AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 01, March, 2022

Immunomodulatory and Anti-Inflammatory Effect of Mentha pulegium L

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Keywords:
Mentha pulegium L, Immunomodulator, Anti-inflammatory, Angiogenesis cytokine

ABSTRACT

Used of Immunomodulator and anti-inflammatory drug is a new application in the treatment disease. The most immunomodulator and anti-inflammatory was from medicinal plants. Mentha pulegium L (MTH) is traditional medicinal plant widely used in the world, in this study investigated the Immunomodulatory and anti-inflammatory activity of MTH leaves ethanol extract and its fractions with the effect of angiogenesis cytokine gene expression and production. Macrophage RAW 264.7 cell was used to investigate the Immunomodulatory and anti-inflammatory effect both MTH leaves extract and fractions. The results were showed significant increase in the viability of treated RAW 264.7 cell with MTH leaves extract and fraction1 and inhibited Nitric oxide production, while enhancement the production of angiogenesis cytokine and increased intracellular production of INF- γ , IL6 and IL8 in dose dependent manner. In conclusion of the outcome the M. pulegium L leaves extract be as new Immunomodulation and anti-inflammatorydrug.

1. Introduction

Recently increased and preferred used medicinal plants or the active compounds isolated in the treatment many for their safety and less in side effect compared with the chemical synthetic drugs. Mentha pulegium L is distributed as native herb in North America, Europe, Asia and Middle East [1]. M. pulegium L leaves were rich in compounds high antioxidant and have antimicrobial activity [2], [3]. The GMCS analysis of M. pulegium leaves essential oil was conducted have compounds as limonene, 1,8-cineol, germacrene-D, β-caryophyllene, β-bourbonene, α-terpineol are important in the pharmaceutical used [4]. In recent study was prepared green synthesis of iron oxide, silver, and copper nanopartical from M. pulegium leaves extract was gave an fast, low cost iron oxide particle from natural source [5], [6]. Polyphenol compounds from chloroform, n-butanol, ethyl acetate and water extract from M. pulegium leaves extract were investigated for antioxidant, anticholinesterase and photoprotective [7]. The aim of this study is investigated Immunomodulatory and anti-inflammatory activity of MTH leaves ethanol extract and its fractions with the effect of angiogenesis cytokine gene expression and production.

2. Methods

2.1 Collection of plant and extraction

The Mentha pulegium L (MTH) leaves purchased from local market, the leaves was cleaned and dried then crushed and extracted by ethanol (purchased by Fisher Scientific, UK) and the MTH was fractionated as done by [8].

2.2 Effect of Mentha pulegium L on the viability of RAW 264.7 Cell

The effect of Mentha pulegium L leaves ethanol extract and its fractions on the a murine macrophage RAW 264.7 cell (ATCC, American type culture collection) viability was investigated as described by [8]. The extract was investigated at final concentration (1000, 500, 250, 125) µg/mL for both leaves extract and fractions.

2.3 Nitric oxide production

To evaluate the effect of Mentha pulegium L (MTH) leaves extract on the nitric oxide production from RAW 264.7 cells, the cells were treated with MTH and the fraction was gave the best viability percentage to RAW264.7 cells in different doses at (25,50,100,200) µg/mL by using Griess method as described [9]. RAW 264.7 cells at concentration of 1 x 10^6 cells/well are incubated with LPS at concentration (100 µg/mL) and tested extract or fractions in different doses in 6 well plate for 24 h at 37^o C in condition (5% CO2). A positive control cells are treated with LPS only. Standard curve was done by prepared dilutions of NaNO2. After 24 h incubation period was end, culture media was aspirated from treated wells and centrifuged at 4000 r.p.m for 10 min., 150 µl from supernatant was mixed with 150 µl of Griess reagent (G4410, Sigma Aldrich,USA), then incubated the mixture at room temperature for 30 min in dark place. The absorbance was read at 546 nm.

2.4 Induction of angiogenesis

For the study the effect of Mentha pulegium L (MTH) leaves extract on the enhanced angiogenesis. For analyzing multiple protein expression or cytokine from inducible macrophage, the RAW 264.7 cells applied to the angiogenesis antibody array methods (RayBio C-Series angiogenesis antibody array, USA). The RAW 264.7 cells were treated with 100 µg/mL MTH leaves extract for 24h. The method was done as manufactured instruction, antibody array membrane was placed in the incubation well, 2 mL of blocking buffer was added after incubation for 30 min. at room temperature aspirated it, then 1 mL from cells treated culture media was added to the well and incubated for 3 hours as room temperature. The sample was aspirated at the end of incubation then was the membrane with 2 mL of 1x wash buffer1, incubate 5 min. at room temperature (repeat 3 times), washed again in the same manner with wash buffer2. Biotinylated antibody cocktail at 1 mL was added to well and incubated for 2 h. at room temperature, Biotinylated antibody cocktail was aspirated and the wash step was repeated. Two mL of 1x HRP-Streptavidin was pipetted to well, incubated 2h. at room temperature after incubation was finished HRP-Streptavidin was aspirated then wash was repeated. The membrane was transfer to plastic sheets and 500 µL of detection buffer was added then the membrane was transferred to chemilluminescence imaging system and expose to get the result.

2.5 Intracellular expression of INF- γ, IL6 and IL8

To estimated effect of Mentha pulegium L (MTH) leaves extract and MTHF1 on the intracellular expression of INF- γ , IL6 and IL8 in the macrophage RAW 264.7 cells. The cells were treated with (50 and 100) µg/mL form each MTH leaves extract and MTHF1 and the method was performed by BD Cytofix/Cytoperm^{TM}Plus Fixation/Permeabilization,BD Golgi Plug^{TM} protein transport inhibitor,Cat.No.555028, the method was done as described previously by [8].

2.6 Statistical Analysis

ISSN: 0005-2523
Volume 62, Issue 01, March, 2022

Results data were presented as mean ± Standard division (SD). The statistical analysis was performed by T-test and the significant of variance was at P value < 0.05. Flow cytometery and cytokine analysis gained with tow dimensional forward and side scatter for the flow cytometer and fluorescent intensity FACS CantoII Flow cytometer and FACSDiva version 6.1.3 software (BD Biosciences). For angiogenesis gene expression and protein array analysis was carried out by using RAYBIOA_ANALYSIS_TOOL-AAH-ANG EXCEL software, the numerical densitometry data was gained, the background subtracted and the result normalized to the positive control signal to analyze the data.

3. FINDINGS AND DISCUSSION

3.1 Results

3.1.1 Viability of cell

The result was showed increasing in the viability percentage of treated macrophage RAW 264.7 cells with both MTH leaves extract and fractions significantly (P < 0.05) in dose depended manner compared with non-treated cell. The viability percentage was 185.2% for MTH leaves extract and 175.4% for MTHF1 is the higher viability percentage among all fractions (Figure1).

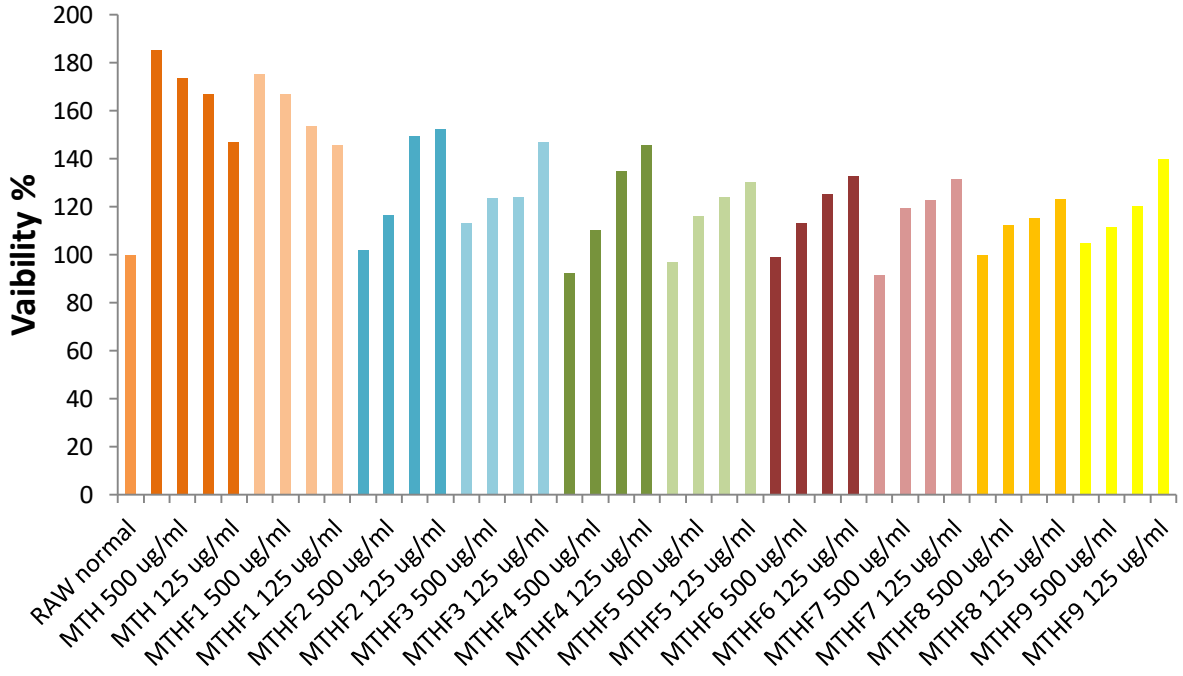

Figure 1: Showed the viability percentage for macrophage RAW 264.7 cells treated with different doses from Mentha pulegium L (MTH) leaves extract and its fraction 1(MTHF1).

3.2 Nitric oxide production

Effect of the Mentha pulegium L (MTH) leaves extract and MTHF1 for the Nitric oxide production from LPS induction macrophage RAW 264.7 cells was investigated and the result was showed that the Nitric oxide production in the treated RAW 264.7 cell with MTH extract and MTHF1 inhibited significantly (P < 0.05) at dose dependent manner at concentration for MTH (587.89 ±0.49, 679.29 ± 0.36, 746.81 ± 0.22, 727.88 ± 0.58) and for MTHF1 (576.94 ± 0.79, 700.13 ± 0.35, 846.98 ± 0.39, 887.01 ± 0.58) for the doses (200,100,50,25) µg/mL respectively for each MTH extract and MTHF1 (Figure 2).

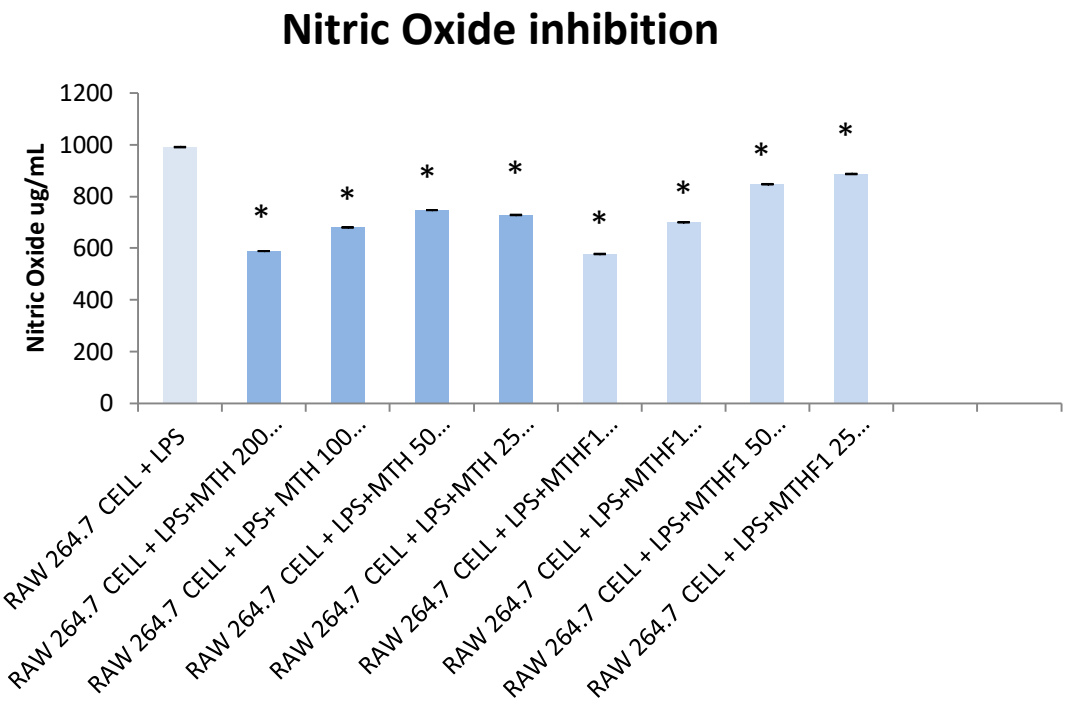

Figure 2: Presented the Nitric oxide production from macrophage RAW 264.7 cells induction with LPS and treated with different doses from Mentha pulegium L (MTH) leaves extract and its fraction 1(MTHF1). Data was presented as mean ± SD, *Significantly at P value < 0.05.

3.3 Enhancement of angiogenesis

To study the effect of Mentha pulegium L (MTH) leaves extract on the expression of angiogenesis cytokines from inducible macrophage RAW 264.7 cells. The result was conducted that treated cell with MTH extract was gave different response for released or production of cytokines as presented in Table 1.

3.4 Intracellular expression of INF- γ, IL6 and IL8

The intracellular expression of INF- γ , IL6 and IL8 in the LPS inducible macrophage RAW 264.7 cells treated with (50 and 100) µg/mL form each MTH leaves extract and MTHF1 result was showed (Figure 3) increased in the intracellular expression percentage of INF- γ , IL6 and IL8 significant ($P < 0.05$) in percentage (33.7, 7.7 & 10.4) and (68.5, 12.4 & 11.4) for INF- γ , IL6 and IL8 at dose (50 and 100) µg/mL to MTH leaves extract. And in percentage is (33.9, 3.4 & 7.2) and (67.7, 11.6 & 7.7) for INF- γ , IL6 and IL8 at dose (50 and 100) µg/mL to MTHF1.

Table 1: Showed fold expression of angiogenesis cytokine in inducible macrophage RAW 264.7 cells treated with 100 µg/mL Mentha pulegium L (MTH) leaves extract.

Target cytokine	*Gene Symbols*	*Fold expression in RWA 26.4 cell*	*Fold expression in RWA 26.4 cell treated 100 µg/mL MTH*	*Fold change %*	*Type for fold change*
Angiopoietin-1	***ANGPT1***	***2762.675***	***0***	***0***	***No expression***
Angiopoietin-2	***ANGPT2***	***4834.975***	***0***	***0***	***No expression***

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Volume 62, Issue 01, March, 2022

Angiostatin	*CCL1*	*559277.1*	*704439.9*	*20.6**	*Increase*
Endostatin	*COL18A1*	*668347.1*	*874671.7*	*23.6**	*Increase*
GCSF	*CSF3*	*33569.43*	*30278.18*	*-10.9**	*Decrease*
GM-CSF	*CSF2*	*31413.93*	*33144.43*	*5.2**	*Increase*
I-309	*(TCA-3/CCL1)*	*99337.08*	*120655.6*	*17.7**	*Increase*
IL-10	*IL10*	*108170.6*	*141567.6*	*23.6**	*Increase*
IL-1 alpha	*IL1A*	*8907.825*	*2397.824*	*-271.5**	*Decrease*
IL-1 beta	*IL1B*	*10479.43*	*4682.66*	*-123.8**	*Decrease*
IL-2	*IL2*	*2.775*	*3.27427*	*15.3**	*Increase*
IL-4	*IL4*	*2.775*	*3.27427*	*15.3**	*Increase*
I-TAC	*(CXCL11)*	*2.775*	*3.27427*	*15.3**	*Increase*
MCP-3	*(MARC/CCL7)*	*2.775*	*0*	*0*	*No expression*
MCP-4	*(CCL13)*	*44840.08*	*35485.28*	*-26.4**	*Decrease*
MMP-1	*MMP1*	*48422.58*	*51920.1*	*6.7**	*Increase*
MMP-9	*MMP9*	*42384.58*	*42384.42*	*-0.0004**	*Decrease*
PECAM-1	*(CD31)*	*42624.58*	*48347.62*	*11.8**	*Increase*
Tie-2	*TEK*	*24275.58*	*19406.1*	*-25.1**	*Decrease*
TNF alpha	*TNF*	*29879.58*	*28717.11*	*-4.1**	*Decrease*
uPAR	*PLG*	*278274.9*	*264733.1*	*-5.1**	*Decrease*
VEGFR2	*KDR*	*319074.9*	*321443.4*	*0.7**	*Increase*
VEGFR3	*FLT4*	*29899.58*	*16734.29*	*-78.7**	*Decrease*

Data was *significant at P value < 0.05

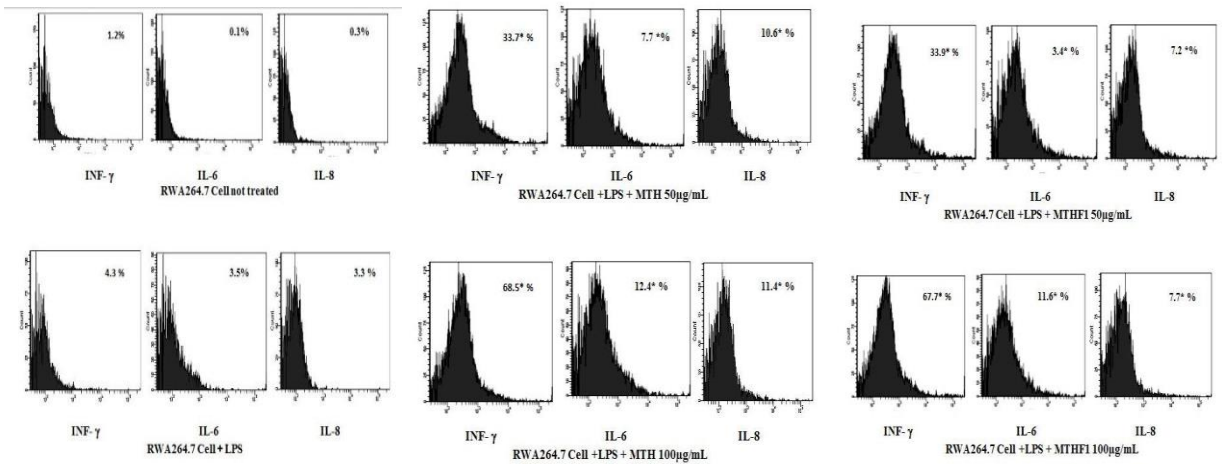

Figure 3: Intracellular expression of INF- γ , IL6 and IL8 in the LPS inducible macrophage RAW 264.7 cells treated with (50 and 100) µg/mL form each Mentha pulegium L (MTH) leaves extract and Mentha pulegium L extract fraction (MTHF1).Data was expression as percentage. *Significant at $P < 0.05$.

3.5 Discussion

Many previous studies was investigated many biological activity of M. pulegium L leaves extract as antioxidant, antimicrobial activity. The results of this study were showed that M. pulegium L leaves extract and its fraction1 was increased the viability of macrophage RAW 264.7 cells and effect in the expression of angiogenesis cytokine production for inducible treatment of RAW 264.7 cells with M. pulegium L leaves extract and its fraction1 and effect on the intracellular expression of inflammatory cytokine, those results were improved the immunomodulatory and anti- inflammatory effect of M. pulegium L leaves extract. The reduction of production of Angiopoietin-1 and Angiopoietin-2 that act for the potential of VEGF2 and VEGF3 that act synergetic to stimulate angiogenesis during remodeling vasculature coupled with Tie receptor control of blood vessel formation [10]. The increasing in the fold change production of Angiostatin was plasminogen have potent to angiogenesis inhibitor with inhibit endothelial cell proliferation and tumor metastasis [11] this result was proven the anti-inflammatory effect of M. pulegium L leaves extract. And increased in fold production Endostatin is potential as anti-cancer agent for human cause tumor regression through complete angiogenesis inhibition [12]. Decreasing of GCSF influence in the survival, differentiation and proliferation all cell for the neutrophil percentage in haemopoietic stem cell at neutrophil maturation [13]. The GM-CSF was increasing in the production that induction of macrophage and granulocyte development and it is crucial role in the autoimmune and inflammatory and act as immunomodulator factor [14]. Human I-309 cytokine is secreted from activated T lymphocyte that encoded inflammatory mediator that stimulated human monocytes [15]. Increasing in the production of Il-10 promote anti-inflammatory by limiting immune response to pathogen [16] so that effect in the modulation of decreasing production ofIL-1 alpha, IL-1 beta and TNF alpha proinflammatory cytokine from stimulate macrophage RAW 264.7 cells treated with M. pulegium L leaves extract lead to increase in the IL2 and IL4 production that responsible to immune system tolerance by effect in the T cell development and differentiation [17], [18]. I-TAC chemokines mediator play critical role in the migration of activated T cells. This chemokine affect by the level of INF-γ [19] so the effect of M. pulegium L leaves in the increasing intracellular expression of INF-γ lead to increase in the fold change production of I-TAC. Addition evidence for immunomodulation effect of M. pulegium L leaves that effect on the production of MMP family, which plays an important role in the modulation of inflammation process by affecting in different way rage from leukocyte recruitment to cytokine production reach to clearance of pathogen [20].

ISSN: 0005-2523
Volume 62, Issue 01, March, 2022

PECAM-1,Platelet endothelial adhesion molecule-1 is a glycoprotein adhesion molecule expressed on the platelet, leucocyte and endothelial cells in to inflammatory site and it is expression affected with level of INFγ and TNF-alpha [21]. uPAR, urokines plasminogen activator receptor is control to macrophage phagocytosis in chronic inflammation disorder and play a role in modulation the development of immunity response through supporting matrix degeneration and regulate migration of the cells, adhesion and proliferation so that influenced in the development of inflammation process [22].

4. Conclusion

The outcome of the result revealed that M. pulegium L leaves extract was influenced in the immune response process as an immunomodulator and anti-inflammatory agent, which could be relies as a natural drug in pharmacological development to discover a new anti-cancer, anti-diabetic and to regulate an autoimmune disease.

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