Piper nigrum and *Lepidium sativum* oils counteract carbon tetrachloride-induced hepatotoxicity and oxidative stress in rats

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Abstract

Background: Carbon tetrachloride (CCl_4) is a potent hepatotoxic agent that has the potential to produce reactive oxidative species (ROS), which can degenerate the liver, kidneys, and other vital organs. However, there is growing interest in the hepatoprotective properties of essential oils, such as those from *Piper nigrum* and *Lepidium sativum*, which show potential biomedical applications. **Aim:** This investigation was conducted to evaluate the impacts of the treatment with *P. nigrum* and *L. sativum* oils (PNO and LSO, respectively), and their combination on hepatotoxicity induced by CCl_4 in rats. **Methods:** Forty-eight male Swiss albino rats were divided into six groups (8 rats/group) as follows: **Group 1**, the Negative control group. **Group 2** the Positive control group, rats administered intraperitoneal (i.p) injections of CCl_4 in corn oil (50% v/v) at a dosage of 1 mg/kg body weight (b.w.), twice a week for 4 weeks. **Group 3** received corn oil only. **Groups 4 to 6** were injected with CCl_4/corn oil as in group 2 and were then treated as follows: **Group 4** received PNO at a dose of 200 mg/kg. **Group 5** received LSO at a dose of 200 mg/kg. **Group 6** received a combination of PNO and LSO at a ratio of 1:1. After 30 days from the start of the experiment, the rats were sacrificed, and blood and liver tissue samples were collected for biochemical and histopathological analyses, respectively. **Results:** The findings of this study indicated that PNO and LSO considerably enhanced the percentage body weight change in CCl_4-treated groups and ameliorated the CCl_4-induced abnormalities in liver function parameters, aspartate aminotransferase, alanine aminotransferase as well as renal functions (creatinine and urea). Furthermore, PNO and LSO treatments noticeably diminished serum levels of total cholesterol, triglycerides, and low-density lipoprotein, while elevating serum high-density lipoprotein levels. Additionally, it reinstated the functions of the antioxidant enzymes superoxide dismutase and catalase, which diminished following CCl_4 administration, and malondialdehyde levels were considerably reduced. Moreover, the notable reduction in vacuolation, hepatocyte disintegration, and monocyte infiltration improved the histological changes in the liver. **Conclusion:** Consequently, it may be said that PNO and LSO may be characterized as possible hepatoprotective agents.

Keywords: *Piper nigrum*, *Lepidium sativum* oils, Carbon tetrachloride, Hepatotoxicity, Serum lipid profile, Oxidative stress, Bioactive compounds, Antioxidant activity.

الزيوت المستخلصة من الفلفل الأسود *(Piper nigrum)* وحب الرشاد *(Lepidium sativum)* تقاوم السُمية الكبدية والإجهاد التأكسدي المستحدثه برابع كلوريد الكربون في الجرذان

المستخلص:

الخلفية :يعتبر رابع كلوريد الكربون (CCl_4) من العوامل السامة القوية للكبد فله القدرة على إنتاج الشائق الحرة(ROS) ، والتي تسبب تدهورًا كبيرا في خلايا الكبد والكليتين والأعضاء الحيوية الأخرى. إلا أنه، هناك اهتمام متزايد بالخصائص الواقية لبعض الزيوت الأساسية على خلايا الكبد، مثل تلك الزيوت المستخلصة من نبات الفلفل الأسود *(Piper nigrum)* وحب الرشاد *(Lepidium sativum)*، والتي لها تطبيقات طبية هامة.

الهدف :أُجريت هذه الدراسة لتقييم تأثير العلاج بزيوت الفلفل الأسود وزيت حب الرشاد ومزيجهما على السمية الكبدية المستحدثة بـ CCl_4 في الفئران.

الطرق :تم تقسيم ٤٨ فأر ذكر من نوع Swiss albino إلى ست مجموعات (٨ فئران لكل مجموعة) كما يلي:

- المجموعة ١: المجموعة الضابطة السالبة.
- المجموعة ٢: المجموعة الضابطة الموجبة، حيث تم حقن الفئران داخل الصفاق (i.p) بـ CCl_4 مذاب في زيت الذرة (٥٠% بالحجم) بجرعة ١ ملغم/كغم من وزن الجسم، مرتين أسبوعيًا لمدة ٤ أسابيع.
- المجموعة ٣: تلقت زيت الذرة فقط عن طريق الحقن داخل الصفاق.

المجموعات من ٤ إلى ٦: *تم حقنها بـ CCl_4 /زيت الذرة كما في المجموعة ٢، ثم عولجت كالتالي:*

- المجموعة ٤ تغذت على زيت الفلفل الأسود بجرعة ٢٠٠ ملغم/كغم.
- المجموعة ٥ تغذت على زيت حب الرشاد بجرعة ٢٠٠ ملغم/كغم.
- المجموعة ٦ تغذت على مزيجًا من زيت الفلفل الأسود وزيت حب الرشاد بنسبة ١:١.

بعد ٣٠ يومًا من بدء التجربة، تم تشريح الفئران وجمع عينات الدم وأنسجة الكبد للتحليل الكيميائي الحيوي والفحص النسيجي، على التوالي.

النتائج :أشارت نتائج الدراسة إلى أن زيوت الفلفل الأسود وحب الرشاد عززا بشكل ملحوظ النسبة المئوية للتغير في وزن الجسم لدى المجموعات المعالجة بـCCl_4 ، كما حسنت التغيرات الغير مرغوبة الناتجة عن الـ CCl_4 في وظائف الكبد (الأسبارتات أمينوترانسفيراز، الألانين أمينوترانسفيراز) وكذلك في وظائف الكلى (الكرياتينين واليوريا). علاوة على ذلك، أدت المعالجة بتلك الزيوت الى انخفاض ملحوظ في مستويات الكوليسترول الكلي، الدهون الثلاثية، والبروتين الدهني منخفض الكثافة في مصل الدم، مع رفع مستويات البروتين الدهني عالي الكثافة. بالإضافة الى ذلك أعاد العلاج بزيوت الفلفل الأسود

وحب الرشاد وظائف إنزيمات مضادات الأكسدة مثل سوبر أكسيد ديسموتاز وألكتاليز والتي كانت قد انخفضت بعد الحقن بـ CCl_4، وتم تقليل مستويات المالونديالديهيد بشكل ملحوظ. علاوة على ذلك، أدى الانخفاض الملحوظ في التجويف، تفتت الخلايا الكبدية، وتسلل الخلايا الوحيدة إلى تحسين التغيرات النسيجية في الكبد.

الاستنتاج :بناءً على ذلك، يمكن القول إن زيوت الفلفل الأسود وحب الرشاد يمكن وصفهما كعوامل محتملة قوية للحماية من السمية الكبدية.

Introduction

Liver injury is regarded as a serious worldwide health issue, attributed to several problems including medication-based treatment, exposure to toxic compounds in the environment, and repeated dosages of medications. Additionally, some illnesses, including heart failure, chronic kidney disease, and non-insulin-dependent diabetes mellitus, can induce it **(Attallah *et al*., 2022 and Byrne and Targher, 2022)**.

One of the potent hepatotoxic toxins is carbon tetrachloride (CCl_4). It is a synthetic colorless gas utilized in manufacturing cleaning fluids, fire extinguishers, degreasing agents, pesticides, stain removers, and refrigerant fluids. Since it is difficult to break down and has accumulated over time from human activity, it is present in the environment even though it does not occur naturally **(Ashour and Mohamed, 2019)**. CCl_4-induced lipid peroxidation causes liver injury, which is well-known and frequently utilized in experimental models to comprehend and assess the potential of therapeutic and dietary antioxidants, as well as the biological mechanisms underlying oxidative damage **(Shenoy *et al.*, 2001)**. It is usually applied to cause free radical poisoning in organs such as liver, kidney, testis, heart, brain, lungs, and in the blood of experimental animals **(Alkreath *et al.*, 2014)**. In addition, it can cause severe liver centrifugal necrosis and steatosis in just one exposure **(Saad *et al.*, 2015)**. The free radical's generation and the changes in tissues antioxidant system are what make CCl_4 harmful **(Fathy and Mohammed, 2021** and **Li *et al*., 2022)**.

The antioxidant-rich natural dietary products provide therapeutic benefits, where phenolic compounds, carotenoids, vitamins, and minerals are antioxidative, anti-stress substances that either stimulate the body's defenses or act on reactive species. Thus, conditions as diabetes, cancer, arthritis, Alzheimer's disease, Parkinson's disease, and cerebrovascular risk are reduced or eliminated **(Ahmad and Ghosh, 2022** and **Almeida *et al.*, 2011)**. Moreover, medicinal plants like herbs and spices have been entirely or partially relied upon to treat illnesses due to their well-documented action, sensory properties, accessibility, acceptance, permission, and environmental impact **(Yuan *et al*., 2016)**. Also, their low toxicity, potential pharmacological activity, and/or viability as an alternative to drugs **(Gillessen and Schmidt, 2020** and **Hasan *et al.*, 2021)**.

Herbs and spices are crucial in cooking, gastronomy, and food technology. Most commonly, spices are described as plant-based products **(Embuscado, 2015).** The king of spices, *Piper nigrum L. or* black pepper, is made from unripe pepper fruit. It has been utilized as a culinary ingredient and a treatment for thousands of year; its main phytochemical components are essential oil and piperine alkaloid, which have been shown to possess substantial biological and pharmacological properties like anti-inflammatory, anticonvulsant, anticancer, antidiabetic, antioxidant, antimicrobial, hepatoprotective and neuroprotective effects **(Bajpai *et al.*, 2013; Mahmoud *et al.*, 2022; Mahmoud *et al*., 2024; Mostafa *et al*., 2021; d Takooree *et al.*, 2019** and **Zhang *et al.*, 2021)**. The pepper species essential oils have exhibited significant antioxidant activity as well as antifungal and antibacterial properties against human infections **(Wanna *et al.*, 2023)**. Additionally, piper species can treat and prevent several chronic illnesses **(Zhang *et al.*, 2021)**. Noteworthy, they are evaluated as a source of potent quorum-quenching molecules that prevent the formation of biofilms by regulating quorum sensing and characterizing particular biochemical interactions. As a result, they are potentially antivirulent against some drug-resistant pathogenic bacteria **(Chatterjee *et al.*, 2025)**.

Hab-alrashed, also known as *Lepidium sativum* Linn. and commonly known as garden cress, is an edible herb that grows annually. It is fast-growing, with an aroma and tangy flavor **(Hussein *et al.*, 2017)**. It belongs to the family of *Cruciferae* (*Brassicaceae*) and is primarily grown for its seeds **(Nehdi *et al.*, 2014)**. The whole fruits and seeds are used as a seasoning and condiment, either fresh or dried, despite their strong odor, because of their spiciness. It is widely used for both culinary and therapeutic purposes.

Nutritionally, its seeds contain 20-25 % oil, and its high oil content makes it suitable for use in the oil industry. Alpha-linolenic acid makes up 32–35% of its major fatty acid content. Also, its inherent antioxidants aid in the oil's defense against rancidity. Sinapic acid, lepidine, imidazole alkaloids, and monomeric alkaloids are the most important phytochemicals present in *L. sativum* **(Rezig *et al.*, 2022; Umesha and Naidu, 2015,** and **Vazifeh *et al*., 2022)**. **Rezig *et al.* (2022)** demonstrated that *L. sativum* seed oil has special chemical, anti-inflammatory, and antioxidant properties, which could be helpful in enhancing human health and averting age-related illnesses. Several phytoconstituents, such as oleic acid, stearic acid, palmitic acid, linoleic acid, vaccenic acid, tocopherols, erucic acid, and sesamolin, were found in garden cress oil. In molecular docking studies, these phytoconstituents demonstrated a high affinity for binding TNF-α and caspase-3 proteins, which could explain the oil's anti-inflammatory and anti-apoptotic functions **(Mabrouk *et al*., 2025)**. Additionally, *L. sativum* seeds possess inherent anti-inflammatory, anti-carcinogenic, hepatoprotective, and antioxidant properties, in addition to the capacity to inhibit cardiovascular diseases. These inhibitory properties stem from the seed oil's remarkable hypolipidemic effect, according to **Abdel-Aty *et al.* (2019) and Abdou e*t al.* (2019).** Consequently, the present study was designed to evaluate the potential impacts of the treatment with *P. nigrum, L. sativum* oils, and their combination on decreasing the hepatotoxicity that CCl_4 causes in rats.

Materials and methods

- *P. nigrum* and *L. sativum* oils were acquired from El Captin Company, Cairo, Egypt.
- Basal diet: Al-Gomhoriya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt, provided the cellulose, casein, vitamins, and minerals, whereas corn oil and starch were purchased from the local market.
- Carbon tetrachloride (CCl_4) was purchased from Al-Gomhoriya Company, Tanta, Egypt.
- The following test kits were acquired from Biodiagnostic Company in Egypt: aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA).
- Forty-eight Swiss albino male rats weighing 20 ± 4 grams were acquired from the National Research Center (NRC, Cairo, Egypt).

Methods:

Animals and experimental protocol

After a week of acclimation in the animal house settings of Tanta University's Faculty of Science, 48 male Swiss albino rats weighing 81± 10g were split up into groups. The experimental design with the numbered ethical requirements was authorized by the institutional animal care committee of Tanta University's Zoology Department in the Faculty of Science.

A day/night cycle was accomplished, and the target temperature and relative humidity were roughly 22 ± 1 °C and 55 ± 5%, respectively. Rats were provided with a conventional experimental pelleted animal diet and drinking tap water *ad* *libitum*. In this manner, the rats were split up into six groups of eight rats each as follows:

- **Group 1:** Negative control group, rats were injected i.p with saline 5 ml/kg/ day
- **Group 2:** Positive control group, rats administered i.p injections of CCl_4 in corn oil (50% v/v) at a dosage of 1 mg/kg b.w., twice a week for 4 weeks **(Wei *et al.,* 2004)**.
- **Group 3:** Corn oil was injected i.p into rats twice a week for four weeks at a dose of 1 ml/kg b.w.

Groups 4 to 6 *were injected with CCl_4 as in the positive control group and were then treated as follows:*

- **Group 4:** Received PNO orally at a dose of 200 mg/kg **(Ncir *et al,* 2020).**
- **Group 5:** Received LSO orally at a dose of 200 mg/kg.
- **Group 6:** Received a combination of PNO and LSO orally at a ratio of 1:1.

Following the 30-day experiment, all groups were sacrificed, and liver and blood samples were collected for histopathological and biochemical analyses. Rats in each group were weighed both at the start of the experiment (I.B. Wt.) and the end (F.B. Wt.). The formula used to get the percentage change in total body weight (% T.B.W.) was [(F.B. Wt – I.B. Wt) / I.B. Wt] × 100. Following the collection of blood samples from the heart chambers and arterial blood vessels, serum was separated by centrifugation for biochemical examination.

Biochemical analysis:

- According to **Reitman and Frankel, (1957)** the liver functions (ALT and AST) were measured. Where, the serum levels for kidney (urea and creatinine) were determined according to **Henry *et al.,* (1974) and (Bartels and Bohmer 1971),** respectively**.**
- According to the enzymatic colorimetric method described by **Allian *et al.,* (1974), Richmond (1973) and Fossati and Principle (1982**), respectively total cholesterol, triglycerides and HDL-cholesterol were determined, while LDL-cholesterol were estimated by **Friedewald *et al*., (1972**).
- SOD activity was measured using the methodology outlined by **Nishikimi *et al.* (1972).**
- CAT activities were quantified using **Aebi's (1984)** technique.
- MDA was evaluated using the procedures outlined by **Li and Chow (1994)**.

Histological investigation

Different experimental groups' liver samples were promptly preserved for 24 hours in 10% buffered formalin. After being dehydrated using increasing ethyl alcohol concentrations, the tissue samples were cleaned with xylene and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed on 5-mm-thick sections using the **Bancroft and Gamble (2008)** technique. Using a light microscope (Olympus, Model: CX21FS2), all slides were inspected for histological changes.

Statistical analysis

Three replicates' means are used for every data point. Utilizing the Kolmogorov-Smirnov test, the data's normality was examined. To determine whether there is a significant difference in the means, Tukey's post-hoc comparisons between the groups were conducted with *p* value < 0.05.

RESULTS AND DISCUSSION

Effect of PNO and/or LSO treatments on the body weight change percentages of CCl_4-injected rats:

The findings in **Table 1** demonstrate that there was no discernible difference in the percentage of body weight change between the corn oil-treated group (CO) and the negative control group. In the CCl4-injected rats, the percentage body weight change decreased considerably to 30.43% ($P < 0.05$) from 42.62% in the negative control group. However, the CCl_4-injected rats that received oral PNO/LSO treatment had the highest percentage of b.wt change (47.25%) ($P < 0.05$). LSO-treated rats revealed a 39.03% b.wt change, whereas PNO-treated rats showed a 45.20% b.wt change **(Table 1)**.

These results align with **Sunday *et al.* (2024)**, who demonstrated a marked reduction in the rat's body weight following CCl_4 induction. They ascribed this weight loss to the mechanism of action of trichloromethyl peroxide radical (CCl_3OO*) and trichloromethyl free radicals (CCl_3), a metabolite of CCl_4 that alkalizes proteins. Additionally, **Elhassaneen e*t al.* (2023)** observed that CCl_4-induced hepatic rats exhibit a considerable decrease in body weight. **de la Fuente (2022)** reported that liver illness can result in malnutrition because of inadequate food intake, poor digestion, malabsorption, as well as anomalies in macronutrient and micronutrient storage and metabolism. However, several studies have demonstrated that by reducing oxidative stress, black pepper oil improves hepatotoxicity in rat models. As rats' body weight significantly increased after being pretreated with piperine alkaloids and a methanolic preparation of *P. nigrum*, a phytochemical that is rich in *P. nigrum*, as compared to the ethanol-CCl_4 group **(Nirwane and Bapat, 2012)**. Additionally, piperine has been shown to enhance body weight loss in rats and reduce acetaminophen-induced hepatotoxicity **(Wali *et al.*, 2021)**. **Shukla and Bigoniya (2013)** reported that CCl4 administration resulted in a significant decrease in body weight, accompanied by reduced food consumption. In contrast, the *L. sativum* alkaloidal fraction demonstrated dose-dependent defense against weight loss.

Table (1): Effect of PNO and/or LSO treatments on the body weight change percentages of CCl_4-injected rats

Groups	Initial body weight (g.)	Final body weight (g.)	Paired t-test p-value	% b.wt change
G1: Negative control	87.14 ± 5.66	124.28 ± 16.18***	< 0.001	42.62%
G2: Positive control CCl_4 alone	83.57 ± 7.88	109 ± 15.65**	0.003	30.43%
G3: Corn oil (CO)	80.71± 6.07	112.25 ± 10.69**	0.007	39.08%
G4: CCl_4/PN O	77.14 ± 6.36	112.01 ± 14.40*	0.017	45.20%
G5: CCl_4/LS O	82.14 ± 6.98	114.20 ± 15.16**	0.001	39.03%
G6: CCl_4/PNO/LSO	81.42 ± 6.26	120.11 ± 12.58**	0.009	47.25%
F-value	0.76	1.03		
p-value	0.597 n.s.	0.444 n.s.		

The values are shown as mean ± SD; **p* value < 0.05 was deemed statistically significant. C.O: Corn oil; L.S.O: *L. sativum* oil; P.N.O: *P. nigrum* oil; CCl_4: Carbon tetrachloride..

Effect of PNO and/or LSO treatments on the CCl_4-induced hepato-renal dysfunctions in rats:

The findings demonstrated that there were no appreciable changes in the ALT, AST, urea, and creatinine levels of the CO administered group; nevertheless, the CCl_4-injected group's ALT, AST, urea, and creatinine levels were significantly higher than the negative control group's equivalent values (P < 0.05). ALT, AST, urea, and creatinine levels were restored toward the normal value in rats given CCl_4 and treated with PNO and/or LSO **(Table 2)**.

According to the results, CCl_4-administered rats developed hepatic injury, which was corroborated by noticeably elevated levels of the liver enzyme markers AST and ALT. Since these enzymes are found naturally within cells and are released into the serum when cell damage occurs, it may be said that CCl_4 damages liver cells **(Eidi *et al.*, 2012).** The breakdown of the carbon-chlorine link in CCl_4 is what causes this toxicity, since it produces the free and unstable radical trichloromethyl. Cell membrane components, particularly unsaturated fatty acids, react quickly with trichloromethyl to generate chloroform and a radical lipid. The breakdown of phospholipids in the endoplasmic reticulum and the release of liver enzymes occur when radially lipids and chloroforms react with oxygen **(Jamshidzadeh and Nikmahad, 2006 and Shokrzadeh *et al.*, 2022)**. These results are according to **Ali *et al.* (2021), Hsu *et al.* (2009), and Lee *et al.* (2007),** who reported that CCl_4 increased serum AST and ALT, which indicated the index of liver injury. Also, **Elhassaneen *et al.* (2023)** and **Sunday *et al.* (20٢4) and Zhai *et al.* (2018)** demonstrated that the enzymatic activities of serum ALT, AST, and alkaline phosphatase (ALP) were considerably elevated by $CCl_{4,}$ which suggested hepatotoxicity. In contrast, **Zhang *et al.* (2021)** demonstrated that rats given black piper oil restored the raised levels of alkaline phosphatase, ALT, AST, and total bilirubin caused by CCl_4. According to **Wali *et al.* (2021)**, CCl_4 is a hepatonecrotic and inflammatory agent. However, serum ALT, AST, and ALP activity as well as serum urea and creatinine concentrations were significantly reduced in rats given CCl_4 in addition to curcumin and black pepper extract **(Shatti, 2023)**. Piperine treatment dramatically restored the increased activities of liver enzymes, suggesting that piperine has hepatoprotective potential and may prevent CCl_4-induced loss of hepatocyte membrane functional integrity and sensitive enzyme leakage. Moreover, the black pepper essential oil prevented liver damage induced by dexamethasone by decreasing vacuolation, hepatocyte degeneration, monocyte infiltration, and ALT and AST levels **(Mahmoud *et al*., 2024)**. They attributed these hepatoprotective effects to their capacity to alter cellular energy metabolism by increasing peroxisome proliferator-activated receptor alpha (PAR-α) activity and inhibiting peroxisome proliferator-activated receptor gamma Coactivator 1-alpha (PGC-1α). Also, black pepper essential oils significantly reduced CCl_4 and dexamethasone-induced hepatic injury, pointing to its possible uses as an agent that promotes liver health **(Mahmoud *et al*., 2024** and **Zhang *et al.*, 2021)**. It has been documented that *Piper nigrum* extract reduces doxorubicin-induced hepatotoxicity by improving AST and ALT levels in rat serum **(Saetang *et al.*, 2022)**. *L. sativum* oil shows hepatoprotective properties against liver damage caused by CCl_4 in this investigation. Consistent with **Zamzami *et al.* (2019),** who approved the hepatoprotective effect of *L. sativum* seeds as significantly repairing the CCl_4-administered rabbits' liver injurious marker enzymes. Garden cress oil treatment markedly decreased ALP, ALT, and AST levels in a dose-dependent manner as compared to methotrexate-induced liver inflammation and apoptosis **(Mabrouk *et al*., 2025)**. *L. sativum* has a possible defense efficacy against aluminum-induced hepatic injury in albino rats **(Balgoon, 2019)**, as evidenced by significant improvement in liver functions ALT and AST. The hepatoprotective effect of *L. sativum* can be explained by the presence of triterpenes, alkaloids, tannins, coumarins, and flavonoids, which may have anti-hepatotoxic properties by preventing damage caused by free radicals **(Shokrzadeh *et al.*, 2022)**. Furthermore, treatment with *L. sativum* extract dramatically reduced plasma ALT and AST and established a hepatic protective effect against CCl_4 **(Ali and Rajab, 2019)**. According to **Raish *et al.* (2016)**, the ethanolic extract of *L. sativum* effectively inhibited the increase in hepatic enzymes caused by D-galactosamine/lipopolysaccharide by alleviating lipid peroxidation and repairing the antioxidant enzymes. Rats exposed to CCl_4 had severe liver damage, as seen by raised serum levels of total and direct bilirubin, AST, ALT and ALP **(Shukla and Bigoniya, 2013).**

Regarding kidney function, these results are consistent with **Abd Elmeged and Alzahrani (2022)** and **Bellassoued *et al.* (2018),** who found that CCl_4 significantly increases the levels of serum urea and creatinine in comparison to the negative control. However, *P. nigrum* extracts have been shown in a recent study to protect rats' kidneys against monosodium glutamate-induced nephrotoxicity **(Onyesife *et al.*, 2023).** The supplementation of diets with ginger or black pepper could decrease uric acid, urea and creatinine concentrations and improve the excretory function of the kidney in the metabolic syndrome-induced rats by the free radical scavenging properties of the spices **(Imam *et al.*, 2023)**. Moreover, *L. sativum* administered either in addition to or following $AlCl_3$ considerably recovered the kidney function indicators close to the normal range. It also counteracted the oxidative stress caused by $AlCl_3$ and, to a certain extent, maintained the normal structure of the liver and kidneys **(Balgoon, 2019)**. Also, in rats with streptozotocin-induced diabetic nephropathy, *L. sativum* decreased serum urea and creatinine levels. Additionally, it diminished the levels of key inflammatory indicators such as nuclear factor-kappa (NF-jB), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) and reduced the kidney tubular damage in the rats, where the hypoglycemic and insulin-releasing properties of *L. sativum* may be responsible for its nephroprotective effects **(Alsuliam *et al.*, 2023)**.

Table 2. Effect of PNO and/or LSO treatments on hepato-renal dysfunctions induced by CCl_4 in rats

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
G1: Negative control	36.16 ± 2.22 [d]	34.36 ± 2.90 [d]	27.01± 2.91 [b]	0.73 ± 0.12 [c,d]
G2: Positive control CCl_4	89.07 ± 3.12 [a]	58.56 ± 3.53 [a]	40.12 ± 4.01 [a]	1.41 ± 0.1 [a]
G3: Corn oil (CO)	37.06 ± 2.02 [d]	35.06 ± 2.02 [d]	25.61 ± 3.81 [b]	0.66 ± 0.13 [d]
G4: CCl_4/PNO	47.15 ± 2.39 [c]	46.20 ± 2.11 [b,c]	34.53 ± 2.2 [a,b]	1.09 ± 0.13 [a,b]
G5: CCl_4/LS O	48.21± 2.61 [c]	47.50 ± 2.95 [b]	36.41 ± 3.73 [a]	1.02 ± 0.14 [b,c]
G6:CCl_4/PNO/LSO	47.02 ± 2.01 [c]	39.76 ± 1.98[c,d]	31.91 ± 3.08 [a,b]	0.95 ± 0.12 [b,c,d]
F-value	194.23	35.88	8.31	13.91
p-value	< 0.001	< 0.001	0.001	< 0.001

The values are shown as mean ± SD; *p value < 0.05 was deemed statistically significant. CO: Corn oil; LSO: *L. sativum* oil; PNO *P. nigrum* oil; CCl_4: Carbon tetrachloride; ALT: Alanine aminotransferase; AST: Aspartate aminotransaminase.

Effect of PNO and/or LSO treatments on lipid profile of CCl_4-injected rats:

As shown in **Table 3**, the results indicated that the parameters of the lipid profile did not alter significantly between the group of rats administered with C.O. alone and the normal control group. Cholesterol (TC), low-density lipoproteins (LDL), and triglyceride (TG) levels, in comparison to the control rat's value, were markedly elevated in the rats who received CCl_4 injection alone (P < 0.05). However, the treatment of CCl_4-injected groups with LSO and/or PNO significantly decreased cholesterol, LDL, and triglycerides levels close to the normal control group (P < 0.05). The rats given CCl_4 alone showed a markedly lower level of high-density lipoprotein (HDL) than the control group. On the other hand, HDL levels in the CCl_4-injected groups recovered to levels near normal (P < 0.05) by administering LSO and/or PNO.

Table 3. Effect of PNO and/or LSO treatments on lipid profile of CCl_4-injected rats

Groups	Biochemical parameters			
	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
G1: Negative control	81.4 ± 4.32[b]	43.5 ±1.11[a]	20.5 ± 3.31[b]	96.1 ±3.52 [b,c]

G2: Positive control CCl_4	134.9 ± 4.01[a]	28.1 ±1.98[b]	55.8 ± 3.12[a]	145.4 ± 5.1[a]
G3: Corn oil (CO)	83.3 ± 3.97[b]	41.2 ± 1.2[a]	23.6 ± 2.91[b]	97.8± 3.89 [b,c]
G4: CCl_4/PN O	90.0 ± 4.41[b]	39.5 ± 2.08[a]	22.5 ± 2.99[b]	100.1 ± 4.0[b,c]
G5: CCl_4/LS O	92.2± 3.89[b]	38.4 ± 2.01[a]	22.9 ±3.11[b]	101.0 ± 4.01[b]
G6:CCl_4/PNO/LSO	89.1 ± 4.31[b]	41.2 ± 2.92[a]	21. ± 2.95[b]	90.2 ± 2.71[c]
F-value	66.01	22.42	9.97	77.17
p-value	< 0.001	< 0.001	< 0.001	< 0.001

The values are shown as mean ± SD; *p value < 0.05 was deemed statistically significant. CO: Corn oil; LSO: *L. sativum* oil; PNO: *P. nigrum* oil; CCl_4: Carbon tetrachloride; low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

Consistent with **Abu *et al.* (2021 and 2022); Dike *et al.* (2023) and Elhassaneen *et al.* (2023)**, the findings of the research revealed that the treatment by CCl_4 significantly reduced HDL levels and increased the lipid profiles (TC, TG, and LDL) as a sign of liver metabolic disruption brought on by the extent of CCl_4-induced hepatic damage. On the other hand, *P. nigrum* raised the plasma HDL level in comparison to the levels of supplemented high-fat fed rats, while decreasing the levels of TC, TG, LDL, very low-density lipoprotein (VLDL) and free fatty acids in the treated rats (**Vijayakumar *et al.*, 2020)**. In comparison to the control animals, it has been found that the treatment of black pepper oil restored blood TG and TC levels to comparable normal values, along with an even superior HDL level **(Mahmoud *et al.*, 2022)**. Moreover, the lipid profile improved with piperine treatment (**Tu *et al.*, 2014 and Wali *et al.*, 2021**). The impact of *L. sativum* on rat lipid profiles has been documented by **Amawi (2012)**, who demonstrated that treating hypercholesterolemic rats with *L. sativum* improved their lipid profiles. Moreover, LSAF significantly reduced the serum TC and TG in contrast to the group treated with CCl_4 **(Shukla and Bigoniya, 2013).** *L. sativum* administration to streptozotocin-induced diabetic nephropathy rats reduced the rise in blood and liver TC, LDL, TG, and free fatty acids. It is thought to have hypolipidemic effects through either independent or cooperative processes that act on gene synthesis in hepatic lipid production and decrease adipose tissue lipolysis by increasing insulin levels **(Alsuliam *et al.*, 2023).**

Effect of PNO and/or LSO treatments on the antioxidants/oxidants status of CCl_4-injected rats:

The results indicated that malondialdehyde (MDA) levels, catalase (CAT) and superoxide dismutase (SOD) activity did not notably change between groups of rats that were administered with C.O-alone and negative control. In the CCl_4-

injected group, CAT and SOD activity were considerably decreased relative to the normal control group's comparable value ($P < 0.05$). However, CCl_4-injected groups that were treated with L.S.O or/and PNO showed significant improvement in the antioxidants/oxidants hemostasis that evidenced by markedly higher SOD and CAT activity levels in comparison to the corresponding value of CCl_4-injected group alone ($P < 0.05$). However, in the CCl_4-injected group MDA level was significantly increased when compared to the normal control group's comparable value ($P < 0.05$). Meanwhile, CCl_4-injected groups treated with LSO and/or PNO demonstrated a noteworthy decrement in the MDA level in comparison to the corresponding value of the CCl_4-injected group alone **(Table 4)**.

CCl_4 is the most common toxic reagent used to induce animal models of hepatic injury **(Scholten *et al*., 2015)**. Its biotransformation into dangerous metabolic products, trichloromethyl free radicals, is brought on by the liver cytochrome P450 enzyme **(Zanger and Schwab, 2013)**. The buildup of superoxide radicals and hydrogen peroxide may be the cause of the decreased CAT activities in the kidneys and liver following CCl_4 injection. Additionally, higher lipid peroxidation can be induced by CCl_4, as indicated by elevated MDA levels **(Zhang *et al.*, 2021)**. In the CCl_4-treated rats, the hepatic GSH, CAT and SOD levels dramatically decreased compared to the control group **(Elhassaneen *et al.*, 2023)**. In this study, treatment with PNO and/or L.S.O. dramatically reinstated the increased in antioxidant enzymes activities, suggesting the hepatoprotective potential of these oils, which may prevent the leaking of sensitive enzymes and the functionality degradation of hepatocyte membranes caused by CCl_4 **(Wali *et al.*, 2021)**. These findings are compatible with a prior study that showed *Ganoderma lucidum's* hepatoprotective effect against CCl_4-induced hepatotoxicity by reducing oxidative stress **(Johra *et al.*, 2023)**. SOD activity is a sensitive indication of hepato-renal damage due to its ability to scavenge superoxide anions and create hydrogen peroxide, where the antioxidant enzymes such as SOD, together with cellular GSH, are essential components of endogenous antioxidant systems that primarily engage in free radical scavenging (**Solanki and Jain, 2011).** The black pepper oil elevated the GSH, SOD, and CAT activities of the kidney and liver in CCl_4-induced liver damage in mice, and the CCl_4-elevated MDA level was reversed (**Zhang *et al.*, 2021)**. Black pepper oil's anti-inflammatory and antioxidant properties are probably its mode of action. It raises the levels of antioxidant enzymes and lowers oxidative stress indicators, which may be a result of its high concentration of phenolic and flavonoid compounds. Additionally, **Hashiesh (2020) and Mahmoud *et al*. (2022)** associated black pepper oil's health effects with its antioxidant capacity, enhancing different antioxidant metrics, and modifying cellular signaling. Antioxidants are one of the more potent effective molecules preventing liver damage, so they have been proposed as adjuvant treatment for a variety of liver illnesses **(Fraschini *et al.,* 2002 and Muriel, 2009)**. Within the research of **Lee *et al*. (2019) and Wali *et al*. (2021)**, significantly reduced levels of GSH, SOD and CAT were observed, and the MDA levels depicted a sharp elevation in CCl_4- treated rats. Nonetheless, the piperine therapy resulted in a notable recovery in the levels of SOD, CAT, and GSH, suggesting that piperine plays a part in facilitating the efficient and prompt consumption of ROS generated by CCl_4-induced hepatotoxicity.

L. sativum seed oil has substantial antioxidants and anti-inflammatory properties because of its high oleic acid concentration and bioactive substances like tocopherols and phytosterols **(Rezig *et al.,* 2022)**. It may help prevent or lessen age-related illnesses frequently linked to dietary deficiencies. *L. sativum* seeds' hepatoprotective effectiveness was validated. where CCl_4 led to a notable decline in CAT, GST and SOD enzymatic activities in rabbits, however, *L. sativum* seeds significantly diminished the MDA levels and repaired their antioxidant status **(Zamzami *et al.,* 2019).** These effects were ascribed to the presence of phenolic and flavonoid compounds **(Chatoui *et al.,* 2020).** Moreover, *L. Sativum* seeds extract significantly increased CAT, SOD, and GSH activities, however, significantly decreasing the MDA level compared to the CCl4-treated group **(Ali and Rajab, 2019).** Also, *L. sativum* extract decreased the MDA (lipid peroxide) concentrations, elevated glutathione (GSH), SOD, and CAT, and activated mRNA for SOD and CAT of rat's kidneys with streptozotocin-induced diabetic nephropathy. These effects were mediated by the antioxidant and hypoglycemic activities of *L. sativum* extract, which also inhibited the activation of transforming growth factor beta 1 (TGF-b1) **(Alsuliam *et al.,* 2023).**

Table 4. Effect of PNO and/or LSO treatments on the antioxidants/oxidants status of CCl_4-injected rats.

Groups	SOD (U/g tissue)	CAT (U/g tissue)	MDA (nmol/g tissue)
G1: Negative control	51.26 ± 4.06^{a}	72.89 ± 5.83^{a}	34.79 ± 2.42^{d}
G2: Positive control CCl_4	22.07 ± 2.91^{d}	32.44 ± 3.57^{c}	104.70 ± 6.96^{a}
G3: Corn oil (CO)	$46.04 \pm 3.87^{a,b}$	71.46 ± 5.55^{a}	39.69 ± 1.93^{d}
G4: CCl_4/PN O	$39.91 \pm 2.88^{b,c}$	$61.88 \pm 4.99^{a,b}$	60.99 ± 4.94^{c}
G5: CCl_4/LS O	36.57 ± 2.69^{c}	51.45 ± 4.59^{b}	75.76 ± 5.38^{b}

G6:CCl_4/PNO/LSO	49.59 ± 3.07^{a}	$63.13 \pm 3.59^{a,b}$	57.11 ± 3.59^{c}
F-value	32.38	29.98	94.9
p-value	< 0.001	< 0.001	< 0.001

The values are shown as mean ± SD; *p value < 0.05 was deemed statistically significant. CO: Corn oil; LSO: *L. sativum* oil; PNO: *P. nigrum* oil; CCl_4: Carbon tetrachloride; SOD: Superoxide dismutase; CAT: Catalase; and MDA: Malodialdehyde. Means that don't have the same letter are markedly different.

Histological investigation:

The histopathological analysis revealed corroborating fragments of supporting evidence for the oxidative markers and enzymes of the liver. Hepatic lobulation and normal hepatocyte organization were observed in the control rat's (G1) liver sections stained with H&E. Hepatic strands were interspersed with Kupffer cells and endothelial cell-lined narrow blood sinusoids. Hepatocytes with centrally located nuclei and normally distributed chromatin, as well as the portal triad, which includes a bile ductule, a branch of the hepatic artery, and a branch of the portal vein, and a lymphatic vessel (Fig. 1). Liver sections of G2 reveal disorganization of the hepatic architecture that is denoted by irregular central vein, few hepatocytes are normal, others are degenerated with vacuolated cytoplasm and nuclear changes like magakarocytic nuclei, also blood sinusoids with activated phagocytic Kupffer cells, portal area exhibits dilated and congested portal vein and proliferated bile ductules (Fig. 2). The figure showing liver sections of Gp3 that reveal normal and regular central veins, mostly hepatocytes are normal with centrally located nuclei, a few with pyknotic nuclei with condensation of its chromatin, others with fat degeneration with aggregation of lipid droplets and typical Kupffer cells (K) in sinusoids of blood (Fig. 3). While liver sections of G4 reveal pronounced improvement of the hepatic architecture that is represented by normal radiating hepatocytes with regular distribution of its cytoplasm and centrally located nuclei, regular central vein and normal sinusoids in the blood with mild Kupffer cell activation (Fig. 4) also, liver sections of G5 exhibit enhancement of the liver's structure that is revealed by regular central vein, mostly hepatocytes are normal, few number with vacuolated cytoplasm, others with darkly stained cytoplasm (eosinophilia) and sinusoids of blood with distinct and Kupffer cell activation (Fig. 5). While liver sections of G6 exhibit more enhancement of the liver's structure indicated by showing regular central vein (Cv), normal hepatic architecture, mostly hepatocytes are normal (H), A small percentage have sinusoids of blood (Bs) and vacuolated cytoplasm (V), along with distinct and normal Kupffer cells (K) (Fig. 6). These results agree with **Abu *et al.* (2022),** who found that the histopathological alterations demonstrated that CCl_4 induced severe vascular congestion, necrosis, vacuolation, and liver lymphocyte infiltrations. According to **Mahmoud *et al.* (2024)**, black pepper essential oil can counteract fibrosis and histological alterations brought on by dexamethasone by lowering vacuolation, hepatocyte degeneration, and monocyte infiltration. CCl_4 led to verified loss of cellular bourgeons, steatosis, ballooning degeneration, and necroinflammation; however, piperine treatment showed a comparatively regular pattern with a modest level of necrosis and infiltration of inflammatory cells (**Wali *et al.*, 2021)**. *L. sativum* seed treatment improved the liver histopathological architecture of rabbits given CCl_4 by decreasing inflammation, hepatocyte regeneration, fibrosis prevention, steatosis or isolated vesicular steatosis prevention, prolonged venous congestion prevention, or simply discrete congestion prevention. This suggests that CCl_4 has a hepatoprotective effect **Zamzami *et al.*, 2019)**.

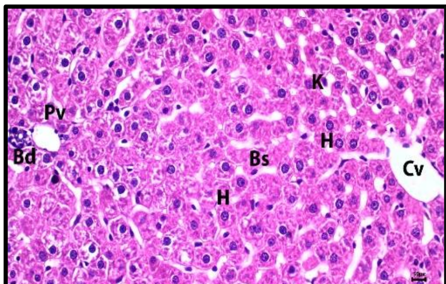

Fig. 1. Low and high magnified liver sections of **G1** exhibit regular central vein (Cv), normal radiating polygonal hepatic cells (H), regular blood sinusoids (Bs) with normal lining Kupffer cells (K), also portal area shows regular portal vein (Pv) and normal bile ductule (Bd) (X 100, 400).

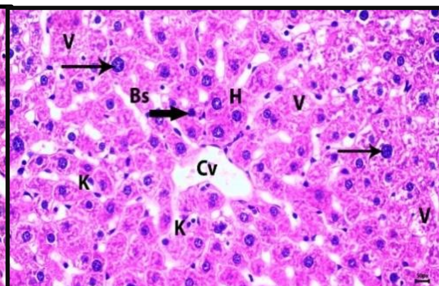

Fig. 2. Low and high magnified liver sections of **G2** reveal irregular central vein (Cv), few hepatocytes are normal (H), others are degenerated (v) having activated Kupffer cells in the blood sinusoids with magakarocytic nuclei (arrows) (K) (X 100, 400) .

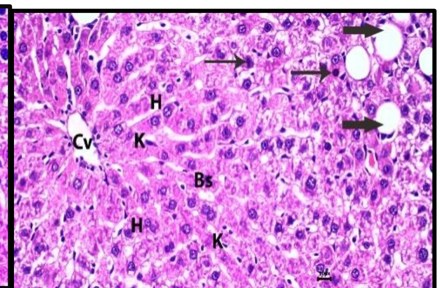

Fig. 3. Low and high magnified liver sections of **G3** showing normal central vein (Cv), mostly hepatocytes are normal (H), few ones with pyknotic nuclei (arrows), others with fat degeneration (thick arrows) and blood sinusoids with normal Kupffer cells (K) (X 100,400).

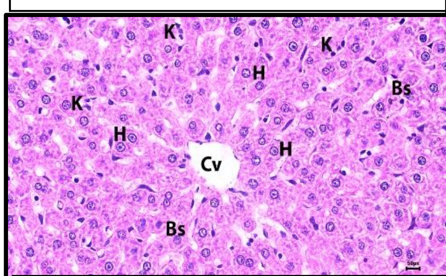

Fig. 4. Low and high magnified liver sections of **G4** show typical cytoplasmic distribution and normal radiating hepatocytes (H) and central nuclei, regular central vein (Cv) and normal sinusoids of blood with moderate activated Kupffer cells (K) (X 100,400).

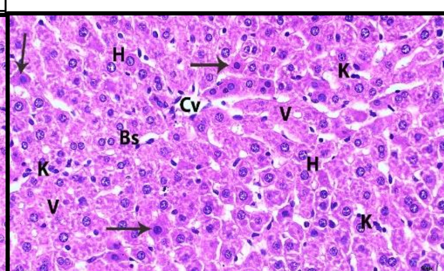

Fig. 5. Low and high magnified liver sections of **G5** showing regular central vein (Cv), normal hepatic architecture, mostly hepatocytes are normal (H), few number with vacuolated cytoplasm (V), Others include blood sinusoids (Bs) with unique and active Kupffer cells and eosinophilic cytoplasm (arrows) (K) (X 100,400).

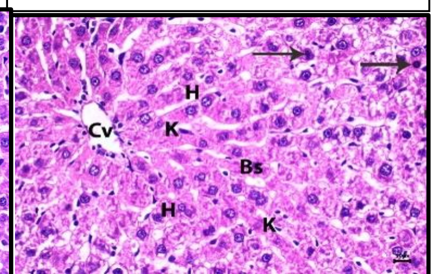

Fig. 6. Low and high magnified liver sections of **G6** showing regular central vein (Cv), normal hepatic architecture, mostly hepatocytes are normal, few have blood sinusoids (Bs) and vacuolated cytoplasm (V), with distinct and normal Kupffer cells (K) (X 100,400).

Conclusion: -

The current study demonstrated that *P. nigrum* and *L. sativum* oils restored the liver damage caused by CCl4. The examined oils, especially their combination, reversed the detrimental effects of CCl_4 on the lipid profile, liver, and kidney functions. Additional research would be necessary to guarantee biosafety, underlying mechanisms of action, and component functional activity.

Interest Conflict:

There are no conflicts of interest, according to the authors' declaration.

References

Abd Elmeged, L.S.M. and Alzahrani, M.S.H. (2022). Magnification of the therapeutic uses of pomegranate fruits and peel in rats injected with carbon tetrachloride (CCl4). *Saudi Journal of Biological Sciences 29 (2022) 103374.*

Abdel-Aty, A.M.; Salma, W.H.; Fahmy, A.S. and Mohamed, S.A. (2019). Impact of germination on antioxidant capacity of garden cress: New calculation for determination of total antioxidant activity. *Sci. Hort. 246, 155–160. https://doi.org/10.1016/j.scienta.2018.10.062*.

Abdou, M. D.; Abass, E. H., Salah, M. R. (2019). Purslane and garden cress seeds as source of unconventional edible oils for prevention of hyperlipidemia. *Pak. J. Biol. Sci. 22, 537–544. https://doi.org/10.3923/pjbs.2019.537.544.*

Abu, M.S.; Yakubu, O.E.; Imo, C.; Yohanna, R.E.; Okpe, O. and Mashi, R.L. (2021). Effect of n-butanol extract of F. glumosa leaves on CCl_4-induced hepatotoxicity and oxidative stress in rats. *Open Sci J Biosci Bioeng 8 (1): 12-18.*

Abu, M.S.; Yakubu, O.E.; Onuche, J.O. and Okpe, O. (2022). Lipid profiles, hematological parameters and histopathological analysis of CCl4-intoxicated wistar albino rats treated with n-butanol extract of Ficus glumosa leaves. *CE LL B IO LOG Y & D E V E L OPM E NT, 6(1): 6-12.*

Aebi H. (1984). Catalase *in vitro*. *Methods Enzymol. 105: 121–126.*

Ahmad S. R. and Ghosh P. (2022). A systematic investigation on flavonoids, catechin, β-sitosterol and lignin glycosides from Saraca asoca (ashoka) having anti-cancer and antioxidant properties with no side effects. *Journal of the Indian Chemical Society. 99: 100293.*

Ali, H.; Samrana, A. J. S.; Ali, A.; Ali, S.; Kabir, N.; Ali, A.; Ullah, R.; Mothana, R.A.; Murtaza, B. N. and Kalim, M. (2021). Hepatoprotective Potential of Pomegranate in Curbing the Incidence of Acute Liver Injury by Alleviating Oxidative Stress and Inflammatory Response. *Front. Pharmacol., 12: 694607.*

Ali, L.H. and Rajab, W.J. (2019). The effect of *Lepidium Sativum* seeds extract on some oxidative stress, antioxidants and histological changes in rats treated with CCL_4. *Pak. J. Biotechnol. Vol. 16 (2) 145-151. http://doi.org/10.34016/pjbt.2019.16.1.22*

de la Fuente. R. (2022). Nutrition and Chronic Liver Disease. *Clinical Drug Investigation (2022) 42 (Suppl 1): S55–S61 https://doi.org/10.1007/s40261-022-01141-x*.

Almeida M. M. B., de Sousa P. H. M., Arriaga Â. M. C. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International. 44(7):2155–2159.*

Alsuliam, S.M.; Albadr, N.A., Alshammari, G.M.; Almaiman, S.A.; Yagoub, A.A.; Saleh A. and Yahya, M.A. (2023). *Lepidium sativum* alleviates diabetic nephropathy in a rat model by attenuating glucose levels, oxidative stress, and inflammation with concomitant suppression of TGF-β1. Saudi Journal of Biological Sciences. 30: 103720.

Amawi K. (2012). Effect of *Lepidium sativum* on lipid profiles and blood glucose in rats. *J. Phys. Pharm. Adv. 2(8): 277-281.*

Attallah, N.G.M.; Mokhtar, F.A.; Elekhnawy, E.; Heneidy, S.Z.; Ahmed, E.; Magdeldin, S.; Negm, W. A., and El-Kadem, A.H. (2022). Mechanistic insights on the in vitro antibacterial activity and in vivo hepatoprotective effects of *Salvinia auriculata aubl* against methotrexate-induced liver injury. *Pharmaceuticals (Basel). 15: 1-26.*

Aebi H. (1984). *Catalase in vitro. Methods Enzymol. 105: 121–126.*

Bajpai, V.K.; Sharma, A. and Baek, K.H. (2013). Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control. 32: 582–590.*

Balgoon, M.J. (2019). Assessment of the Protective Effect of Lepidium sativum against Aluminum-Induced Liver and Kidney Effects in Albino Rat. *Biomed Res Int. 2019: 4516730.*

Bancroft, J. D. and Gamble, M. (2008). Theory and Practice of Histological Techniques. *6th ed. Churchill Livingstone/Elsevier, Philadelphia, PA, USA, 2008; pp. 725.*

Bartels, H. and Bohmer, M. (1971). Eine mikrometode zur kreatininbestimmung. *Clin. Chim. Acta 32, 81–85.*

Bellassoued, K.; Ben Hsouna, A.; Athmouni, K.; van Pelt, J.; Fatma Makni Ayadi, F.M.; Rebai, T. and Elfeki, A.(2018). Protective effects of Mentha piperita L. leaf essential oil against CCl4 induced hepatic oxidative damage and renal failure in rats. *Lipids in Health and Disease (2018) 17:9 DOI 10.1186/s12944-017-0645-9.*

Byrne C.D. and Targher G. (2022). Non-alcoholic fatty liver disease-related risk of cardiovascular disease and other cardiac complications. *Diabetes Obes Metab. 24: 28-43.*

Chatoui,K.; Harhar, H.; Kamli, T. and Tabyaoui, M. (2020). Chemical Composition and Antioxidant Capacity of Lepidium sativum Seeds from Four Regions of Morocco. *Evidence-Based Complementary and Alternative Medicine Volume 2020, Article ID 7302727, 7 pages https://doi.org/10.1155/2020/7302727.*

Chatterjee, B.; Renganathan, A. and Vittal, R.R. (2025). Phytoconstituents from (*Piper nigrum L.*) Black pepper essential oil (BPEO) acts as anti-virulent to fight quorum sensing, an analysis through in-vitro and in-silico studies. *Industrial Crops & Products 225 (2025) 120469.*

Dike, C. C.; Nwoko, S. O.; Ezeonu, F. C.; Ejiogu, L. C. and Kasarachi, O.D. (2023). Effects of *Sida corymbosa* Leaf-Extract on the Lipid Profile of Carbon Tetrachloride-Injected Male Rats. *Int. J. Biochem. Res. Rev., 32(7): 16-30.*

Eidi, A.; Mortazavi, P.; Bazargan, M. and Zaringhalam, J. (2012). Hepato protective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. *Excli J. 11:495-507.*

Elhassaneen, Y. A.; Khader, S.A. and El-aslowty, M.A. (2023). Potential Ameliorative Effects of Graviola *(Annona muricata L.)* Fruits on Carbon-tetrachloride Induced Hepatic

Injury in Rats: Antioxidant, Apoptotic, Anti-inflammatory Markers, and Histopathological Studies. *International Journal of Healthcare and Medical Sciences ISSN(e): 2414-2999, ISSN(p): 2415-5233, 9(2):17-31.*

Embuscado, M.E. (2015). Spices and herbs: Natural sources of antioxidants—A mini review. *J. Funct. Foods. 18: 811-819.*

Fathy, S.M. and Mohammed, M. S. (2021). Moringa oleifera Lam. Leaf extract mitigates carbon tetrachloride-mediated hepatic inflammation and apoptosis via targeting oxidative stress and toll-like receptor 4/nuclear factor kappa B pathway in mice. *Food Sci Hum Wellness. 10: 383–391.*

Fraschini, F.; Demartini, G. and Esposti, D. (2002). Pharmacology of silymarin. *Clinical drug investigation, 22(1), 51-65.*

Gillessen, A. and Schmidt H.H. (2020). Silymarin as supportive treatment in liver diseases: A narrative review. *Adv Ther. 37: 1279-1301.*

Hasan, M.K.; Ara, I.; Mondal, M.S.A. and Kabir, Y. (2021). Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra. *Heliyon, 7(6), e07240.*

Hashiesh, H. M. (2020). Therapeutic potential of β-caryophyllene: A dietary cannabinoid in diabetes and associated complications. *Nutrients12, 2963.*

Henry, R. J. (1964). Calorimetric methods of total protein. *J. Clin. Chem.; Haepre, Row Publishers, New York. P. 181.*

Hsu, Y. W.; Tsai, C. F.; Chen, W. K. and Lu, F. J. (2009). Protective effects of seabuckthorn (*Hippophaerhamnoides* L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. *Food Chem Toxicol., 47(9): 2281-2288.*

Hussein, H. J., Hameed, I. H. and Hadi, M. Y. (2017). Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Compounds of Methanolic Leaves extract of Lepidium sativum. *Research Journal of Pharmacy and Technology 10: 3981-3989.*

Imam, N. U.; Osibemhe, M.; Nura, L.; Ganiyu, B. I. and Idoko, A. S. (2023). Ginger or Black Pepper Supplements Attenuate Oxidative Stress and Renal Damage in Metabolic Syndrome-induced Wistar Rats. *Nigerian Journal of Parasitology, ISSN 1117 4145:* 44(1):148-154.

Jamshidzadeh, A. and Nikmahad, H. (2006). [Hepatoprotective effects of Fumaria parviflora L. on CCl4-induced hepatotoxicity (Persian)]. *J Med Plants. 5 (19):34-39.*

Jansen, P.C.M. (2004). Lepidium sativum L. In: Grubben, G.J.H., Denton, O.A. (Eds.), Plant resources of Tropical Africa 2: Vegetables. *PROTA Foundation, Backhuys Publishers, Netherlands, pp. 365–367.*

Johra, F.T.; Hossain, S. and Jain, P. (2023). Amelioration of CCl4-induced oxidative stress and hepatotoxicity by Ganoderma lucidum in Long Evans rats. *Sci Rep. 13: 9909.*

Lee, C. P.; Shih, P. H.; Hsu, C. L. and Yen, G. C. (2007). Hepatoprotection of tea seed oil (*Camellia oleifera* Abel.) against CCl4-induced oxidative damage in rats. *Food Chem Toxicol., 45(6): 888-895.*

Lee, Y.S.; Cho, I.J.; Kim, J.W.; Lee, M.K.; Ku, S.K.; Choi, J.S. and Lee H.J. (2019). Hepatoprotective effects of blue honeysuckle on CCl4-induced acute liver damaged mice. *Food Sci Nutr. 7: 322-338.*

Li, R.; Yang, W.; Yin, Y.; Ma, X.; Zhang, P. and Tao, K. (2022). 4-OI Attenuates Carbon Tetrachloride-Induced Hepatic Injury via Regulating Oxidative Stress and the Inflammatory Response. *Front Pharmacol. 12: 651444.*

Li, X.Y. and Chow, C.K. (1994). An improved method for the measurement of malondialdehyde in biological samples. *Lipids. 29: 73–75.*

Mabrouk, D. M.; Afifi, H. R.; Sharaf, A.F and makawy, A. I. (2025). In vivo and in silico studies on the potential role of garden cress oil in attenuating methotrexate-induced inflammation and apoptosis in liver. *Scientific Reports, 15(1), 1-17. https://doi.org/10.1038/s41598-025-89550-8*

Mahmoud, M.F.; Elmaghraby, A.M.; Ali, N.; Mostafa, I.; El-Shazly, A.M.; Abdelfattah, M.A.O. and Sobeh, M. (2022). Black pepper oil (*Piper nigrum* L.) mitigates dexamethasone induced pancreatic damage via modulation of oxidative and nitrosative stress. *Biomed Pharmacother. 153:113456.*

Mahmoud, M.F.; Mahdi, I.; Ali, N.; Dib, H.; El-Shazlyd, A.M.; Mostafa, I.; Abdelfattah, M.A.O.; Hasan, R. and Sobe, M. (2024). Black pepper (Piper nigrum L.) essential oil counteracts dexamethasone-induced hepatic injury via modulating PGC-1α/ PPAR-α pathway in rats. *Arabian Journal of Chemistry, 17 (2024) 105690.*

Mostafa, N.M.; Mostafa, A.M.; Ashour, M.L. and Elhady, S.S. (2021). Neuroprotective effects of black pepper cold-pressed oil on scopolamine-induced oxidative stress and memory impairment in rats. *Antioxidants (Basel). 10: 1993.*

Muriel, P. (2009). Role of free radicals in liver diseases. *Hepatol Int, 3(4), 526–536. https://doi.org/10.1007/s12072-009-9158-6.*

Ncir, M.; Ali, B.M.; Hanen, S.; Mohamed, S.A.; Amina, L.; Fatma, M.A.; Tahia, B., Noureddine, A.; Abdelfattah, E.F; Mongi, S. (2020). Protective effects of Allium sativum essential oil rich in disulfides against deltamethrin induced oxidative stress and hepatotoxicity in rats. *Journal of Food Measurement and Characterization 14: 2667–2675.*

Nehdi, I.A.; Sbihi, H.; Tan, C.P. and Al-Resayes, S.I. (2014). Garden cress (*Lepidium sativum* Linn.) seed oil as a potential feedstock for biodiesel production. *Bioresour Technol. 126: 193-197.*

Nirwane A. M. and Bapat A. R. (2012). Effect of methanolic extract of Piper nigrum fruits in Ethanol-CCl4 induced hepatotoxicity in Wistar rats. *Der Pharmacia Lettre, 4 (3):795-802.*

Nishikimi M., Rao N. A., and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun. 46: 849–853.*

Onyesife, C.O.; Chukwuma, I.F.; Okagu, I.U.; Ndefo, J.C.; Amujiri, N.A. and Ogugua V.N. (2023). Nephroprotective effects of *Piper nigrum* extracts against monosodium glutamate-induced renal toxicity in rats. *Scientific African. 19: e01453.*

Raish, M.; Ahmad, A. and Alkharfy, K. M. (2016). "Hepatoprotective activity of *Lepidium sativum* seeds against D-galactosamine/ lipopolysaccharide induced hepatotoxicity in animal model." *BMC Complementary and Alternative Medicine, 16(1):501.*

Reitman, S. and S. Frankel, (1957). Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. *Am. J. Clin. Path., 28(1): 56-63.*

Rezig, P.; Chemkhi, H.; Gharsallah, K'; Mokbli, S.; B'chirf, F.; Ben Achour, N.; Bourgou S. and Chouaibi, M. (2022). Profile characterization and biological activities of cold pressed Garden Cress (Lepidium sativum) seed oil. *Arabian Journal of Chemistry, 15 (8): August 2022, 103958.*

Saetang, J.; Tedasen, A.; Sangkhathat, S.; Sangkaew, N.; Dokduang, S.; Prompat, N.; Taraporn S. and Graidist P. (2022). The attenuation effect of low piperine Piper nigrum extract on doxorubicin-induced toxicity of blood chemical and immunological properties in mammary tumour rats. *Pharm Biol. 60: 96-107.*

Scholten, D.; Trebicka, J.; Liedtke, C. and Weiskirchen, R. (2015). The carbon tetrachloride model in mice. *Lab Anim. 49: 4-11.*

Shatti, N.A.M. (2023). Hepato-renal Protective Effect of Antioxidants (Curcuma and Black Pepper) Against CCL_4 Induced Toxicity in Male Albino Rats. *Thesis submitted to the University of Zawia for the degree of Master of Sciences in Zoology.*

Shenoy, K. A.; Somayaji, S. and Bairy, K. (2001). Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride-induced hepatic injury in rats. *Indian Journal of pharmacology, 33(4), 260–266.*

Shokrzadeh, M.; Khalvati, R.; Hosseinzadeh, M. H.; Ayatifard, M. and Habibi, E. (2022). Effects of Hydroalcoholic Extract of *Lepidium Sativum L*. on Carbon Tetrachloride-Induced Hepatotoxicity in Mice. *Pharmaceutical and Biomedical Research. 8(3):225-232. http://dx.doi.org/10.18502/pbr.v8i3.11036*

Shukla, A. and Bigoniya, P. (2013). Hepatoprotective Effect of Lepidium Sativum Linn *(Cruciferae)* Total Alkaloid Fraction against CCl4-Induced Hepatotoxicity on Rats. *Research J. Pharmacognosy and Phytochemistry. 5(2): 94-99*

Solanki, Y. B. and Jain, S. M. (2011). Hepatoprotective effect of *Clitoria terenatea* and *Vigno mungo* against acetaminophen and carbon tetrachloride-induced hepatotoxicity in rats. *J. Pharmacol. Toxicol. 6, 30–48.*

Sunday G.A.; Dikwa K.B.; Alhaji A.I.; Vantsawa P.A.; Nwankwo C.T. and Muhammed, M. (2024). Hepatoprotective effects of aqueous and methanolic leaves extracts of *azadiracta indica* against carbon tetrachloride (CCL_4) induced hepatotoxicity in Wistar rats. *Academy Journal of Science and Engineering 18(1): 99-107.*

Takooree, H., M. Z. Aumeeruddy, K. R. R. Rengasamy, K. N. Venugopala, R. Jeewon, G. Zengin and M. F. Mahomoodally (2019). A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. *Crit. Rev. Food Sci. Nutrit. 59(sup1): S210-S243.*

Tu, Y.; Sun, D. and Zeng, X. (2014). Piperine potentiates the hypocholesterolemic effect of curcumin in rats fed on a high-fat diet. *Exp. Ther. Med. 8(1):260–266.*

Umesha, S.S. and Naidu, K.A. (2015). Antioxidants and antioxidant enzymes status of rats fed on n-3 PUFA rich Garden cress (*Lepidium Sativum L*) seed oil and its blended oils. *J Food Sci Technol. 52(4): 1993-2002.*

Vazifeh, S.; Kananpour, P.; Khalilpour, M.; Eisalou, S.V. and Hamblin M.R. (2022). Anti-inflammatory and immunomodulatory properties of *Lepidium sativum*. *Biomed Res Int. 2022: 3645038.*

Vijayakumar, R.; Surya, D.; Rajagopal, S. and Nalini N. (2020). Hypolipidemic effect of black pepper (*Piper nigrum* Linn.) in rats fed high fat diet. *Journal of Clinical Biochemistry and Nutrition. 32: 31-42.*

Wali, A.; Ali S.; Rashid S.; Alsaffar R.; Arafah A.; Qamar W.; Ahmad A.; Paray B.; Ahmad Sheikh B.; Alsuwayni B.; and Rehman M. (2021). Attenuation of oxidative damage-associated hepatotoxicity by piperine in CCl_4-induced liver fibrosis. *Journal of King Saud University - Science. 33: 101629.*

Wanna, R.; Darika Bunphan, D. and Wongsawas, M. (2023). Chemical composition and bioactivity of essential oils from *Piper nigrum L*. and *Piper retrofractum Vahl*. against *Callosobruchus maculatus (F.). AJCS 17(8):664-670.*

Wei, Y.H.; Jun, L. and Qiang, C.J. (2004). Effect of losartan, an angiotension II antagonist, on hepatic fibrosis induced by CCl4 in rats. *Dig. Dis. Sci. 49:1589-1594.*

Zamzami, M.A.; Baothman, O.A.S.; FatmaSamy, F. and Abo-Golayel, M.K. (2019). Amelioration of CCl_4-Induced Hepatotoxicity in Rabbits by *Lepidium sativum* Seeds. *Evidence-Based Complementary and Alternative Medicine, Article ID 5947234, 17 pages. https://doi.org/10.1155/2019/5947234*

Zanger, U.M. and Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther. 138(1):103-41.*

Zhai, X., Zhu, C., Zhang, Y., Sun, J., Alim, A., and Yang, X. (2018). Chemical Characteristics, Antioxidant Capacities and Hepatoprotection of Polysaccharides from Pomegranate Peel. *Carbohydr. Polym., 202: 461–469.*

Zhang, C.; Zhao, L.; Famous, E.; Pan, S.; Peng, X. and Jun Tian, T. (2021). Antioxidant, hepatoprotective and antifungal activities of black pepper *(Piper nigrum L.)* essential oil. *Food Chemistry 346 (2021) 128845.*