

Original Article

Comparison between MPO, CD177 and NF- κ B Gene Expression of Septic Rats Treated with *Mentha longifolia* Essential Oils

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Abstract

Background and Aim: Incidences range of sepsis-associated liver dysfunction and lung injury are observed in patients with sepsis. *Mentha longifolia* as a native Iranian plant have many properties such as antimicrobial and antioxidant activity. In this regard, the anti-inflammatory effect of *Mentha longifolia* essential oil (E.O) on liver and lung injury induced by sepsis was investigated.

Materials and Methods: Gene expression of three genes (MPO, CD177 and NF- κ B) as inflammatory factors was evaluated by real-time PCR in four experimental groups (Laparotomy (LAP), cecal ligation and puncture (CLP) and essential oil groups at 50 and 100 mg/kg body weight doses). The treatment was carried out orally for two weeks before CLP surgery. 24 hours after CLP, samples were immediately transferred to -80°C to maintain for further experiments.

Results: Expression of genes increased in the CLP group as compared to the LAP group. Treatments of rats with essential oil have been effective in decreasing genes expression levels in two dosages at two tissues.

Conclusion: Our results demonstrated that *M. longifolia* essential oil treatment could protect against sepsis-induced liver and lung injury by reducing inflammatory factor.

Keywords: Myeloperoxidase, Gene expression, Sepsis

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Introduction

The liver is an organ that plays an important role in metabolic, homeostatic and host-defense activities (1). Hepatocytes play an axial role in the capture and clear invading bacteria and their products in sepsis. Liver injury occurs frequently during the sepsis by several proinflammatory mediators (2, 3). Because of various functions of the organ, liver failure in sepsis has a vital effect on the severity and outcome of sepsis in patients (4).

The lung is another organ at risk of failing during consecutive development of multiple organ dysfunction in sepsis. Acute lung injury is a primary cause of death in septic patients. Excessive cytokine-mediated inflammation plays a crucial role in the pathogenesis of sepsis-induced acute lung injury (5, 6). Furthermore, immunosuppression is signed at the onset of sepsis (7).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is a ubiquitous transcription factor, which plays a key role in regulating immune response

against infection. Increased and/or prolonged activation of NF- κ B has been linked to cancer, inflammatory, autoimmune diseases, septic shock and viral infection (7, 8).

Myeloperoxidase (MPO) is a hallmark of the systemic inflammatory disease and oxidative stress. MPO is one of the main pillars in neutrophils attack of bacteria. Neutrophils are the first immune cell type to react in the host against the pathogen (9, 10). CD177 is a glycosylphosphatidylinositol-anchored glycoprotein expressed by neutrophils. Measurement of CD177 mRNA levels has become a useful diagnostic tool for distinguishing some infectious diseases such as sepsis (11, 12). Accordingly, considering the importance of these enzymes, they are considered as effective markers for sepsis.

Natural resources especially plant as the richest bio-resource of drugs have been used in traditional and modern medicines (13). Labiatae family especially the genus *Mentha* is one of the most employed medicinal plants with antioxidant properties. *Mentha longifolia* is used for the treatment of many diseases as antispasmodic, choleric, antiemetic, emmenagogue, diaphoretic, carminative and anti-inflammatory (14-16). Therefore, the aim of the present study was to evaluate the anti-inflammatory effects of *M. longifolia* essential oils by checking the gene expression profile of MPO, CD177, and NF- κ B on liver and lung tissues of septic rats in cecal ligation and puncture (CLP) model.

Materials and Methods

Animal trials

Forty male Wistar rats (250 \pm 20 g) were purchased from the Pasteur Institute of Iran in these experiments and rats divided into 3 groups including laparotomy (LAP) as control, CLP, and essential oil treatment groups. Animals were kept under standard condition (12 h light/ 12 h dark) at 20 - 25 $^{\circ}$ c for two weeks. Cecal ligation and puncture were used to induce septic liver and lung injury. CLP has been performed as previously described (17). Animals were anesthetized by IP injection of ketamine and xylazine mixture. The incision was made 2-3 cm midline and cecum was exposed. The cecum was ligated by 3-0 silk, punctured twice with a 20-gauge needle. The cecum was repositioned, and

two layers of the abdomen were then closed with 3-0 silk thread. For LAP group the same surgical procedures were done but without ligation and puncture. Then, animals were allowed to recover. This ethics committee was based on the world medical association declaration of Helsinki (Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964). Treatment groups were received essential oil 50 and 100 mg/kg body weight orally for two weeks before CLP surgery. Essential oil purchased from Barij Essence Company with a voucher specimen (Batch No. 3138-031-93/8 (707051) and sample serial No. BE930347). Rats were killed 24 hrs after CLP and liver and lung tissues were collected and processed for Real-time PCR.

Gene expression assay

RNA was isolated from target tissues using RNA isolation kit according to the manufacturer's protocol (BioBasic Inc, Canada). Quality of the total RNA was assessed by NanoDrop 2000 spectrophotometer (Thermo Scientific). Complementary DNA (cDNA) was synthesized with PrimeScriptTM RT reagent kit (Takara Bio Inc, Japan) and oligo dt primers (Takara Bio Inc, Japan). Primers used for PCR and real-time PCR are listed in Table 1. Primers were designed with the Gene Runner Software Version 3.05 and primer 3 servers. cDNA samples were amplified by PCR amplification and then checked by 2.5% agarose gel electrophoresis. Relative-expression carried out that described by Dadkhah *et al.* (18). All reactions were performed in triplicate. GAPDH expression as the internal standard was used to normalize all expression data. COX-2 gene expression was calculated by the formula $2^{-\Delta\Delta Ct}$ as following: $\Delta\Delta Ct = (\Delta Ct \text{ of the experimental test}) - (\Delta Ct \text{ of control test})$. The ΔCt was calculated by subtracting the Ct of the target gene from the Ct of the reference gene (GAPDH).

Statistical analysis

Statistical analyses were performed with one-way ANOVA followed by Tukey's HSD using the SPSS 22.0 software. The significance was considered as $P < 0.05$. All samples were tested in triplicate.

Results and Discussion

Gene expression levels of MPO, CD177, and NF- κ B for liver and lung tissues are shown in Figure 1 and 2. Levels of genes expression were markedly increased

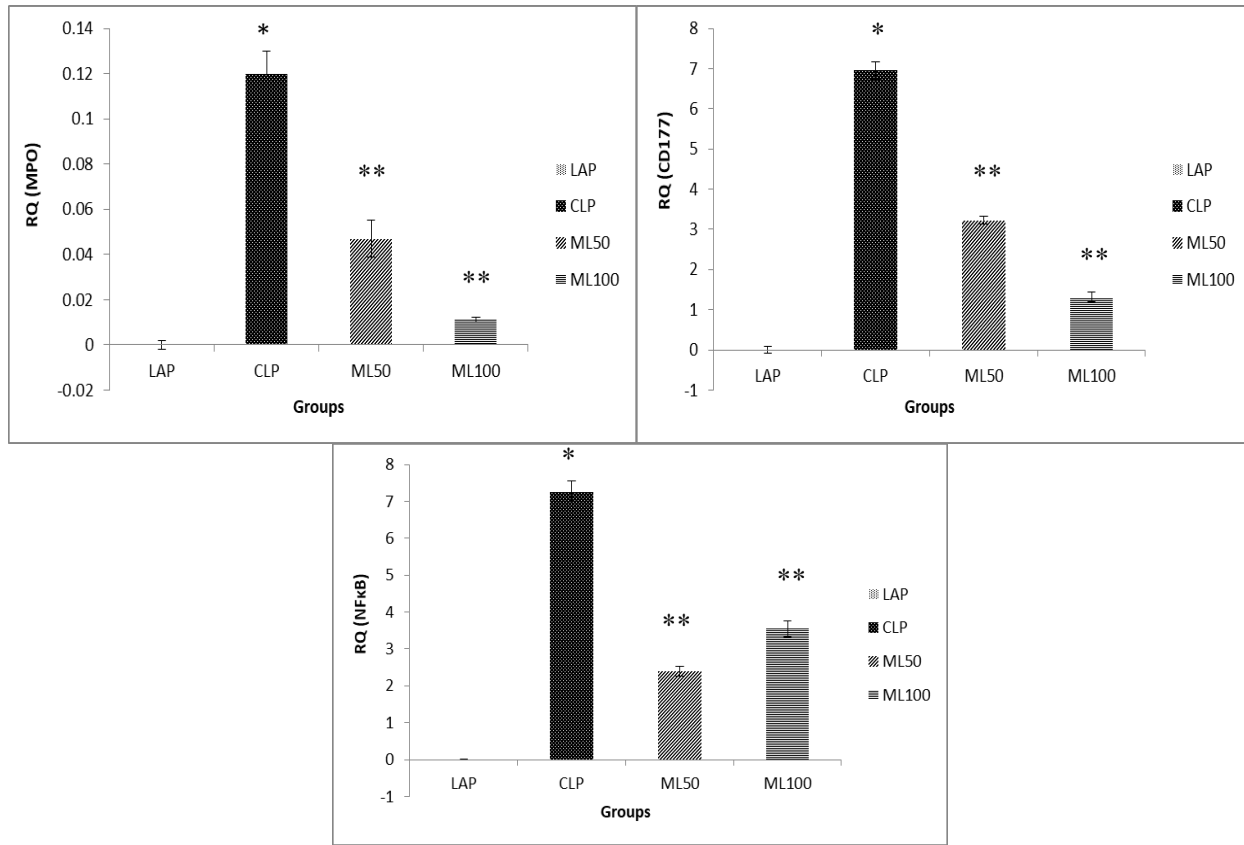


Figure 1. The effects of *M. longifolia* E. Os at 50 and 100 mg/kg b.w doses (ML50 and ML100) on liver MPO, CD177 and NFκB genes expression. *P<0.05 is significantly considered between LAP and CLP group. **P<0.05 is significantly considered between CLP and treated groups. Data are presented as mean ± SD.

in the CLP group compared with the LAP group which decreased (P=0.00). In previous studies mentioned that sepsis could create oxygen-free radicals. The radicals are **led to** cause oxidative stress and multi-organ failure such as liver and lung (18). Our results showed that CLP could alter antioxidant enzymes (GSH, LP, FRAP and MPO) and liver biomarkers (AST and ALT). Furthermore, CLP increased PGE2 and COX-2 as an inflammatory factor (18). Same results confirmed that on antioxidant enzymes and COX-2 expression in the lung. On the other hand, inflammation leads to multiple organ failure and therefore to higher mortality (19).

Sepsis is characterized by both pro and anti-inflammatory responses to an infection with an occurrence of sepsis-induced immunosuppression (20). Neutrophils are essential for innate immunity and response to pathogens. The morbidity and mortality from infections increase in patients with

quantitative or qualitative neutrophil defects, providing clinical confirmation of the important role of neutrophils for human health (21). Lill et al. reported that CD177 is up-regulated on the neutrophil surface upon stimulation and in some conditions associated with increased neutrophil counts (during severe bacterial infections) (22). Kovach and Standiford represented that relationship between sepsis and elevated MPO levels could be explained by leakage of MPO into the plasma when neutrophils phagocyte bacteria (23). The recent study has demonstrated that MPO and CD177 gene expression could change after 48 hrs of CLP surgery (17). Furthermore, NF-κB is an important regulator of pro-inflammatory gene expression. Ding et al. demonstrated that inhibition of NF-κB could be a promising therapeutic target in sepsis (24). Many medicinal plants are used to improve immunological complaints and be used as a replacement for synthetic therapy, for treatment of

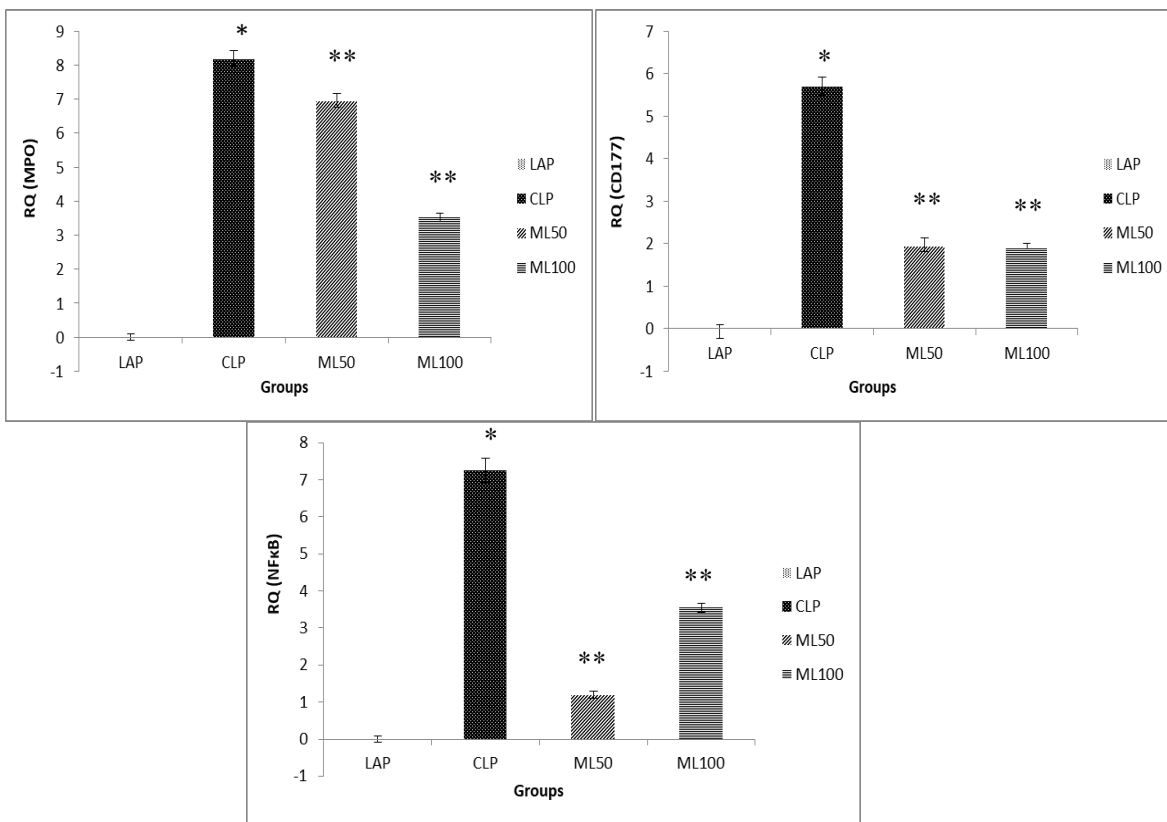


Figure 2. The effects of *M. longifolia* E. Os at 50 and 100 mg/kg b.w doses (ML50 and ML100) on lung MPO, CD177 and NFκB genes expression. *P<0.05 is significantly considered between LAP and CLP group. **P<0.05 is significantly considered between CLP and treated groups. Data are presented as mean ± SD.

Table 1: Primers used in the PCR and Real-time PCR.

Primer	Sequence (5' → 3')	product (bp)
Forward CD177	ATACCAGTGCTGACCCTTCTG	
Reverse CD177	CCTCGCAGGTTTTCTCACC	145
Forward MPO	GCGATAGGTTTTGGTGGGAG	
Reverse MPO	AGCTCACAAAGTCTCGGGG	165
Forward NF-Kβ	CGCAAAGGACCTACGAGAC	
Reverse NF-Kβ	TGGGGAAAACATCAAAG	193
Forward GAPDH	TGCCAGCCTCGTCTCATAG	
Reverse GAPDH	ACTGTGCCGTTGAACTTGC	197

immunological diseases. In some studies have been reported that immunomodulatory activities of medicinal plants could be beneficial in infections and

immune-related diseases (25, 26). *M. longifolia* is an aromatic plant with antioxidant activities (27). This plant has been reported as a useful therapy without

adverse effects to be used in many conditions such as diabetes, amenorrhea and inflammation (28). In the present study, we documented that treat with *M. longifolia* essential oil protected liver and lung against the CLP-induced injury via inhibition of inflammatory response. With *M. longifolia* essential oil treatment, inflammatory gene expression levels were markedly reduced compared with the CLP group ($p=0.00$). It is noteworthy that *M. longifolia* essential oil at a dose of 100 mg/kg b.w was better in diminishing of MPO and CD177 gene expression in liver and MPO expression in lung as compared with the dose of 50 mg/kg b.w (Figs 1, 2). Our results were consistent with previous findings, which showed that *M. longifolia* E.O was able to protect liver injuries against sepsis by suppression of inflammatory reactions and modulating the oxidative stress parameters. Carvone and limonene were major compounds in *M. longifolia* essential oil with antioxidant activity. One study indicated that the *M. longifolia* essential oil with piperitone oxide and piperitenone oxide or carvone and dihydrocarvone compounds possessed strong antimicrobial activity against bacteria, fungi and a yeast species (14). In addition, Liu *et al.* suggested that baicalein treatment could protect against sepsis-induced liver injury (3). Another study indicates that berberine (extracts from Chinese traditional herbs) treatment improved the rate of survival and cognitive disorders in septic rats (29).

Conclusion

Overall, our study provides a new outlook of using natural products and medicinal plants. *Mentha longifolia* essential oil with anti-inflammatory effects could be as a potential therapeutic agent for liver and lung injury in sepsis. Further investigation of other genes that are involved in sepsis seems to be needed for the development of new therapeutic targets and strategies.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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