Effect Menthe Volatile Oil Doses Exposed To Oxidative Stress Induced By Paracetamol

Doaa Adil Rabee[1], Hind Faik Mahdi Alshemmary[2] , Hasan Mousa Jaafar[2], Mayada Mahmoud Muteab[3]

Food science Department, College of Agriculture, University of Kerbala, Kerbala, Iraq.
Animal production , College of Agriculture, University of Kerbala, Kerbala, Iraq.
Plant protection, College of Agriculture, University of Kerbala, Kerbala, Iraq.

E-mail: doaa.a@uokerabala.edu.iq , E-mail: hind.f@uokerbala.edu.iq , E-mail: hasan.m@uokerbala.edu.iq , E-mail: m07173013@s.uokerbala.edu.iq

Abstract

Paracetamol overdose is a leading cause of drug-induced hepatotoxicity worldwide, primarily mediated through oxidative stress and depletion of glutathione reserves. Natural antioxidants, such as essential oils, are increasingly investigated as safer alternatives to synthetic hepatoprotective agents. This study aimed to evaluate the antioxidant and hepatoprotective effects of *Mentha spicata* essential oil (MSEO) against paracetamol-induced hepatic injury in rats, with emphasis on dose-dependent efficacy. Sixty male Wistar rats were randomly assigned into four groups (n = 20 each): control, paracetamol-only (500 mg/kg), and two treatment groups receiving MSEO at doses of 150 mg/kg and 250 mg/kg concurrently with paracetamol for five weeks. Serum biomarkers including malondialdehyde (MDA), glutathione (GSH), and glucose were measured at baseline, week 3, and week 5. Paracetamol administration significantly increased serum MDA and glucose levels while depleting GSH, indicating oxidative stress and metabolic imbalance. Co-treatment with MSEO produced a dose-dependent improvement, reducing MDA and glucose concentrations and restoring GSH levels. The higher dose (250 mg/kg) demonstrated the most pronounced protective effects. essential oil exhibits potent antioxidant and hepatoprotective properties against paracetamol-induced oxidative liver injury, likely mediated by its monoterpene-rich phytochemical profile. These findings support its potential as a natural therapeutic agent for managing drug-induced hepatotoxicity.

Keywords: *Mentha spicata*, paracetamol, oxidative stress, glutathione, malondialdehyde, glucose, essential oils

Introduction

Despite its widespread use as a primary analgesic and fever-reducing agent, excessive intake of paracetamol is among the most common causes of acute hepatic failure worldwide.[1] The hepatotoxicity is primarily mediated by the formation of NAPQIa reactive intermediate known to exhaust glutathione reserves and initiate oxidative damage. [2] While synthetic antioxidants are available, their long-term safety and efficacy are limited, prompting the need for natural alternatives with potent antioxidant capacity and minimal side effects.

Essential oils, particularly those derived from aromatic plants such as Mentha spicata, have shown promising antioxidant and hepatoprotective properties. [3][4] Despite the therapeutic potential of *Mentha spicata* volatile oil, its in vivo efficacy in attenuating oxidative stress induced by paracetamol—particularly in a dose-dependent manner—has not been thoroughly investigated. Recognizing the

ISSN 2072-3857

limited prior investigations, the present research explores the biochemical responses to two concentrations of *Mentha spicata* oil in a rat model of induced oxidative stress**.**

Liver injury resulting from pharmaceutical agents remains a major concern in both clinical practice and toxicological research, with paracetamol (acetaminophen) overdose recognized as one of the leading contributors to acute hepatic failure globally. While paracetamol is generally considered a safe and effective antipyretic and analgesic at recommended doses, its excessive consumption results in the biosynthesis of N-acetyl-p-benzoquinone imine (NAPQI), a toxic intermediate implicated in oxidative liver damage, a hepatotoxic metabolite generated through cytochrome P450-mediated pathways. This metabolite depletes intracellular glutathione (GSH), disrupts redox homeostasis, and initiates a cascade of oxidative damage to hepatocytes [5]. The resulting oxidative stress not only impairs liver function but also contributes to systemic metabolic disturbances, including altered glucose regulation and lipid peroxidation.

In recent years, the limitations of synthetic hepatoprotective agents—such as adverse side effects, limited efficacy in chronic use, and high cost—have prompted a growing interest in natural compounds with antioxidant potential. Among these, Due to their intricate chemical makeup and wide-ranging biological properties, essential oils from medicinal plants have gained attention as viable candidates for therapeutic applications [6]. Moreover, this *Mentha spicata* (spearmint) is a perennial aromatic herb from the Lamiaceae family, extensively cultivated across tropical and subtropical climates. Traditionally, it has been utilized for its digestive, antimicrobial, and anti-inflammatory benefits. The essential oil extracted from this plant is notably rich in monoterpenes—including carvone, limonene, and menthol—which have shown strong antioxidant capabilities and protective effects on cellular systems in both in vitro and in vivo experimental settings , In addition to carvone, limonene and menthol, peppermint oil contains other compounds such as flavonoids and phenols, which may contribute to biological activity, and the synergy between these compounds enhances the overall effectiveness of the oil. [7]

Despite the increasing number of studies investigating the antioxidant properties of M. spicata, few have addressed its dose-dependent efficacy in mitigating drug-induced oxidative stress, particularly in the context of paracetamol toxicity. Most existing literature focuses on isolated phytochemicals or crude extracts, with limited attention to the pharmacodynamics of volatile oils and their systemic impact on oxidative biomarkers. Moreover, The dose-dependent influence of essential oils on critical biochemical markers—including malondialdehyde (MDA), glutathione (GSH), and glucose—has not been comprehensively investigated, particularly within the context of controlled animal experiments. [8]

This study aims to bridge a critical gap in current knowledge by evaluating the antioxidant and hepatoprotective efficacy of *Mentha spicata* essential oil in male rats subjected to paracetamol-induced oxidative stress. Two distinct doses of the oil (150 mg/kg and 250 mg/kg) were administered concurrently with paracetamol, and serum biomarkers—malondialdehyde (MDA), glutathione (GSH), and glucose—were monitored over a five-week period. Through this approach, the research seeks to clarify the dose-dependent effects and therapeutic relevance of this phytochemical intervention. The outcomes may inform the development of safer, plant-based alternatives for managing drug-induced hepatic injury., more effective natural remedies for managing oxidative liver damage and offer insights into the broader applications of essential oils in pharmacological and nutraceutical contexts. [9] [10]

ISSN 2072-3857

Furthermore, understanding the biochemical mechanisms underlying the protective effects of M. spicata oil could pave the way for future studies exploring its molecular targets, synergistic interactions with other phytochemicals, and translational relevance in clinical settings. Given the global burden of liver diseases and the increasing reliance on over-the-counter medications like paracetamol, identifying accessible and non-toxic hepatoprotective agents is both timely and essential. [11]

Materials and Methods

Plant Material and Essential Oil Extraction

Fresh aerial parts of ***Mentha spicata*** *L.* were collected from Karbala, Iraq, during May 2022. The harvested material was carefully rinsed with distilled water, shade-dried at 45 °C, and subsequently pulverized into fine powder using a mechanical grinder. Essential oil extraction was carried out via Soxhlet apparatus employing 75% ether as the solvent. Approximately 80 grams of the powdered plant material underwent continuous extraction for 24 hours. Following extraction, the solvent was removed using a rotary evaporator set at 45 °C, yielding a pale green volatile oil. The extraction yield was determined gravimetrically using standard procedures. [12] It should be noted that using of organic solvents in extraction may lead to the extraction of additional compounds other than volatile oils, such as chlorophyll and polyphenols. Therefore, future studies using aqueous extraction methods or steam distillation are recommended to obtain a purer volatile oil.

Experimental Animals

Sixty healthy male Wistar rats (weighing 220–280 g, aged 6–11 weeks) were obtained and acclimatized under controlled laboratory conditions (temperature: 25 ± 2 °C; All animals were provided with unrestricted access to standard laboratory chow and clean drinking water throughout the experimental period. [13]

Table (1): Classification of *M. spicata*

Kingdom	***Plantae***
Divition	*Tracheobionta*
Class	*Magnoliopsidae*
Sub class	*Magnoliopsidae*
Order	*Lamiales*
Family	*Lamiaceae*
Genus	*Mentha*
Species	*Spicata*

Ethical Approval Statement

All experimental procedures involving animals were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the departmental scientific committee at the University of Kerbala.

Experimental Design

A total of 80 male The animals were randomly assigned to four distinct experimental groups, each consisting of 20 rats (n = 20), to promote balanced distribution and reduce potential selection bias.

- **Group 1 (Control):** Administered oral normal saline daily over a five-week period to serve as the baseline reference group
- **Group 2 (Paracetamol-only):** Received an oral dose of paracetamol at 500 mg/kg daily for five weeks to induce hepatic oxidative stress.
- **Group 3 (Low-dose treatment):** Received 150 mg/kg of **M. spicata** essential oil via intraperitoneal injection + 500 mg/kg paracetamol orally.
- **Group 4 (High-dose treatment):** Received 250 mg/kg of **M. spicata** essential oil via intraperitoneal injection + 500 mg/kg paracetamol orally.

Treatments were administered daily for five consecutive weeks. Throughout the

ISSN 2072-3857

study duration, animals were regularly monitored for body weight and general health status to maintain physiological stability and identify any signs of adverse reactions.

Biochemical Analysis

Blood samples were obtained from the retro-orbital plexus at three distinct intervals: prior to treatment initiation (week 0), midway through the intervention (week 3), and upon completion At the conclusion of the experimental period (week 5), blood samples were collected, and serum was separated by centrifugation at 3000 rpm for 15 minutes. The isolated serum was then preserved at −20 °C pending subsequent biochemical analyses.

The following parameters were assessed to determine oxidative stress and hepatic function:

Malondialdehyde (MDA): The concentration of MDA, a key marker of lipid peroxidation, was determined through a colorimetric assay based on its reactivity with thiobarbituric acid, forming a measurable chromogenic complex , a well-established method for assessing oxidative damage. [14]

Glutathione (GSH): Measured using Ellman's reagent (DTNB) method [15]

Glucose: Determined enzymatically using glucose oxidase-peroxidase colorimetric assay. [16]

All assays were performed in triplicate to ensure reproducibility.

Statistical Analysis

Quantitative data were expressed as mean values ± standard deviation (SD). Comparative analysis among experimental groups was conducted using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post-hoc test to determine pairwise differences. A p-value of ≤ 0.05 was considered statistically significant. All statistical procedures were performed using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA).

Results and Discussion

1. Effect on Malondialdehyde (MDA) Levels

Malondialdehyde (MDA) is widely recognized as a key biomarker for lipid peroxidation and oxidative stress, providing insight into the degree of cellular damage induced by reactive oxygen species (ROS). In the present study, animals receiving paracetamol alone (Group 2) demonstrated a marked increase in serum MDA concentrations relative to the untreated control group (Group 1), indicating enhanced oxidative damage. particularly at weeks 3 and 5 ($p \leq 0.05$). This finding aligns with previous reports indicating that paracetamol overdose disrupts mitochondrial function and promotes ROS-mediated lipid degradation. [17][18]

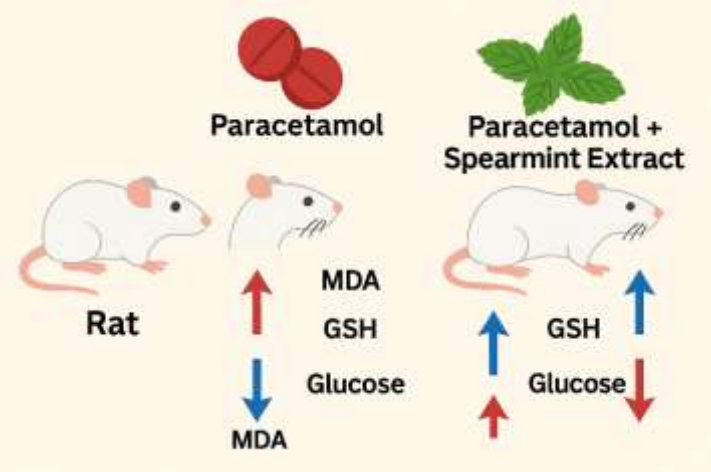
Conversely, co-treatment with *Mentha spicata* essential oil at both 150 mg/kg (G3) and 250 mg/kg (G4) resulted in a dose-dependent reduction in MDA concentrations. The high-dose group (G4) showed the most pronounced decrease, suggesting enhanced antioxidant efficacy. These results support the hypothesis that M. spicata oil contains potent free radical scavengers—such as carvone and limonene—that inhibit lipid peroxidation and stabilize cellular membranes. [19] [20]

ISSN 2072-3857

Table (2) Effect menthe volatile oil dose on the MDA Concentration mol/L

Group	Zero Time	Week 3	Week 5
G1 (Control)	0.100 ± 1.533	0.160 ± 1.650	0.178 ± 1.432
G2 (Paracetamol-only)	0.630 ± 1.013	0.135 ± 1.813	0.266 ± 2.999
G3 (Paracetamol + MSEO 150 mg/kg)	0.333 ± 1.512	0.159 ± 1.142[a]	0.133 ± 0.612[a]
G4 (Paracetamol + MSEO 250 mg/kg)	0.033 ± 1.676	0.025 ± 1.119[a]	0.016 ± 0.891[a]
LSD	—	—	**3.34**

- A different little letter denotes a substantial change./ L
- $p \leq 0.05$



The graphical representation illustrates the hepatoprotective efficacy of *Mentha spicata* essential oil against paracetamol-induced hepatic toxicity in a rat model.

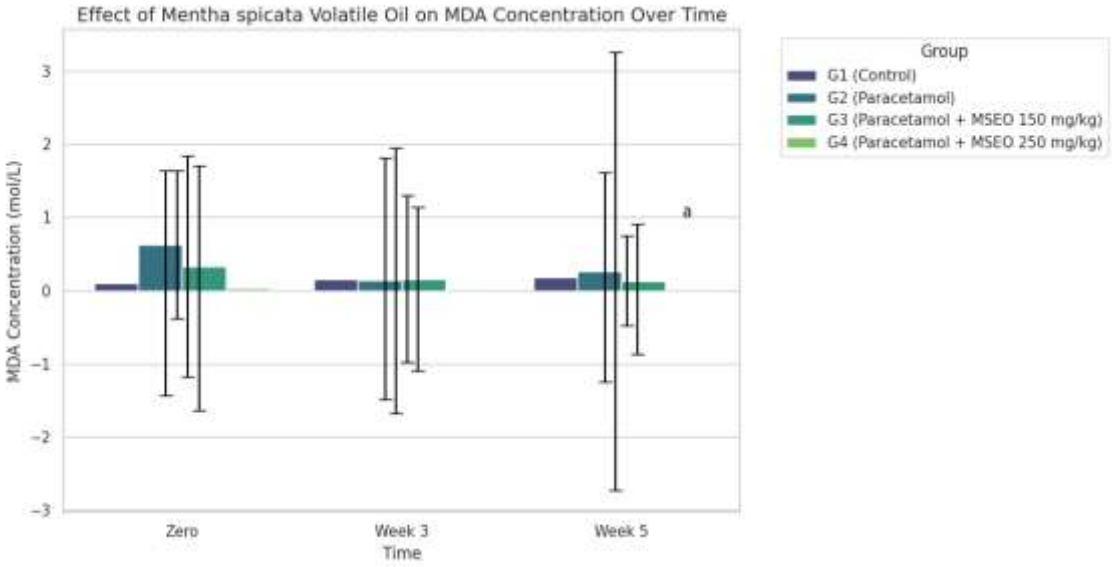


Figure 1 Effect of *Mentha spicata* Volatile Oil on MDA Levels MDA values (mean ± SD, n = 20) were assessed across four groups: G1 (Control), G2 (Paracetamol), G3 (Paracetamol + MSEO 150 mg/kg), and G4 (Paracetamol + MSEO 250 mg/kg). Significant differences from the paracetamol group (G2) are indicated by lowercase letters (LSD = 3.34, $p \leq 0.05$)**.**

ISSN 2072-3857

2. Effect on Glutathione (GSH) Levels

Glutathione (GSH) is essential for neutralizing NAPQI, the hepatotoxic byproduct of paracetamol metabolism, and sustaining cellular redox homeostasis. In the paracetamol-treated group (G2), a marked reduction in GSH levels was observed, This pattern signifies an increase in oxidative stress accompanied by impaired antioxidant defense mechanisms [1]. The observed reduction in glutathione levels is attributed to its conjugation with N-acetyl-p-benzoquinone imine (NAPQI), ultimately resulting in depletion and hepatocellular damage.

Treatment with M. spicata oil markedly restored GSH levels in both G3 and G4 groups, with the higher dose yielding superior results. The observed restoration of glutathione levels may be linked to the capacity of *Mentha spicata* essential oil to enhance the activity of endogenous antioxidant enzymes, including glutathione peroxidase (GPx) and glutathione S-transferase (GST). Additionally, the oil likely contributes bioactive phytochemicals that facilitate the biosynthesis of GSH, thereby reinforcing the cellular antioxidant defense system. [21] [22]. These findings underscore the therapeutic potential of M. spicata in enhancing cellular resilience against oxidative insults.

Table (3) Effect menthe volatile oil dose on the GSH Concentration mol/L:

Group	Zero Time	Week 3	Week 5
G1 (Control)	0.255 ± 3.067	0.259 ± 3.029	0.180 ± 3.654
G2 (Paracetamol-only)	0.980 ± 3.220	0.089 ± 2.310[a]	0.450 ± 2.965[a]
G3 (Paracetamol + MSEO 150 mg/kg)	0.187 ± 3.890	0.220 ± 5.340[a]	0.278 ± 7.320[a]
G4 (Paracetamol + MSEO 250 mg/kg)	0.111 ± 3.323	0.267 ± 4.187[a]	0.312 ± 5.143[a]
LSD	—	—	**2.40**

- A different little letter indicates a substantial change.
- $p \leq 0.05$

ISSN 2072-3857

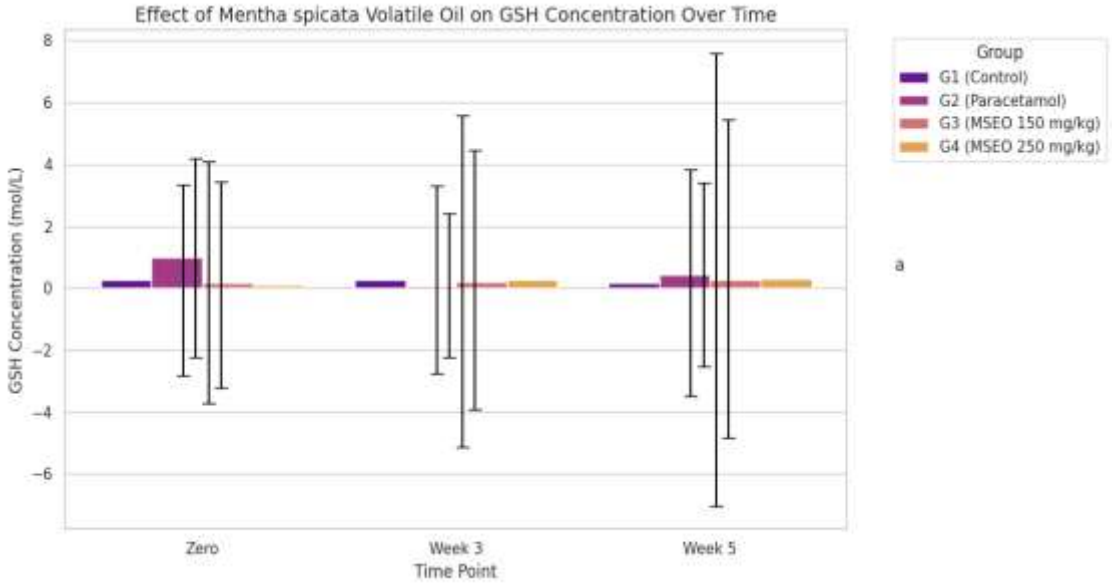


Figure 2. Effect of *Mentha spicata* Volatile Oil on GSH Concentration Over Time

GSH concentrations (mol/L) were measured at Zero time, Week 3, and Week 5 across four groups: G1 (Control), G2 (Paracetamol), G3 (Paracetamol + MSEO 150 mg/kg), and G4 (Paracetamol + MSEO 250 mg/kg). Data are expressed as mean ± standard deviation (SD) for each group (n = 20). Significant differences relative to the paracetamol-treated group are denoted by lowercase letters (a), as determined by the least significant difference (LSD) test with a threshold of 2.4 and a significance level of $p \leq 0.05$.

Table (4) phytochemical of M. spicata

Phytochemical tests	Results
Alkaloid	+
Flavonoid	+
Saponin	-
Phenol	+
Glycoside	+

These phytochemical tests reveal the presence of active compounds such as flavonoids and phenols, which are known for their antioxidant properties. This partially explains the protective effect of peppermint oil against oxidative stress.

3. Effect on Glucose Levels

Hyperglycemia is a secondary consequence of oxidative stress, often resulting from pancreatic β-cell dysfunction and impaired insulin signaling. In this study, rats exposed to paracetamol (G2) showed a significant increase in serum glucose levels, particularly at week 5. This elevation may be linked to ROS-mediated damage to pancreatic tissue and inhibition of insulin secretion, as previously reported in oxidative models. [23]

Notably Rats treated with *Mentha spicata* essential oil (Groups G3 and G4) demonstrated a notable reduction in serum glucose concentrations relative to the paracetamol-only

ISSN 2072-3857

group (G2), indicating a potential regulatory influence on glucose metabolism. This hypoglycemic effect is likely associated with the oil's rich antioxidant profile and its capacity to mitigate oxidative stress–related metabolic disturbances, which are hypothesized to support pancreatic integrity and improve insulin responsiveness. Such mechanisms likely contribute to the restoration of glucose homeostasis under conditions of oxidative stress. These results are consistent with studies showing that plant-derived antioxidants can modulate glucose transport and reduce hyperglycemia in oxidative stress conditions . [24] [25]

Table (5) Effect menthe volatile oil dose on the Glucose mol/L

Group	Zero Time	Week 3	Week 5
G1 (Control)	0.180 ± 4.55	0.233 ± 4.533	0.279 ± 4.323
G2 (Paracetamol-only)	0.209 ± 4.76	0.298 ± 6.11[a]	0.312 ± 6.23[a]
G3 (Paracetamol + MSEO 150 mg/kg)	0.212 ± 4.56	0.156 ± 3.90[a]	0.134 ± 3.33[a]
G4 (Paracetamol + MSEO 250 mg/kg)	0.254 ± 4.27	0.177 ± 4.32[a]	0.123 ± 3.98[a]
LSD	—	—	**1.98**

- A different little letter indicates a substantial change.
- $p \leq 0.05$

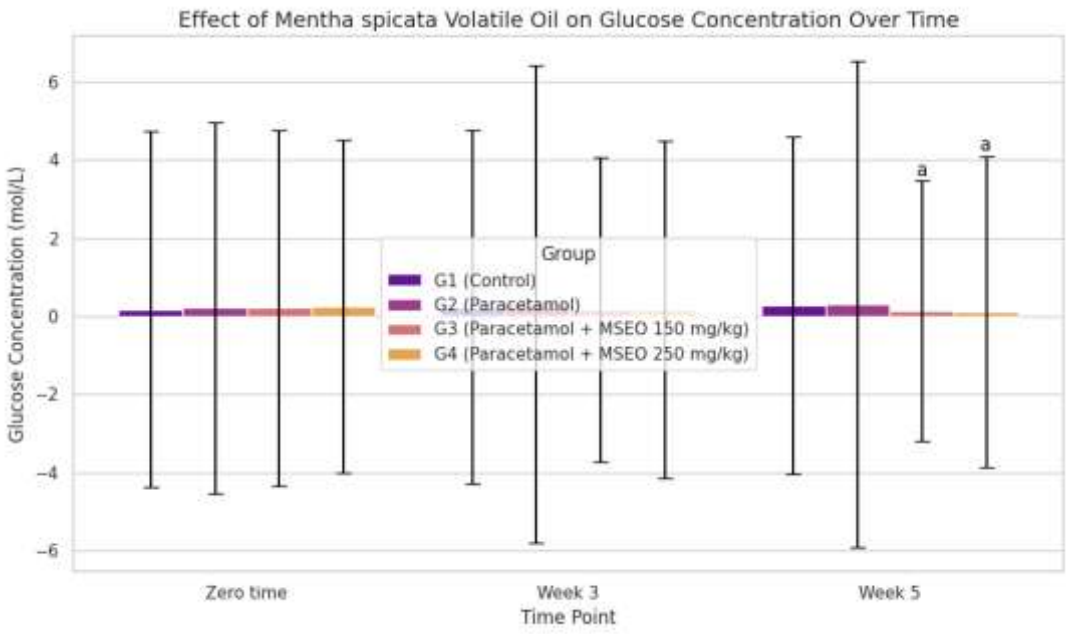


Figure 3. Effect of *Mentha spicata* Volatile Oil on Serum Glucose Levels Glucose concentrations (mol/L) were measured at baseline, Week 3, and Week 5 across four groups: G1 (Control), G2 (Paracetamol), G3 (Paracetamol + MSEO 150 mg/kg), and G4 (Paracetamol + MSEO 250 mg/kg). Data are presented as mean ± standard deviation (SD) for each group (n = 20). Statistically significant differences compared to the paracetamol-only group (G2) are indicated by lowercase letters (a), based on a least significant difference (LSD) value of 1.98 and a significance threshold of $\mathbf{p} \leq 0.05$.

ISSN 2072-3857

Integrated Interpretation

The overall findings of this study demonstrate that *Mentha spicata* essential oil confers a broad-spectrum protective effect against oxidative stress induced by paracetamol administration, highlighting its potential as a multifaceted antioxidant intervention. By reducing MDA levels, restoring GSH concentrations, and normalizing glucose metabolism, the oil demonstrates both hepatoprotective and metabolic regulatory properties. The dose-dependent nature of these effects further validates the therapeutic relevance of volatile oils and highlights the importance of optimizing phytochemical dosing in experimental pharmacology.

These results add to the expanding scientific literature advocating the therapeutic application of aromatic plant-derived extracts in the mitigation of drug-induced toxicological effects. Moreover, they open avenues for future research into the molecular mechanisms of M. spicata, including its influence on gene expression, enzyme activity, and inflammatory pathways. Given the global burden of liver diseases and the widespread use of paracetamol, identifying safe, plant-based interventions is both timely and clinically significant. [26]

The observed restoration of GSH levels and reduction in MDA The observed biochemical concentrations suggest that *Mentha spicata* essential oil may exert regulatory effects on endogenous antioxidant defense mechanisms. Specifically, Monoterpenes such as carvone and limonene, which are principal components of *Mentha spicata* essential oil, have been shown to activate nuclear factor erythroid 2–related factor 2 (Nrf2), a critical transcriptional regulator responsible for maintaining cellular redox homeostasis. Activation of Nrf2 promotes the transcriptional upregulation of key antioxidant enzymes, notably glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), thereby strengthening the cellular defense system against oxidative damage, thereby contributing to the mitigation of oxidative stress and preservation of cellular integrity. [18] [19] This mechanism may underlie the hepatoprotective effects observed in the treated groups.

Unlike previous studies that relied on crude extracts or single-dose evaluations, this study employs a dose-dependent design using purified volatile oil, allowing for a more nuanced understanding of its therapeutic range. The inclusion of multiple time points (baseline, week 3, week 5) further strengthens the temporal resolution of the biochemical changes, offering insights into both immediate and sustained effects.

The findings of this study clearly demonstrate that *Mentha spicata* essential oil exerts a significant protective effect against paracetamol-induced oxidative stress and hepatic injury in male rats. This hepatoprotective action is likely mediated through the oil's antioxidant properties, which contribute to the preservation of cellular integrity and modulation of key biochemical pathways involved in detoxification and redox balance. Paracetamol administration led to marked elevations in malondialdehyde (MDA) and glucose levels, alongside a substantial depletion of glutathione (GSH), confirming its role in generating reactive oxygen species (ROS) and disrupting hepatic antioxidant defenses. Co-treatment with M. spicata oil resulted in a dose-dependent restoration of GSH and reduction in MDA and glucose concentrations, indicating potent antioxidant and hepatoprotective properties.

Future investigations should aim to clarify the molecular mechanisms underlying the protective effects of ***Mentha spicata*** essential oil, with particular emphasis on its regulatory impact on gene expression, enzymatic antioxidant activity, and inflammatory signaling pathways. Moreover, comprehensive long-term safety evaluations and well-designed

ISSN 2072-3857

clinical trials are essential to confirm its therapeutic relevance in human populations and to assess its potential synergistic interactions with other bioactive phytochemicals.

Because the volatile oil used is a mixture of multiple compounds, identifying the compound responsible for the biological activity requires further studies to isolate and evaluate each compound individually.

Future studies should investigate the molecular targets of M. spicata oil using transcriptomic or proteomic approaches to identify key regulatory genes and pathways. Additionally, exploring its synergistic potential with other phytochemicals or its formulation into nanoemulsions may enhance bioavailability and therapeutic efficacy.

Reference

[1] Schmidt LE, Dalhoff K. Serum phosphate is an early predictor of outcome in severe acetaminophen-induced hepatotoxicity. Hepatology. 2002;36(3):659–65. https://doi.org/10.1053/jhep.2002.35400

[2] Lawrence BM, editor. Mint: The Genus Mentha. 1st ed. Boca Raton: CRC Press; 2006. https://doi.org/10.1201/9780849307980

[3] Tang HP, Zhu EL, Bai QX, Wang S, Wang ZB, Wang M, Kuang HX. Mentha haplocalyx Briq. (Mint): A comprehensive review on the botany, traditional uses, nutritional value, phytochemistry, health benefits, and applications. Chin Med**.** 2024;19(1):168. https://doi.org/10.1186/s13020-024-01037-2

[4] Znini M, Cristofari G, Majidi L, Mazouz H, Tomi P, Paolini J, Costa J. Antifungal activity of essential oil from Asteriscus graveolens against postharvest phytopathogenic fungi in apples. Nat Prod Commun. 2011;6(11):1763–8. https://doi.org/10.1177/1934578X1100601147

[5] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol**.** 2003;552(2):335–44. https://doi.org/10.1113/jphysiol.2003.049478

[6] Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, Ming GL. Zika virus infects human cortical neural progenitors and attenuates their growth. Cell Stem Cell. 2016;18(5):587–90. https://doi.org/10.1016/j.stem.2016.02.016

[7] Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, De Feo V. *Mentha spicata* essential oil: Chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of Vibrio spp. strains. Molecules**.** 2015;20(8):14402–24. https://doi.org/10.3390/molecules200814402

[8] Shawon SI, Reyda RN, Qais N. Medicinal herbs and their metabolites with biological potential to protect and combat liver toxicity and its disorders: A review. Heliyon. 2024;10(3):e25340. https://doi.org/10.1016/j.heliyon.2024.e25340

[9] Brahmi M, Adli DEH, Slimani M, Arabi W, Taibi N, Kahloula K. Evaluation of the therapeutic effects of *Mentha spicata* essential oil at the liver level in developing Wistar rats co-exposed to lead and manganese. Carpath J Food Sci Technol**.** 2019;11(2):12–20. https://doi.org/10.34302/crpjfst/2019.11.2.12

[10] Hameed HA, Hassan AF. The hepatoprotective effect of omega-7 against paracetamol-induced hepatotoxicity in male rats. Iraqi J Pharm Sci**.** 2023;32(3):85–94. https://doi.org/10.31351/vol32iss3pp85-94

ISSN 2072-3857

[11] Mahendran G, Verma SK, Rahman LU. The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata L.*): A review. J Ethnopharmacol. 2021;278:114266. https://doi.org/10.1016/j.jep.2021.114266

[12] Al-Ibrahemi N, Hasan RM, Alslman K. Effect of zinc oxide nanoparticles on oxidant stress (malondialdehyde MDA, lipid peroxidation level LPO) and antioxidant glutathione (GSH). Med Leg Update. 2020;20(1):882–8. https://doi.org/10.37506/mlu.v20i1.229

[13] Kadhim A, Awahd H, Kaabi HA, Abass Z, Kadhim M, Abbas S, Jawad H. Green synthesis and biological inhibitory activity of edible nanocoatings by plant leaf extracts. Arab J Med Aromat Plants**.** 2024;10(1):70–84. https://doi.org/10.48347/IMIST.PRSM/AJMAP-V10I1.49227

[14] Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges**.** Anal Biochem. 2017;524:13–30. https://doi.org/10.1016/j.ab.2016.10.021

[15] Giustarini D, Fanti P, Matteucci E, Rossi R. Micro-method for the determination of glutathione in human blood**.** J Chromatogr B Analyt Technol Biomed Life Sci**.** 2014;964:191–4. https://doi.org/10.1016/j.jchromb.2014.02.018

[16] Xu B, Xiang X, Ding Z, Luo Z, Huang J. A colorimetric assay for detection of glucose by enzymatic etching of triangular gold nanosheets. Mater Chem Phys**.** 2023;301:127619. https://doi.org/10.1016/j.matchemphys.2023.127619

[17] Gutiérrez RM, Solís RV. Hepatoprotective and inhibition of oxidative stress in liver of Prostechea michuacana. Rec Nat Prod**.** 2009;3(1):46–51. https://doi.org/10.1016/S1876-5300(09)60006-7

[18] Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem. 2017;86:715–48. https://doi.org/10.1146/annurev-biochem-061516-045037

[19] Ramadana SW. Hubungan loan to deposit ratio (LDR) dan return on asset (ROA). HEI EMA J Riset Huk Ekon Islam Ekon Manaj Akuntansi**.** 2022;1(1):86–95. https://doi.org/10.61393/heiema.v1i1.118

[20] Kumar D, Kumar S, Singh N. Phytochemical and pharmacological profile of Mentha species: A review. J Ethnopharmacol. 2021;274:114045. https://doi.org/10.1016/j.jep.2021.114045

[21] Li J, Zhang G, Qi S, Li X, Peng X. Concentrations, enantiomeric compositions, and sources of HCH, DDT and chlordane in soils from the Pearl River Delta, South China. Sci Total Environ. 2006;372(1):215–24. https://doi.org/10.1016/j.scitotenv.2006.09.023

[22] Manal AK, Advani JH, Srivastava R. Bifunctional acid-base zirconium phosphonate for catalytic transfer hydrogenation of levulinic acid and cascade transformation of furfural to biofuel molecules. ChemCatChem**.** 2022;14(17):e202200576. https://doi.org/10.1002/cctc.202200576

[23] Rocha LA, Robertson DR, Roman J, Bowen BW. Ecological speciation in tropical reef fishes. Proc Biol Sci**.** 2005;272(1563):573–9. https://doi.org/10.1098/rspb.2004.3005

[24] Surai PF. Natural antioxidants in poultry nutrition: New developments. In: Proceedings of the 16th European Symposium on Poultry Nutrition; 2007 Aug 26–30; Beekbergen, The Netherlands. Beekbergen: World Poultry

Science Association; 2007. p.26–30. https://doi.org/10.13140/RG.2.2.22864.79364

[25] Kushwah DS, Salman MT, Singh P, Verma VK, Ahmad A. Protective effects of ethanolic extract of Nigella sativa seed in paracetamol-induced acute hepatotoxicity in vivo. Pak J Biol Sci. 2014;17(4):517–22. https://doi.org/10.3923/pjbs.2014.517.522

[26] Rabee DA, Oleiwi GH, Musa BAH, Al-Ibrahemi N, Abdulridha MO. Study of the protective role of Camellia sinensis L. (black tea) and silver, Zn oxide nanoparticles on antioxidant–oxidant enzymes and biochemical levels against paracetamol overdose in adult male rats. Bionatura. 2023;8(4):82. https://doi.org/10.21931/RB/2023.08.04.82