Portal area (PA) shows dilated portal vein (v) with thickened wall and distorted bile ducts (d). Note normal hepatic artery (a). areas of apparent loss (bifid arrow) of hepatocytes are noticed. (g& h) CV+GA3 group marked improvement which appear as most hepatocytes have acidophilic cytoplasm and vesicular nuclei (↑) and an apparent increase of binucleated cells (arrowhead) are seen. Notice few cellular infiltration (dot arrow), small areas of loss hepatocytes (bifid arrow), and vacuoles (v) in the hepatocytes are noticed.

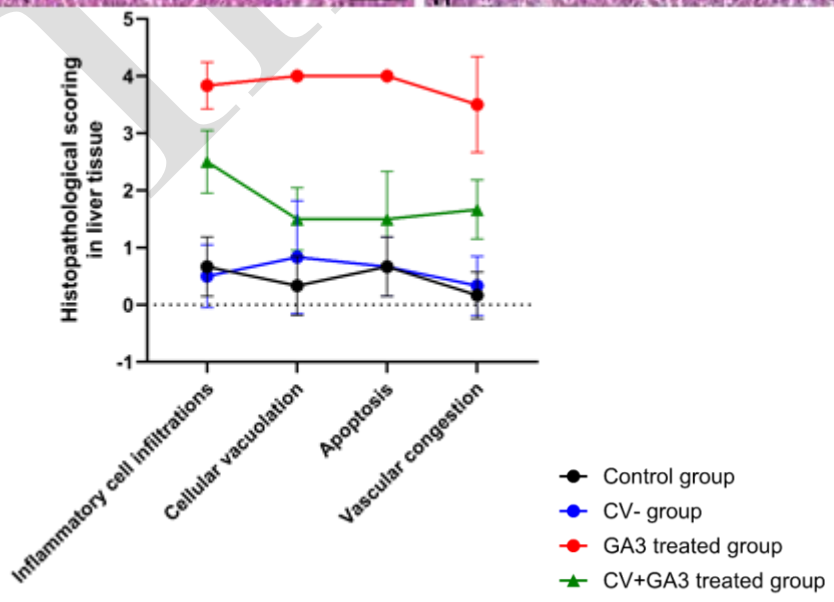


Fig. 3: Mean± Std. Deviation (S.D) of Histopathological scoring in liver tissue in all experimental groups (n=10 rat/group). (GA3): Gibberellic acid, (CV): *Chlorella vulgaris*

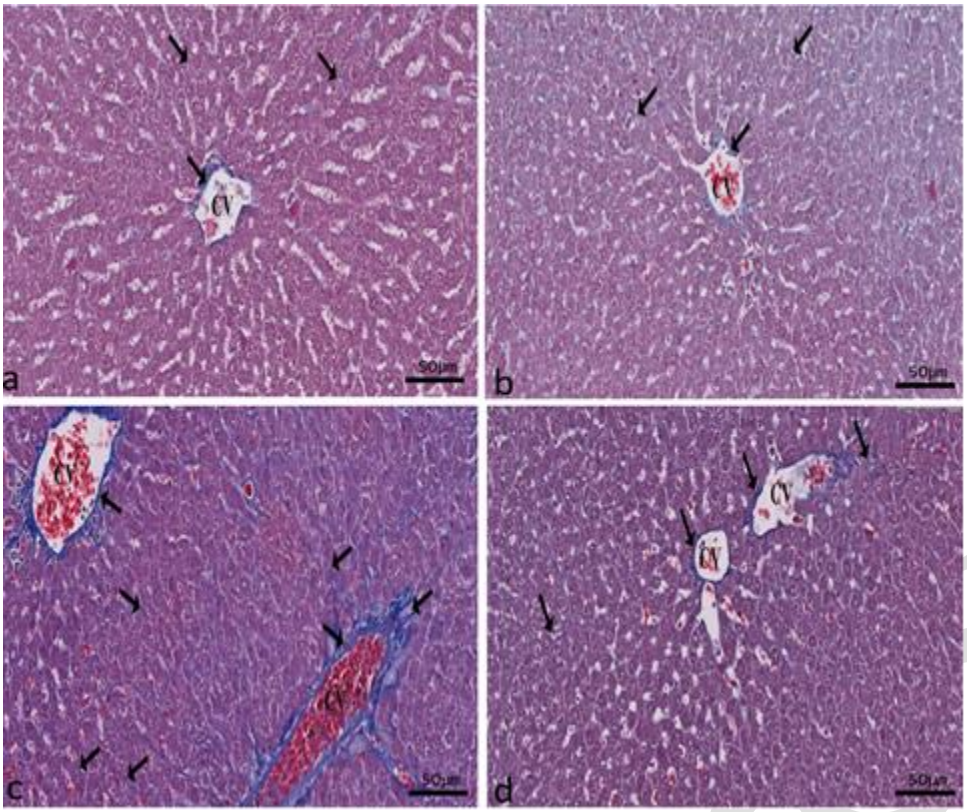
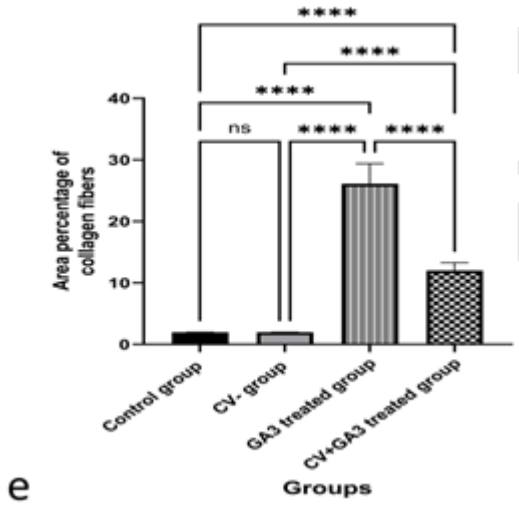


Fig. 4: A photomicrograph of a liver section stained with Masson trichrome (20X) stain showing: (a) Control group & (b) *Chlorella vulgaris* (CV-group) normal distribution of collagen fibers (↑) around central vein (CV) and in in-between hepatocytes. (c) Gibberellic acid (GA3) marked an increase of collagen fibres (↑) around the central vein (CV) and in in-between hepatocytes. (d) CV+GA3 group: mild distribution of collagen fibers (↑) around central vein (CV) and in in-between hepatocytes. (e) Mean± Std. Deviation (S.D) of area percentage of collagen fibers in the liver tissue stained with Masson trichrome stain in all experimental groups (n=10 rat/group). ns= non-significant. Significant at ****P <0.0001.



(Fig. 6C). Remarkably, co-administration of *Chlorella vulgaris* with GA3 restored PAS-positive glycogen granule distribution in hepatocytes to levels comparable to the control group (Fig. 6D).

IHC analysis: Caspase-3 did not express in control, CV-only, and GA3+CV groups, however, was found to be markedly elevated in hepatocytes, especially around the central vein and sinusoids, following GA3 treatment (Fig. 5A-E). The GA3-treated group showed a significant increase (P<0.0001) in caspase-3 positive cell area % (53.16) compared to the control (5.27) and CV-only (5.47) groups. Co-treatment with CV significantly reduced caspase-3 expression (20.21, P<0.0001) versus GA3 alone but remained higher than control and CV groups. No significant difference was observed between the control and CV-only groups.

DISCUSSION

The study explores the effects of gibberellic acid, a plant growth hormone widely used in agriculture, on liver health and the potential mitigating role of *Chlorella vulgaris*. GA3, recognized as an environmental pollutant, poses significant risks to human health, mainly through long-term exposure (Alatawi *et al.,* 2023). The liver, central to detoxification and biotransformation, is especially vulnerable to GA3-induced toxicity (Tekade *et al.,* 2023). This research investigates the impact of GA3 on oxidative stress, liver function enzymes, and histological changes in the liver while also examining the protective effects of *Chlorella vulgaris*.

The result of the current study indicates that rats treated with *Chlorella vulgaris* after GA3-induced liver damage showed significantly lower levels of AST and ALT, indicating a lower degree of inflammation or liver damage. This may explain why treatment with *Chlorella vulgaris* was better and had more positive outcomes. The study establishes the connection between liver injury and biomarkers such as aspartate AST and ALT. These enzymes, crucial for amino acid metabolism and gluconeogenesis, are released into the bloodstream when hepatocytes are damaged (Aboraya *et al.,* 2022). These findings align with prior research, confirming that GA3 significantly elevates AST and ALT serum levels, indicating hepatocellular toxicity (Hussein *et al.,* 2011). This toxicity results from increased membrane permeability due to loss in functional integrity of liver cells(Soliman *et al.,* 2022). However, when CV was administered alongside GA3, a notable reduction in AST and ALT levels was observed, suggesting that CV helps maintain normal enzyme levels and protects liver cells from damage (Latif *et al.,* 2021).

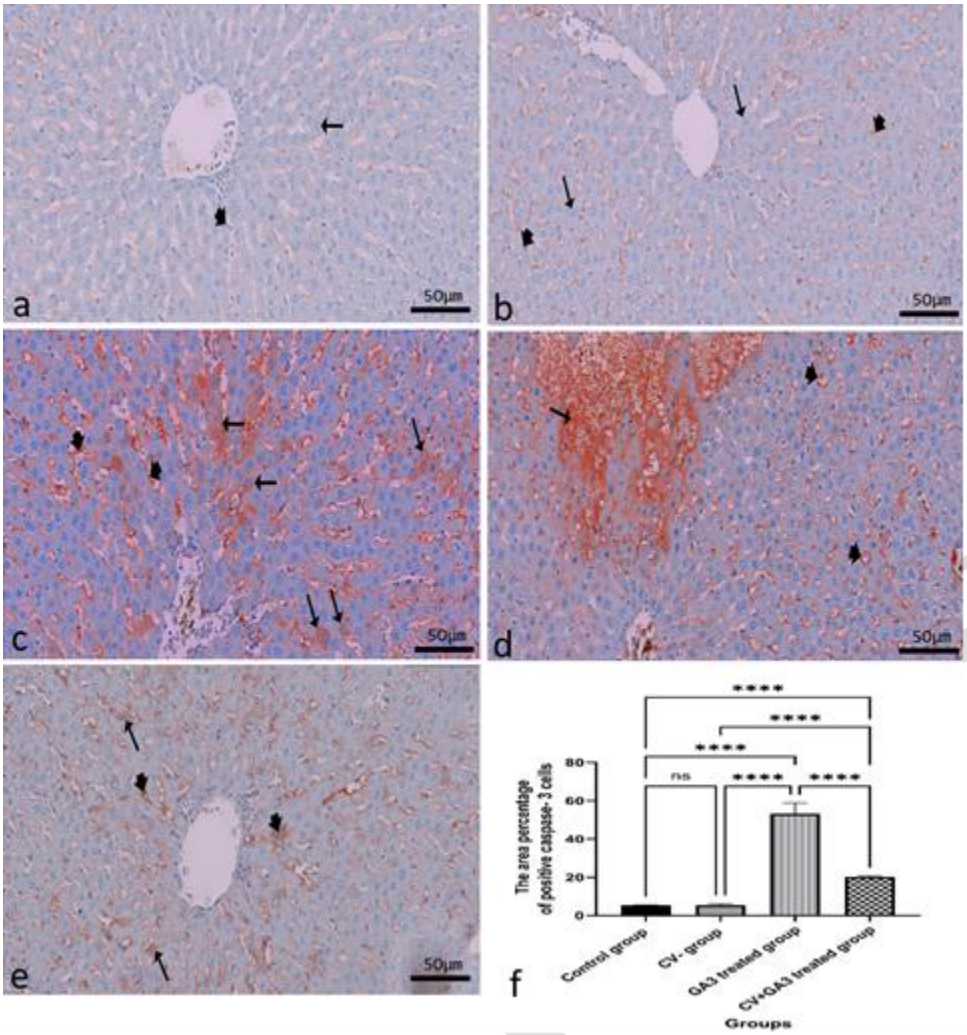


Fig. 5: A photomicrograph of a liver section stained with immuno histochemical reaction of caspase - 3 (20X) stain showing: (a) Control group & (b) *Chlorella vulgaris* (CV-group) negative immuno histochemical reaction of caspase - 3 in hepatocytes (↑) and in cells lining blood sinusoids (arrowhead). (c &d) Gibberellic acid (GA3) strong positive immuno histochemical reaction of caspase - 3 in hepatocytes (↑) and in cells lining blood sinusoids (arrowhead). (e) CV+GA3 group: moderate positive immuno histochemical reaction of caspase -3 in hepatocytes (↑) and in cells lining blood sinusoids (arrowhead). (f) Mean± Std. Deviation (S.D) of positive brownish cytoplasmic reaction of caspase -3 in the liver tissue stained in all experimental groups (n=10 rat/group). ns= non-significant. Significant at ****P <0.0001.

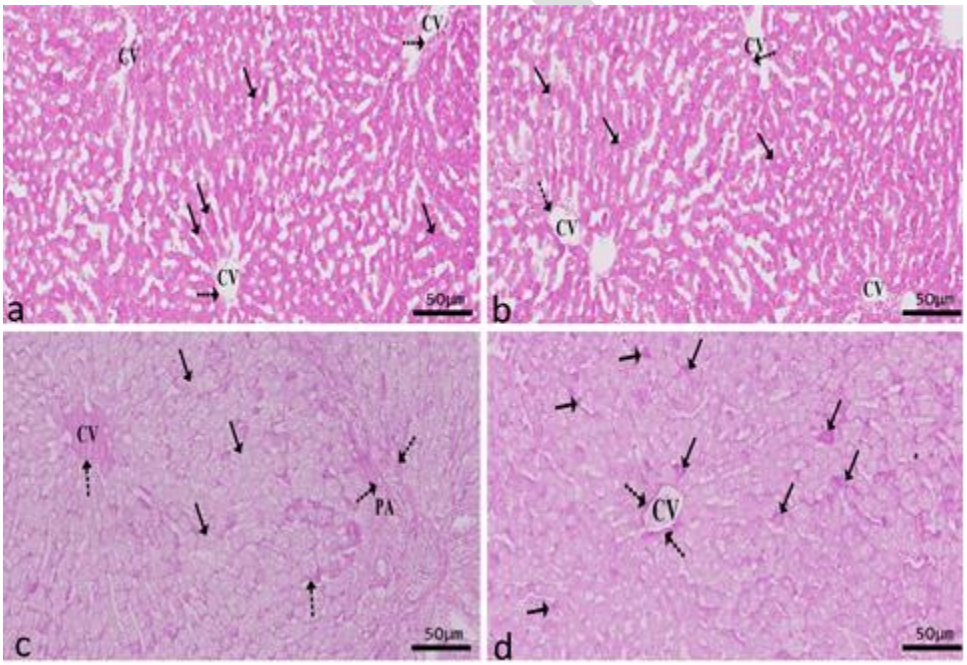


Fig. 6: A photomicrograph of a liver section stained with Periodic acid Schiff (20X) stain showing: (a) Control group & (b) *Chlorella vulgaris* (CV-group) the greatest PAS distribution in all (↑) hepatocytes and surrounding the central vein (dot arrow). (c) Gibberellic acid (GA3) a weak and uneven distribution of PAS distribution in most of the (↑) hepatocytes with an apparent increase of the distribution in the basement membrane (dot arrow) lining the central vein and in the portal area (PA). (d) CV+GA3 group: moderate PAS reaction in most of the (↑) hepatocytes with an apparent increase of the distribution in the basement membrane (dot arrow) linin the central vein.

Oxidative stress and the subsequent generation of oxidative stress markers such as MDA are suggested to be the fundamental mechanisms underlying the pathogenesis of tissue damage. The current study showed that GA3 is associated with an increase in oxidative stress markers like MDA and a concomitant reduction in antioxidant enzyme activities (SOD and CAT), indicating enhanced oxidative damage and impaired antioxidant defense. Interestingly, rats receiving *Chlorella vulgaris* as treatment exhibited a significant reduction in the levels of oxidative stress markers and significant attenuation of antioxidant enzymes. According to the results of this

study, *Chlorella vulgaris* can repair damaged tissues caused by GA3 because of its antioxidant properties. Oxidative stress is critical in liver disease, with GA3 exposure producing reactive oxygen species (ROS) and hydrogen peroxides (Conde de la Rosa *et al.,* 2022). This particular imbalance between free radical production and the antioxidant defense system could result in tissue damage, which is consistent with the previous study byAbdel-Aty & Masoud, (2016). Hussein *et al.* (2011) reported that oxidative stress and lipid peroxidation are linked to the production of hydroxyl radicals, which damage cellular lipids. CV, however, has been reported to demonstrate antioxidant properties by increasing SOD and CAT activity while decreasing MDA levels, thereby protecting cell membranes from oxidative damage (Noguchi *et al.,* 2013; Abdel-Tawwab *et al.,* 2022).

The histological findings further support the biochemical data revealing that GA3 treatment leads to significant hepatic degeneration, characterized by cytoplasmic vacuolations, pyknotic nuclei, and inflammatory infiltrates in line with the previous reports (Hussein *et al.,* 2011). Caspase-3 staining intensified in GA3-treated rats, indicating elevated apoptosis, particularly in hepatocytes around the central vein and blood sinusoid area consistent with previous findings (Ghonimi *et al.,* 2022). These changes are considered relevant to oxidative stress-induced damage (Bayoumy *et al.,* 2023). However, CV treatment partially restored the liver structure and significantly reduced fibrosis and collagen distribution key markers of liver damage (Ramos-Tovar & Muriel, 2020). *Chlorella vulgaris* believed to inhibit the activation of hepatic stellate cells, which are responsible for excessive collagen production during liver fibrosis (Ramos-Tovar & Muriel, 2020). By reducing collagen fiber distribution and maintaining the ultrastructure of liver cells, CV prevents the progression of fibrosis and promotes tissue repair (Kumar *et al.,* 2018). This antifibrotic effect, combined with its antioxidant and anti-inflammatory actions, underscores CV's multifaceted hepatoprotective potential. Additionally, these hepatoprotective effects of *Chlorella vulgaris* are largely attributed to its high carotene content and antioxidant properties, which collectively facilitate in mitigating the inflammation and oxidative stress (Kumar *et al.,* 2018; El-Fayoumy *et al.,* 2021).Additionally, it is reported that this microalga contains bioactive compounds such as lutein, α- and β-carotene, ascorbic acid, and α-tocopherol, which help to neutralize reactive oxygen species and inhibit oxidative damage (Elsheikh *et al.,* 2018). These antioxidants disrupt the chain reactions of lipid peroxidation in cellular membranes, thereby preserving membrane integrity and preventing the leakage of liver enzymes such as AST and ALT into the bloodstream (Noguchi *et al.,* 2013; Abdel-Tawwab *et al.,* 2022). It is further reported that by enhancing the activity of endogenous antioxidant enzymes like SOD and CAT, *Chlorella vulgaris* restores the balance between free radical production and the antioxidant defense system, reducing oxidative stress and its associated cellular damage (Khadrawy *et al.,* 2023). *Chlorella vulgaris* is known to protect the liver by activating the Nrf2 pathway, a critical regulator of cellular antioxidant responses. Nrf2, a transcription factor, translocate to the nucleus under oxidative stress and binds to antioxidant response elements (ARE), initiating the transcription of antioxidant genes such as heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1), and glutathione S-transferase (GST) (Ma, 2013). CV's bioactive compounds, including carotenoids, ascorbic acid, and tocopherols, activate Nrf2, enhancing the expression of these antioxidant enzymes (Elsheikh *et al.,* 2018). This activation neutralizes reactive oxygen species (ROS) and reduces oxidative stress, thereby protecting hepatocytes from GA3-induced damage (Khadrawy *et al.,* 2023).

According to the literature, in addition to its antioxidant effects, *Chlorella vulgaris* also exhibits anti-inflammatory properties that contribute to its hepatoprotective role. *Chlorella vulgaris is proposed to* modulate the NF-κB pathway, a key regulator of inflammation. GA3-induced liver injury activates NF-κB, promoting the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β and COX2 (Ghonimi *et al.,* 2022; Khadrawy *et al.,* 2023). *Chlorella vulga* inhibits NF-κB activation, reducing the production of these inflammatory mediators and suppressing the expression of iNOS and COX-2, which contribute to oxidative stress and tissue injury (Kumar *et al.,* 2018; El-Fayoumy *et al.,* 2021). Furthermore, CV reduces caspase-3 expression, a key effector protein in the apoptotic pathway, thereby preventing GA3-induced hepatocyte apoptosis (Eissa *et al.,* 2021). This antiapoptotic effect is mediated through CV's ability to reduce oxidative stress and inflammation and its potential modulation of the Bcl-2 family of proteins (Abdel-Tawwab *et al.,* 2022; Khadrawy *et al.,* 2023).

Furthermore, *Chlorella vulgaris* has been shown to enhance glycogen storage in liver cells, which is often depleted due to GA3-induced energy impairment (Abu Amra *et al.,* 2020). This restoration of glycogen levels supports energy metabolism and aids in the detoxification process, further protecting the liver from damage. Additionally, *Chlorella vulgaris* protects mitochondrial integrity by enhancing the activity of mitochondrial antioxidant enzymes such as SOD2 and GPx, ensuring adequate ATP production and supporting energy metabolism (Noguchi *et al.,* 2013). By restoring hepatocyte glycogen levels, *Chlorella vulgaris* further mitigates the energy depletion caused by GA3 (Abu Amra *et al.,* 2020). These mechanisms highlight CV's multifaceted hepatoprotective effects, making it a promising natural agent for combating GA3-induced liver damage.

These findings suggest that *Chlorella vulgaris* could be a valuable natural supplement for protecting against environmental toxins like GA3 and improving liver health. While the current study highlights *Chlorella vulgaris*'s ability to mitigate acute hepatotoxicity, the effects of chronic GA3 exposure on liver function and the potential for cumulative damage remain areas of concern. Additionally, further investigation is needed to explore the mechanisms by which *Chlorella vulgaris* exerts its protective effects at the molecular level, particularly in relation to its impact on gene expression, cellular signaling pathways, and mitochondrial function. Understanding these mechanisms could pave the way for the development of targeted therapies using *Chlorella vulgaris* or its bioactive compounds.

Conclusions: Hepatic function enzyme impairment and oxidative stress were among the harmful histological, morphometric, histochemical, immunohistochemical, and biochemical changes brought about by gibberellic acid (GA3). These changes highlight the significant risks posed by GA3, particularly through its ability to induce oxidative damage, disrupt liver enzyme activity, and trigger apoptosis in hepatic tissues. However, *Chlorella vulgaris* demonstrates remarkable efficacy in mitigating these adverse effects. CV improves oxidative stress markers, reduces caspase-3 protein expression in hepatic tissues, and normalizes the secretion of liver function enzymes, thereby decreasing hepatotoxicity caused by GA3. These findings underscore *Chlorella vulgaris*'s potential as a powerful mitigating agent against GA3-induced liver damage. The hepatoprotective effects of *Chlorella vulgaris* are likely attributed to its anti-inflammatory, antioxidant, and antiapoptotic properties, which collectively counteract the detrimental impacts of GA3 on liver health. Future research should focus on the long-term implications of GA3 exposure and the sustained use of *Chlorella vulgaris* as a protective agent.

Funding: This work was funded by the University of Jeddah, Jeddah, Saudi Arabia, under grant No. (UJ-24-DR-2225-1). Therefore, the authors thank the University of Jeddah for its technical and financial support.

Authors contribution statement: SJM: conceptualization, project administration, funding acquisition, writing the original draft, writing – review, and editing.

Declaration of interests: The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements: This work was funded by the University of Jeddah, Jeddah, Saudi Arabia, under grant No. (UJ-24-DR-2225-1). Therefore, the authors thank the University of Jeddah for its technical and financial support.

REFERENCES

Abdel-Aty OA, Masoud RA, 2016. Potential toxicity of plant growth regulator gibberellic acid (ga3) on the pancreatic structures and functions in the albino rat. Acad Anat Int 2:11–26.

Abdel-Tawwab M, Mousa MAA, Mamoon A, *et al.,* 2022. Dietary *Chlorella vulgaris* modulates the performance, antioxidant capacity, innate immunity, and disease resistance capability of Nile tilapia fingerlings fed on plant-based diets. Anim Feed Sci Technol 283:115181.

Abdulrazik AM, Eldeib KM, Abd El-Azeem RM, 2024. Assessment of the ameliorative effect of Beta-Carotene against Gibberellic acid toxicity in kidneys of rats using comet assay. Res J Appl Biotechnol 10:24-42.

Aboraya DM, El Baz A, Risha EF, *et al.,* 2022. Hesperidin ameliorates cisplatin induced hepatotoxicity and attenuates oxidative damage, cell apoptosis, and inflammation in rats. Saudi J Biol Sci 29:3157–3166.

Abu Amra E-S, Lashein FEDM, Seleem AA, *et al.,* 2020. The protective role of olive oil against gibberellic acid-induced embryotoxicity at prenatal stages of mice. J Basic Appl Zool 81:1–13.

Aebi H, 1984. [13] Catalase *in vitro*. In *Methods in enzymology*. Vol. 105, pp. 121–126). Elsevier.

Alatawi A, Wang X, Saleem MH, *et al.,* 2023. Individual and synergic effects of phosphorus and gibberellic acid on organic acids exudation pattern, ultra-structure of chloroplast and stress response gene expression in Cu-stressed jute (*Corchorus capsularis* L.). J Plant Growth Regul 42:1186–1211.

Alsemeh AE, Moawad RS, Abdelfattah ER, 2019. Histological and biochemical changes induced by gibberellic acid in the livers of pregnant albino rats and their offspring: ameliorative effect of *Nigella sativa*. Anat Sci Int 94:307–323.

Bancroft JD, Gamble M, 2008. Theory and practice of histological techniques (Elsevier health sciences (ed.); 7th ed). Elsevier health sciences.

Bauer LM, Costa JAV, da Rosa APC, *et al.,* 2017. Growth stimulation and synthesis of lipids, pigments and antioxidants with magnetic fields in *Chlorella kessleri* cultivations. Bioresour Technol 244:1425–1432.

Bayoumy AA, Abd El Salam MH, Ali AAER, *et al.,* 2023. Toxic effects of chronic use of gibberellic acid on liver and kidney and hematological study in male albino rats. *Zagazig* J Forensic Med Toxicol 21:208–222.

Bergmeyer HU, Bernt E, 1974. Colorimetric assay of reitman and frankel. In *Methods of enzymatic analysis* (pp. 735–739). Elsevier.

Conde de la Rosa L, Goicoechea L, Torres S, *et al.,* 2022. Role of oxidative stress in liver disorders. Livers 2:283–314.

Draper HH, Hadley M, 1990. [43] Malondialdehyde determination as index of lipid Peroxidation. In *Methods in enzymology* (Vol. 186, pp. 421–431). Elsevier.

Eissa MM, Ahmed MM, Abd Eldaim MA, *et al.,* 2021. *Chlorella vulgaris* ameliorates sodium nitrite-induced hepatotoxicity in rats. Environ Sci Pollut Res 28:9731–9741.

El-Fayoumy EA, Shanab SMM, Gaballa HS, *et al.,* 2021. Evaluation of antioxidant and anticancer activity of crude extract and different fractions of *Chlorella vulgaris* axenic culture grown under various concentrations of copper ions. BMC Complement Med Ther 21:1–16.

Elsheikh S, Galal AAA, Fadil R, 2018. Hepatoprotective impact of Chlorella vulgaris powder on deltamethrin intoxicated rats. Zagazig Vet J 46:17–24.

Ghonimi WAM, Alferah MAZ, Dahran N, *et al.,* 2022. Hepatic and renal toxicity following the injection of copper oxide nanoparticles (CuONPs) in mature male Westar rats: histochemical and caspase 3 immunohistochemical reactivities. Environ Sci Pollut Res Int 29:81923–81937.

Gikas GD, Parlakidis P, Mavropoulos T, *et al.,* 2022. Particularities of fungicides and factors affecting their fate and removal efficacy: A review. Sustainability 14:4056.

Hussein WF, Farahat FY, Abass MA, *et al.,* 2011. Hepatotoxic potential of Gibberellic Acid (GA3) in adult male albino rats. Life Sci J 8:373–383.

Iftikhar A, Rizwan M, Adrees M, *et al.,* 2020. Effect of gibberellic acid on growth, biomass, and antioxidant defense system of wheat (*Triticum aestivum* L.) under cerium oxide nanoparticle stress. Environ Sci Pollut Res Int 27:33809–33820.

Khadrawy SM, Mohamed DS, Hassan RM, *et al.,* 2023. Royal Jelly and *Chlorella vulgaris* mitigate gibberellic acid-induced cytogenotoxicity and hepatotoxicity in rats via modulation of the PPARα/AP-1 signaling pathway and suppression of oxidative stress and inflammation. Foods 12:1223.

Kumar M, Jeon J, Choi J, *et al.,* 2018. Rapid and efficient genetic transformation of the green microalga *Chlorella vulgaris*. J Appl Phycol 30:1735–1745.

Latif AA, El Assar DH, Elkaw EM, *et al.,* 2021. Protective role of *Chlorella vulgaris* with Thiamine against paracetamol induced toxic effects on haematological, biochemical, oxidative stress parameters and histopathological changes in Wistar rats. Sci Rep 11: 3911.

Lin Y-H, Chen G-W, Yeh CH, *et al.,* 2018. Purification and identification of angiotensin I-converting enzyme inhibitory peptides and the antihypertensive effect of *Chlorella sorokiniana* protein hydrolysates. Nutrients, 10:1397.

Ma JQ, 2024. Gibberellic acid induced oxidative stress, endoplasmic reticulum stress, and apoptosis in the livers of gibel carp (*Carassius auratus* gibelio). Aqu Toxicol 267: 106807.

Murcia G, Alonso R, Berli F, *et al.,* 2024. Quantitative proteomics analysis of ABA-and GA3-treated malbec berries reveals insights into H_2O_2 Scavenging and anthocyanin dynamics. Plants 13:2366.

Nishikimi M, Rao NA, Yagi K, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46:849–854.

Noguchi N, Konishi F, Kumamoto S, *et al.,* 2013. Beneficial effects of *Chlorella* on glucose and lipid metabolism in obese rodents on a high-fat diet. Obes Res Clin Pract 7:e95–e105.

Panda D, Mohanty S, Das S, *et al.,* 2022. The role of phytochrome-mediated gibberellic acid signaling in the modulation of seed germination under low light stress in rice (*O. sativa* L.). Physiol Mol Biol Plants 28:585–605.

Ramos-Tovar E, Muriel P, 2020. Molecular mechanisms that link oxidative stress, inflammation, and fibrosis in the liver. Antioxidants 9:1279

Sherafati N, Bideshki MV, Behzadi M, *et al.,* 2022. Effect of supplementation with *Chlorella vulgaris* on lipid profile in adults: A systematic review and dose-response meta-analysis of randomized controlled trials. Complement Ther Med 66: 102822.

Soliman MM, Aldhahrani A, Gaber A, *et al.,* 2022. Ameliorative impacts of chrysin against gibberellic acid-induced liver and kidney damage through the regulation of antioxidants, oxidative stress, inflammatory cytokines, and apoptosis biomarkers. Toxicol Res 11(1): 235–244.

Tekade M, Pingale PL, Wani SP, *et al.,* 2023. Clinical detoxification of the body from chemical toxicants. In Essentials of Pharmatoxicology in Drug Research, Volume 1 (pp. 469–505). Elsevier.

Tietz NW, Burtis CA, Duncan P, *et al.,* 1983. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 29:751–761.

Wu J-P, 2020. *Chlorella* sp. protective effect on acetaminophen-induced liver toxicity in ICR mice. Int J Prev Med 11:111.