Ameliorative Role of Psyllium Husk on Hepatotoxicity Induced by CCL₄ in Male Albino Rats: A Histological and Immunohistochemical Study

Amna M. Mostafa¹, Eman A. Allam*, ¹, and Alshaimaa A.I. Alghriany²
¹Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt.
²Department of Zoology, Faculty of Science, Assiut University, Assiut, Egypt.
E-mail*: eman.allam6@sci.svu.edu.eg

Carbon tetrachloride (CCl₄) is a strong hepatotoxin that is commonly used to induce experimental liver injury to investigate a substance's potential hepatoprotective effects. Plantago ovata husk (PSH) contains a variety of primary and secondary metabolites, as well as a number of bioactive compounds that have strong antioxidant properties. The goal of this study was to determine whether PSH powder could protect and treat adult male albino rats' livers from CCl₄-induced damage. Five groups were created: A: (Control group) received nothing. Oil was administered in B (Oil treated group). C: (CCl₄ treated) group. D: (PS+CCl₄) treated group, which received daily PSH for six weeks and CCl₄ for four weeks. E: (CCl₄+PS) treated group, after giving them CCl₄, they received daily PSH. Histological and immunohistochemical examinations were carried out. Homogenized liver samples were analyzed for antioxidant enzymes; statistical analysis was performed. The intraperitoneal injection of CCl₄ resulted in a significant rise in SOD, GPx, and MDA, as well as various lesions in hepatocytes, including cytoplasmic vacuolations, leukocyte infiltration, necrosis, collagen deposition, and a high level of caspase-3. Treatment by PSH in both groups ameliorated the effect of CCl₄ on decreasing antioxidant enzymes. PSH in the PS+CCl₄ group showed nearly normal histological structure of the liver, collagen deposition, and Caspase-3 levels. It could be concluded that PSH powder acts as a strong antioxidant that can be used in daily life for protection and curation from liver diseases.

INTRODUCTION

The liver acts as a crucial primary organ in a number of physiological functions. These include lipid and cholesterol balance, blood volume regulation, immune system support, endocrine modulation of growth signaling pathways, and macronutrient metabolism (Trefts et al., 2017). The liver is susceptible to harm since it is crucial for the process of eliminating foreign chemicals from the body (Hassanen, 2012). Liver damage is a common condition that typically involves oxidative stress (Bourogaa et al., 2014).

When the equilibrium between oxidant and antioxidant agents shifts in favor of the oxidant, this is referred to as oxidative stress (Sanchez-Valle et al., 2012). The main targets of ROS and reactive nitrogen species in hepatocytic cells include DNA, proteins, and lipids (Cichoz-Lach & Michalak, 2014). Strong anti-oxidant and free radical scavenging activities are usually found in edible or medicinal plants, which act as natural antioxidants. The most
popular substance used to explore how antioxidants/plants affect the liver's histopathological alterations is carbon tetrachloride (CCl₄) (Li et al., 2015).

Experimental liver injury is frequently induced by the xenobiotic carbon tetrachloride (CCl₄) (Cosgun et al., 2019). A wide variety of pathological outcomes are covered by the type and severity of liver damage brought on by CCl₄. (Weber et al., 2003). In the liver, cytochrome P450 catalyzes the conversion of CCl₄ to other toxic metabolites, releasing free radicals (Al Amin & Menezes, 2020).

*Plantago ovata* belongs to the family Plantaginaceae. The common names Psyllium and Isphaghula are used to refer to a number of Plantago species. Psyllium husk is Isabgol's principal export (Verma & Mogra, 2013). Natural, concentrated, soluble fiber is found in *Plantago ovata* husk, which is made from the seed's outer, green, membrane-like covering (Solà et al., 2007). Psyllium husk is a potential hepatoprotective medicinal plant due to its antioxidant properties (Wahid et al., 2020). It has flavonoids, ash, protein, and polysaccharides that can help fight numerous diseases (Khan et al., 2021).

This study aims to clarify the role of PSH powder as a hepatoprotective and hepatotherapeutic natural agent against liver damage induced by CCl₄ in adult male albino rats.

### MATERIALS AND METHODS

**Materials:**
- Forssk-growing plant, *psyllium husk.*
- CCl₄ carbon tetrachloride with a 99.9% concentration
- In Qena, olive oil was purchased at a local market.
- Chemicals that are used to evaluate oxidative stress:
  - Glutathione peroxidase (GPx) determination kit
  - Superoxide Dismutase (SOD) determination kit
  - Malondialdehyde (MDA) determination kit

**Animals:**
Thirty white male albino rats, weighing between 144 and 176 g, were obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt.

The rats were housed in South Valley University's Faculty of Science's animal house in Qena, Egypt, where they had free access to food and water. They were kept in plastic cages with mesh wire covers under ideal lighting, humidity, and temperature conditions.

Under the national institutes of health criteria for the use of experimental animals, the medical ethics committee of the faculty of veterinary medicine at South Valley University in Egypt reviewed and accepted the research protocol used in this study. (N.43/05.07.20224)

**Preparation of Doses:**

**Plantago ovata** husk dosage preparation:
PSH was ground into powder. Each rat got 0.5g/kg body weight (Ahmed et al., 2010; Mostafa et al., 2022) orally in 2ml of water, and weights were taken every week.

CCl₄ and olive oil solution preparation:
Two mg of CCl₄ were mixed in two milliliters of olive oil, and each rat received 0.5 mg/kilogram of body weight of the prepared solution twice/week (EL Sayed et al., 2019; Mostafa et al., 2022).

**Experimental Protocol:**
Prior to the experiment, adult male rats were kept under observation for two weeks for adaptation to the new environment, then divided into five groups, each with six rats:
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- G: A (Control group): included rats that received no treatment.
- G: B (Control Oil group): They received an intraperitoneal injection of an equivalent volume of olive oil at a dosage of (0.5 mg/kg b. wt.) in the third week for four consecutive weeks.
- G: C (CCL₄-treated group): CCL₄ was administered intraperitoneally at a dose of (0.5 mg/kg body weight) in the third week for four consecutive weeks.
- G: D (PS+CCL₄-treated group): For six weeks, they received PSH orally daily at a dose of (0.5g/kg b. wt.). CCL₄ was subsequently administered intraperitoneally at a dose of (0.5 mg/kg body weight) in the third week for four consecutive weeks.
- G: E (CCL₄+PS-treated group): CCL₄ was administered intraperitoneally at a dose of (0.5 mg/kg body weight) in the third week for four consecutive weeks, after that, they received a daily oral dose of PSH for six weeks at a level of (0.5g/kg b. wt.).

At the end of the experiments, all groups were anesthetized, the animals were sacrificed, and the liver was immediately dissected for different studies.

Assessment of Hepatic Oxidant-Antioxidant Markers:

The right lobe of the liver was homogenized directly after dissection in (4-8) volumes of cold phosphate buffer (e.g., 50 mM buffer, pH 7.0, containing 5 mM EDTA and 1 mM 2-mercaptoethanol) /per weight tissue then, centrifuged at 4000 rpm for 10-20 minutes at 2-8 °C. The supernatant fluid containing the enzyme was removed, and collected in labeled Eppendorf’s tubes and stored at -70 °C until used for determination of hepatic GPx, SOD, and MDA contents (Nishikimi et al., 1972; Ohkawa et al., 1979; Paglia, 1989) according to the commercial bio-diagnostic kit pamphlet.

Histological and Immunohistochemical Studies:

The liver was dissected and fixed in 10% neutral buffered formalin (NBF) at a pH of 7.2 for 24 hours, dehydrated in an ascending series of alcohols, cleared in xylene, and embedded in paraffin wax. Paraffin sections were cut at (6) um and stained by the following:

1- Harris haematoxylin and eosin (H&E) stain for general histology (Bancroft and Gamble, 2008).
2- Masson's trichrome stain for collagenous fiber demonstration (Bancroft and Gamble, 2008).
3- Immunohistochemical analysis of caspase-3 (Atia & Alghriany, 2021).

Morphometrical Study:

Images were taken using a research microscope and image analysis system (Image J) in the central laboratory of South Valley University's Faculty of Science. Twelve random fields from each slide were analyzed, and the area% of collagen fibers in sections stained with Masson trichrome and the optical density of hepatocytes that reacted to caspase-3 were measured (López-reyes et al., 2008).

Statistical Analysis:

The findings were presented as mean ± SEM. The statistical analysis utilized a one-way analysis of variance (ANOVA), which was followed by the student Newman-Keuls T-test, prism, and image analyzer software. Statistics were considered significant at P< 0.05.

RESULTS

Histological and Histochemical Examination:

1. Histological Examination by (H&E):

In the control group, liver sections showed normal architecture. Polygonal hepatocytes contain granulated cytoplasm with one or more nucleoli, which are organized in hepatic cords that surround the central vein and radiate toward the periphery, forming...
the hepatic lobule. The space between hepatic cords forms the blood sinusoids, which are lined with an irregular layer of endothelial cells. The branches of the hepatic artery, portal vein, and bile duct were noticed at the corners of hepatic lobules, forming the portal areas (Figs. 1a-c). The results of the olive oil group were similar to those of the control group.

Liver sections of rats exposed to CCl\(_4\) showed a loss of liver tissue architecture, inflammatory cell infiltration, features of hepatocyte necrosis (Pyknosis, Karyorrhexis, and Karyolysis), cytoplasmic vacuolation, and necrotic areas (Figs. 2a-d).

Protected rats subjected to PS+CCl\(_4\) showed nearly normal organization of normal hepatic cord morphology and uniform sinusoidal arrays. Hepatocytes with homogenous acidophilic cytoplasm and central nuclei are visible in most cells compared to the CCl\(_4\) group; vacuolated cytoplasm is still present in some hepatocytes; some necrotic cells are present; and very few inflammatory cells infiltrate in the portal area and central vein (Figs. 3a-c).

Liver sections of therapeutic rats subjected to CCl\(_4\) + PS showed a slight reduction in pathological alterations compared to CCl\(_4\). Mild infiltration of some inflammatory cells around the portal area, pyknotic nuclei, and vacuolated cytoplasm was seen (Figs. 4a-d).

2. Masson Trichrome Stain for Collagen Fibers:

Masson trichrome stain sections of the liver of control groups showed scant amounts of collagen fibers among liver lobules and around the central vein (Fig. 5a). The liver of rats subjected to CCl\(_4\) showed extensive collagen fiber deposition around the central vein and in the perisinusoidal spaces, as well as steatofibrosis, which is characterized by lipid accumulation in hepatocytes in the form of macrovesicles (Fig. 5b). While the livers of rats subjected to both PS+CCl\(_4\) and CCl\(_4\) + PS showed a few collagen fiber depositions (Figs. 5c-d).

Quantitative morphometric analysis showed a significant increase in collagen deposition in the CCl\(_4\) group by the total percentage of collagen fibrosis (93.8%) in the liver section when compared with control rats. While in both GPs (PS+CCl\(_4\) and CCl\(_4\) + PS) this percentage had a significant decrease in percentage (62.2 and 79.2 %) as compared to the CCl\(_4\) group, a slight increment was noticed in the last group (CCl\(_4\) + PS) (Fig.5e).

Immunohistochemical Detection of Caspase-3:

Immunohistochemical detection of Caspase-3 level in control male rats, showed a negative reaction of Caspase-3 antigen level in the vast majority of hepatocytes (Fig. 6a). It was observed that most hepatocytes treated with CCl\(_4\) revealed a sharp increase in fine, homogenous brown patches and a variable degree of their localization in the cytoplasm (Fig. 6b). Furthermore, the PS+ CCl\(_4\) group showed a remarkable negative reaction of Caspase-3 compared with the CCl\(_4\) group (Fig. 6c), while the CCl\(_4\)+ PS group showed a positive reaction like the CCl\(_4\) group but with a smaller degree (Fig. 6d).

The quantitative intensity of caspase-3 was 199.9% higher in the CCl\(_4\) group than in the control group. However, as compared to the CCl\(_4\) group, the PS+CCl\(_4\) group dropped by 70.2%, while the CCl\(_4\) +PS group had a non-significant decrease of 14.8% as compared with the control (Fig. 6).
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Fig. 1(a-c): Photomicrographs of liver sections from control group showed: central vein (CV), endothelial cell (E), hepatocytes (H), blood sinusoids (S), portal area containing portal vein (PV), hepatic artery (HA) and bile duct (BD). (H&E stain, Bar = 200 & 50 µm).

Fig. 2(a-d): Photomicrographs in liver sections from CCL₄ group showed: inflammatory cells infiltration (yellow arrow), necrotic areas (black arrow), pyknotic nuclei (green arrows), karyorrhexis (blue arrow), karyolysis (orange arrow) and vacuolated cytoplasm (red arrow) (H&E stain, Bar = 200, 50 µm).
Fig. 3 (a-c): Photomicrographs in liver sections from PS+CCl₄ group showed normal morphology of hepatic contents, normal hepatic structure and hepatocytes with well-defined cell boundaries and contains homogenous acidophilic cytoplasm with central nuclei (violet arrow). Few hepatocytes with vacuolated cytoplasm (red arrow) pyknotic cells (green arrows). (H&E stain, Bar = 200 & 50 µm).

Fig. 4 (a-c): Photomicrographs in liver sections from CCl₄+PS group showed mild infiltration of some inflammatory cells in portal area (yellow arrow), pyknotic cells (green arrows) and vacuolated cytoplasm (red arrow) (H&E stain, Bar = 200 & 50 µm).
Figure 5(a-d): Photomicrographs in liver sections showed collagen fibers in all experimental groups. Control group showed normal distribution of collagenous fibers around the central vein and hepatic cords (a). CCl₄ group, showed extensive collagen fibers accumulation extending between hepatocytes and around the central vein region (asterisk). Macro-steatosis is extensive (orange arrow) (b). PS+CCl₄ & CCl₄+PS groups, showed few collagenous fibers accumulation around the central vein (c-d). Masson's trichrome stain, bar = 50µm. Percentage of area of collagen fibers in all experimental groups. Values in the column with unlike superscript letters are significantly different at (P < 0.05). Data represents mean ± S.E.M. (e).
Fig. 6(a-d): Photomicrographs showed Immunohistochemical detection of Caspase-3 protein expression in liver of all the experimental groups (Scale bar =50μm). Showed control group with negative reaction (a). CCl4 group, showed sharp positive reaction (arrows) in hepatocytes (b), PS+CCl4 group, showing faint staining reaction (c) CCl4+PS group, showing positive reaction (d). Densitometric levels of positive Caspase-3 patches. Values in the column with unlike superscript letters are significantly different at (P<0.05). Data represents mean ± S.E.M(e).

Biochemical Results:
1. Determination of GPx:

After CCl4 administration, GPx was raised (by 150%) in the CCl4 group compared to the control group. PS+CCl4 and CCl4+PS groups showed significantly reduced GPx (70% and 60%), and the most decrement was observed in the last one (Fig. 7).
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2. Determination of SOD:

After 30 days of CCl₄ administration, SOD was higher in the CCl₄ group than in the control group (53.9%). The protective group (PS+CCl₄) significantly reduced SOD (by 62.4%). While this reduction by percentage was 50.6% in a non-significant manner in the CCl₄+PS group (Fig. 8).

3. Determination of MDA:

Following CCl₄ administration, MDA was elevated (90.6%) in the CCl₄ group compared to the control group. MDA was significantly reduced (47.5%) in the PS+CCl₄ group.
group. MDA was reduced significantly (62.8%) in the CCl4+PS group when compared with the control group (Fig. 9).

![Graph showing MDA levels](image)

**Fig. 9.** Showed variations in the mean values ±S.E.M. of the MDA (nmol/g.tissue) level in both control and different treatments in male rats. Values in the same column with unlike superscript letters are significantly different at P < 0.05.

**DISCUSSION**

The purpose of the current study was to investigate the preventive and possibly therapeutic effects of psyllium husk powder (PSH) on carbon tetrachloride (CCl₄)-induced liver toxicity in male albino rats. Histopathological, immunohistochemical, and biochemical investigations were used to carry this out.

Our histopathological results were in agreement with (Amin et al., 2010; Saber et al., 2017), who found that the rat liver is affected in a variety of pathological ways by CCL₄; These alterations manifested as an inflammatory leucocytic infiltration, enlargement of the sinusoids, activation of Kupffer cells, necrosis, hepatocyte vacuolization, dilating and congested blood vessels, loss of normal hepatic tissue architecture, and loss of normal organization. According to (Liu et al., 2001; Cichoz-Lach & Michalak, 2014), the conversion of CCl₄ into the trichloromethyl free radical by the endoplasmic reticulum's mixed function cytochrome P450 oxygenase system is assumed to be the mechanism by which CCl₄ harms the liver. Numerous essential biological components, including proteins, nucleic acids, lipids, amino acids, and nucleotides, interact with the trichloromethyl free radical. The trichloromethyl peroxy radical is produced from the free trichloromethyl radical (Mostafa et al., 2022). The trichloromethyl peroxy radical is far more likely than the trichloromethyl free radical to take hydrogen from polyunsaturated fatty acids, which starts the process of lipid peroxidation.

Free radicals produced by CCl₄ are hypothesized to increase the concentration of leukocytes in the tissue, which in turn activate neutrophils, which in consequence may indirectly damage the tissue. Activated neutrophils that have accumulated in the inflammatory site and are creating lipid peroxidation and free oxygen radicals, among other things, are thought to be the pathogenic culprits behind fatty and infiltrative liver diseases (Gharagozloo et al., 2015).
Cellular apoptosis and necrosis are caused by the CCl₄ free radicals and the activation of Kupffer cells, which produce inflammatory and profibrogenic mediators. A cascade of events starting with an increase in free radical generation eventually leads to membrane lipid peroxidation, which in turn causes cellular apoptosis and necrosis (Ebaid et al., 2013).

The most common cause of liver congestion, heart failure brought on by CCl₄, may be responsible for the dilated and congested blood vessels in the liver that were mentioned in our study. In addition, sinusoid dilatation causes an increase in venous blood in the organs, which raises blood pressure in the veins and capillaries (Naji et al., 2017).

With PSH (PS+CCl₄) treatment, the level of inflammation and vacuolar degeneration was greatly reduced. This suggests that giving husk powder to rats could bring the tissue markers back to normal levels and protect the hepatocellular architecture from oxidative damage. Also, the PSH-achieved anti-lipid peroxidation halted the damaging effects of CCl₄ free radicals, resulting in the healing of the hepatic parenchyma and the regeneration of hepatocytes. Moreover, the enhancement of the hepatic integrity in the PSH-treated groups was very clear in our histopathological results, in which decreased fibrosis, vacuolization, and apoptosis was observed compared to that of the CCl₄ group. Certainly, the reduced hepatic injury observed in the hepatic histology was in agreement with a previous study (Wahid et al., 2020; Rafiee, 2022). The antioxidant and anti-inflammatory properties of Plantago ovata can be attributed to the effects of PSH. Plantago ovata has antioxidant features because of bioactive compounds such as phenols, flavonoids, tannins, and fibers. Due to flavonoids' capacity to chelate metal ions like iron and copper, investigations have shown that the reducing action of plant extracts can be substantially related to their concentration. Reducing substances have the ability to act as primary and secondary antioxidants by reducing the oxidized intermediates of lipid peroxidation processes and showing an electron donor property (Khedher et al., 2024).

In the current study, the CCl₄ group's collagen fiber density and dispersion significantly increased, primarily in the portal tract area. Our histopathological findings concurred with those of (Nishikimi et al., 1972; Ohkawa et al., 1979; Paglia, 1989), who discovered that lipotoxic hepatocytes can activate Kupffer cells (KCs), which release the transforming growth factor TGF-1. TGF-1 also directly increased the activity of hepatic stellate cells (HSCs), which lost their ability to store lipids and vitamin A and differentiated into myofibroblast-like cells, which are the primary source of extracellular matrix secretion. Along with increasing type I and type III collagen deposition in the perisinusoidal region, these factors also enhance tissue matrix metalloproteinase I and II expressions, which causes liver fibrosis to proceed. Treatment with PSH powder in the (PS+CCl₄) group resulted in marked alleviation in liver structure. This alleviation appeared as a significant decrease in the area percentage of collagen in comparison with CCl₄-group, the antioxidant activity of PSH may be responsible for this outcome since it is well-known that antioxidants are able to prevent the development of liver fibrogenesis, the mechanism by which collagen fibers secreted in the liver as we mentioned before can be summarized in the activation of HSCs by TGFβ-1 and secretion of collagen. TGFβ-1 is recognized as a major profibrogenic cytokine in the progression of liver fibrosis, it is responsible for hepatic stellate cells (HSCs) activation and migration which is suggested to be the central event of liver fibrosis. In recent studies it was found that antioxidants have effects on the TGFβ-1, these effects represented in decreased TGF- expression levels and inhibition of collagen synthesis where TGF- was blocked by antioxidants decreasing collagen fibre production, compared with the CCl₄ group (Yuan et al., 2008; Morsy et al., 2012; Wei et al., 2015; Cachón et al., 2017; Farrag et al., 2017; Rafiee et al., 2021).

Our study found that CCl₄ increased the protein expression of the caspase-3 protein, showing that CCl₄ can induce liver cell apoptosis. Our result agrees with (Deniz et
Amna M. Mostafa et al., 2019; Wang & Wu, 2019; Amer et al., 2022), who found that CCl₄ increased caspase 3 activity, which indicates that apoptosis initiated the development of liver damage. Mitochondria are the main organelle that regulates apoptosis because they are the major site for producing energy and generating ROS. Pathological cascade events, such as inflammation, activation of myofibroblasts, and liver fibrosis, are induced by apoptosis of injured liver cells, which indicates that CCl₄ triggers apoptosis via the mitochondrial and death receptor pathways.

We discovered that the PSH powder-treated rats in the (PS+CCl₄) group had considerably decreased caspase 3 activity, which shows that PSH's ability to preserve the liver is attributable to its ability to prevent apoptosis. These results suggest that PSH can suppress hepatocyte apoptosis and are consistent with other studies that linked the rescue effect on mitochondrial oxidative damage in the liver to the free radical scavenging, metal chelating, and antioxidant potentials of antioxidant compounds.

Rats post-treated with PSH in the CCl₄+PS group showed significant improvements in quantitative histochemistry and the avoidance of collagen deposition, but no improvements in immunohistochemical investigations. This could be explained by the fact that the intoxicated rats only got PSH for a brief period of time and that the amount of psyllium given to the CCl₄+PS group was lower than that given to the PS+CCl₄ group. (Sharma & Shukla, 2011; Mostafa et al., 2022) found that the antioxidant was ineffective in treating the liver at low doses and that histology findings indicated modest generation.

There is an effective defense mechanism used by the body to prevent and neutralize free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD and GPx. These enzymes constitute a mutually supportive team of defense against ROS (Venukumar & Latha, 2002; Shankar et al., 2008). The antioxidant enzymes SOD and GPx limit the effects of oxidant molecules on tissues, they are activated to defend against oxidative cell injury by means of their acting as free radical scavengers. SOD is the first line of defense against oxygen-derived free radicals and functions by dismutating two superoxide (O₂⁻) ions into H₂O₂. (Dhawan et al., 2006). GPx is an enzyme with peroxidase activity that assures the protective effect from oxidative damage. GPx catalyzes the reduction in H₂O₂ or organic peroxide to water or alcohol (Taamalli et al., 2020). In our study, SOD and GPx activity considerably increased in the CCl₄-treated group. It might indicate an increase in oxygen-free radical production and hydrogen peroxide levels produced by the body's metabolism of CCl₄; these substances promote SOD and GPx activity to decrease the toxicity of these free radicals under oxidative stress. Also, it may attribute to the greater tolerance of the animals in reducing the toxic stress the agreement with (Liu et al., 2001; Dhawan et al., 2006; Gowri Shankar et al., 2008; Cosgun et al., 2019; Taamalli et al., 2020).

The groups were treated orally with PSH at a dose of 0.5g/kg b.wt. Showed a significant decrease in the level of these enzymes and/or activate enzyme activity in CCl₄-damaged liver tissue, which indicates the free radical scavenging property of plantago ovata husk and, suggests that it can restore enzymes to the normal ranges and it can be used as protective as a therapeutic agent. These results are in agreement with a previous report which showed a significant restoration of SOD and GPx activity by treatment with an antioxidant in CCl₄-intoxicated animals.

In our investigation, MDA products were observed to significantly rise in the CCl₄-treated rats. If an antioxidant is not present, oxidative stress can result in cell damage and finally cell death. The aetiology of cancer, liver disorders, and toxic cellular damage is caused by MDA, one of the byproducts of lipid peroxidation and a marker of lipid peroxidation. Free radicals produced by CCl₄ may be responsible for the accumulation of MDA (Lee et al., 2017; Cosgun et al., 2019). this result agrees with (Liu et al., 2001; Dhawan et al., 2006; Lee et al., 2017; Cosgun et al., 2019).
On the other hand, there is a significant decrease in MDA product in groups treated with PSH + CCl₄ group, these results suggest that PSH has inhibitory effects against CCl₄-induced liver hepatotoxicity, which may be associated with hepatic antiperoxidation.

In the present study, post-treatment with PSH powder reduced lipid peroxidation by decreasing MDA levels, indicating the free radical scavenging activity of this plant and also the curative properties of PSH. This result was in agreement with (Vuda et al., 2012; Ibrahim & Al-Azawi, 2018) who found that the post-treatment of rats intoxicated with CCl₄ with antioxidant substance reduce the MDA to the normal value.

**Conclusion:**

In conclusion, our findings support the application of PSH powder for combating oxidative damage to hepatic cells induced by CCl₄, preserving liver function, and preventing fibrotic disorders. In addition, PSH may also be an effective therapeutic agent against hepatic injury. We suggest that PSH, which is potentially safe and inexpensive for clinical use, may be considered an effective antioxidant agent against liver disease with more protective rather than therapeutic action, which may be enhanced by increasing the period of post-treatment in future searches.

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