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Protective effects of *Mentha* leaves' extract against ethanol-induced liver damage and inflammatory response in mice

Eman Mobder Nayif[1], Hashim Abed Abass[2], Aamer Mousa Ali[3,*], Hayder H. Abed[4]

[1]Department of Biology, College of Science, University of Babylon, Hillah, Iraq
[2]Department of Pathology, College of Medicine, University of Diyala, Baqubah, Iraq
[3]Department of Pharmaceutics, College of Pharmacy, University of Babylon, Hillah, Iraq
[4]Department of Chemistry and Biochemistry, College of Medicine, Al-Muthanna University, Samawah, Iraq

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*** CORRESPONDING AUTHOR:**
Aamer Mousa Ali, Department of Pharmaceutics, College of Pharmacy, University of Babylon, Hillah, Iraq; e-mail: pharm.aamer.mousa@uobabylon.edu.iq

ABSTRACT

The mint (*Mentha* spp.) leaf extract plays a pivotal role in enhancing immunological functions, notably by stimulating the activity of macrophages and natural killer cells; key components in pathogen recognition and elimination. This study aimed to investigate the anti-inflammatory properties of mint leaf extract in a murine model of ethanol-induced inflammation, with particular emphasis on its modulatory effects on major inflammatory cytokines: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). Male mice were randomly assigned to four groups: control, ethanol-only, and two experimental groups receiving mint extract at doses of 100 mg/kg and 200 mg/kg, respectively, in combination with ethanol for 30 consecutive days. Serum cytokine concentrations were quantified using ELISA. Ethanol administration resulted in a significant elevation of the TNF-α, IL-6, and IL-1β levels. Co-treatment with the mint extract at both 100 mg/kg and 200 mg/kg attenuated these cytokine levels, with the higher dose demonstrating a more pronounced effect. These findings indicate that the mint extract exerts a dose-dependent anti-inflammatory response and may hold therapeutic promise for mitigating ethanol-induced inflammation and other cytokine-mediated inflammatory conditions.

1. Introduction

Mint (*Mentha* spp.) is a medicinal plant belonging to the Lamiaceae family, widely recognized for its aromatic properties and diverse

PHARMAKEFTIKI, 37, 2S, 2025 | 367-371

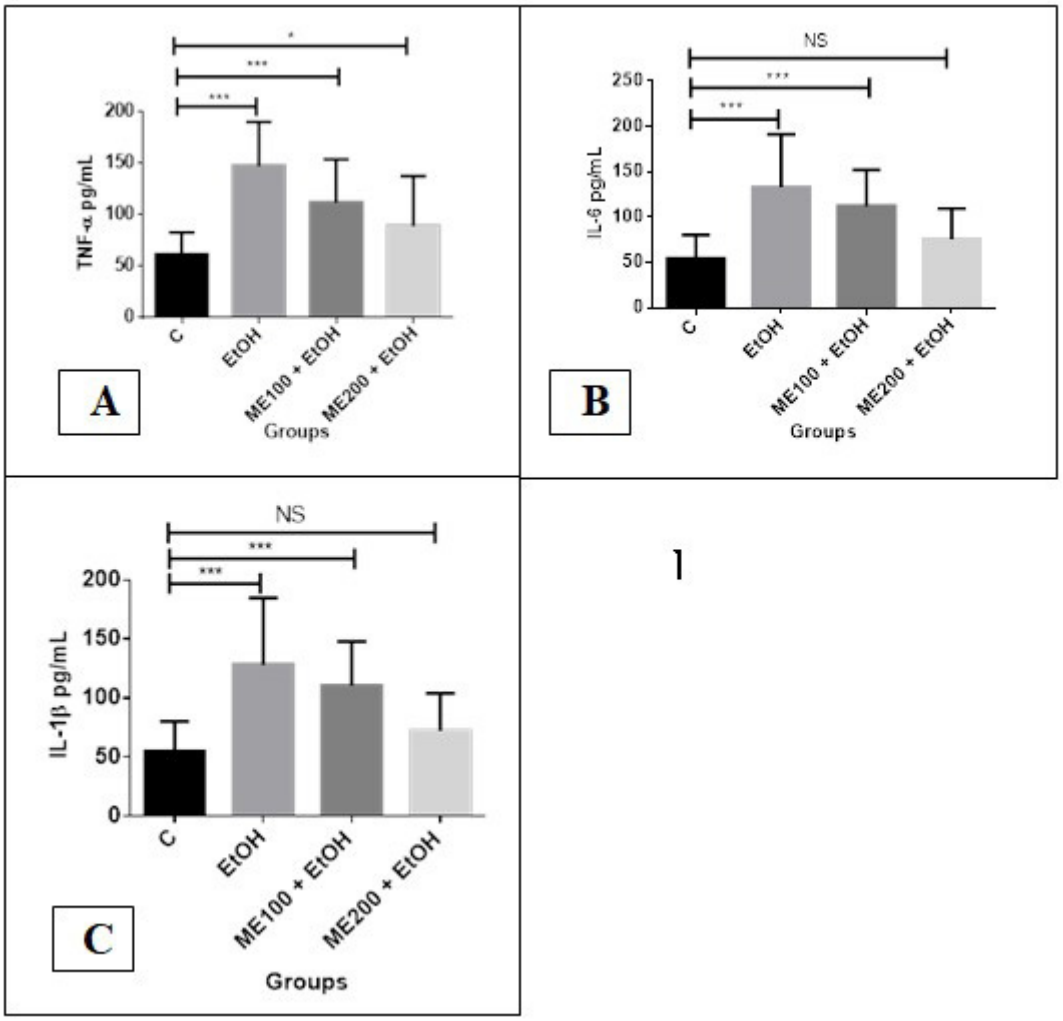

Figure 1. *Cytokine levels across experimental groups: TNF-α (A), IL-6 (B), and IL-1β (C). Statistical significance was determined by one-way ANOVA: *, p<0.05; **, p<0.01; ***, p<0.001; NS, non-significant. Group definitions: EtOH, ethanol (20%) only; ME100 + EtOH, mint extract (100 mg/kg) with ethanol (20%); ME200 + EtOH, mint extract (200 mg/kg) with ethanol (20%). Other abbreviations used: IL-1β, interleukin 1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha.*

health benefits. Mint leaves are extensively used for both culinary and therapeutic purposes[1]. They contain bioactive compounds such as rosmarinic acid and flavonoids, which exhibit antioxidant and antibacterial activities[2]. These constituents are particularly effective against respiratory infections, as mint inhibits bacterial proliferation within the body.

Moreover, the anti-inflammatory components of mint – especially rosmarinic acid – help attenuate inflammation, promote a balanced immune response, and prevent chronic inflammatory disorders[3]. Mint also contains antioxidant vitamins, notably vitamin C and vitamin A, which protect immune cells from oxidative damage, thereby preserving their function and enhancing overall immunological efficacy[4].

Mint modulates immune cell activity, including the

PHARMAKEFTIKI, 37, 2S, 2025 | 367-371

activation of macrophages and natural killer cells, both of which are essential for pathogen recognition and clearance[5]. Additionally, mint promotes the production of immune-enhancing cytokines, thereby strengthening the host's defence against infections[6].

This study investigates the anti-inflammatory effects of mint leaf extract in a murine model of ethanol-induced hepatic inflammation, with a focus on its regulatory impact on key pro-inflammatory cytokines: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β).

2. Methodology

Dried mint leaves were pulverized into a fine powder using a blender. A total of 250 g of powdered leaves was added to a flask containing 250 mL of ethanol. The mixture was heated to 50°C and stirred continuously for 18 h. The solution was then filtered through a Büchner funnel, and the filtrate was concentrated using a rotary evaporator.

A total of 120 male mice (weighing 30–40 g) were housed under controlled environmental conditions. The study protocol was approved by the College of Pharmacy of the University of Babylon, under ethical clearance number A-0048 (April 2024).

Mice were randomly allocated into four experimental groups (n=30 per group): (i) group 1 (C) acting as a negative control group (standard feeding), (ii) group 2 (EtOH) receiving ethanol (20% v/v) in the drinking water for 30 days in order to induce hepatic and systemic inflammation, (iii) group 3 (ME100 + EtOH) receiving the mint Extract (at 100 mg/kg) co-administered with ethanol, and (iv) group 4 (ME200 + EtOH) receiving the mint extract (at 200 mg/kg) co-administered with ethanol. All treatments were administered orally once daily for 30 consecutive days.

Serum levels of TNF-α, IL-6, and IL-1β were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabscience), following the manufacturer's protocol. Cytokine concentrations were expressed in pg/mL[7].

Statistical analyses were performed using GraphPad Prism (version 6) and Microsoft Excel 2020. Intergroup differences were assessed using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons.

3. Results and Discussion

This study evaluated the anti-inflammatory efficacy of mint extract in a murine model of ethanol-induced hepatic inflammation, focusing on its impact on the serum TNF-α, IL-6, and IL-1β levels (Figure 1).

Analysis of TNF-α levels revealed significant intergroup differences ($p<0.001$; Figure 1.A). The control group (C) exhibited a mean serum TNF-α concentration of 61.77 ± 20.94 pg/mL. The ethanol group (EtOH) showed a marked elevation to 147.6 ± 42.72 pg/mL ($p<0.001$ *vs.* control). A co-treatment with mint extract at 100 mg/kg (ME100 + EtOH) reduced the serum TNF-α levels to 112.1 ± 41.76 pg/mL ($p<0.001$ *vs.* ethanol group), while the 200 mg/kg dose (ME200 + EtOH) further reduced the serum TNF-α levels to 89.07 ± 48.50 pg/mL ($p<0.05$ *vs.* ethanol group). These findings suggest a dose-dependent anti-inflammatory effect of the mint extract, with the higher dose offering greater protection against ethanol-induced TNF-α elevation.

The bioactive constituents of the mint extract – particularly terpenoids, flavonoids, and phenolic acids – are likely responsible for these effects. These compounds possess antioxidant properties that neutralize reactive oxygen species, thereby mitigating oxidative stress. Previous studies have demonstrated that phenolic and flavonoid compounds in *Mentha piperita* suppress pro-inflammatory cytokine production and reduce oxidative damage. The present findings support the potential of the *Mentha* extract as a therapeutic agent for ethanol-induced hepatic inflammation, with a clear dose-response relationship[5,8].

IL-6 levels were also significantly affected ($p<0.001$; Figure 1.B). The control group (C) exhibited an average serum IL-6 concentration of 54.50 ± 25.71 pg/mL. Ethanol exposure elevated the serum IL-6 levels to 132.8 ± 58.52 pg/mL ($p<0.001$ *vs.* control). Treatment with ME100 + EtOH reduced the serum IL-6 levels to 112.1 ± 40.06 pg/mL ($p<0.001$ *vs.*

PHARMAKEFTIKI, 37, 2S, 2025 | 367-371

ethanol group), while ME200 + EtOH yielded a mean of 75.83 ± 33.47 pg/mL, though this reduction was not statistically significant.

The undertaken IL-1β analysis revealed significant intergroup differences (p<0.001; Figure 1.C). The Control Group (C) exhibited mean serum IL-1β levels of 55.03 ± 25.25 pg/mL. Ethanol administration increased the serum IL-1β levels to 128.4 ± 56.51 pg/mL (p<0.001 *vs.* control). The ME100 + EtOH co-administration reduced the serum IL-1β levels to 110.8 ± 37.28 pg/mL (p<0.001 *vs.* ethanol group), while the ME200 + EtOH co-administration achieved a further reduction to 72.17 ± 31.71 pg/mL, though this was not statistically significant.

The observed reductions in IL-6 and IL-1β may be attributed to the antioxidant and anti-inflammatory properties of phenolic acids and flavonoids in *Mentha piperita*. These compounds likely modulate immune responses, attenuate oxidative damage, and interfere with inflammatory signalling pathways.

4. Conclusion

This study demonstrates the anti-inflammatory potential of the mint extract in a murine model of ethanol-induced hepatic inflammation. Administration of mint extract at 100 mg/kg significantly reduced the serum levels of TNF-α, IL-6, and IL-1β, indicating its capacity to modulate inflammatory responses. These findings suggest that the mint extract may serve as a natural therapeutic agent for ethanol-induced inflammation, although further research is warranted to optimize dosing and evaluate long-term efficacy.

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Conflicts of interest

None exist.

ORCIDs

0000-0002-1228-2960 (E.M. Nayif); 0009-0003-9879-7117 (H.A. Abass); 0000-0002-1390-9200 (A.M. Ali); 0000-0001-7764-3037 (H.H. Abed)

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PHARMAKEFTIKI, 37, 2S, 2025 | 367-371

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