

CHAPTER VII

CONCLUSION AND SUGGESTIONS

7.1 Conclusion

From the research result, it can be concluded that:

7.1.1 Extract of *Thymus vulgaris* increases the level of IL-12 in mice blood infected by *S. Typhimurium*.

7.1.2 Extract of *Thymus vulgaris* decreases bacterial in liver the infected with *S. Typhimurium*.

7.2 Suggestions

Thymus vulgaris have antibacterial activity against *S. Typhimurium*.

Thymus vulgaris in male mice will provide of the increase IL12 blood level and decreases bacterial colonies in liver.

From this experiment, suggestion or opinions in the future researcher:

1. Future detailed research should be conducted to determine the lowest dosage with the *Thymus vulgaris* effect on male immunity.
2. The duration of research should be maintained to discover the exact duration for *Thymus vulgaris* to optimize its effect on immune response agonists

REFERENCES

- A.Zarzuelo and E. Crespo, "The medicinal and non-medicinal uses of thyme," in *Thyme: The Genus Thymus. Medicinal and Aromatic Plants—Industrial Profiles*, E. Stahl-Biskup and F. Saez, Eds., pp. 263–292, Taylor & Francis, New York, NY, USA, 2002.
- Abbas A K. , Lichtman A H, 2004. *Basic Immunology Function And Disorders Of Immune System*. ISBN: 0-7216-0241-X
- Achouri S, Wright JA, Evans L, Macleod C, Fraser G, Cicuta P, et al. The Frequency And Duration of Salmonella–Macrophage Adhesion Events Determines Infection Efficiency .*Phil.Trans. R. Soc.* 2015. B 370: 20140033
- Achouri S, Wright JA, Evans L, Macleod C, Fraser G, Cicuta P, et al , The Frequency And Duration of Salmonella- Macrophage Adhesion Events Determines infection Efficiency. *Phil.Trans.R. Soc.* 2015. B 370. 20140033.
- AL-Saimary I, S Bakr, B Khudaier, Y Abass (2006). Efficiency of antibacterial agents extracted from *Thymus vulgaris* L. (*lamiaceae*). *The Internet Journal of Nutrition and Wellness*. 4 (1):1-5.
- Alun C. Kirby, Ulf Yrlid, And Mary Jo Wick . *The Innate Immune Response Differs In Primary And Secondary Salmonella Infection*. 2002. 169:4450-4459
- Amiri, H. (2012). "Essential oils composition and antioxidant properties *Thymus* species. *Evidence-Based Complementary and Alternative Medicine*.:1-8.
- Arcila-Lozano CC, Loarca-Pina G, Lecona-Urbe S, Gonzalez EM (2004). Oregano properties, composition and biological activity. *ALAN*. 54(1): 100-111.
- Bahman Nickavar, Faraz Mojab, Reza Dolat-Abadi (2005). Analysis of the essential oils of two *Thymus* species from Iran. *Food Chemistry*. 90(4):609–611
- Ben Arfa, S. Combes, L. Preziosi-Belloy, N. Gontard, P. Chalier (2006). Antimicrobial activity of carvacol related to its chemical structure. *Lett. Appl. Microbiol.* 43(2):149-54.
- Benjamin, W. H., Jr., P. Hall, S. J. Roberts, and D. E. Briles. 2008. The primary effect of the *Ity* locus is on the growth rate of *Salmonella typhimurium* that are relatively protected from killing. *J. Immunol.* 144:3143-3151.
- Birgitta J, Yvonne A, Carl E (2005). Effect of regulation and education and reptile associated salmonellosis. *Emerg. Infect. Dis.* 11: 398-403
- Braga P.C, M. D. (2006). Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil elastase. *pharmacology*.
- Braga PC, Dal Sasso M, Culici M, Bianchi T, Bordoni L, Marabini L (2006). Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil Elastase. *Pharmacology*. 77(3): 130- 136.

- Braga PC, Dal Sasso M, Culici M, Bianchi T, Bordoni L, Marabini L (2006). Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil Elastase. *Pharmacology*. 77(3): 130- 136.
- Bukovska A, Cikos S, Juhas S, Il'kova G, Rehak P, Koppel J (2007). Effects of a combination of thyme and oregano essential oils on TNBS-induced colitis in mice. *Mediators Inflamm*. 23296.
- Carter, P.B., and F.M. Collins. 1975. Peyer's patch responsiveness to *Salmonella* in mice. *J. Reticuloendothel. Soc.* 17:38–46.
- Cebra JJ 2006 Influences of microbiota on intestinal immune system development. *Am J Clin Nutr* 69:1046S–1051S 8
- Dalleau S, Cateau E, Bergès T, Berjeaud JM, Imbert C. (2008). In vitro activity of terpenes against *Candida* biofilms. *Int J of Antimicrobial agents*.31(6):572-6.
- Dorman HJ, Deans SG (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*88(8): 308–316.
- El-Nekeety AA, Mohamed SR, Hathout AS, Hassan NS, Aly SE, Abdel- Wahhab MA (2011). Antioxidant properties of *Thymus vulgaris* oil against aflatoxin-induce oxidative stress in male rats. *Toxicon*. 57(7- 8): 984–991.
- El-Nekeety AA, Mohamed SR, Hathout AS, Hassan NS, Aly SE, Abdel- Wahhab MA (2011). Antioxidant properties of *Thymus vulgaris* oil against aflatoxin-induce oxidative stress in male rats. *Toxicon*. 57(7- 8): 984–991.
- Eman A. Al-Timimi, B. M.-C. (2012). Effect of *thymus vulgaris* extract on streptococci and mutans streptococci, in comparison to chlorhexidinegluconate (in vivo study) .*J Bagh College Dentistry*. 24(3):116-121.
- F. C. Fachini-Queiroz, R. Kummer, C. F. Estev~ao-Silva et al., “Constituents of *Thymus vulgaris* L. Essential Oil, on the inflammatory response,” *Evid Based Complement Alternat Med*, vol. 2012, Article ID 657026, 2012.
- Fierer J, Swancutt M A, Heumann D, Golenbock D. The Role of Lipopolysaccharide Binding Protein In Resistance To *Salmonella* Infections In Mice, *Journal of Immunology*, pp. 6396–6403.
- Filomena Nazzaro, Florinda Fratianni, Laura De Martino, Raffaele Coppola and Vincenzo De Feo (2013). Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals*. 6 (12):1451-1474
- Finnemore, H.,. *The Essential Oils*. Ernest Benn Limited, London Gouyon, P.H., Ph. Vernet, J.L. Guillermin and G. Valdeyron, 1986. Polymorphisms and environment: the adaptive value of the oil polymorphisms in *Thymus vulgaris* L. *Heredity*, 57: 59–66.

- Gabius, H.J., *Probing The Cons And Pros of Lectin-Induced Immunomodulation: Case Studies For The Mistletoe Lectin And Galectin-1*. *Biochemie*, 2003,.83: 659-666. PMID: 11522395.
- Garai, D. P. P. Gnanadhas, and D. Chakravorty, "Salmonella enterica serovars Typhimurium and Typhi as model organisms: revealing paradigm of host-pathogen interactions," 2012. *Virulence*, vol. 3, no. 4, pp. 377–388
- Grimont P. A. and F. X. Weill, *Antigenic Formulae of the Salmonella Serovars*, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2007
- Guydish B.S.M., Olomu I.N., Zhao H., Wong R.J., Stevenson D.K., Contag C.H. *Monitoring Age-related susceptibility of young mice to oral Salmonella enterica serovar typhimurium infection using an in vivo murine model*. 2005. *Pediatr. Res.* 58,153-158
- Hamza T, John B. Barnett and Bingyun Li. *Interleukin 12 a Key Immunoregulatory Cytokine in Infection Applications*. *International Journal of Molecular Sciences*. Int. J. Mol. Sci. 2010, 11, 789-806; doi:10.3390/ijms11030789
- Haynie, D.T.; Palath, N.; Liu, Y.; Li, B.; Pargaonkar, N. *Biomimetic nanotechnology: Inherent reversible stabilization of polypeptide microcapsules*. *Langmuir* 2005, 21, 1136–1138
- Helander I.M., H.-L. Alakomi, E. Skyttä, M. Saarela, T. Mattila-Sandholm, and K. Latva-Kala, (2014). *characterization of the action of selected essential oil components on gram negative bacteria*. *J.Agric.Food Chem.*46(9):3590–3595.
- Hurley D , Matthew P. M , Fanning S , And Martins M . *Salmonella–Host Interactions – Modulation Of The Host Innate Immune System*. *Immunology* 2014 Oct 7. doi: 10.3389/fimmu.2014.00481.
- Jiang, B.; Li, B (2009). *Tunable drug incorporation and release from polypeptide multilayer nanofilms*. *Int. J. Nanomed.*, 4, 37–53
- Jones BD, Falkow S (2010). *Salmonellosis: Host Immune responses and bacterial virulence determination*. *Ann. Rev. Immunol.* 14:533- 556
- Juhás S, Cikos S, Czikková S, Veselá J, Il'ková G, Hájek T, Domaracká K, Domaracký M, Bujnáková D, Rehák P, Koppel J (2008). *Effects of borneol and thymoquinone on TNBS-induced colitis in mice*. *Folia Biol (Praha)*. 54(1): 1-7.
- Juhás S, Cikos S, Czikková S, Veselá J, Il'ková G, Hájek T, Domaracká K, Domaracký M, Bujnáková D, Rehák P, Koppel J (2008). *Effects of borneol and thymoquinone on TNBS-induced colitis in mice*. *Folia Biol (Praha)*. 54(1): 1-7.
- Khan KH, Ganjewala D, Rao KVB. *Recent advancement in typhoid research- a review*. *Advanced Biotech* 2008; 7 (4): 35-40.

- Kortylewski, M., Xin, H., Kujawski, M., et al. 2009. Regulation of the IL-23 and IL-12 balance by STAT-3 signaling in the tumor microenvironment. *Cancer Cell*. 15:114–123.
- Kotton C (2007). Typhoid fever medicine plus <http://www.nlm.nih.gov/medlineplus/cartridge/001332.htm>. Retrieved 04/05/2007.
- Kumar SV, Kumar SP, Rupesh D, Nitin K. Immunomodulatory Effects Of Some Traditional Medicinal Plants , *J. Chem. Pharm.*2011 Res. 3 (1):675-684.
- Lam, H.Y., Yeap S.K., Alitheen N.B., Ho W.Y, Omar A.R. et al. Understand the role of natural killer. *Am. J. Immunol.*, 2010, 6: 54-61. DOI: 10.3844/ajisp.2010.54.61.
- Lapaque N, Walzer T, Me´resse S, Vivier E, Trowsdale J. Interactions between human NK cells and macrophages in response to *Salmonella* infection. *J Immunol* 2009; 182: 4339-48.
- Ley RE, Peterson DA, Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837–848.
- Liang, D., Li, F., Fu, Y., et al. 2014. Thymol inhibits LPS-stimulated inflammatory response via down-regulation of NF- κ B and MAPK signaling pathways in mouse mammary epithelial cells. *Inflammation* 37:214–222.
- Lima, M. D., Quintans-Ju ´nior, L. J., de Santana, W. A., et al. 2013. Anti- inflammatory effects of carvacrol: Evidence for a key role of IL-10. *Eur. J. Pharmacol.* 699:112–117.
- Lis-Balchin, M. and S.G. Deans, 2005. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Bacteriol.*, 82: 759–762
- Ma´kela¨, P. H., and C. E. Hormaeche. 2007. Immunity to salmonella. In *Host Response to Intracellular Pathogens*. S. H. E. Kaufmann, ed. R. G. Landes, Austin, TX, pp. 143–166.. Immunity to salmonella. In *Host Response to Intracellular Pathogens*. S. H. E. Kaufmann, ed. R. G. Landes, Austin, TX, pp. 143–166.
- MaksimovićZoran,Danilo Stojanović,Ivan Šoštarić,Zora Dajić,Mihailo Ristić (2008). Composition and radical-scavenging activity of *Thymus glabrescens* Willd.(Lamiaceae) essential oil. *J Sci Food Agr* . 88(11):2036–2041
- Marino M, Bersani C, Comi G (1999). Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. *J. Food Prot.* 62(9): 1017–1023.
- Marino M, Bersani C, Comi G (1999). Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. *J. Food Prot.* 62(9): 1017–1023.
- Mastroeni P and Me´nager N. Development of Acquired Immunity to *Salmonella*, *Journal of Medical Microbiology*, 2003, 52, 453–459.

- Meeran, M.F., P.S. Prince (2012) Protective effects of thymol on altered plasma lipid per-oxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. *J Biochem Mol Toxicol* 26: 368–373.
- Mittrücker H and Kaufmann S H E ., Immune response to infection with *Salmonella Typhimurium* in mice,. *Journal of Leukocyte Biology* 2000.475- 461
- Mittrücker H, Raupach B, Köhler A, Kaufmann S.H.E. Cutting Edge: Role of B Lymphocytes in Protective Immunity Against *Salmonella Typhimurium* Infection,.*J Immunol*, 2000, 164:1648-1652
- Miura K, Kikuzaki H, Nakatani N (2002). Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *J. Agric. Food Chem.* 50 (7): 1845–1851.
- Mukherjee P K, Nema N K, Bhadra S, Mukherjee D, Braga C F, and Matsabisa M G. *Immunomodulator Leads From Medicinal Planta, Indian Journal Of Traditional Knowledge* 2014, p. 235-256.
- Muotiala, A., And P. H. Makela. *The Role Of IFN- γ In Murine Salmonella Typhimurium Infection.*2008.*Microb. Pathog.* 8:135-141
- Murphy K . 2012, *Janeway's Immunobiology Diseases And Immunological Deficiencies.* ISBN 978-04243-4-8153-.
- Murphy K . 2012, *Janeway's Immunobiology Diseases And Immunological Deficiencies.* ISBN 978-04243-4-8153-.
- Nasser Gholijani ,and Marjan Gharagozloo (2015) *Modulatory effects of thymol and carvacrol on inflammatory transcription factors in lipopolysaccharide-treated macrophages.*
- Nweze E.L, Okafor.J.I.(2010). *Antifungal activities of a wide range of medicinalplants extracts and essential oils against Scedosporiumapiospermum isolates.**American-Eurasian J of Scientific research.* 5(3): 161-9.
- Ocaña, A., G. Reglero (2012) *Effects of thyme extract oils (from Thymus vulgaris, Thymus zygis, and Thymus hyemalis) on cytokine production and gene expression of oxLDL-stimulated THP-1-macrophages.* *J Obes* 2012: 1–11.
- on these cytokines (Guimaraes et al., 2012; Lima et al., 2013; Samara et al., 2014).
- Ozkaya H , Abdullah Baris, Akcan Etc .*Salmonella Typhimurium Infections In Balb/C Mice: A Comparison Of Tissue Bioluminescence, Tissue Cultures And Mice Clinical Scores ,* 2011.
- Palaniappan Kavitha & Richard A Holley (2010). *Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria.**International Journal of Food Microbiology.* 140(2-3):164-8.
- Papalia, A. N. (2012). *Antimicrobial Activity of Carvacrol: Current Progress and Future Prospectives.* *Recent Patents on Anti-Infective Drug Discovery.*7: 28-35.

- Park, J. S., Lee, J., Lim, M. A., et al. 2014. JAK2-STAT3 blockade by AG490 suppresses autoimmune arthritis in mice via reciprocal regulation of regulatory T-cells and TH17 cells. *J. Immunol.* 192: 4417–4424.
- Parkhill, J., G. Dougan, K.D. James, N.R. Thomson, D. Pickard, J. Wain, C. Churcher, K.L. Mungall, S.D. Bentley, M.T. Holden, et al. 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. *Nature.* 413:848–852.
- Picone, G., Laghi, L., Gardini, F., Lanciotti, R., Siroli, L., & Capozzi, F. (2013). Evaluation of the effect of carvacrol on the *Escherichia coli* 555 metabolome by using ¹H-NMR spectroscopy. *Food Chem.* 141(4) :4367–4374.
- Pina-Vaz C, Gonçalves C, Rodrigues E, Pinto S, Costa-de-Oliveira C, Tavares L, Salgueiro, C., Cavaleiro MJ, Martinez-de Oliveira J (2004). Antifungal activity of Thymus oils and their major compounds. *J. Eur. Acad. Dermatol. Venereo.* 18(1): 73–78.
- Prabuseenivasan, S., M. Jayakumar and S. Ignacimuthu, 2006. *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary Altern. Med.*, 6: 39
- R. Baranauskiene, P. R. Venskutonis, P. Višskelis, and E. Dambrauskiene, “Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*),” *Journal of Agri- cultural and Food Chemistry*, vol. 51, no. 26, pp. 7751–7758, 2003.
- Rofaiil, S.K., Ragaa A. Soliman, Ola F. Abo Elmenem and M.H., Mahmoud. *Studies On Some Medicinal Plant Extracts as Immunostimulant In Rats Experimentally Infected With Salmonella Typhimurium Egypt.* 2011. *J. Agric. Res.*, 89 (1).
- Rossi GA, Zocchi E, Sacco O, et al. - Alveolar macrophage stimulation of T-cell proliferation in autologous mixed lymphocyte reaction. Role of HLA-DR antigens. *Am Rev Respir Dis*, 2008; 133: 78-82.
- Rubin, R.H., and L. Weinstein. 1977. *Salmonellosis: Microbiologic, Pathogenic, and Clinical Features.* Stratton Intercontinental Medical Book Corp., New York. 137 pp.
- Sahelian, R. (2016). *Thyme herb health benefit leaf research and medical uses.* <http://www.raysahelian.com/thyme.html>. (March 12 2016).
- Salcedo S P, Noursadeghi M, Cohen J, Holden D W. Intracellular Replication of *Salmonella Typhimurium* Strains In Specific Subsets of Splenic Macrophages In Vivo, *Cellular Microbiology*, 2001, 3 (9), 587–597
- Salzman, N. H., H. de Jong, Y. Paterson, H. J. M. Harmsen, G. W. Welling, and N. A. Bos. 2002. Analysis of 16S libraries of mouse gastrointestinal microflora reveals a large new group of mouse intestinal bacteria. *Microbiology* 148:3651–3660.

- Samara, R. B., Damasceno, F. R., Nathalia, S., et al. 2014. Carvacryl acetate, a derivative of carvacrol, reduces nociceptive and inflammatory response in mice. *Life. Sci.* 14:58–66.
- Santos, R.L., S. Zhang, R.M. Tsois, R.A. Kingsley, L.G. Adams, and A.J. Baumler. 2001. *Animal models of Salmonella infections: enteritis versus typhoid fever. Microbes Infect.* 3:1335–1344.
- Stanilove, S.A., Dobrova Z.G, Slavov E.S. and Mitera L.D, . C3 binding glycoprotein from *Cuscuta Europea* induced different cytokine profiles from human PBMC compared to other plant and bacterial immunomodulators. *Int. Immunopharmacol*, 2005, 5: 723-734. PMID: 15710341
- Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, et al. (2004) Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc Natl Acad Sci U S A* 101: 1981–1986
- Toews GB, Vial WC, Dunn MM, et al. - The accessory cell function of human alveolar macrophages in specific T-cell proliferation. *J Immunol*, 1984; 132: 181- 186.
- Ugrinovic S, Me ´nager N, Goh N, and Mastroeni P. Characterization and Development of T-Cell Immune Responses in B-Cell-Deficient (*Igh-6*^{-/-}) Mice With *Salmonella enterica* Seroovar Typhimurium Infection. *Infection and immunity*, 2003, p. 6808–6819.
- Ultee, A., Bennik, M., & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* 68(4):1561–1568.
- Ündeğer, Ü., Başaran, A., Degen, G. H., & Başaran, N. (2009). Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 Chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. *Food and Chemical Toxicology*. 47(8):2037–2043.
- van der Poll T, Lowry SF.(1995). Tumor necrosis factor in sepsis: mediator of multiple organ failure or essential part of host defense? *Shock*. 1995;3:1-12.
- Valle Balbi B, MT, Oddera S, et al. - Thymomodulin increases release of granulocyte-macrophage colony stimulating factor and of tumour necrosis factor in vitro. *Eur Respir J*, 1992; 5: 1097- 1103.
- Vigo E, Cepeda A, Gualillo O, Perez-Fernandez R (2004). In-vitro anti-inflammatory effect of *Eucalyptus globulus* and *Thymus vulgaris*: nitric oxide inhibition in J77RA.1 murine macrophages. *J. Pharm. Pharmacol.* 56(2): 257–263.
- WHO (2003). *Manual for the Laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world.* pp. 103-162.

- Wilcock C, Manson-Bahr PEC (1972). *Manson's tropical disease* 17th edn. Macmillan, Macmillan, Tindall, pp. 630-632.
- Willi M Hans , Cker And Stefan H. E. Kaufmann . *Immune Response To Infection With Salmonella Typhimurium in Mice*. 2000. *Journal Of Leukocyte Biology* Volume 67
- Y. Mariana and R. Setiabudy, "Sulfonamid, Kotrimoksazol dan Antiseptik Saluran Kemih," in *Farmakologi Dan Terapi (Sulfonamides, Cotrimoxazole and Urinary Tract Antiseptic)*, S. G. Ganiswarna et al., Ed., pp. 584–596, Faculty of Medicine, Indonesia University, Jakarta, Indonesia, 1995
- Zarrini Gholamreza , Zahra Bahari Delgosha, Kamyar Mollazadeh Moghaddam & Ahmad Reza Shahverdi (2010). *post antibacterial effect of thymol. Pharmaceutical biology*. 48(6):633-636.