Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Evaluation of the protective effect of liquorice, curcumin, and marjoram on oxidative stress in CCL4 hepatotoxicity in rats

Fatima El zahra M. Fathi[1]*, Afaf D. Abd El-Magid[1], Mohammed K. Mahfouz[1], Elsayed I. Salim[2]

[1]*Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University, Egypt*

[2]*Department of Zoology, Faculty of Science, Tanta University, Egypt*

ARTICLE INFO

Keywords

CCL4

Oxidative stress

Liqurice

Curcumin

Marjoram

Received 23/02/2025
Accepted 19/07/2025
Available On-Line 01/10/2025

ABSTRACT

This work was carried out to evaluate the protective effect of Liquorice, Curcumin, and Marjoram on CCl4 hepatotoxicity in rats. A total of 60 male rats were separated into 6 equal groups. Group 1: control negative, rats received 1 mL/kg olive oil (i.p). Group 2: rats received CCl4 (1 ml/kg B.W i/p) twice weekly for 8 weeks. Group 3: rats received CCl4 as group 2 twice weekly and treated with extract of Liquorice orally at dose 200 mg/kg B.W. Group 4: were received CCl4 as group 2 twice weekly and treated with extract of Curcumin and given orally at a dose of 200 mg /kg B.W. Group 5: received CCl4 as group 2 twice weekly and treated with extract of Marjoram administered orally at dose of 375 mg/ kg B.W. Group 6: rats received CCl4 as group 2 and treated with Liquorice, Curcumin and Marjoram extracts and administered orally at the dose of 200, 200, and 375 mg /kg B.W. Every rat received treatment for 2 months. At the end of the experiment, rats were sacrificed. Serum and liver tissue samples were collected. CCl4 hepatotoxicity causes significant increase in serum of γGT, ALP, and MDA, with significant decrease in GSH level, SOD and CAT activities. However, administration of Liquorice, Curcumin, and Marjoram returned these biochemical parameters to nearly their normal values. On conclusion, the strong antioxidant activity of these plants may be the main factor in mediating their hepatoprotective effects in CCl4 hepatotoxicity

1. INTRODUCTION

The liver is one of the body's major organs responsible for energy production, toxic material excretion, and homeostasis regulation. Oxidative stress is an imbalance between free radicals and antioxidants. As a result, the excess free radicals start to harm the body's cells and tissues. It is caused by several agents, including alcohol, medications, chemicals, and environmental pollutants, resulting in the development of liver injury (Allameh et al., 2023). CCl4 is a well-known chemical hepatotoxicant that causes liver damage by exacerbating oxidative stress (Abdulmohsen et al., 2024).

Natural products provide a resource for identification of novel leads that can be utilized for the treatment a diversity of illnesses, including: (cancer, inflammation, and liver disease). More than half of all pharmaceutical products have been discovered from natural compounds or their derivatives. The vital roles that bioactive chemicals from plants play in combating various human diseases have led to their widespread usage in conventional and botanical medicine (Jinhong et al., 2024). Acute liver damage can be induced in a wide variety of laboratory animals by a well-known hepatotoxic drug, CCl4. The principal causes of CCl4 are induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes, and generation of free radicals. The liver damage is induced by the ROS formation, which causes oxidative stress and finally cellular damage (Almatroodi et al., 2020; Sonali, 2023).

Liquorice is a traditional medicinal plant that grows in many places of the world and has ethnobotanical origins. Its roots have some nutritional and therapeutic characteristics. Traditional medical systems in Nepal, India, China, and other nations have utilized glycyrrhizin, a triterpenoid glycoside that was extracted from the root of Glycyrrhiza glabra L., commonly referred to as Liquorice root, to treat jaundice. Glycyrrhizinic acid, the main component of Liquorice, displays hepatoprotective action by limiting changes in cell membrane permeability and boosting the survival rate of hepatocytes (Nouri-Vaskeh et al., 2020). Furthermore, several research and experimental experiments have shown that curcumin effectively reduces liver damage caused by CCl4 poisoning.

Curcumin is the primary component of turmeric, a rhizome of Curcuma longa. It is extensively utilized because of its therapeutic efficacy and acceptable safety profile. Curcumin has a number of biological actions, including anti-inflammatory, anticancer, and antioxidant. Acute liver injury is induced by the formation of ROS, which causes oxidative stress and ultimately cellular damage (Sonali, 2023).

Marjoram is a significant aromatic plant, its leaves, stems, and flowers contain essential oils and polyphenols that are frequently used as a spice and condiment in cooking. Polyphenols have significant antioxidant properties and are commonly utilized as food additives (Cinbilgel and Kurt 2019; Bouyahya et al., 2021). Marjoram serves several therapeutic purposes in addition to its use in the food industry. This plant has bacteriostatic, anticancer, antioxidant, and insecticidal properties in its methanol extracts and essential oils. Increased plasma insulin, increased hepatic glycogen formation, and improved glucokinase activity are some of the antidiabetic effects of the phenolic chemicals found in marjoram. Additionally, marjoram has been shown to have anti-inflammatory, analgesic, antipyretic, and nephrotoxicity-protective effects (Lombrea et al., 2020).

* Correspondence to: fm69883@gmail.com

The present study aims to evaluate the hepatoprotective effect of Liquorice, Curcumin, and Marjoram extracts on CCL4 hepatotoxicity in rats.

2. MATERIAL AND METHODS

2.1. Experimental Animals

Sixty male Wister rats (150 ± 10 grams) were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University. Rats were housed for 12 hours in separate stainless steel cages with a light/dark cycle, at 25 °C and 50 ± 5% relative humidity. They were provided with unlimited food and water. Before starting the experiment, rats were housed for two weeks to get used to the environment. The use committee and institutional animal care requirements were followed when creating the experimental protocol, which was authorized by the Benha University Faculty of Veterinary Medicine's Research Ethics Committee (BUFV 03-11-24).

2.2. Chemicals and plant materials

All of the other analytical-grade chemicals and reagents used in this study were also acquired from El-Gomhoriya Company for Trading Drugs, Chemicals, and Medical Appliances, Cairo, Egypt, while CCL4 was acquired from AL-Gomhoriya Company for Med-preparations Chemicals and Medical Equipment, Cairo, Egypt.

The selected plant leaves were obtained from the Local Company for Herbs and Medicinal Plants in Cairo, Egypt, and utilized to prepare crude extraction.

2.3. Experimental Design

Rats were randomly divided into 6 main groups, and each group contained 10 rats. After 1week of acclimatization, the groups were classified as follows: Control negative group: rats received 1 mL/kg olive oil (i.p) and supplied with standard pellet diet and fresh water for 8 weeks. Group 2: CCL4 intoxicated group, received a dose of CCl4 (1 ml/kg B.W i/p) twice weekly for induction of hepatotoxicity for 8 weeks. Group 3: CCL4 + Liquorice extract group, received a dose of CCl4 (1ml/kg B.W i/p) twice weekly for induction of hepatotoxicity and treated with extract of Liquorice dissolved in distilled water and given at a dose of 200 mg/kg B.W. Group 4: CCL4 + Curcumin extract group, received acute dose of CCl4 (1 ml/kg B.W i/p) twice weekly and treated with extract of Curcumin and given orally via at a dose of 200 mg /kg B.W in distilled water. Group 5: CCL4 + Marjoram extract group, received CCl4 (1 ml/kg B.W i/p) twice weekly and treated with extract of Marjoram administered orally at dose of 375 mg/ kg B.W. Group 6: CCL4 + Mixture of 3 previous extracts groups, received CCl4 (1 ml/kg B.W i/p) twice weekly for induction of hepatotoxicity and treated with Liquorice, Curcumin and Marjoram extracts and administered orally at the dose of 200, 200, and 375 mg /kg B.W in distilled water. Every rat received treatment for 2 months.

2.4. Sampling

Blood and tissue samples were collected at the end of the experiment.

2.4.1. Blood Samples

The blood samples were collected from the retro-orbital vein located at the medial canthus of the eye using heparinized capillary tubes. The blood samples were collected in dry, clean and screw capped test tubes and incubated for 1/2 hr. at room temperature to allow clotting for serum separation. Clear sera were separated by centrifugation for 15 minutes and then collected in Eppendorf tubes. Part of the serum samples was used immediately for measuring the levels of γGT and ALP.

2.4.2. Liver Tissue Samples

Rats from each group were decapitated, the abdomen opened, and the liver was gently excised, cleaned with ice-cold isotonic saline, cleared of blood, and immediately put back into ice-cold isotonic saline before being blotted between two filter sheets. A sample of liver tissue was promptly frozen at -20 °C for further biochemical studies (hepatic MDA, GSH, SOD, and CAT).

2.5. Biochemical analysis

Serum γGT level was estimated according to Heersink (1980), and ALP level was estimated according to Kind and King (1954). The hepatic MDA and GSH concentrations were estimated according to Ohkawa et al. (1979) and Beutler et al. (1963), respectively. The hepatic SOD and Catalase activities were measured according to Nishikimi et al. (1972) and Aebi (1984), respectively

2.6. Statistical analysis

The results were expressed as mean ± SEM. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. P-values < 0.05 were considered statistically significant.

3. RESULTS

As shown in Table 1, the CCl4-intoxicated rats (Group 2) had a significant increase (P≤ 0.05) in γGT and ALP enzyme levels when compared to the control negative group (Group 1). The CCl4-intoxicated groups, treated with extracts of Liquorice, Curcumin, Marjoram, and a mixture of previous extracts in groups (3, 4, 5, 6), demonstrated a substantial (P<0.05) reduction in levels of γGT and ALP.

Although, The CCl4-intoxicated rats (Group 2) showed a significant increase (P< 0.05) in the hepatic MDA level when compared to the control negative group (Group 1). While, the CCl4-intoxicated groups treated with extracts of Liquorice, Curcumin, Marjoram, and the mixture of previous extracts in groups 3,4,5,6 demonstrated a substantial (P<0.05) reduction in the concentration of the MDA (Fig. 1). A significant decrease (P<0.05) has been recorded in the hepatic level of GSH and the activities of SOD and CAT enzymes compared to control negative group. In contrast, the hepatic level of GSH, and the activities of SOD and CAT enzymes are significantly elevated (P<0.05) in these treated groups compared to CCL4-intoxicated group (Fig. 2, 3, and 4).

Table 1: Impact of Liquorice, Curcumin, and Marjoram treatment on γGT and ALP enzymes in rats intoxicated with CCL4 (Mean± SEM)

Groups	γGT (U/L)	ALP (U/L)
Group 1	12.56±0.92^{d}	138.00±1.53^{c}
Group 2	70.41±0.85^{a}	625.33±54.34^{a}
Group 3	32.76±2.97^{bc}	235.67±2.96^{b}
Group 4	39.27±5.19^{b}	251.00±6.35^{b}
Group 5	37.37±2.12^{b}	246.41±2.23^{b}
Group 6	25.04±2.79^{c}	205.82±6.17^{b}

Values with different superscript letters within the same column are significantly different at P < 0.05. Group 1 (Control negative), Group 2 (CCL4 intoxicated group), Group 3 (CCL4+ Liquorice extract), Group 4 (CCL4+Curcumin extract), Group 5 (CCL4+Marjoram extract), Group 6 (CCL4+mixture of 3 previous extracts; L+C+M).

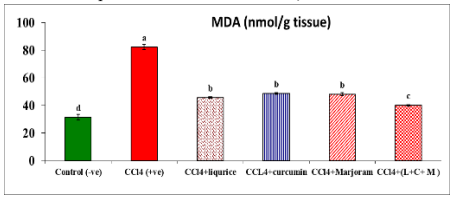

Fig. (1). Impact of treatment with Liquorice, Curcumin, and Marjoram on hepatic MDA level in rats intoxicated with CCL4

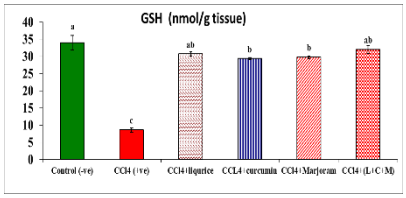

Fig. (2). Impact of Liquorice, Curcumin, and Marjoram treatment on hepatic GSH level in rats intoxicated with CCL4

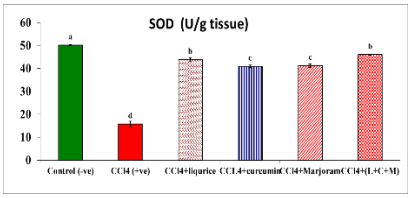

Fig. (3). Impact of Liquorice, Curcumin, and Marjoram treatment on hepatic SOD activity in rats intoxicated with CCL4

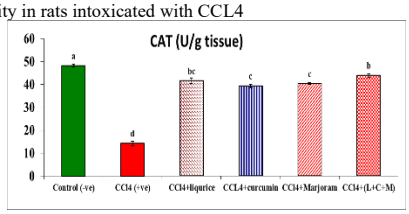

Fig. (4). Impact of Liquorice, Curcumin, and Marjoram treatment on hepatic CAT activity in rats intoxicated with CCL4

4. DISCUSSION

The current study assessed the preventive impact of Liquorice, Curcumin, and Marjoram against CCl4-induced liver damage. In this study, the CCl4-intoxication increased the level of γGT and ALP enzymes, which is consistent with that reported by Li et al. (2015) and Sun et al. (2018). The CCL4 significantly increases serum enzyme levels by causing leakage from liver cells, indicating membrane damage (Sun et al., 2018). The elevated γGT and ALP suggest impaired liver function. CCl4 induces liver injury through mechanisms like fatty degeneration, fibrosis, cell death, and carcinogenicity, all of which raise liver biomarkers, especially ALP (Lijun et al., 2023). The ALP is a homodimer mainly found in the liver, is involved in bile production, and its elevation signals (Minisola et al., 2025) In the present study, a marked restoration of γGT and ALP enzyme levels after administration of Liquorice in group 3 (CCL4+ Liquorice extract) has been recorded, which is coordinated with that of Abdel-Kader et al. (2018) and Abdel Maksoud et al. (2018) who reported that a significant decrease in γGT and ALP levels in Liquorice extract treated group in comparison with CCL4 treated group might be due to Glycyrrhizinic acid, the major component of Liquorice preventing changes in cell membrane permeability and increasing survival rate of hepatocyte.

Although the present results revealed an improvement in γGT and ALP activity in Group 4 (CCL4+ curcumin extract), which is in agreement with AL-Mashhadani et al. (2023), curcumin preventing the overproduction of γGT and ALP. Curcumin treatment reduces inflammation and steatosis, offering protection by inhibiting lipid peroxidation and oxidative chain reactions. Moreover, Rahman et al. (2020) reported that serum ALP activities are related to the function of hepatic cells. Elevated serum ALP reflects impaired bile excretion due to liver injury. Curcumin's ability to regulate ALP levels may be due to improved liver cell secretory function.

In this study, the Marjoram extract (Group 5, CCl4+Marjoram extract) decreased the γGT and ALP levels, which is consistent with Hikal et al. (2018) who noted that the Marjoram extract improves liver enzyme function in CCl4 intoxicated rats, with enhanced effects in treated groups due to its synergistic antioxidant action. It protects liver tissue by reducing free radical formation and preventing enzyme leakage into the blood. Although, the present results revealed an improvement in γGT and ALP activity in Group 6 (CCL4+ mixture of Liquorice, Curcumin and Marjoram extracts) compared to CCL4 intoxicated group. Based on the previous results from the other groups, it was found that the combination of these extracts work to protect and repair liver tissue from damage casued by CCL4. Similar to our results, Sarhan et al. (2019) and Almatroodi et al. (2020) reported that MDA significantly increased, while parameters, SOD, GPx, GSH and CAT were decreased in CCl4-intoxicated rats. Additionally, it has been noted that antioxidant agents like SOD and GSH play crucial roles in defense mechanisms against the damaging effects of ROS and free radicals in biological systems. These findings may be explained by the way that CCl4 damages tissues, which is oxidative damage brought on by lipid peroxidation, which begins after CCl4 is converted by the cytochrome P450 enzyme into free radicals of the extremely toxic trichloromethyl radicals (•CCl3) and trichloromethyl peroxyl radical (•CCl3O2). These harmful free radicals cause lipid peroxidation and chain reaction in membrane-like structures like the endoplasmic reticulum and mitochondria that are high in phospholipids and damage the cell membrane by changing how it normally functions, and this leads to an increase in lipid peroxidation marker (MDA) (Velid et al., 2021; Pelvan et al., 2022).

In this study, CCl4 significantly reduced the hepatic antioxidant levels of GSH, SOD and CAT, which in turn led to an increase in MDA levels. This is because CCl4 causes liver cells to produce less endogenous antioxidants, which in turn reduces the level of GSH, and enzymatic activity of (SOD and CAT) (Pelvan et al., 2022). Moreover, Albaqami et al. (2022) reported that the deactivation of their isoenzymes due to oxidation of a cysteine residue close to the active center may also be the cause of the decrease in antioxidant enzyme activity. By regulating the physiological generation of ROS, redox homeostasis promotes immunological responses and balance through antioxidant mechanisms. Exogenous substances, on the other hand, are known to disrupt redox equilibrium and result in damage mediated by oxidative stress.

We investigated how CCl4 affected the hepatic antioxidant state. Glutathione directly inhibits ROS, like lipid peroxides, and is essential for the metabolism of xenobiotics (Ogbuagu et al., 2019). Hydrogen peroxide (H_2O_2) and lipid peroxide are detoxified by glutathione, which gives H_2O_2 an electron and transforms it into water and oxygen while shielding macromolecules like lipids from oxidation (Airaodion et al., 2019). Interestingly, endogenous antioxidants like GSH, SOD and CAT have been included into the biological system to combat the negative effects of free radicals (Singh and Jambunathan, 2017). Glutathione- s-transferase, SOD, CAT maintaining the balance between ROS and antioxidant enzymes. Dismutation of superoxide anion (O_2-) to H_2O_2 and O_2 catalyzes by SOD. Although H_2O_2 is still toxic to cells, catalase catalyzes its breakdown into water, and this process is crucial to preventing oxidative stress-related damage (Pelvan et al., 2022).

However, our study's findings demonstrated that group 3 (CCL4+ Liquorice extract) experienced significant decrease in MDA levels and increase in production of the antioxidant system (GSH, SOD and CAT). These findings were corroborated by Sun et al., (2018) who discovered that the antioxidants enhanced the activity of SOD, CAT, GSH-Px antioxidant enzymes in the rats' liver treated with Liquorice

and lowered MDA, and ROS. This could be explained by Glycyrrhizin has the capacity to scavenge ROS to counteract oxidative damage caused by CCl4. The treatment of glycyrrhizin increased antioxidants activity (CAT, SOD and GSH), which served to mitigate all of these cellular alterations (Rasool et al., 2014).

Although, our results showed that in group 4 (CCL4 + Curcumin extract) there is decrease in MDA level while, showed significant increase in hepatic level of GSH and activities of SOD and CAT compared to CCL4 intoxicated group. These findings are consistent with research by Abd-Alfattah and Abdelraouf (2018) who found that curcumin, a strong cytochrome P450 inhibitor, decreased levels of MDA and significantly increased in GSH level and activities of antioxidant enzymes (SOD and CAT). Because curcumin suppresses the development of lipid peroxide and lysosomal enzymes, its administration reduced the liver damage produced by CCL4 by triggering the Nrf2-Keap1 pathway and boosting the activity of antioxidant enzymes, it reduces oxidative stress during inflammation. It scavenges ROS and boosts the activity of serum antioxidants including GSH and SOD (Lin et al., 2019; Saleh et al., 2021).

Furthermore, in the present investigation, the findings in Group 5 (CCL4 + Marjoram extract) showed significant decrease in MDA level while, on other hand there are significant increase in level GSH and activities of SOD and CAT. These results agree with study by Hikal et al., (2018) who observed that CCl4-intoxicated group treated with Marjoram extract had a substantial rise in serum TAC, liver CAT, GSH and SOD activity compared to CCL4-intoxicated group. Although, Abdelhamid et al., (2020) demonstrated that treatment with Marjoram extract it significantly decreased gastric MDA while, increased activities of gastric CAT, SOD and GSH level compared to ulcerative gastritis group. This is due to Marjoram can either increase GSH biosynthesis or oxidative stress reduction leading to less degradation of GSH, and increase SOD which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen, hence diminishing the toxic effects caused by their radicals.

In addition to that, in Group 6 (CCL4+mixture of Liquorice, Curcumin and Marjoram extracts) observed that significant decreased in MDA levels while, showed significant increase in antioxidant level of GSH and activities of SOD and CAT enzymes in compared to the CCL4 intoxicated group. The supplementation with mixture of previous extracts attenuated the formation of ROS, which leads to restoring the redox status in the cell by increasing the levels and activities of antioxidant agents and this finding was further substantiated by the results obtained from the other groups treated individually with each of these extracts. This finding suggests that these herbs work to protect and repair liver tissue from damage caused by CCL4.

5. CONCLUSIONS

According to the results of our investigation, administering Liquorice, Curcumin and Marjoram may help attenuate CCL4-induced hepatotoxicity as natural antioxidants from these plants. It protects the liver from oxidative damage and modulates biochemical parameters to physiological limits. Therefore, it is recommended to conduct more research on this topic.

ACKNOWLEDGENTS

The authors express their gratitude to the entire staff members in the Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

5. REFERENCES

1. Abd-Alfattah, A. and Abdelraouf, Y.M., 2018. Study the effect of curcumin on hepatic DNA damage in an experimental model of hepatic fibrosis. (Official Journal of Egyptian Society for Physiological Sciences) Bull. Egypt. Soc. Physiol. Sci., 38,2, 100- 110
2. Abdel Maksoud, H.A., Abdel Magid, A.D., Mostafa, Y.M., Sorour, R.I., Mohamed, G.E., 2018. Biochemical and Genetical evaluation of ameliorative role of liquorice extract on experimentally induced Hepatitis. International Journal of Current Research10 ,9, 73150-73160.
3. Abdelhamid, O. M., Said, A.M., Hussein, R.S., 2020. Biochemical Effects of Basil and Marjoram on Ulcerative Gastritis Induced Experimentally In Rats, World Journal Of Pharmacy And Pharmaceutical Sciences, 9 ,1, 424-435.
4. Abdel-Kader, M.S., Abulhamd, A.T., Abubaker, A.M., Hamad, A.H., Alanazi, R. A., Saleh, I.A., 2018. Evaluation of the hepatoprotective effect of combination between hinokiflavone and glycyrrhizin against CCl_4-induced toxicity in rats. Saudi Pharmaceutical Journal, 26,4, 496–503.
5. Abdulmohsen, I.A., Alfwuaires, M., Famurewa, A.C., Elsawy, H., Sedky, A., 2024. Geraniol prevents CCl_4-induced hepatotoxicity via suppression of hepatic oxidative stress, pro-inflammation and apoptosis in rats. Toxicology Reports 12, 128-134.
6. Aebi, H., 1984. Catalase in vitro. Methods in enzymology 105, 121-126.
7. Airaodion, A.I., Ogbuagu, U., Ekenjoku, J.A., Ogbuagu, E.O., Airaodion, E.O., Okoroukwu, V.N., 2019. Hepato-protective efficiency of ethanol leaf extract of Moringa oleifera against hydrocarbon exposure. Int J Adv Herbal Alternat Med, 3,1, 32–41
8. Albaqami, J.J., Hamdi, H., Narayanankutty, A., Naduvilthara, U.V., Sasidharan, A., Kuttithodi, A.M., Famurewa, A.C., Pathrose, B., 2022. Chemical composition and biological activities of the leaf essential oils of Curcuma longa, Curcuma aromatica, and Curcuma angustifolia. Antibiotics 11,11, 1547.
9. Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastiani, G., Pantopoulos, K., 2023. Oxidative Stress in Liver Pathophysiology and Disease. Antioxidants 12, 1653.
10. AL-Mashhadani, F.A., Nabee, N.W., Abdulrasol, S.A., 2023. Effect of Annona Muricata Fruit and Turmeric Combination on Some Biophysiological and Histological Parameters in CCl4-Induced Toxic Liver in Rats, 1st International & 4th Local Conference for Pure Science (ICPS-2021) AIP Conf. Proc. 2475, 040002-1–040002-12.
11. Almatroodi, S.A., Anwar, S., Almatroudi, A., Khan, A. A., Alrumaihi, F., Alsahli, M.A., Rahmani, A.H., 2020. Hepatoprotective effects of garlic extract against carbon tetrachloride (CCl_4)-induced liver injury via modulation of antioxidant, anti-inflammatory activities and hepatocyte architecture. Applied Sciences, 10,2, 295.
12. Beutler, E., Duron, O., Mikus, K.B., 1963. Improved method for the determination of blood glutathione. Journal of laboratory and clinical Medicine 61, 882-888.
13. Bouyahya, A., Chamkhi, I., Benali, T., Guaouguaou, F.E., Balahbib, A., El Omari, N., 2021. Traditional use, phytochemistry, toxicology, and pharmacology of Origanum majorana L. J Ethnopharmacol, 265, 113318–113367.
14. Cinbilgel, I. and Kurt, Y., 2019. Oregano and/or marjoram: Traditional oil production and ethnomedical utilization of Origanum species in southern Turkey. J. Herb Med, 16, 100257.
15. Heersink, R., 1980. Some generalizations of an elliptic boundary value problem of Hadamard. Acta Physica Austriaca, 52,3-4, 277- 291.
16. Hikal, A.H., Abd El-Fattah, H.M., El-Sheikh, N.M., Refaie, A.A., 2018. Comparative Study of Marjoram (Origanum Majorana L.) and Silymarin (Silybum Marianum L.) extract against carbon tetrachloride induced hepatic Injury. World Journal of Pharmacy and Pharmaceutical Sciences 7, 8, 1369-1392.

17. Jinhong, G., Wenjie, Y., Hao, D., Diandian, W., Yaxi, Z., Duo, F., Yue, Z., Shiqi Zhou, G., Liu, X. Q., 2024. Therapeutic Effects of Natural Products on Liver Cancer and Their Potential Mechanisms. Nutrients 16,11 , 1642.
18. Kind, P.R. and King, E.G., 1954. Colorimetric determination of alkaline phosphatase activity. J Clin Pathol, 7, 322.
19. Li, C., Yi, L.T., Geng, D., Han, Y.Y., Weng, L.j., 2015. Hepatoprotective effect of ethanol extract from Berchemia lineate against CCL4- induced acute hepatotoxicity in mice. Pharmaceutical biology 53, 767-772.
20. Lijun, Z., Chuhe, L., Liufang, Y., Cheng, H., Shengjie, F. (2023). Mangiferin relieves CCl_4-induced liver fibrosis in mice, Scientific Reports (Nature): 13, 1 :4172.
21. Lin, X, Bai, D., Wei, Z., Zhang, Y., Huang, Y., Deng, H., 2019. Curcumin attenuates oxidative stress in RAW264. 7 cells by increasing the activity of antioxidant enzymes and activating the Nrf2-Keap1 pathway. PloS One, 14, 5 , e0216711.
22. Lombrea, A., Antal, D., Ardelean, F., Avram, S., Pavel, I.Z., Vlaia, L., 2020. A recent insight regarding the phytochemistry and bioactivity of Origanum vulgare L. essential oil. Int J Mol Sci, 21, 9653–9733.
23. Minisola, S., Cipriani, C., Colangelo, L., Labbadia, G., Pepe, J. 2025. Diagnostic Approach to Abnormal Alkaline Phosphatase Value, Mayo Foundation for Medical Education and Research. Published by Elsevier Inc. All rights are reserved, including those for text and datamining, AI training, and similar technologies. Mayo Clin Proc. 2025;100, 4 :712-728.
24. Nishikimi, M., Appaji, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Common, 46, 849-854.
25. Nouri-Vaskeh, M., Malek, M.A., Afshan, H., Alizadeh, L., Zarei, M., 2020. Effect of curcumin supplementation on disease severity in patients with liver cirrhosis: a randomized controlled trial. Phytother Res, 34, 1446–1454.
26. Ogbuagu, E.O., Airaodion, A.I., Ogbuagu, U., Airaodion, E.O., 2019. Prophylactic propensity of methanolic extract of Vernonia amygdalina leaves against acute ethanol-induced oxidative stress in Wistar rats. Int J Bio-Sci Bio- Technol, 11, 7, 37–46
27. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry 95, 351-358.
28. Pelvan, E., Karaoğlu, Ö., Fırat, EÖ., Kalyon, K.B., Ros, E., Alasalvar, C., 2022. Immunomodulatory effects of selected medicinal herbs and their essential oils: a comprehensive review. J Funct Foods 94,105-108.
29. Rahman, F., Sarma, J., Mohan, P., Choudhury, C.B., Nath, R., Rahman, S., Sarma, M., 2020. Role of Nano-curcumin on carbon tetrachloride , CCl4 induced hepatotoxicity in rats. Journal of Pharmacognosy and Phytochemistry 9, 2 , 2168-2176
30. Rasool, M., Iqbal, J., Malik, A., Ramzan, H.S., Qureshi, M.S., Asif, M., Qazi, M.H., Kamal, M.A., Chaudhary, A.G., Al-Qahtani, M.H., et al., 2014. Hepatoprotective Effects of Silybum marianum , Silymarin and Glycyrrhiza glabra , Glycyrrhizin in Combination: A Possible Synergy. Evidence-Based Complementary and Alternative Medicine 641597; 1-9.
31. Saleh, H.A., Yousef, M.H., Abdelnaser, A., 2021. The Anti-Inflammatory Properties of Phytochemicals and Their Effects on Epigenetic Mechanisms Involved in TLR4/NF-kB-Mediated Inflammation Front. Immunol, 12, 606069.
32. Sarhan, H., Farid, A., Mostafa, K., 2019. Hepatoprotective and antioxidant effects of Ocimum basilicum extract in CCl4-induced hepatotoxic rats compared with Silymarin. Benha Veterinary Medical Journal 36, 2 , 282-292.
33. Singh, H. and Jambunathan, S., 2017. Evaluation of the hypoglycemic effect of Sphenostylis stenocarpa , seeds in mice. Indian Journal 36,10–23.
34. Sonali, R., 2023. Hepatoprotective Effects of Curcumin from Curcuma Longa L.A Comprehensive Account, Trends in Chemical and Biological Research, 84-97.
35. Sun, J., Wu, Y., Long, C., He, P., Gu, J., Yang, L., 2018. Anthocyanins isolated from blueberry ameliorates CCl4 induced liver fibrosis by modulation of oxidative stress, inflammation and stellate cell activation in mice. Food Chem Toxicol; 120, 491-500.
36. Velid, U., Mustafa, C., İlhan, S., 2021. Toxicity of carbon tetrachloride, free radicals, and role of antioxidants. Rev Environ Health 36, 2, 279–295.