SCHOLASTIC:
Journal of Natural and Medical Education

Volume 4, Issue 1, Year 2024
https://univerpubl.com/index.php/scholastic

Article

Protective Effects of 1,8-Cineole on Liver Function in Rats Exposed to Cadmium Chloride

Abdulmutalb B. M. Al-Khaleeli[1], Hussein Ali Al-Bahrani[2] and Nahlah Jaber Hussein[3]

1 Department of chemistry, College of Education for pure science, University of Kerbala, Karbala, Iraq.
* Correspondence: abdulalmutalib.b@uokerbala.edu.iq , nahla.j@uokerbala.edu.iq , hamg.al1991@yahoo.com

Abstract: Background: The liver, an essential organ for metabolic control and detoxification, is particularly vulnerable to harm from environmental contaminants like cadmium chloride ($CdCl_2$). Significant liver damage results from the oxidative stress, lipid peroxidation, and inflammation that $CdCl_2$ causes. Natural monoterpene 1,8-cineole is known to have anti-inflammatory and antioxidant qualities, which makes it a viable option for reducing cadmium-induced liver toxicity.

Objectives: This research looked at how 1,8-cineole affected oxidative stress, lipid metabolism, and inflammatory markers in order to assess its hepatoprotective properties against $CdCl_2$-induced liver damage in rats.

Keywords: 1,8-Cineole, Cadmium chloride, Liver function, Oxidative stress, Hepatoprotection

1. Introduction

The liver is exposure to damage from different environmental effect, as heavy metals especially the cadmium chloride ($CdCl_2$), since it is a major organ for detoxification and metabolic of the body. The oxidative stress, inflammation, and other cellular damage are the major harmful to liver due to toxic effects of $CdCl_2$, which subsequently lead to degeneration of liver cells and a loss of metabolic and detoxification function (Jaishankar, 2014; Renugadevi & Prabu, 2010).

The previous research illustrated the $CdCl_2$ usage in many industrial applications, especially in nickel-cadmium, batteries, pigments, and chemical coatings, which the cadmium has significantly expanded globally lead to increased to body exposure (Abu-El-Zahab et al., 2019). Furthermore, due to its oxidative stress, which results in excessive lipid peroxidation, mitochondrial dysfunction, and DNA damage, $CdCl_2$ is regarded as a highly harmful substance that causes deferent risks effect to both people and animals (Rani et al., 2014).

In other hand the eucalyptol, which known as 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), is a monoterpene that is present in different plants; Eucalyptus globulus, Rosmarinus officinalis, and Cinnamomum camphora (Abdollahi et al., 2024). Deffrent stuidy illustrated that 1,8-cineole has bronchodilatory and anti-inflammatory properties, since effect in reducing respiratory, digestive and nervous systems. Additionally, lasts studies shown its antidiabetic, neuroprotective, and cardioprotective properties, which potentially used as a multipurpose medicinal (Abdollahi et al., 2024).

Citation: Abdulmutalb B. M. Al-Khaleeli. Protective Effects of 1,8-Cineole on Liver Function in Rats Exposed to Cadmium Chloride. Scholastic: Journal of Natural and Medical Education 2025, 4(1), 1-8.

Received: 10th Dec 2024
Revised: 20th Dec 2024
Accepted: 1th Jan 2025
Published: 7h Jan 2025

The last researchers provided that $CdCl_2$ leads to oxidative stress which induces damage and leads to elevated the different biomarkers as alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides, and malondialdehyde (MDA), on other hand 1,8-cineole's has a protective role that is attributed to its antioxidative stress and antiinflammatory effects by its scavenging role of free radicals, inhibiting lipid peroxidation, and regulating signaling pathways (Al-musawi et al., 2020; Di et al., 2022; Honório et al., 2015; Yang et al., 2024).

Thus the present study aimed to proved the protective effects of 1,8-cineole against $CdCl_2$ on liver function in male rats. Which, its evaluates the ability of 1,8-cineole to decreased oxidative stress, lipid peroxidation, and inflammation by study the biochemical markers, including ALT, AST, cholesterol, triglycerides, and malondialdehyde.

2. Materials and Methods

Animals

The study used forty Wister male rats, weighing between 200 to 250 g, were purchased from the College of Science/ University of Kufa's and housed in a controlled animal house before 1- week to the experiment, furthermore, the rats were allowed to access to food and water as they were in the laboratory environment (Bustani et al., 2022).

Study design

The animals was randomly divided into four equal groups (n=10) included; control (C) group provide placebo for 14 day; $CdCl_2$ (CD) group was intraperitoneally injection $CdCl_2$ at dose of [10 mg/kg] for 14 days; the 1,8-Cineole (Ci) Group was provided 1,8-cineole orally at a dose of 100 mg/kg-day for 14 days according to (Abdollahi et al., 2024) and finally, the protective (P) group that received 1,8-cineole at the same dose and duration prior of Cd and Ci group (Ali Hameed et al., 2022). At the end of experimental at 60^{th} day the blood samples was collection by heart puncture, and serum was separated for the biochemical test of triglycerides, cholesterol, ALT, and AST. Malondialdehyde (MDA) levels (Al-garawi et al., 2022).

Animal Preparation

For sample collection the rat was anesthetize by Ketamine (90 mg/kg body weight) and Xylazine (40 mg/kg body weight) on the 60th day.

Biomedical parameters

The Analysis of ALT and AST:

The Enzyme-Linked Immunosorbent Assay (ELISA) methods are used to measure the levels of ALT and AST, which are markers of liver function. Each experimental group's serum samples are gathered, and the amounts of ALT and AST are quantitatively measured using ELISA kits (chain, Sunlong®) tailored for these enzymes.

The Sandwich-ELISA method is used in the ELISA kit to measure AST and ALT levels. Whole blood must be clotted before the clots are removed by centrifugation. Standard dilutions are made via a series of dilution stages that range from 3 ng/ml to 0.25 ng/ml. After adding 10 µl of the sample and 40 µl of the sample dilution buffer to the appropriate wells on a microplate, the plate is incubated for 30 minutes at 37°C. 50 µl of HRP-Conjugate reagent is added to each well after the washing buffer has been diluted and a washing operation has been completed. This is followed by another incubation. The subsequent steps involve washing and coloring using Chromogen Solutions A and B. After that, there is a 15-minute incubation time at 37°C. The reaction is terminated by adding stop solution, resulting in a color change from blue to yellow. Absorbance is then measured at 450nm using a Microtiter Plate Reader, with the OD value of the blank control well set as zero. It is crucial to complete the assay within 15 minutes after adding the stop

solution. This comprehensive protocol ensures precise quantification of ALT and AST levels in serum samples, contributing to the evaluation of liver function in experimental settings.

Evaluation of Triglycerides and Cholesterol

Triglyceride and cholesterol levels were measured with enzymatic colorimetric test kits (brand must be specified).

Cholesterol: By using enzymatic hydrolysis and oxidation processes to produce a colorimetric product that could be detected at 500 nm, the levels of serum cholesterol were determined.

Triglycerides: by using enzymatic lipase, were that converted to glycerol and free fatty acids which serum were measured by calorimetrically at 505 nm.

Analysis of Malondialdehyde (MDA)

The Thiobarbituric Acid Reactive Substances assay was used to check liver homogenates for the lipid peroxidation indicator MDA. phosphate-buffered saline was used to homogenize the liver tissues, which were then centrifuged at 3,000 rpm for 10 minutes. Following the addition of thiobarbituric acid reagent, the supernatant was incubated at 95°C for 30 minutes. A spectrophotometer was used to measure the absorbance of the pink chromogen at 532 nm after the reaction mixture had cooled and been centrifuged. nmol/mg of tissue protein was the unit of measurement for MDA levels.

Statistical Analysis

Data were analyzed using **one-way ANOVA**, followed by Tukey's post hoc test to compare the groups. Results were presented as **mean ± standard deviation (SD)**, and a p-value <0.05 was considered statistically significant.

3. The result and discussion

The results in Table 1 revealed a significant increase in serum ALT (IU/g) with their standard errors were: Control (121.5 ± 4.026), $CdCl_2$ (173.8 ± 4.429), 1,8-Cineole (107.7 ± 2.599), and Protective (132.7 ± 2.716). The Cadmium chloride exposure significantly increased ALT levels in the CD group compared to the Control group, confirming hepatocellular damage. Cadmium-induced liver toxicity is primarily mediated by oxidative stress, as cadmium generates reactive oxygen species (ROS) that result in lipid peroxidation, mitochondrial dysfunction, and DNA damage. These effects compromise cellular membrane integrity, causing leakage of intracellular enzymes such as ALT into the bloodstream (Jaishankar, 2014; Renugadevi & Prabu, 2010). Treatment with 1,8-cineole alone Ci group resulted in a significant reduction in ALT levels compared to both the Control and CD groups, highlighting its hepatoprotective properties. As a natural monoterpene, 1,8-cineole exerts its effects through potent antioxidant and anti-inflammatory mechanisms, scavenging free radicals and reducing oxidative stress-induced damage in hepatic tissues. It also inhibits lipid peroxidation and modulates key signaling pathways, preventing the activation of inflammatory mediators that exacerbate liver injury (Abdollahi et al., 2024). The treatment with 1,8-cineole and cadmium chloride exposure in the Protective group partially mitigated the hepatotoxic effects of cadmium. The ALT levels in the Protective group were significantly lower than those in the $CdCl_2$ group but remained elevated compared to the Control and 1,8-Cineole groups. This partial reduction indicates that while 1,8-cineole reduces the severity of cadmium-induced liver injury, it cannot fully neutralize the damage.

These findings align with prior studies demonstrating the hepatoprotective effects of natural antioxidants against heavy metal-induced liver toxicity. In rats exposed to cadmium, for example, supplementing with Chlorella vulgaris was shown to reduce oxidative stress and improve membrane integrity, hence mitigating ALT increases (Yang et al., 2024). In a similar vein, nanoparticles produced by Crassocephalum rubens have

shown protective benefits against cadmium-induced hepatotoxicity via antioxidant pathways (Di et al., 2022).

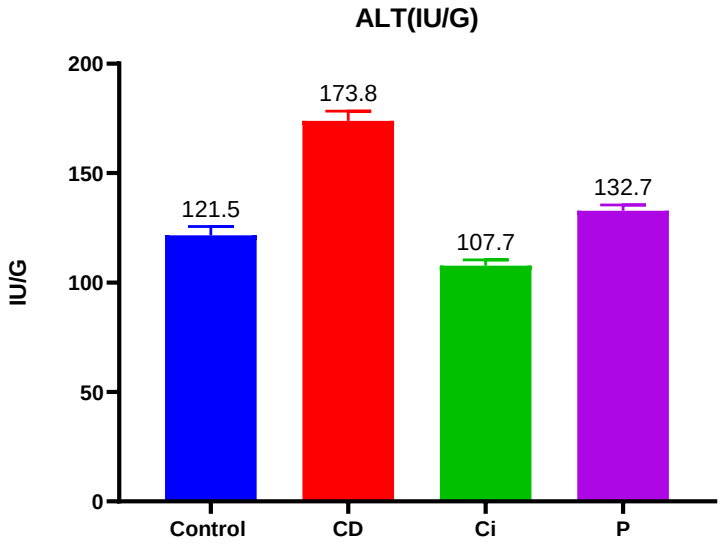

Figure 1: Effects of 1,8-Cineole on ALT Levels in $CdCl_2$ Exposed Rats.

The result mean of AST levels (IU/g) Table 2 with their standard errors across the four experimental groups were as follows: Control (55.7 ± 1.033), $CdCl_2$ (201.8 ± 14.3), 1,8-Cineole (50.6 ± 2.202), and Protective (125.2 ± 6.088). Severe hepatic damage was indicated by the considerably higher AST values in the $CdCl_2$ group as compared to the Control group. Elevated levels of aspartate aminotransferase in blood indicate mitochondrial malfunction and intracellular enzyme leakage as a result of damaged cell membranes. This enzyme is found in the cytoplasm and mitochondria of hepatocytes (Jaishankar, 2014). The treatment of 1,8-cineole (Ci group) demonstrated its strong hepatoprotective effects by producing AST levels that were similar to those of the Control group (50.6 ± 2.202). Because 1,8-cineole scavenges free radicals, it reduces oxidative stress and preserves mitochondrial and cellular integrity. The substance also lessens the production of ROS and suppresses lipid peroxidation, two processes that are essential to cadmium-induced liver damage (Abdollahi et al., 2024; Ghobadi et al., 2017). In the Protective group (125.2 ± 6.088), the 1,8-cineole therapy considerably reduced AST levels in comparison to the $CdCl_2$ group, but it did not completely return them to Control levels. Although 1,8-cineole cannot completely prevent the harmful effects of cadmium, this partial reduction suggests that it may considerably reduce the hepatocellular damage induced by cadmium. According to Di et al. (2022) and Rusco et al. (2022), the protective mechanism most likely involves maintaining mitochondrial membranes and altering inflammatory and apoptotic signaling cascades. According to recent studies, natural antioxidants may protect the liver from cadmium-induced damage. For example, Chlorella vulgaris significantly reduced AST levels in mice exposed to cadmium by enhancing antioxidant defenses and preventing mitochondrial damage (Yang et al., 2024). A research on gold nanoparticles made from Crassocephalum rubens also demonstrated the importance of natural chemicals in reducing the toxicity of heavy metals via antioxidant mechanisms, demonstrating that rats exposed to cadmium had much lower AST levels (Yang et al., 2024).

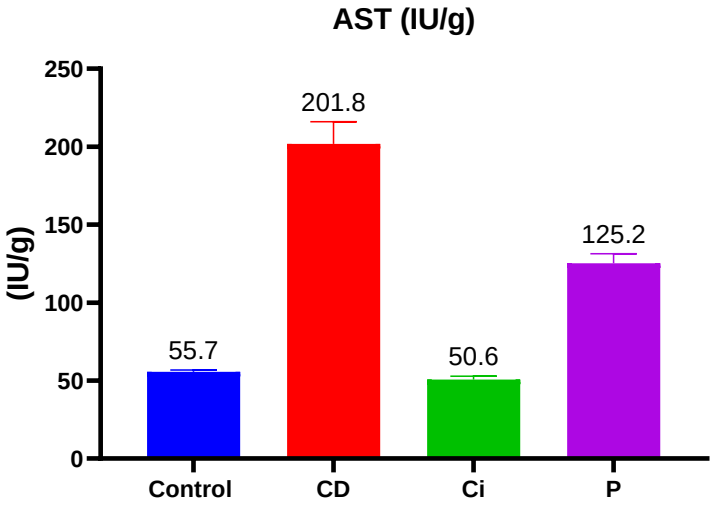

Figure 2: Effects of 1,8-Cineole on Serum AST Levels Across Experimental Groups.

The four experimental groups' mean triglyceride levels (mg/dL) were as follows: Protective (97.9 ± 3.076), 1,8-Cineole (90.2 ± 1.772), $CdCl_2$ (142.6 ± 4.377), and Control (118.8 ± 2.691). When compared to the Control group, exposure to cadmium chloride ($CdCl_2$) markedly increased triglyceride levels, suggesting disturbances in lipid metabolism. Exposure to cadmium is known to cause mitochondrial dysfunction and oxidative stress, which hinder lipid metabolism and cause an abnormal buildup of triglycerides in the blood. Cadmium-induced disruption of the production of very-low-density lipoproteins (VLDL) and decreased hepatic lipase activity are also responsible for the increase in triglyceride levels (Jaishankar, 2014; Renugadevi & Prabu, 2010). When 1,8-cineole alone (Ci group) was administered, triglyceride levels significantly decreased (90.2 ± 1.772 mg/dL) in comparison to both the Control and $CdCl_2$ groups, suggesting a potent lipid-lowering impact. According to this decrease, 1,8-cineole improves lipid metabolism by modifying important enzymes involved in the production and degradation of lipids. Because of its antioxidant qualities, the hepatic mitochondria are probably shielded from oxidative damage, maintaining the regular metabolic processes that control triglycerides. Additionally, 1,8-cineole may enhance the removal of VLDL from the circulation and prevent ROS-induced disruption of hepatic lipase (Abdollahi et al., 2024; Ghobadi et al., 2017). The Protective group's pre-treatment with 1,8-cineole (97.9 ± 3.076 mg/dL) considerably reduced the rise in triglyceride levels brought on by $CdCl_2$. Nonetheless, this group's triglyceride levels were marginally greater than those of the 1,8-cineole group, suggesting that the harmful effects of cadmium were somewhat lessened. Because 1,8-cineole preserves mitochondrial integrity, lowers lipid peroxidation, and increases the activity of enzymes involved in lipid metabolism, it probably has a protective impact in this situation. The study's results on 1,8-cineole's lipid-lowering effects are in line with other research. Research has shown that by lowering oxidative stress and boosting mitochondrial function, natural antioxidants like 1,8-cineole and other terpenes enhance lipid metabolism. For instance, Yang et al. (2024) found that by regulating oxidative stress and reestablishing regular lipid metabolic pathways, supplementing rats exposed to cadmium with Chlorella vulgaris decreased their triglyceride levels. Similarly, in models of cadmium-induced liver damage, Al-Garawi et al. (2020) found that plant-derived antioxidants significantly decreased triglyceride levels (Al-garawi et al., 2022).

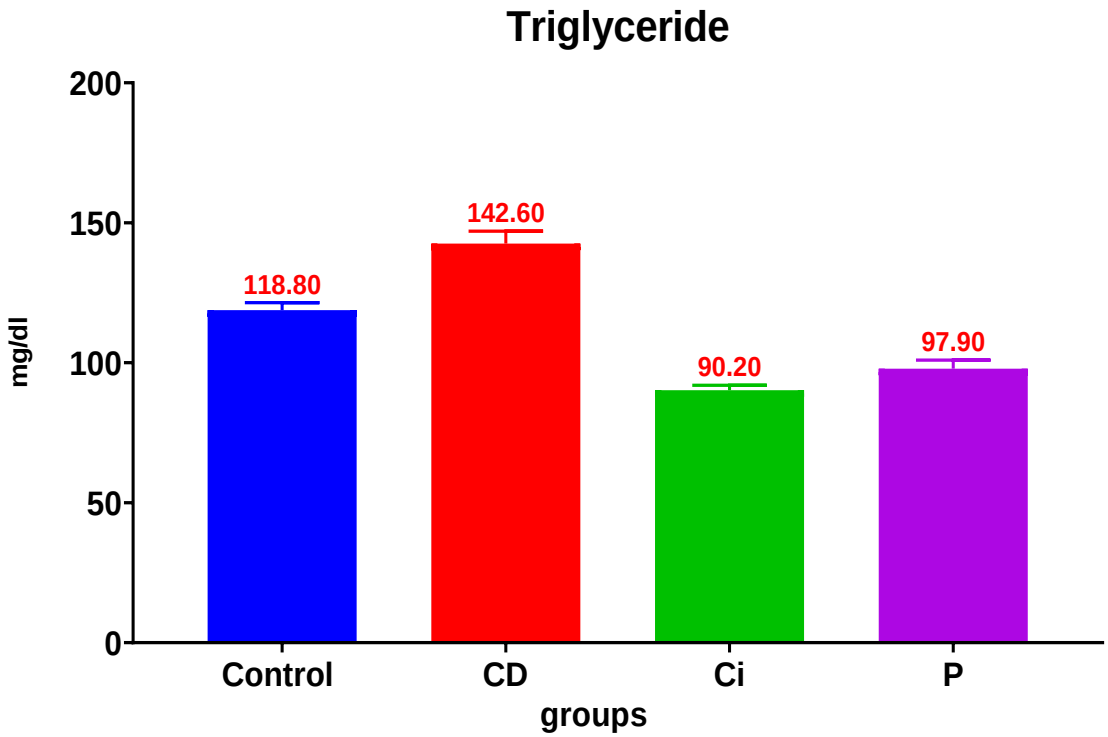

Figure 3: Effects of 1,8-Cineole on Serum Triglyceride Levels Across Experimental Groups: Triglyceride levels were compared between the Control group (normal saline), $CdCl_2$ group (cadmium chloride-induced dyslipidemia), 1,8-Cineole group (treated with 1,8-Cineole), and Protective group (treated with 1,8-Cineole before $CdCl_2$ exposure). **Values are expressed as mean ± SEM**

The four experimental groups' mean cholesterol levels (mg/dL) were as follows: Protective (70.4 ± 1.99), 1,8-Cineole (61.0 ± 5.779), $CdCl_2$ (117.8 ± 4.224), and Control (73.6 ± 2.441). Cadmium exposure caused dyslipidemia, as seen by the considerably higher cholesterol levels in the $CdCl_2$-treated group as compared to the Control group. By causing oxidative stress and mitochondrial malfunction in hepatic cells, cadmium is known to hinder lipid metabolism. Because these disturbances impact the control of important lipid metabolism-related enzymes, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the production and clearance of cholesterol are changed (Jaishankar, 2014). When 1,8-cineole alone (Ci group) was administered, cholesterol levels were considerably lower (61.0 ± 5.779 mg/dL) than in the Control and $CdCl_2$ groups. This discovery emphasizes 1,8-cineole's cholesterol-lowering action, which could include modifying lipid metabolic pathways. By stabilizing mitochondrial activity and maintaining the regulatory processes of cholesterol generation and clearance, 1,8-cineole probably lessens oxidative stress in hepatic cells. Its anti-inflammatory properties may also reduce the production of inflammatory cytokines that interfere with lipid metabolism (Abdollahi et al., 2024; Al-musawi et al., 2020; Di et al., 2022; Honório et al., 2015).

treatment with 1,8-cineole before cadmium exposure significantly reduced the cholesterol increase caused by $CdCl_2$ in the Protective group (70.4 ± 1.99 mg/dL). The cholesterol levels in this group were similar to those in the Control group, though slightly higher than in the 1,8-cineole group. This demonstrates that 1,8-cineole effectively reduces the dyslipidemic effects caused by cadmium. The antioxidant properties of 1,8-cineole reduce lipid peroxidation and preserve the function of enzymes associated with cholesterol homeostasis, likely contributing to the partial restoration of cholesterol levels. The results are consistent with existing studies on the hepatoprotective and lipid-lowering effects of natural antioxidants. Al-Garawi et al. (2020) demonstrated that plant-derived antioxidants, such as those from rosemary, reduced cholesterol levels in rats subjected to cadmium by modulating oxidative stress and inflammatory responses. Yang et al. (2024) similarly demonstrated that chlorella vulgaris supplementation, with its antioxidant and

anti-inflammatory properties, restored cholesterol levels in cases of cadmium-induced hepatotoxicity (Al-garawi et al., 2022; Yang et al., 2024).

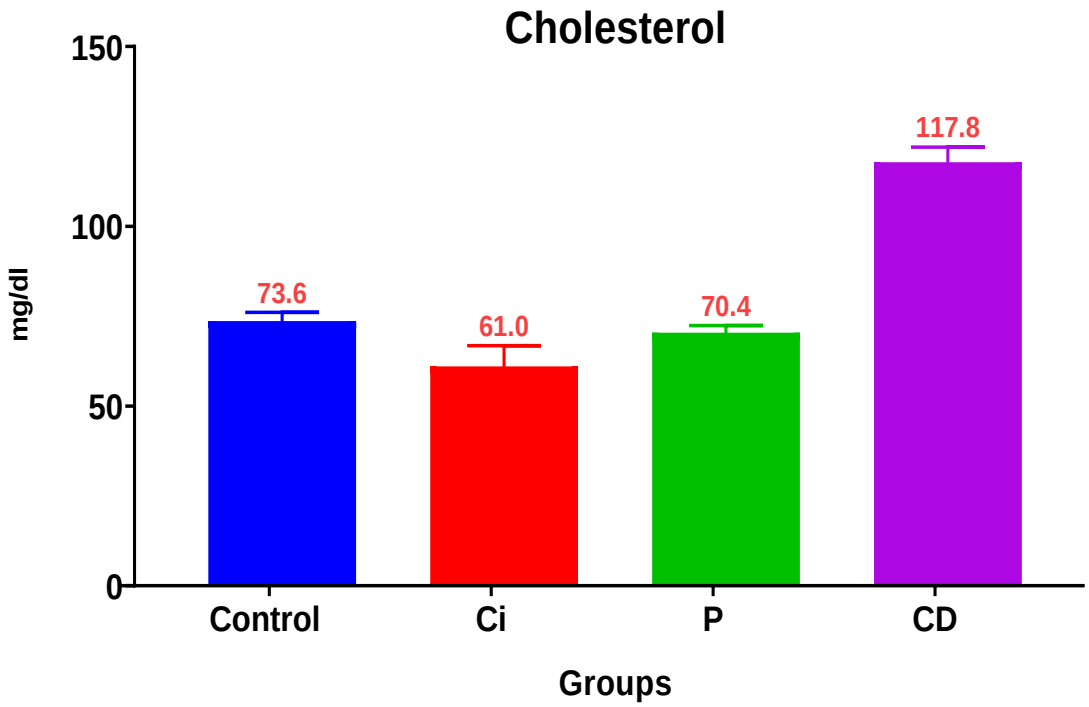

Figure 4: Impact of 1,8-Cineole on Serum Cholesterol Levels Among Experimental Groups: Cholesterol levels were analyzed in the Control group (normal saline), $CdCl_2$ group (exposed to cadmium chloride), 1,8-Cineole group (treated with 1,8-Cineole only), and Protective group (pre-treated with 1,8-Cineole before $CdCl_2$ exposure). Values are presented as mean ± SEM.

This study illustrated the hepatotoxic effects of cadmium chloride ($CdCl_2$) and the preventive efficacy of 1,8-cineole in alleviating its detrimental effects on liver function. Increased blood concentrations of ALT, AST, cholesterol, triglycerides, and malondialdehyde (MDA) indicate substantial liver damage attributable to pronounced oxidative stress, lipid peroxidation, and inflammation caused by $CdCl_2$ exposure. 1,8-cineole had a protective impact by regulating biochemical markers, diminishing oxidative damage, and maintaining liver function via its robust antioxidant and anti-inflammatory characteristics.

Although complete normalization was not attained, pretreatment with 1,8-cineole in the Protective group substantially alleviated the harmful effects of $CdCl_2$. Conversely, treatment with 1,8-cineole alone restored biochemical markers to almost normal levels. The results demonstrate that 1,8-cineole has therapeutic characteristics as a natural antioxidant, possibly alleviating hepatotoxicity caused by heavy metals.

To evaluate 1,8-cineole's effectiveness in clinical settings and investigate the intricate molecular processes behind its hepatoprotective benefits, further study is advised. This research emphasizes the significance of creating natural substances like 1,8-cineole as effective treatments for liver damage brought on by environmental toxins.

4. Conclusion

The study demonstrated the protective effects of 1,8-cineole on liver function in rats exposed to cadmium chloride, a heavy metal known for its hepatotoxic properties. Cadmium chloride exposure resulted in significant oxidative stress, inflammation, and liver damage, as evidenced by elevated liver enzyme levels, histopathological changes, and increased markers of oxidative damage. However, the administration of 1,8-cineole effectively mitigated these adverse effects.

1,8-Cineole exhibited potent antioxidant and anti-inflammatory properties, reducing oxidative stress markers and preserving the structural integrity of liver tissue. The normalization of liver enzyme levels and improved histopathological findings in treated groups indicate the compound's ability to counteract cadmium-induced toxicity. These results suggest that 1,8-cineole has potential therapeutic value in protecting liver function against heavy metal-induced damage. Further studies are recommended to explore its mechanisms of action and potential applications in clinical settings

REFERENCES

1. Abdollahi, M., Asle-Rousta, M., & Mahmazi, S. (2024). Protective effect of 1, 8-cineole (eucalyptol) against lead-induced liver injury by ameliorating oxidative stress and inflammation and modulating TLR4/MyD88/NF-κB signaling. Iranian Journal of Basic Medical Sciences, 27(10), 1293.
2. Abu-El-Zahab, H. S. H., Hamza, R. Z., Montaser, M. M., El-Mahdi, M. M., & Al-Harthi, W. A. (2019). Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. Ecotoxicology and Environmental Safety, 173, 419–428.
3. Al-garawi, N. A. D., Suhail, A. A., Kareem, H. A., & Bustani, G. S. (2022). Study of Lipid Profile and Leptin hormone and Adiponectin hormone hypertensive patients in Najaf Governorate. Revista Electronica de Veterinaria, 45–51.
4. Ali Hameed, M., Sabah AL-Khalidi, Z., Abdulhussein Hasan, M., & Sabah Bustani, G. (2022). Effect of Kisspeptin-54 on Testicular Degeneration Induced by Cadmium Chloride. Archives of Razi Institute, 77(1), 461–466.
5. Al-musawi, M. T., Ali, A. E. H., Humadi, A. A., & Al-Kaisei, B. I. (2020). Antioxidant effects of 1, 8-cineole against long term dl-polychlorinated biphenyls (PCBs) toxicity in domestic Hen's Liver. SRP. Sys Rev Pharm, 11(10), 1050–1057.
6. Bustani, G. S., Jabbar, M. K., AL-Baghdady, H. F., & Al-Dhalimy, A. M. B. (2022). Protective effects of curcumin on testicular and sperm parameters abnormalities induced by nicotine in male rats. AIP Conference Proceedings, 2386(1).
7. Di, Y., Cao, A., Zhang, Y., Li, J., Sun, Y., Geng, S., Li, Y., & Zhang, L. (2022). Effects of Dietary 1, 8-Cineole supplementation on growth performance, antioxidant capacity, immunity, and intestine health of broilers. Animals, 12(18), 2415.
8. Ghobadi, E., Moloudizargari, M., Asghari, M. H., & Abdollahi, M. (2017). The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. Expert Opinion on Drug Metabolism & Toxicology, 13(5), 525–536.
9. Honório, V. G., Bezerra, J., Souza, G. T., Carvalho, R. J., Gomes-Neto, N. J., Figueiredo, R. C. B. Q., Melo, J. V, Souza, E. L., & Magnani, M. (2015). Inhibition of Staphylococcus aureus cocktail using the synergies of oregano and rosemary essential oils or carvacrol and 1, 8-cineole. Frontiers in Microbiology, 6, 1223.
10. Jaishankar, M. T. (2014). T., Anbalagan, N., Mathew, BB, Beeregowda, KN. Toxicity, Mechanism and Health Effects of Some Heavy Metals.
11. Rani, A., Kumar, A., Lal, A., & Pant, M. (2014). Cellular mechanisms of cadmium-induced toxicity: a review. International Journal of Environmental Health Research, 24(4), 378–399.
12. Renugadevi, J., & Prabu, S. M. (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Experimental and Toxicologic Pathology, 62(2), 171–181.
13. Rusco, G., Słowińska, M., Di Iorio, M., Cerolini, S., Maffione, A. B., Ciereszko, A., & Iaffaldano, N. (2022). Proteomic analysis of rabbit fresh and cryopreserved semen provides an important insight into molecular mechanisms of cryoinjuries to spermatozoa. Theriogenology, 191, 77–95.
14. Yang, H., Chen, Y., Linghu, K., Ren, P., Yao, Y., Jiang, F., Wu, G., Chen, T., Ji, Y., & Tao, L. (2024). 1, 8-Cineole alleviates Nrf2-mediated redox imbalance and mitochondrial dysfunction in diabetes mellitus by targeting Sirt1. Phytomedicine, 135, 156099.