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Research Article

The Biochemical Effect of Leaf's Alcoholic Extract *Mentha-Pulegium* Active Compound on Angiogenesis, Intracellular Expression Of INF- γ , IL6, IL8, And Inflammation Response For RAW.264.7. Cell.Line

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Abstract

Increasing attention is paid to evaluating the immunostimulant and anti-inflammatory effects of the newly created plant preparations, including *Mentha pulegium* L., the anti-inflammatory drugs that can be utilized in modern disease therapy. MTH's immunomodulatory anti-inflammatory property is believed to be superior to the other plants. TNF- α , IL-1 β , and IL-6 are involved in wide-ranging physiological processes and pathological states. MTH is one of the common traditional medicinal plants widely used in different parts of the globe. This work aimed at assessing the biological attributes of immunomodulation and anti-inflammatory effects on seed ethanol extract from MTH leaves and its effects on angiogenesis, as well as the effects of cytokine gene expression and synthesis. RAW 264.7 Macrophage cells were employed to study the impacts of MTH leaf extracts and fractions in various situations. This suggested a dose-dependent and statistically significant positive cell activity of RAW 264.7 cells as well as considerably reduced nitric oxide synthesis, up-regulated angiogenic cytokines, and elevated intra-cellular INF- γ IL6, IL8 expression after MTH leaf extracts and different fraction treatment. First of all, this work suggests that the studied *M. pulegium* L. leaf extract can be considered a potent new immunomodulatory and anti-inflammatory agent.

Keywords: Extracts, *Mentha-pulegium* L.-; Immunomodulator; Anti-inflammatory; -Angiogenesis-cytokines.

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Introduction

More precisely, the present study aims to explore the immunomodulating and anti-allergic effects of the ethanol obtained from *Mentha Pulegium* L. leaves. In the context of the present work, particular importance is attributed to learning about the efficiency of this extract and its components in angiogenesis, as well as tendencies concerning the genes responsible for cytokine production (1).

The objective in this case is to determine the immunomodulatory and anti-allergic activities of these peptides without translating or opening the original website content (2). *M. Pulegium* including Asia and the Middle East, has rich antioxidant and antimicrobial constituents. Earlier studies on its oil highlighted compounds including limonene, 1,8-cineole, germacrene-D, β -caryophyllene, β -bourbonene, and α -terpineol (3) medications (4). low-cost and green

synthesis of Iron-silver-copper oxide nanoparticles has been synthesized by using the extract of *M. Pulegium* that confirms the future application (5.6). Also, the antioxidant and anticholinesterase of polyphenolic compounds alcoholic of leaves of this plant are discussed (7).

Methods

Collection of plant and extraction:

Mentha Pulegium L. (MTH) leaves were collected from local commercial sources and dried, cleaned, and ground before extraction in ethanol, 95% obtained from Fisher Scientific from the United Kingdom. After the extraction process was fractionation and in doing so the percentage content of MTH was retained.

Mentha Pulegium L. extract on the viability

The impact of ethanol extract from *Mentha Pulegium* L. leaves macrophage, RAW.264.7 from ATCC investigated results were documented (8). In this study, the extract was tested at various final concentrations of embryo extract, and their respective fractions were set at 100, 50, 250, and 12.5 $\mu\text{g/ml}$ for the extracts from the leaf and the fractions of the extract.

Nitric oxide Production

As a measure of the effect of MTH zero production in RAW 264.7, MTH treated cell line was used to determine the maximum nontoxic concentration of MTH for RAW 264.7 cells exposed to MTH (25, 50, 100, and 200 $\mu\text{g/milliliter}$) as previously explain Vasicek et al. (9) RAW 264.7 Cells was plated at 1 The extract or the fraction in different dilutions was placed on 6 well plates for 24 hr at 37 °C, 5% CO₂. Only LPS was added to cells in the positive control media. A standard curve was plotted using dilutions of sodium nitrite (NaNO₂) in the concentration range of 0.5 $\mu\text{g/mL}$ to 4 $\mu\text{g/mL}$. After 24h incubation, the media from treated wells were carefully aspirated off and cells were centrifuged at 3500 rpm for 15 min. After, 150 μl of the upper layer of the supernatant was allowed to react with 150 μl of Griess reagents (GR4410). To stand for 30 minutes at room temp. in Lack of light before the absorbance was recorded using 547nm as the appropriate wavelength.

Induction of angiogenesis production:

In the investigation of the angiogenesis and erythropoiesis factors that MTH stimulates, RAW 264.7 cells were used and the proteins or cytokines of the stimulated macrophages were evaluated using angiogenesis antibody array techniques (Ray-Bio c-Series Angiogenesis Antibody Array, United States). RAW264. 7 cells were treated with 100 $\mu\text{g/mL}$ MTH for 24 hours. The procedure followed the manufacturer's instructions to the letter the antibody array membrane was first washed then 2 mL of

blocking buffer was applied and incubated at room temperature, 25°C for thirty min. The medium aspirated and 1 ml of the cell-treated media was added to each well and incubated at room temperature for three hours. Following incubation, the sample was passed through aspiration and the membrane was washed in 2 ml of 1x wash buffer 1 at room temperature for 5 minutes. This was performed 3 times and then followed by a wash with wash buffer 2. Next, 1 mL of biotinylated antibody cocktail was pipetted into the corresponding well of the respective plate and then the plate was incubated for 2 hours at RT, 25°C. After this incubation, the cocktail was aspirated, and the washing step. 2 mL of 1x HRP-Streptavidin was pipetted into each and the plates were incubated for a further 2h with RT (25°C). Later on, the plate was washed the HRP-Streptavidin was removed and the washing process was repeated. The membrane was then placed on the plastic plates 500 μL of detection buffer was then applied to each plate.

Expression Intracellular INF- γ ,IL6 and IL8 as markers

To determine the effect of MTH and MTHF1 on cytokine synthesis in RAW 264.7 macrophages, cells were exposed to MTH and MTHF1 leaf extracts at 50 and 100 $\mu\text{g/mL}$. The experimental procedure was carried out according to the BD Cytotfix/Cytoperm™ Plus Fixation/Permeabilization protocol and the BD Golgi Plug™ Protein Transport Inhibitor (550028) as explained in the methodology section (8). This approach enabled the evaluation of MTH and MTHF1 interference with cytokine expression in RAW 264.7 macrophages and the elucidation of their possible immunomodulatory activity.

Statistical Analysis

The given data were expressed in mean SD form. Descriptive statistics used consisted of T-tests, while the variance of the test was considered significant at P-value < 0.05. Blood vessel formation gene products were analyzed on the angiogenesis gene expression and protein array using software. The standardized based on the positive control signal to analyze data.

Results

Viability.of.cell

The improvement of treated phagocytic cells with RAW 264.7, alcoholic extracts from the leaves of MTH, and its various fractions. The enhancement herein established was established to be significant at P<0.05 relying on dose-response relationship as compared to untreated cells. MTH leaf extract's viability rate recorded the highest value of about 185.2% while MTHF1 which is a fraction showed an even much better value of about 175.4% Fig-1.

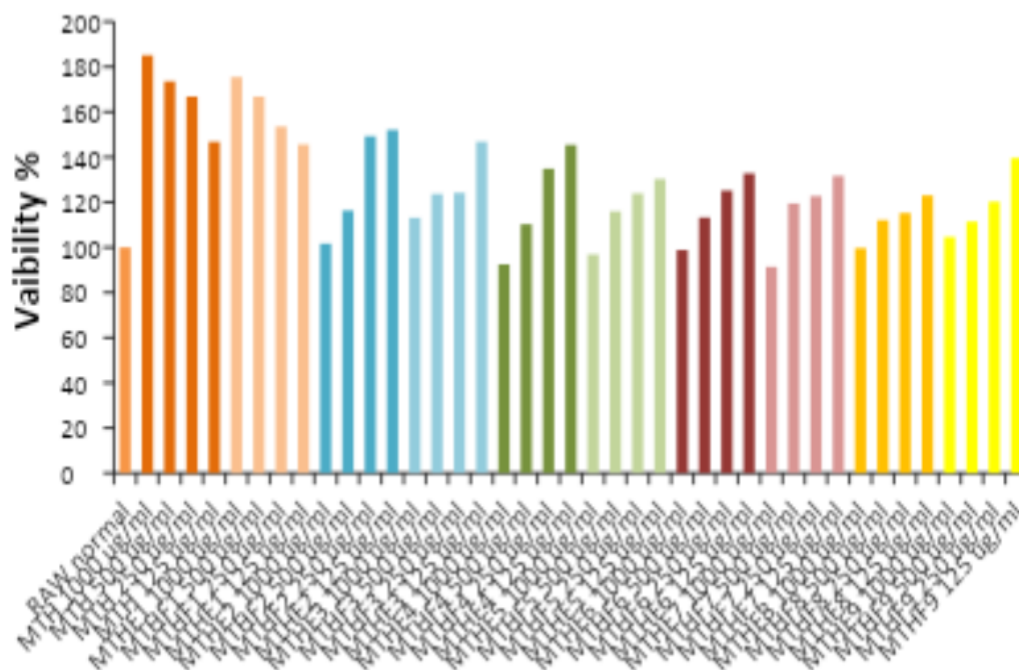


Figure-1: The results imply that the optimal percentage for RAW macrophages is 264.7, which has been exposed to different concentrations of *Mentha Pulegium L.* leaf extraction (MTH) and (MTHF1).

NO.production

The study investigated the impact of *Mentha pulegium* L leaf extract and MTHF1 on nitric oxide production in LPS-stimulated RAW 264.7 cells. The results revealed a significant reduction in nitric oxide levels ($P < 0.05$) in a dose-dependent manner following treatment with

the extract and MTHF1. Specifically, the nitric oxide levels for MTHF1 were 89 ± 0.49 , 679.29 ± 0.36 , 746.81 ± 0.22 , and 727.88 ± 0.58 , while the levels for *Mentha pulegium* L extract were 576.94 ± 0.79 , 700.13 ± 0.35 , and 846.98 . These findings are detailed in Table 1.

TABLE-1:(NO) Productions, from.Macrophages RAW,264.7.Cells induction with.LPS and Treated with Different Doses from (MTH) leaf Extract and (MTH-1).

samples	Nitric.Oxide inhibitions	SE,
(RAW.264.7 .CELL.+ LPS.	1991.13	10.52
(RAW.264.7 .CELL.+ LPS+MTH 200.µg /ml	1967.84	10.68
(RAW 264.7.CELL.+ LPS+ MTH 100 .µg/ml	1968.07	11.81
(RAW 264.7.CELL.+ LPS+MTH 50 .µg/ml.	1986.11	12.99
(RAW 264.7.CELL.+ LPS+MTH 25 .µg/ mL.	1991.73	15.64
(RAW 264.7.CELL.+ LPS+MTHF1.200 µg/ mL.	1877.14*	116.91
(RAW 264.7.CELL.+ LPS+MTHF1.100 µg/ mL.	1909.54*	10.82
(RAW 264.7.CELL.+ LPS+MTHF1.50 µg/ mL.	1924.39*	10.36
(RAW 264.7.CELL.+ LPS+MTHF1 .25 µg/ mL.	1877.93*	11.77

Enhancement.of.angiogenesis

A study was performed to determine the effect of *Mentha pulegium* L. (MTH) leaf extract on angiogenesis cytokines secreting RAW 264.7 inducible macrophage cells. The present study were more complex and reflected various reactions of cells treated with MTH extract about cytokines and the cytokines produced or synthesized by cells, as illustrated in Table 1.

Cell-based expression of INF- γ , IL6 and IL8

This work focused on the effect of MTH leaf and MTHF1 on the INF- γ , IL6, and IL8 in LPS-induced RAW 264.7 macrophage and used different doses for

analysis. The findings revealed that cytokine/expression percent in all the treated groups had a higher significant ($P < 0.05$) increase than the control group. In particular, the INF- γ , IL6, and IL8 expression percentages were (33.7, 7.7, and 10.4) percent and (68.5, 12.4, and 11.4) percent at 50 and 100 Micrograms per milliliter. concentrations of MTH leaving extract. Compared to the control group, percentage fold change of INF- γ , IL6, and IL8 at 50 and 100 $\mu\text{g/mL}$ concentrations of MTHF1 were 33.9%, 3.4%, and 7.2% and 67.7%, 11.6%, and 7.7%, respectively. Altogether, both MTH leaf extract and MTHF1 appeared to exert significant impacts on

intracellular INF- γ , IL6, and IL8 expression in the macrophage cells under consideration.

Table 2: Fold expression of angiogenesis cytokines in inducible macrophage RAW 264.7 cells treated with 100 Micrograms per milliliter *Mentha pulegium* L. (MTH) leaf extract.

'Target Cytokine	Gene Symbol	Fold Expression in "RAW 264.7 Cells"	Fold Expression in RAW 264.7 Cells Treated with (100 μ g/mL MTH)	Fold Changes (%)	Type of Fold Changes
Angiopoietin-1	ANGPT1	2762.675	0	0*	No expression
Angiopoietin-2	ANGPT2	4834.975	0	0*	No expression
Angiostatin	CCL1	559277.1	704429.9	20.6**	Increase
Endostatin	COL18A1	668347.1	875671.7	23.6**	Increase
GCSF	CSF3	33569.43	30378.18	-10.9**	Decrease
GM-CSF	CSF2	31413.93	33244.43	5.2**	Increase
I-309	TCA-3/CCL1	99337.07	120755.6	17.7**	Increase
IL-10	IL10	108170.65	141767.6	23.6**	Increase
IL-1 α	IL1A	8907.824	2398.824	-271.5**	Decrease
IL-1 β	IL1B	10479.42	4681.66	-123.8**	Decrease
IL-2	IL2	2.775	3.28427	15.3**	Increase
IL-4	IL4	2.775	3.28427	15.3**	Increase
I-TAC	CXCL11	2.775	3.37427	15.3**	Increase
MCP-3	MARC/CCL7	2.775	0	0*	No expression
MCP-4	CCL13	44840.00	35495.28	-26.4*	Decrease
MMP-1	MMP1	48421.58	51930.1	6.7*	Increase
MMP-9	MMP9	42386.58	42394.42	-0.0004*	Decrease
PECAM-1	CD31	42623.58	48357.62	11.8*	Increase
Tie-2	TEK	24275.58	19416.1	-25.1*	Decrease
TNF- α	TNF	29879.58	28727.11	-4.1*	Decrease
uPAR	PLG	278274.9	264733.2	5.1*	Decrease
VEGFR2	KDR	319074.9	321443.4	0.7*	Increase
VEGFR3	FLT4	29899.58	16734.29	-78.7*	Decrease'

(significant at P -value < 0.05*)

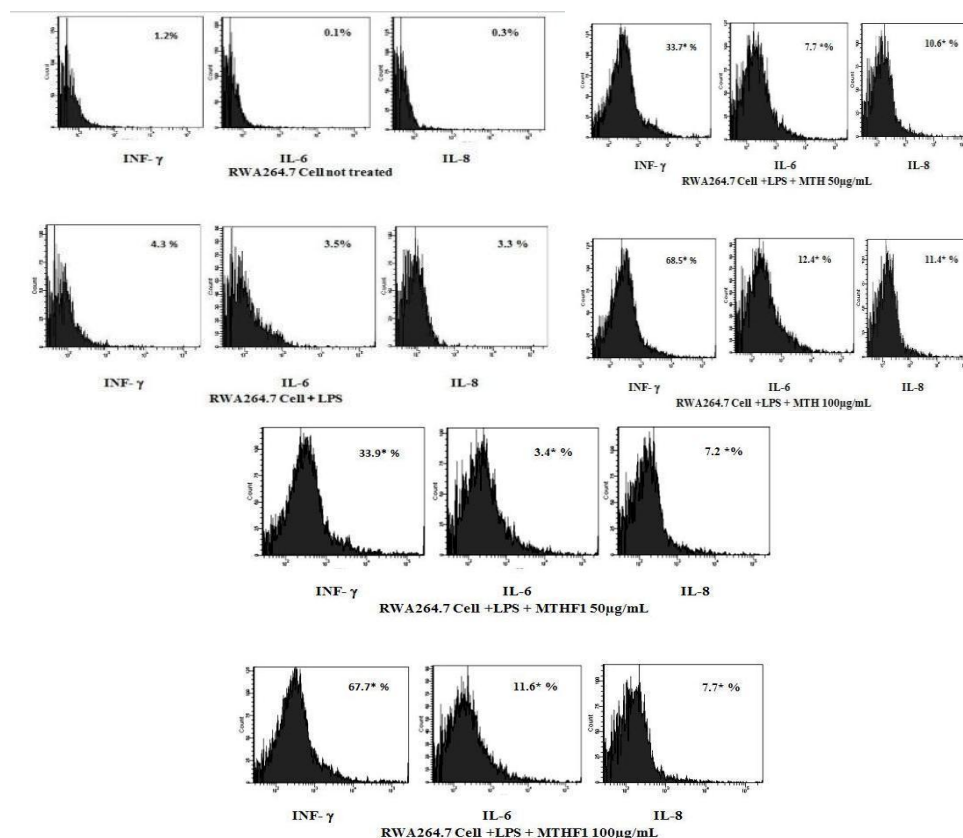


Figure 2: In this study, INF- γ , IL6, and IL8 and concentrations of 50 and 100 Micrograms per milliliter of MTH and MTHF1 on the intracellular expression in LPS-inducible macrophage RAW 264.7 cells. $p < 0.05$.

Discussion

This study, *Mentha pulegium* L. leaf extract exerts immunomodulatory and anti-inflammatory effects on cytokines and angiogenic factors that are essential in physiological and pathological processes. The present study indicates that *M. pulegium* L. leaf extract and its fraction 1 help increase the survival of RAW 264.7 macrophage cells and affect cytokines concerning inflammation. This effect was seen in the regulation of inflammatory cytokines including TNF- α , IL-1 β , and IL-6 which are well-targeted in autoimmune and inflammatory diseases. Furthermore, the extract also decreased the angiopoietins (Ang 1 and Ang 2) that are central to angiogenesis and blood vessel formation and gave an inkling about the extract's possible use in cancer cell growth and metastasis suppression by way of anti-angiogenic properties (10). The increase of angiostatin and plasminogen also indicated a strong potential of *M. pulegium* L. leaf extract to suppress blood vessel formation endothelial cell proliferation and tumor metastasis, which makes it a potential candidate for cancer treatment (11). Further, the upregulation of endostatin expression substantiates its role as an anti-cancer molecule as it inhibits angiogenesis and induces tumor regression (12). In addition, the immunomodulatory effects of *M. pulegium* L. leaf extract are supported by the increased levels of IL-2 and IL-4 that modulate cell development and immune tolerance (13,14). The T-cell-attracting chemokine I-TAC was also increased, suggesting that the extract might affect the migration and activation of

immune cells (15,16). These results are also consistent with the enhancement of INF- γ synthesis and the increase in I-TAC expression, indicating a more general effect on the immune system (17,18). The study also describes the regulation of the MMP family, PECAM, and uPAR in inflammatory processes, as well as the up-regulation of these molecules by the *M. pulegium* L. leaf extract to prepare immune cells to eliminate pathogens (19,20). *M. pulegium* L. leaf extract enhances the expression of these molecules, further contributing to immune cell preparation for pathogen removal (21,22). These findings give evidence of the complex roles of *M. pulegium* L. leaf extract in regulating immune response and therefore could be useful management of inflammatory disorders, autoimmune diseases, and even cancer (23). This process is tightly regulated loss of regulation results in acute or chronic inflammatory diseases and autoimmune diseases. Organ failure, the promotion of tumor cell proliferation, and even cancer. (24,25).

Conclusions

As a result of the study, it was established that the extract from the leaves of the *M pulegium* L plant influences the Responses of the immune system it is immunomodulatory and anti-inflammatory. Which natural medicine the advancement of the pharmaceutical industry and the discovery of new cancer and inflammation-fighting compounds for diabetes and managing autoimmune diseases.

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