Immunomodulatory effect of *Moringa oleifera* and *Marrubium vulgare* leaf aqueous extracts in BALB/c mice infected with *Salmonella typhimurium*

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**ABSTRACT**

**Aim:** The current study aimed to evaluate the immunomodulatory effect of *Moringa oleifera* and *Marrubium vulgare* leaf aqueous extracts as single and as a combination in *Salmonella typhimurium*-infected mice. The immunomodulatory effect was evaluated as the curing agent (in mice infected with *S. typhimurium*) and as protective agent (in mice infected with *S. typhimurium* at the end of treatment period). **Methods:** A total of 50 female BALB/c mice, aged 7–9 weeks, were divided into 10 groups: Positive control (C+), normal mice (C–), protective groups (P1, P2, and P3), and cure groups (P4, P5, P6, P7, and P8). Different concentrations (100, 500, and 1000 mg/kg body weight [BW]) of each plant extract were used as single and a combination for 14 days. At the end of the treatment period, all mice were sectioned and mice splenocytes were isolated. Immunomodulatory markers were checked using flow cytometry which include CD4⁺CD8⁺, CD4⁺CD62L⁺, CD8⁺CD62L⁺, CD8⁺ interleukin (IL)-17, and CD8⁺ interferon-gamma (IFN)-γ. **Results:** The results showed that *M. oleifera* leaf aqueous extract had potential immunomodulatory effects as protective and curing agent. The single treatment of *M. oleifera* as curing agent (P4) leading to a significant decrease in the double positive (DP) (CD4⁺CD8⁺) T cells activation and production of IL-17 and IFN-γ. Furthermore, the level of naïve CD4⁺ and CD8⁺ T cells was increased significantly (P < 0.05) after the single treatment with *M. oleifera* as protective agent (P1). Combination treatment of *M. oleifera* and *M. vulgar* as cure agent also showed immunomodulatory effect in the level of DP T cells. Whereas protective treatment with the combined extracts increase the level of IL-17 and IFN-γ produced by CD8 especially at the moderate dose (P7, 500 mg/kg BW). In addition, the level of naïve CD4⁺ and CD8⁺ T cells also increased at the high dose of *M. oleifera* and *M. vulgar* combination when it used as protective agent. However, the immunomodulatory effects were higher during single treatment with *M. oleifera*. **Conclusion:** These results suggest that single treatment with *M. oleifera* extract is more effective than combination treatments with *M. vulgar* in *S. typhimurium*-infected mice. Protective treatment with *M. oleifera* and *M. vulgar* aqueous extracts was more effective than cure treatments. Dosage with low concentration of combined extracts showed high immunomodulatory activity than those dosages with moderate and high concentration. These findings have proven that *M. oleifera* and *M. vulgar* will be a very good material for future herbal medicine.

**KEY WORDS:** Immunomodulatory, *Marrubium vulgare* L., *Moringa oleifera*, *Salmonella* infection

**INTRODUCTION**

The World Health Organization reported that a massive 80% of the global population depends on traditional medicine for their primary health-care needs. Plants used in traditional medicine contain various substances that can be used to treat chronic and infectious diseases. However, the majority of the substances which used in modern medicine are produced synthetically.[1,2] The current research has shown that extracts of different medicinal plant parts were reported to have wide spectrum of antimicrobial activities and immunomodulatory effects against pathogenic organisms. The most significant bioactive compounds from plants include alkaloids, flavonoids, tannins, and phenolic compounds.[3,4] The effect of *Moringa oleifera* leaves as immunomodulator was studied in normal and immunosuppressed mice models. Pre-treatment with *Moringa* extract inhibited cyclophosphamide bone marrow suppressive effect.
on phagocytic activity in mice.[5] Furthermore, various doses of *M. oleifera* caused a significant increase in the level of white blood cell counts and immunoglobulin levels.[6,7]

*Marrubium vulgare* L. is generally known as white or common horehound which belongs to the family Lamiaceae. It is an evergreen aromatic herb that grows naturally in North Africa, Southern Europe, and Central and Western Asia. Since ancient Egypt, this species has been known as a remedy for upper respiratory tract ailments. Nowadays, horehound is used in herbal medicine for the treatment of liver diseases, biliary tract disorders, and for increasing the appetite and supporting the function of the stomach. The main biologically active substances in *M. vulgare* organs are marrubiin, tannins, essential oils, and ursolic acid. Phytochemicals present in the plant include caryophyllene oxide, trans-caryophyllene, caffeoyl-l-malic acid, acteoside, phenylethanoid glycoside, and marrubioside.[8,9]

Typhoid fever is the main problem in many developing countries that caused by *Salmonella typhimurium* infection. The main actors in the immune response to *S. typhimurium* are CD4+ and CD8+ T cells. CD4+ T lymphocytes act to eliminate bacterial cells, regulate cellular and humoral immune activity, and induce CD8+ T cells through interferon (IFN) and these cells play the major part in attacking these microorganisms and the virulence factors. *S. typhimurium* infections induce a significant reduce in absolute CD4+CD8+ T lymphocyte levels and their secretions.[10,11]

The present study was conducted to evaluate the immunomodulatory effect of two different medicinal plants, *M. oleifera* from Indonesia and *M. vulgare* L. from Libya. Aqueous extract of these plants was used as immunomodulatory agents as single and as combination on the immune response of BALB/c mice which infected with *S. typhimurium*. The changes in the immunological markers which include CD4+CD8+, CD4+CD62L+, CD8+interleukin (IL)-17+, and CD8+IFN-gamma (IFN)-γ were analyzed using flow cytometry *in vivo* mice models to evaluate the changes in the levels of T cells activation, naïve T cells, and pro-inflammatory cytokine markers.

**METHODS**

**Plant Sample Collection**

The plant materials used in this study consist of *M. oleifera* which is growing in Indonesia and *M. vulgare* L. which is growing in Libya. These plants collected from different area in Indonesia and Libya. The plant leaves and stem were washed thoroughly with tap water followed by sterile distilled water and shade dried at room temperature for 10–15 days.

**Aqueous Extraction**

*M. oleifera* leaf was obtained from UPT Materia Medica Batu in September 2017 and *M. vulgare* L. leaf was collected in August 2017 from K. Khiar village in Libya where it is part of the natural vegetation in that region.

For aqueous extraction, 20 g of air-dried leaf powder was added to 150 ml of distilled water and boiled on slow heat for 2 h, filtered through eight layers of muslin cloth and centrifuged at 5000 rpm for 10 min. Then, the supernatant was collected. The procedure was repeated twice. After 6 h, the supernatant was collected at an interval of 2 h, pooled together, and concentrated to make the final volume one-fourth of the original volume.

**Animals and Experimental Design**

*S. typhimurium* stock was obtained from Faculty of Medicine, Brawijaya University. The study was conducted on female mice strain BALB/c, 7–9 weeks old with body weight [BW] 30–40 g. The animals were acclimatized for a week before the experiment. The total of 50 subjects mice were divided into 10 groups, two control groups which include negative control group (control) (normal untreated mice) and positive control group infected with *S. typhimurium* but untreated (Control+) and eight treated groups (P1–P8) which orally treated with *M. oleifera* and *M. vulgare* L. aqueous extract as single and combination for 14 days. From these eight treated groups, there were three groups (P1, P2, and P3) infected with *S. typhimurium* prior treatment with 500 mg/kg per day of *M. oleifera* aqueous extract for P1 group, with 500 mg/kg per day of *M. vulgare* aqueous extract for P2 group, and with combination of 500 mg/kg of *M. oleifera* and 500 mg/kg of *M. vulgare* aqueous extract per day for P3 group to check the effect of choice extracts as curing agent. The other treated groups which referred as P4–P8 were infected with *S. typhimurium* at the end of the treatment period to check the effect of the chosen single and combination extracts at different doses as protecting agent. The P4 group was treated with 500 mg/kg per day *M. oleifera* aqueous extract. The P5 group was treated with 500 mg/kg per day *M. vulgare* aqueous extract. A combination of 100 mg/kg *M. oleifera* and 100 mg/kg *M. vulgare* per day was given to P6 group. The P7 group was treated with combination of 500 mg/kg *M. oleifera* and 500 mg/kg *M. vulgare* per day. Moreover, the P8 was treated with the highest dose with combination of 1000 mg/kg *M. oleifera* and 1000 mg/kg *M. vulgare* per day.

The effectiveness of *M. oleifera* and *M. vulgare* L. as single and combination at different concentrations to the immune system of treated mice was analyzed. The variable parameters which indicate the mice immune response such as CD4+CD8+, CD4+CD62L, CD8+CD62L, CD8+IL17, and CD8+INF g were
analyzed using flow cytometry. The blood samples were obtained from in vivo mice models to evaluate the changes in the levels of pro-inflammatory, macrophage activation, and regulatory T-cell markers.

**Lymphocyte Isolation**

Both pre- and post-treatment of mice BWs were recorded. At the end of the study, the mice were sacrificed by cervical dislocation. The spleens were removed, weighed, and placed in 15 ml sterile centrifuge tubes containing 1 ml RPMI-1640 medium then stored at 4°C for further analyses.

**Flow Cytometry Analysis**

Each spleen was placed on a sterile culture plate and crushed by compressing between two sterile glass slides. Then, the homogenized suspension was collected from the tissue plates into a new sterile centrifuge tube. The tubes were centrifuged at 1500 rpm for 5 min at 10°C. The supernatant was discarded, and the pellet was resuspended with 1 ml of phosphate-buffered saline (PBS). The resuspension cell was prepared and aliquoted 100 μl into a microtube containing 300 μL of PBS, then centrifuged at 1500 rpm, in temperature 10°C, for 5 min. The supernatant was discarded; the pellets were formed then added with one μl of antibody (for detection of cell surface molecules) which had been diluted with 50 μL PBS. The suspension was incubated in an icebox for 20 min.

The cell suspension was added to 50 μL fixation buffer (BioLegend), and it was incubated in the icebox for 20 min. Next, the cell suspension was added to 500 μL of Intracellular Staining Permeabilization Wash Buffer (×1) (BioLegend) and centrifuged at 1500 rpm, in 10°C, for 5 min. Supernatant was discarded, and the pellets were resuspended with 1 μL of antibody (for detection of intracellular molecules) which had been diluted with 50 μL PBS. The suspension was incubated in an icebox for 20 min. Next, the suspension was added with 500 μL PBS and moved into a flow cytometry round-bottom tubes. Then, samples will be analyzed using FACSCalibur™ flow cytometry to identify lymphocyte cell subsets distribution. Cell counting will be performed using hemocytometer for viability cell with the Trypan blue dye.

**Statistical Analysis**

The obtained result was analyzed by CellQuest Pro™ software and the data were analyzed using one-way ANOVA with α = 0.05 by SPSS 16.0 for Windows with complete randomized design.

**RESULTS**

**The Expression Level of Naïve T Helper Cells (CD4+CD62L+)**

This study also found that M. oleifera extract as a cure agent (after S. typhimurium infection) showed significant effect on the relative number of naïve helper T cells (CD4+CD62L+) [Figure 2b]. A high increase in the level of naïve help T cells was found in P1 (53.466%) compared to positive control (23.101%). However, treatment with M. oleifera extract as protective agent (before S. typhimurium infection) decrease the relative number of naïve T cells (CD4+CD62L+) (P4: 0.198%) compared to the positive control group (C+: 1.708% and P1: 1.792%). In contrast, M. ulifera leaf aqueous extract had potential immunomodulatory effects when it uses as protective agent. The treatment with M. oleifera extract as protective agent (before S. typhimurium infection) decrease the relative number of CD4+CD8+ DP T cells significantly (P<0.05) (P4: 0.198%) compared to the positive control group [Figure 1b].

**The Level of CD4+CD8+ Double Positive (DP) T Cells**

This study found that the treatment with M. oleifera extract as cure agent (after S. typhimurium infection) showed no significant effect on the relative number of CD4+CD8+ DP T cells [Figure 1a, P1]. The relative number of CD4+CD8+ T-cell in the P1 group was almost equal with positive control group (C+: 1.708% and P1: 1.792%). However, treatment with M. vulgare extract as protective agent showed no significant effect on the relative number of CD4+CD8+ DP T cells significantly (P2: 1.792%) compared to the positive control group (C+: 1.708%) [Figure 2a, P1]. A high increase in the level of naïve help T cells was found in P1 (53.466%) compared to positive control (23.101%). However, treatment with M. oleifera extract as protective agent (before S. typhimurium infection, P4) showed a decrease in the relative number of naïve T cells (CD4+CD62L+) compared to the positive control group which had 23.101% [Figure 2a, P4].

The administration of M. oleifera extract as curing agent may lead to significant (P < 0.05) increase to the relative number of naïve T cells (P1 = 53.466%). However, using the same concentration of M. oleifera extract as protective agent leads to decrease in the relative number of naïve T cells (P4 = 5.151%). Treatment with M. vulgare extract as curing and as protective agent showed no significant effect on the relative number of CD4+CD62L- T cells (P2, P5). Combination treatment of M. oleifera and M. vulgare aqueous extracts showed no significant difference in the relative number of CD4+CD62L- T cells between curing and protective groups. However, using low concentration (100 mg/kg) of combination extracts leads to the decrease in the relative number of CD4+CD62L- [Figure 2b, P6 = 11.282%].
The Level of Naive CD8⁺ T Cells (CD8⁺CD62L⁺)

Treatment with *M. oleifera* extract as curing agent revealed a high increase in relative number of T cytotoxic cells expressing CD62L or naive CD8⁺ T cells [Figure 3a, P1 = 55.845%]. However, using same dose of *M. oleifera* extract as protective agent showed decrease on the relative number of CD8⁺CD62L⁺ (P4 = 14.197%) compared to the positive control data (control⁺ =32.518%). Treatment with *M. vulgare* extract as curing agent showed no significant effect on the relative number of CD8⁺CD62L⁺ (P2 = 34.799%), but treatment with same dose of *M. vulgare* extract as protective agent showed significant increase (P < 0.05) on the relative number of T-cytotoxic...
Figure 2: (a and b) The level of naïve T cells (CD4+CD62L+) in Salmonella typhimurium-infected mice after treatment with Moringa oleifera and Marrubium vulgare L. leaf aqueous extracts. (a) The percentage of naïve T cells is shown in each panel and (b) quantitative data of the percentages of naïve T cells (CD4+CD62L+) after 14 days treatment with M. oleifera and M. vulgare L. leaf aqueous extracts. Data are mean ± standard deviation of five independent experiments. Control (+): S. typhimurium-infected mice; Control (−): Healthy mice; P1: S. typhimurium-infected mice treated with 500 mg/kg of M. oleifera aqueous extract; P2: S. typhimurium-infected mice treated with 500 mg/kg of M. vulgare aqueous extract; P3: S. typhimurium-infected mice treated with combination of 500 mg/kg M. oleifera and 500 mg/kg M. vulgare; P4: Mice were treated with 500 mg/kg of M. oleifera aqueous extract and infected with S. typhimurium at the end of treatment; P5: Mice were treated with 500 mg/kg of M. vulgare aqueous extract and infected with S. typhimurium at the end of treatment; P6: Mice were treated with combination of 100 mg/kg M. oleifera and 100 mg/kg M. vulgare; P7: Mice were treated with combination of 500 mg/kg M. oleifera and 500 mg/kg M. vulgare; and P8: Mice were treated with combination of 1000 mg/kg M. oleifera and 1000 mg/kg M. vulgare. P1–P3 as cure treatment and P4–P8 as protective treatment.

Cells expressing CD62L (P5 = 52.025%) compared to positive control [Figure 3a, control+ = 32.518%]. Combination treatment of M. oleifera and M. vulgare aqueous extracts as curing agent showed no significant effect on the relative number of CD8+CD62L+ (P3 = 25.835). Nevertheless, there was a significant effect on the relative number of CD8+CD62L+ when the combination use as protective agent (P7 = 70.946). However, using high concentration (1000 mg/kg) of combination extracts leads to high increase on the relative number of CD8+CD62L+ (P8 = 74.392%). Low concentration of combination extract (100 mg/kg) showed no significant effect on the relative number of CD8+CD62L+ [Figure 3b, P6 = 12.040%].
The Level of IL-17-Producing CD8 T Cells (CD8^IL17^)

Treatment with *M. oleifera* and *M. vulgar* extract (single and combination) as curing agent had no effect in the relative number of IL-17-producing CD8 T cells (CD8^IL17^) compared to the positive control [Figure 4a, P1, P2, and P3]. Treatment with *M. oleifera* extracts as protective agent caused a decrease in the relative number of IL-17-producing CD8 T cells (P4 = 10.279%). Whereas using same concentration of *M. vulgar* extract as protective agent had no effect on the relative number of IL-17-producing CD8 T cells compared to the positive control (P5 = 37.968%). Combination treatment with
low concentration of 100 mg/kg of *M. oleifera* extract and 100 mg/kg *M. vulgare* extracts as protective agent caused a decrease in the relative number of CD8\(^+\)IL17\(^+\)T cells (P6 = 13.333%), but using moderate concentration 500 mg/kg and high concentration 1000 mg/kg of *M. oleifera* and *M. vulgare* extract combination as protective agents had no significant effect on the relative number of CD8\(^+\)IL17\(^+\) T cells [Figure 4b, P7 and P8].

**The Production of IFN-\(\gamma\) by CD8\(^+\) T Cells (CD8\(^+\)IFNg\(^+\))**

Treatment with *M. oleifera* and *M. vulgare* L. aqueous extracts as curing agent had no significant effect on the relative number of CD8\(^+\)IL17\(^+\) T cells [Figure 4b, P7 and P8].
Figure 5: (a and b) The level of interferon-gamma (IFN-γ) production by CD8⁺ T cells (CD8⁺IFN-γ⁺) in *Salmonella typhimurium*-infected mice after treatment with *Moringa oleifera* and *Marrubium vulgare* L. leaf aqueous extracts. (a) The percentage of IFN-γ production by CD8⁺ T cells is shown in each panel and (b) quantitative data of the percentages of naïve CD8⁺ T cells (CD8⁺CD62L⁺) after 14 days treatment with *M. oleifera* and *M. vulgare* L. leaf aqueous extracts. Data are mean ± standard deviation of five independent experiments. Control (+): *S. typhimurium*-infected mice; Control (−): Healthy mice; P1: *S. typhimurium*-infected mice treated with 500 mg/kg of *M. oleifera* aqueous extract; P2: *S. typhimurium*-infected mice treated with 500 mg/kg of *M. vulgare* aqueous extract; P3: *S. typhimurium*-infected mice treated with combination of 500 mg/kg *M. oleifera* and 500 mg/kg *M. vulgare*; P4: Mice were treated with 500 mg/kg of *M. oleifera* aqueous extract and infected with *S. typhimurium* at the end of treatment; P5: Mice were treated with 500 mg/kg of *M. vulgare* aqueous extract and infected with *S. typhimurium* at the end of treatment; P6: Mice were treated with combination of 100 mg/kg *M. oleifera* and 100 mg/kg *M. vulgare*; P7: Mice were treated with combination of 500 mg/kg *M. oleifera* and 500 mg/kg *M. vulgare*; and P8: Mice were treated with combination of 1000 mg/kg *M. oleifera* and 1000 mg/kg *M. vulgare*. P1–P3 as cure treatment and P4–P8 as protective treatment.
the production of IFN-γ by CD8+ T cells [Figure 5b, P1, P2, and P3]. Treatment with M. oleifera extracts as protective agent caused the huge decrease to the IFN-γ production by CD8+ T cells compared to the positive control (P4 = 10.279%). Using the same concentration of M. vulgare extract as protective agent had no effect on the IFN-γ production by CD8+ T cells compared to the positive control [Figure 5b, P5 = 26.142%]. Combination treatment with low concentration of 100 mg/kg of M. oleifera extract and 100 mg/kg M. vulgar extract as protective agent caused a decrease in the IFN-γ production by CD8+ T cells (P6 = 11.307%). The moderate concentration 500mg/kg and high concentration 1000 mg/kg of M. oleifera and M. vulgar combination as protective agents had opposite effect where it increased the production of IFN-γ by CD8+ T cells [Figure 5a, P7 = 30.068% and P8 = 29.306%]. This study also showed a significant difference in the level of IFN-γ between mice treated with M. oleifera and M. vulgar groups, cure and protective groups, and single and combination groups. Based on our findings, the leaf aqueous extract of M. oleifera produced potential immunomodulatory effects, especially as protective agent than as the curing agent. The single treatment of M. oleifera extracts more effective than combination treatments with M. vulgar extract.

**DISCUSSION**

*Salmonella* contributes to a variety of infections such as gastroenteritis, enteric fever, bacteremia, endovascular infections, and focal infections such as osteomyelitis and abscesses. The numbers of studies have documented that the infection of *Salmonella* induces the T1 response. A subset of DP T cells expressing high levels of both CD4 and CD8 molecules has been identified in chronic inflammatory conditions.[12,13] In this study, we found that the expression of CD4+CD8+ DP T cells is strongly expressed during the infection of *S. typhimurium* [Figure 1]. It was indicated that *Salmonella* infection induces antigen-specific CD4 and CD8 T cells activation which can contribute to protective immunity. The DP T cells generally represent a very small population, but it can be increased in current reactive condition such as bacterial infection. Thus, the DP T cells expression can be a sign of chronic inflammatory condition. Despite indications from their expression in *S. typhimurium*-infected mice, the level of DP T cells was not increased in mice after *S. typhimurium* infection and treatment with M. oleifera as a protective agent. M. oleifera leaf aqueous extract had potential immunomodulatory effects when it administered before *S. typhimurium* infection. This suggests that single treatment with M. oleifera leading to a decrease in the percentage of DP T cells activation due to the increasing of naïve CD4+ T cells [Figure 2].

Recent studies investigated that *Salmonella*-specific CD4 T cells were activated as an early response to *Salmonella* infection. During the infection process, dendritic cells as antigen-presenting cells present antigens to naïve T cells and then causing expansion of *Salmonella*-specific Th1 cells. Th1 cells have the ability to produce the effector cytokine IFN-γ and migrate to the infected tissues. Under normal condition, the level of naïve T cells is very low and there is an inadequate amount of *Salmonella* epitope in major histocompatibility complex Class I and II.[14] These results of this study proved that the level of naïve T cells was increased significantly (P < 0.05) in mice with *S. typhimurium* infection and administered M. oleifera extract as curing agent. However, using M. oleifera extract as protective agent leads to decrease in the relative number of naïve T cells. The CD8+ T cells also activated rapidly during *Salmonella* infection and implying that CD8+ T cells memory is early developed after infection. These memory CD8+ T cells are segregated based on their CD62L expression.[15] Treatment with M. oleifera extract as curing agent revealed a high increase in the level of T cytotoxic cells expressing CD62L or naïve CD8+ T cells, but using same dose of M. oleifera extract as protective agent showed a decrease compared to the positive control. Furthermore, using high concentration (1000 mg/kg) of M. oleifera and M. vulgar combination extracts leads to increase to a high number of naïve CD8+ T cells. CD8+ T cells are involved in destroying infected host cells through cytolysis activity and production of IFN-γ, tumor necrosis factor-α, and IL-17 cytokines. The ability of expanded CD4 and CD8 T-cell levels to secrete IFN-γ in response to *Salmonella* antigen persists.[16] Mayuzumi et al.[17] showed that the expression of IFN-γ is increased around 3 days after *S. typhimurium* infection. IL-17 cytokines also participate in the early stage of infection, but IFN-γ is participating at the later stage of the infection. It has been revealed in the present study that the level of IFN-γ and IL-17 production by CD8+ T cells was increased significantly after *Salmonella* infection. These cytokines cooperate in protective immunity against *S. typhimurium* during the early period of *S. typhimurium* infection. Treatment with M. oleifera and M. vulgar extract as protective agent may be a promising agent for modulating the level of IFN-γ and IL-17 production after *S. typhimurium* infection. Administration of M. oleifera extract for 14 days caused a noticeable decrease in the level of IFN-γ and IL-17 production by CD8+ T cells. Furthermore, combination treatment with 100 mg/kg of M. oleifera and 100 mg/kg of M. vulgar extracts as the protective agent also caused a decrease in the IFN-γ and IL-17 production by CD8+ T cells. These effects might be caused by the bioactive compound in each extract of M. oleifera and M. vulgar which has immunomodulatory properties.
activity. The possible mechanism of *M. oleifera* and *M. vulgare* effect in this study might be caused by the ability of the phytochemical compound to regulate inflammatory genes. *M. oleifera* aqueous leaf extract downregulates a pro-inflammatory transcription factor NF-κB and increases the cytotoxic effect.[10] Downregulation of NF-κB is followed by decreasing of pro-inflammatory cytokines such as IL-17 and IFN-γ. *M. oleifera* and *M. vulgare* as curing agent also showed an immunomodulatory effect in the level of DP T cells, IL-17, and IFN-γ at low dose (P6, 100 mg/kg BW), whereas the level of naïve CD4+ and CD8+ T cells increased at the high dose of *M. oleifera* and *M. vulgare*. However, the immunomodulatory effects were higher during single treatment with *M. oleifera*. The various biological activities of *M. oleifera* including antiproliferation, hepatoprotective, anti-inflammatory, antiinociceptive, antiatherosclerotic, oxidative DNA damage protective, antiperoxidative, and cardioprotective. This effect is attributed to the presence of bioactive compounds such as phenolic acids, flavonoids, alkaloids, phytosterols, natural sugars, vitamins, minerals, and organic acids.[11] The previous research in Brazil was conducted to evaluate the antibacterial effect of aqueous and ethanolic *M. oleifera* leaf extracts to the growth of Gram-positive and Gram-negative bacteria. The result shows promising potential for aqueous and ethanolic *M. oleifera* leaf extracts as the alternative treatment to cure infections caused by the tested strains especially *Salmonella*.[12] The plant constituent phytochemical screening of *M. oleifera* was analyzed using qualitative methods by Imhoisien et al.[13] The ethanol extract of *M. oleifera* leaf was active against *E. coli* and *Salmonella typhi*. It was indicated by the presence of bioactive components, which proves *Moringa* potency to treat some bacterial infections.[14]

Another immunomodulatory study evaluated the effect of 50, 100, and 200 mg/kg BW of the alcoholic and hydroalcoholic extract of the leaves of *M. oleifera* on various immune paradigms. The hydroalcoholic extract of *M. oleifera* substantially enhanced cellular immune response, humoral immune response, neutrophil index, and phagocytic activity in doses of 100 and 200 mg/kg BW. The ethanolic extract (200 mg/kg BW) was efficient in improving immune response. The results suggest that *M. oleifera* has a significant role to play as an immune stimulator.[15]

The antibacterial effect of *M. vulgare* L. leaf extracts was evaluated against 30 *Klebsiella pneumoniae* strains isolated from urine culture of hospitalized patients. The essential oil and extract of this plant could serve as an antibacterial agent in pharmaceutical industry.[9] Zarai et al. revealed *M. vulgare* essential oil possesses a potent antimicrobial effect against some Gram-positive pathogenic bacteria and Botrytis cinerea fungi.[21] The methanolic extract of *M. vulgare* whole plant had *in vitro* antibacterial.[22]

**CONCLUSION**

The results suggest that single treatment with *M. oleifera* extract is more effective than combination treatments with *M. vulgare* in *S. typhimurium*-infected mice. The findings have proven that *M. oleifera* and *M. vulgare* will be a very useful material for future herbal medicine.

**REFERENCES**

17. Mayuzumi H, Inagaki-Ohara K, Uyttenhove C, Okamoto Y, Matsuzaki G. Interleukin-17A is required to suppress invasion
of *Salmonella enterica* Serovar *typhimurium* to enteric mucosa. Immunology 2010;131:377-85.


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