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Synergistic effect of basil, mint extracts and zinc oxide nanoparticles on biochemical parameters in rats reduced by methotrexate

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ABSTRACT

The basil and mint plants were collected from local markets, the plants were extracted using a soxhlet apparatus with 70% methanol alcohol. zinc nanoparticles with a typical size of 33–40 nm were acquired from nanomaterials. design Eighty adult male rats from the University of Kerbala animal facility were used in this investigation. they were given food. rats groups were divided into eight groups.G1: control received saline solution (0.90%), G2:100 mg of Basil and 10 mg of MTX, G3:150 mg of Basil and 10 mg of MTX , G4:100 mg of mint and 10 mg of MTX , G5: 150 mg of mint and 10 mg of MTX ,G6: 0.5 mg of zinc nanoparticles and 10 mg of MTX ,G7 :0.8 mg of zinc nanoparticles and 10 mg of MTX ,G8:synergistic effect between basil and mint extracts 150 mg and zinc oxide nanoparticles 0.8mg MTX, Indicate the results synergistic treatment (basil + mint + nanoparticles zinc) achieved the best results compared to the individual treatments, indicating a true synergistic effect that enhances liver protection through the reduction of MDA and LPO levels, an increase in GPX activity, and a relative improvement in total cholesterol and triacylglyceride levels. this suggests the effectiveness of plant antioxidants in reducing lipid per oxidation and enhancing the efficiency of cellular defenses.

Keywords: Basil, Mint, Zinc oxide nanoparticles, Methotrexate, Hepatotoxicity

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1.Introduction

Methotrexate (MTX) common medication used to treat cancer and inflammatory autoimmune disorders like leukemia and rheumatoid arthritis, works by blocking the enzyme dihydrofolate reductase, which prevents the synthesis of folic acid and restricts cell growth [1]. However, the therapeutic use of this drug is limited due to its high hepatotoxicity, which manifests as elevated liver enzyme levels (such as ALT, AST, ALP), increased oxidative stress and inflammation within liver tissue, in addition to harmful histological changes in hepatocytes in various animal models [2].The liver's response to acute exposure to methotrexate is a clear example of oxidative damage and inflammatory stimulation that can lead to programmed cell death (apoptosis) and damage to the liver tissue structure, highlighting the need for preventive and therapeutic strategies to reduce these side effects[3].natural compounds with anti-inflammatory and antioxidant qualities have drawn more attention recently as a means of shielding tissues against medication toxicity, for example, studies have shown that certain plant compounds such as naringin and rosmarinic acid have the ability to reduce liver damage and oxidative stress caused by MTX in rats [4].among the y, basilmedicinal plants with known biological efficac(and mint (*Ocimum basilicum*(*Mentha* spp.) stand out. With a composition rich inphenolcompounds and flavonoids that act as antioxidants and stimulate cellular defence systems against free radicals, which can help mitigate used by various toxinsphysiological changes ca[5] Previous studies ha indicatethat basil leaf extract may have a protective effect on liver functions in models of liver toxicity, although studies directly linking this toMTX-inducedtoxicity are still insufficient [6].On the other hand, nanoparticle, especially zinc oxide nanoparticles (ZnO NPs), represent an emerging field in medical applications due to their antioxidant activity and simultaneous immune stimulation. Some studies have shown their ability to enhance cellular defense and reduce oxidative stress in the liver, depending on the dose and method of administration, supporting their potential use as an adjunct in reducing the toxicity of hepatocyte drug[7]. based on that, the combination of basil and mint extracts with zinc nanoparticles may represent a unique synergistic strategy, combining antioxidant and anti-inflammatory properties with nano defensive stimulants to enhance the biological response against liver toxicity caused by methotrexate overdose in adult rats.

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therefore, this research aims to evaluate this synergistic effect on the biochemical and histologist indicators of liver functions, in addition to analysis the possible protective or preventive mechanisms at the molecular and cellular levels [8,9].The aim of this research is synergistic effect basil, mint extracts and zinc oxide nanoparticles on antioxidant enzymes, total cholesterol and triglyceride in reducing methotrexate.

2.Material and method

The mint and basil plants were gathered from nearby markets, cleaned of dust and debris using distilled water, and then allowed to air dry. an electric grinder was used to grind the plants, which were then put in airtight containers the plants were extracted using a Soxhlet apparatus with 70% methane alcohol. the fine powder was placed in the thimble, and the device was operated at a temperature of 65 °C for 24 hours[10,11]. The alcohol was separated from the extract using a rotary evaporator, and then the Crude extract was stored in opaque, airtight Container in the refrigerator until use [12]. Zinc nanoparticles were purchased from the nanomaterials, pratical size 33-40 nm. design eighty mature male rats between the ages of ten and twelve weeks and weighing between 200 and 300 g were used in this investigation. They were housed at the University of Kerbala animal facility in a somewhat controlled environment with a temperature of 25°C. they were given food rats groups were divide in to eight groups.

G1: saline solution (0,90%).

G2: 100 mg of Basil and 10 mg of MTX per K.B W.

G3: 150 mg of Basil and 10 mg of MTX per K.B W.

G4: 100 mg of mint and 10 mg of MTX per K.B W.

G5: 150 mg of mint and 10 mg of MTX per K.B W.

G6: 0.5 mg of zinc nanoparticles and 10 mg of MTX per K.B W.

G7 :0.8 mg of zinc nanoparticles and 10 mg of MTX per K.B W.

G8:synergistic effect between basil and mint extracts 150 mg and zinc oxide nanoparticles 0.8 mg MTX per K.B W, given intravenously, after taking all dosages orally once a day for 14 days, the blood bled for 30 days [13] the cardiac puncture method was used to take blood, which was then filtered after being spun at 3000 rpm for ten minutes to separate the blood serum. blood was drawn after 30 days, and the serum was kept for enzyme tests at 40 °C [14]. Total cholesterol and

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triacylglycerides were determined by using analysis kit (Biomaghreb company) [15] MDA, GPX, and LPO assays utilizing a Chin Bio-assay Technology Laboratory BT LAB kit [16].

Statistical Analysis:

Mean was used to express the data.One-way analysis of variances was used to assess the statistical significance of differences between control and other groups (ANOVA), P values of 0.05 or less were deemed significant when statistical analysis was performed using SPSS (SPSS, Inc., Chicago, Illinois) [17].

3. Results

Table (1) and Figure (1) in the current study, the total cholesterol level of groups G8, rats identified a significant increase 14.54± 1.84 mg/dL as an alternative to the control 99.34± 1.65 mg/dL , G2 to G7 showed a noticeable decline 99.34±1.65, 134.46±0.54, 134.46±0.54, 122.25± 0.45, 118.23± 0.63, 121.54±1.76 and 116.65± 0.64 mg/dL correspondingly compared to G8 114.54± 1.84 mg/dL. The triglyceride level of groups G8 indicated a significant decrease 32.74± 1.75 mg/dL compared with control 67.34 mg/dL G2 to G7 showed a noticeable decline 90.34± 0.35, 88.53± 0.74, 77.64± 0.46,69.53± 0.73, 59.63± 0.46,and 44.73±0.36 mg/dL correspondingly compared to G8 32.74± 1.75 mg/dL.

Table 1. Synergistic effect of basil, mint extracts and zinc oxide nanoparticles on triacylglycerid and total cholesterol

Groups	total cholesterol	Triacylglycerid
	Mean ± SD	
G 1	99.34± 1.65	77.47± 0.65
G2	142.54±1.54	90.34± 0.35
G3	134.46±0.54	88.53± 0.74
G4	122.25± 0.45	77.64± 0.46
G 5	118.23± 0.63	69.53± 0.73
G6	121.54±1.76	59.63± 0.46
G7	116.65± 0.64	44.73±0.36
G8	114.54± 1.84	32.74± 1.75
L.S.D	1.76	1.45

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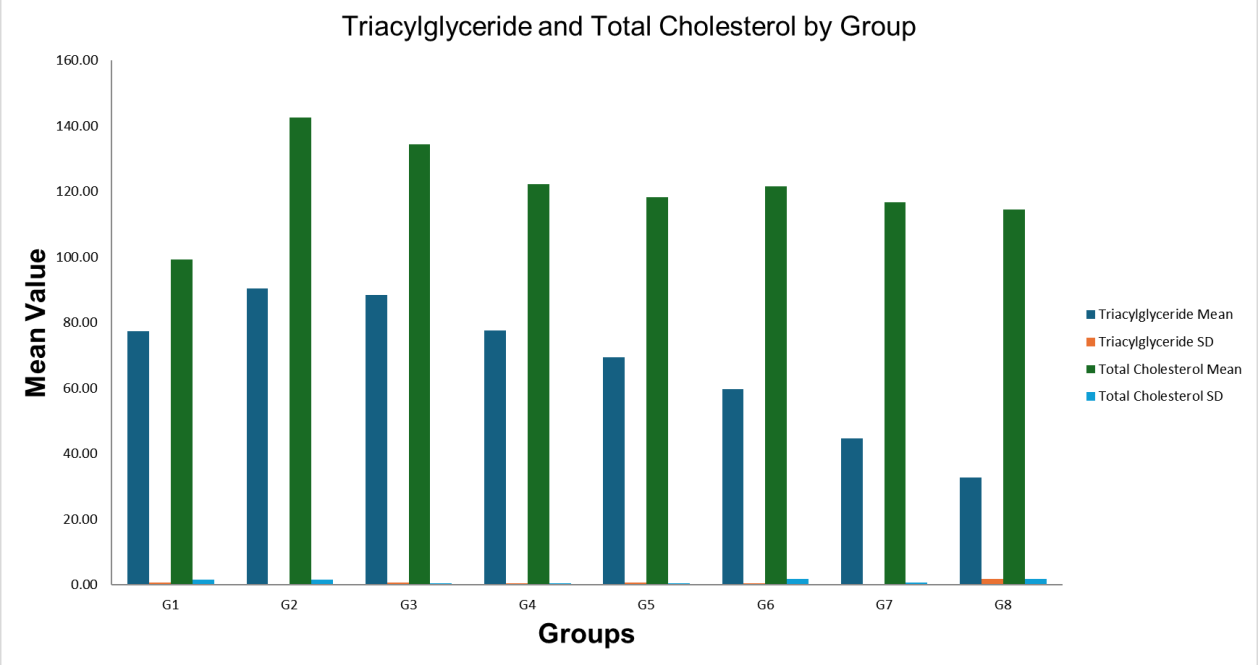

Figure 1.Synergistic effect of basil, mint extracts and zinc oxide nanoparticles on triacylglycerid and total cholesterol

Table (2) and Figure (2) show that the MDA level of groups G8, rats identified a possible increase 0.254± 0.22µmol/L as an alternative to the control 0.634µmoL, while G2 to G7 demonstrate a noticeable decline 0.654±0.66, 0.594±0.55, 0.456±0.45, 0.334± 0.40, 0.329±0.36 and 0.301± 0.26µmol/L correspondingly compared to G8 0.254± 0.22µmol/L. The LPO level of groups G8 show a significant increase (3.45± 0.33µmol/L) as an alternative to the control 7.43± 0.88 µmoL , G2 to G7 show a noticeable decline (8.54± 0.76,7.76± 0.66,6.93± 0.57,5.87± 0.45,4.92± 0.40 and 4.43± 0.39µmol/L) correspondingly compared to G8 3.45± 0.33µmol/L. The GPX level of groups G8 show a noticeable decline 99.64± 0.93µmol/ L as an alternative to the control 55.65± 0.34 µmoL/ while G2 to G7 indicate a significant increase 41.64±0.44, 59.64±0.54, 64.75±0.64,79.63± 0.75,85.15± 0.85 and 88.64±0.88µmol/L respectively compared with G8 99.64± 0.93µmol/L.

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Table 2. Synergistic effect of basil, mint extracts and zinc oxide nanoparticles on the MDA , LPO and GPX mol/L.

Groups	MDA mol/L	LPO mol/L	GPX mol/L
		Mean ± SD	
G 1	0.534±0.76	7.43± 0.88	55.65± 0.34
G2	0.654±0.66	8.54± 0.76	41.64± 0.44
G3	0.594±0.55	7.76± 0.66	59.64± 0.54
G4	0.456±0.45	6.93± 0.57	64.75± 0.64
G 5	0.334± 0.40	5.87± 0.45	79.63± 0.75
G6	0.329± 0.36	4.92± 0.40	85.15± 0.85
G7	0.301± 0.26	4.43± 0.39	88.64± 0.88
G8	0.254± 0.22	3.45± 0.33	99.64± 0.93
L.S.D	1.87	1.55	1.45

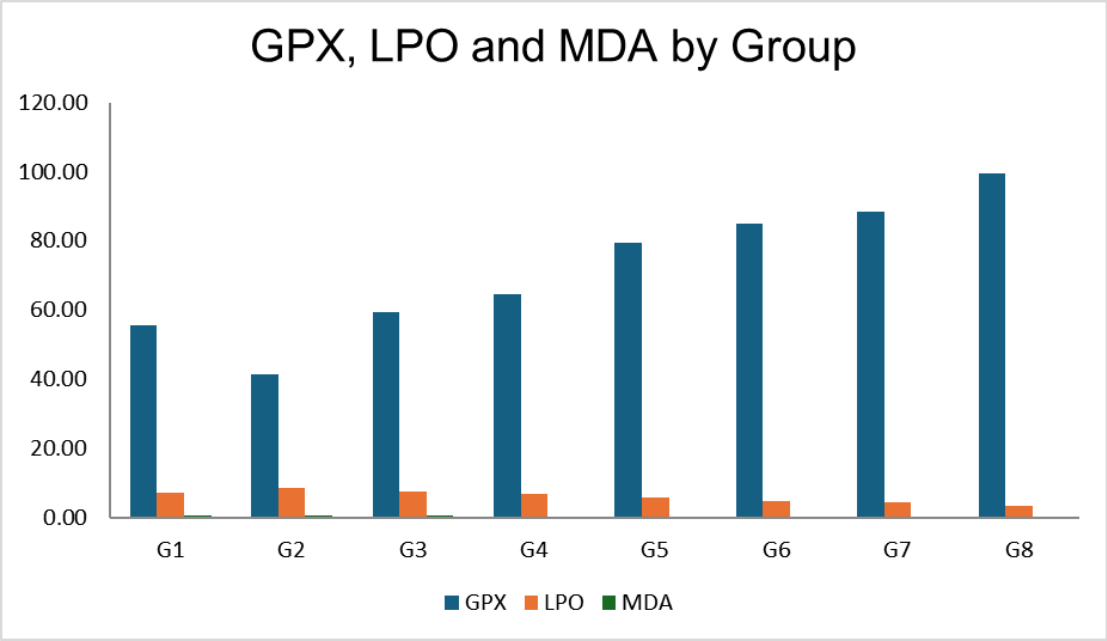

Figure 2. Synergistic effect of basil, mint extracts and zinc oxide nanoparticles on the MDA , LPO and GPX Concentration mol/L.

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4. Discussion

Malondialdehyde (MDA) levels were significantly higher, LPO and GPX enzymatic activity was significantly lower, and total serum cholesterol levels were higher than in the control group. These findings are consistent with the known toxicity mechanism of methotrexate, which is characterized by the production of reactive oxygen species (ROS) and the depletion of the cellular antioxidant system. They also show the presence of acute oxidative stress and disturbance of hepatic lipid metabolism [18]. MDA is a direct indicator of increased per-oxidation of membrane lipids, leading to damage to cellular membranes and loss of hepatic cell integrity [19]. Previous studies have supported that the hepatocyte resulting from MTX is associated with increased oxidative stress and inhibition of antioxidant enzymes, leading to exacerbation of hepatic tissue and functional damage [20]. The decrease in MDA and LPO activity indicates the depletion of the first line of defense against free radicals, Therefore, the decrease in their activity enhances the accumulation of ROS and increases the severity of oxidative damage [21]. Regarding the total cholesterol level, its increase in the MTX group may be attributed to a liver dysfunction responsible for regulating lipid metabolism and lipoprotein synthesis. The liver is the main organ in lipid homeostasis, and any disruption in its structure or functions directly affects blood lipid levels [22]. The treatment with basil extract (*Ocimum basilicum*) showed a significant improvement in the studied indicators, as MDA decreased and LPO and GPX activities increased compared to the MTX group. This is attributed to the presence of phenol compounds and flavonoid in basil, which have strong antioxidant activity that neutralizes free radicals and stimulates the expression of antioxidant enzymes [23] the mint extract (*Mentha* spp.) also showed a similar protective effect, which is attributed to its richness in compounds such as menthol and rosmarinic acid, that possess antioxidant and anti-inflammatory properties, contributing to the reduction of lipid per oxidation and improving cellular defense efficiency [24]. As for zinc oxide nanoparticles (ZnO NPs), they have contributed to enhancing antioxidant enzymatic activity and reducing MDA levels, possibly due to the role of zinc as a co factor in many antioxidant enzymes and its regulation of the gene expression of proteins that protect against oxidative stress. However, their effect largely depends on the dosage used, as they may turn into an oxidizing agent at high concentrations [25]. More importantly, the

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combined treatment (basil + mint + nano zinc) show greater improvement than the individual effects, indicating a synergistic effect that enhances liver protection.

Conclusions

It can be concluded that the combination of plant extracts rich in phenolic compounds and zinc oxide nanoparticles represents a promising strategy to reduce methotrexate-induced hepatotoxicity. This study also recommends conducting future research to elucidate the precise molecular mechanisms of the synergistic effect, determine the optimal safe doses, and assess the applicability of these results in clinical models.

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References

1. Elsawy, H., El-Sayed, Y.S., Ibrahim, S.R. and Mahmoud, A.M. (2020) Naringin alleviates methotrexate-induced liver injury in male albino rats. Bioscience Reports, 40(7), p. BSR20201179. doi:10.1042/BSR20201179.
2. Jafaripour, L., Ahmadvand, H., Babaei, H. and Khosravi, A. (2021) Effects of rosmarinic acid on methotrexate-induced nephrotoxicity and hepatotoxicity in Wistar rats. Indian Journal of Nephrology, 31(3), pp.252–258. doi:10.4103/ijn.IJN_275_20.
3. Abdel-Wahab, W.M., Al-Harizy, R.M. and Abd-Ellah, M.F. (2018) Protective effect of zinc oxide nanoparticles against liver injury. Toxicology Reports, 5, pp.307–312. doi:10.1016/j.toxrep.2018.02.003.
4. Mahmoud, Y.I., Hegazy, H.G. and El-Banna, S.G. (2014) Protective effect of Mentha piperita against oxidative liver injury. Journal of Medicinal Plants Research, 8(10), pp. 450–457.
5. Rehman, M.U., Rashid, S., Arafah, A., Qamar, W., Alsaffar, R.M., Ahmad, A. and Ahmad, S.B. (2019) Multi-targeted approaches for hepatoprotection against drug-induced toxicity. Biomedicine & Pharmacotherapy, 109, pp. 1332–1342. doi:10.1016/j.biopha.2018.10.090.

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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2026; 4(1):*299-309 **Vol. 4, No.1, 2026**
www.zjhms.alzahraa.edu.iq

6. Sakr, S.A. and Al-Amoudi, W.M. (2012) Effect of Ocimum basilicum extract on liver injury in experimental rats. Saudi Journal of Biological Sciences, 19(3), pp. 325–331. doi:10.1016/j.sjbs.2012.02.002
7. Uzar, E., Koyuncuoglu, H.R., Uz, E., Yilmaz, H.R., Kutluhan, S., Kilbas, S. and Gultekin, F. (2011) Methotrexate-induced oxidative stress in liver tissue. Human & Experimental Toxicology, 30(5), pp. 349–356. doi:10.1177/0960327110379243.
8. Vardi, N., Parlakpinar, H., Ates, B., Otlu, A. and Cetin, A. (2010) Protective effects of antioxidants on methotrexate-induced liver injury. Human & Experimental Toxicology, 29(6), pp. 485–492. doi:10.1177/0960327109358733.
9. Al-Ibrahemi, N. and Hasan, R.M. (2019) Identification of artemisinin compound in Artemisia herba alba belong to the Asteraceae by HPLC and GC/MS. Al-Kufa University Journal for Biology, 11(2), p. 32.
10. Al-Ibrahemi, N., Hasan, R.M. and Alslman, K. (2020) Effect of zinc oxide nanoparticles on oxidative stress (malondialdehyde, lipid peroxidation and glutathione). Medico-Legal Update, 20(1), pp. 882–888. doi:10.37506/v20/i1/2020/mlu/194398.
11. Al-Ibrahemi, N., Al-Yassiry, A., Al-Laith, Z.N. and Al-Musawi, B.H. (2022) Chemical analysis of phytochemicals for Anethum graveolens L. fresh and commercial dry by gas chromatography–mass spectrometer. IOP Conference Series: Earth and Environmental Science. 1060(1), p.012089.
12. Naser, N.K., AlMasoody, I.H. and Al-Ibrahemi, N. (2022) Antibiotic and chemical study for Petroselinum sativum L. that belong to Umbellifera family. International Journal of Health Sciences, 6(S6), pp. 6066–6073. doi:10.53730/ijhs.v6nS6.11559.
13. Al-Ibrahemi, N., Al-Laith, Z.N., Al-Yassiry, A. and Al-Masaoodi, N.H. (2022) Phytochemical study of volatile oil for Ocimum basilicum L. and Mentha spicata by gas chromatography technique. IOP Conference Series: Earth and Environmental Science. 1158(6), p. 062004).
14. Al-Jowari, S.A.K. and Alhussainy, I.A.A. (2020) Effect of orange peel powder (Citrus sinensis L.) on lipid profile in normal and experimentally induced oxidative stress albino mice. Faculty of Basic Education Journal, 19(79), pp. 847-855.

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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2026; 4(1):*299-309 **Vol. 4, No.1, 2026**
www.zjhms.alzahraa.edu.iq

15. Uzar, E., Koyuncuoglu, H.R., Uz, E., Yilmaz, H.R., Kutluhan, S., Kilbas, S. and Gultekin, F. (2006) The activities of antioxidant enzymes and the level of malondialdehyde in cerebellum of rats subjected to methotrexate: Protective effect of caffeic acid phenethyl ester. Molecular and Cellular Biochemistry, 291, pp. 63–68. doi:10.1007/s11010-006-9193-7.
16. Babiak, R.M., Campello, A.P., Carieri, E.G. and Oliveira, M.B. (1998) Methotrexate: pentose cycle and oxidative stress. Cell Biochemistry and Function, 16, pp. 283–293. doi:10.1002/(SICI)1099-0844(199812)16:4<283::AID-CBF795>3.0.CO;2-2.
17. Şener, G., Ekşioğlu-Demiralp, E., Çetiner, M., Ercan, F. and Yeğen, B.Ç. (2006) β-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. European Journal of Pharmacology, 542, pp.170–178. doi:10.1016/j.ejphar.2006.05.042.
18. Hassan, W.S., Rabee, D.A., Atallah, A.H., Al-Kaykanee, A. and Jassim, Y.A. (2025) Study the antioxidant of phenol compounds isolated from black tea Camellia sinensis L. in male rats exposed to methotrexate. Edelweiss Applied Science and Technology, 9(4), pp. 677–683.
19. Rabee, D.A., Oleiwi, G.H., Musa, A.H., Al-Ibrahemi, N. and Abdulridha, M.O. (2023) Study protective role of Camellia sinensis L. (black tea) and silver and zinc oxide nanoparticles on antioxidant-oxidant enzymes and biochemical levels against paracetamol overdose in adult male rats. Bionatura, 8(4). doi:10.21931/RB/2023.08.04.82.
20. Salman, T.A., Ahmed, A.T., Oleiwi, G. and Al-Ibrahemi, N. (2023) Study of the effect of oil extract of dill (Anethum graveolens L.) plant on oxidative stress parameters of liver enzymes in male rats. IOP Conference Series: Earth and Environmental Science, 1215(1), p. 012059. doi:10.1088/1755-1315/1215/1/012059.
21. Saleem, M.M.N.M., Mohammad, A.A.W., Al-Amiery, A.A.H. and Hussan, Y.K. (2010) In vivo study of cherry stick effect on concentration of serum total cholesterol, triglyceride and total protein in white albino male mice. Journal of Faculty of Medicine Baghdad. 52(3), pp.342-345.

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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2026; 4(1):*299-309 **Vol. 4, No.1, 2026**
www.zjhms.alzahraa.edu.iq

22. Yokozawa, T., Nakagawa, T. and Kitani, K. (2005) Role of oxidative stress in lipid metabolism disorders. Experimental and Toxicologic Pathology, 57(2), pp. 95–103. doi:10.1016/j.etp.2005.05.002.
23. Wang, Q.X., Lin, H., Jia, H., Zhang, Z. and Wang, C. (2012). Effects of Lactobacillin on serum biochemical indices and antioxidant function in broilers. Chinese Journal of Animal Nutrition, 24(1), pp.131-136.
24. Su, K., Yu, C.Y., Chen, Y.W., Huang, T.Y., Chen, C.T., Wu, H.F. and Chen, S.Y. (2014) Rutin, a flavonoid and principal component of *Saussurea involucrata*, attenuates physical fatigue in a forced swimming mouse model. International Journal of Medical Sciences, 11(5), pp. 528–537. doi:10.7150/ijms.6990.
25. Wang, I. (1993) The protection action of glycyrrhiza flavonoids against carbon tetrachloride hepatotoxicity in mice. Yao Xue Xue Bao, 28, pp. 572–576.