CHAPTER6

DISCUSSIONS

It took 2 months to complete the study of *Thymus vulgarise* tha extract effect on blood level of IL-12 and bacterial colonies in liver in 20 male rats infected with Salmonella Typhimurym.

There were 20 mice categorised into 5 groups with 4 mice in each, given different doses with *Thymus vulgaris* D1 extract (250mg / kg B.wt), D2 (500mg / kg Bwt), D3 (750mg / Kg B.wt) . With a positive C1 group, rats were infected with Salmonella typhimurym. While the negative group C2 is still normal with infection and treatment (such as control group)

The treatment groups (D1, D 2, D3) were treated (250,500 and 750 mg / kg Bwt rats) each for 7 days and the bacteria were inoculated at the same time. In this study, we used ELISA to measure L-12 levels and measure the number of bacterial colonies in the liver.

Thymus vulgaris extract increased IL-12 at the dose of 250 mg/kgETV (D1), 500 mg/kgETV (D2) 750 mg/kgETV (D3). IL-12 more increased when given higher doses,After the treatment was given in the form of *Thymus vulgaris* extract started at a dose of 250 mg/kg. Then the colony of *Salmonella Typhimurium* becomes zero when given higher doses of 500 mg/kg (D2), and decrease again when given higher doses of 750 mg/kg (D3).

6.1. Effect of *Thymus vulgaris* Extract on Levels of blood IL-12 and bacterial colonies in liver in mice infected with *Salmonella Typhimurym*.

IL-12 possesses many biological activities which is a main factor which simulates responses of Th1 and the production of IFN. Initial need or production of IL-12 when the bacteria infect can cause macrophages activated and enhance immunity mediated by the cells during the formation of a final response of immune which is antigen-specific. Consequently, IL-12 can be the main player in the bacterial viral infection protection. Besides, immunotherapy of IL-12 can be essential to treat sicknesses in which the Th1 response is needed. Whilst, the cytokines which involve IL-12 are commonly half-life in vivo and in the establishment of more modern medicine delivery mechanisms *(Haynie et al., 2005; Jiang et al., 2009).*

Thymus vulgaris extract affects or different effects on IL-12. The existence of the effect of extracts of Thymus vulgaris starting to look where bacteria IL-12 on the bacterium Salmonella typhimurium induces becomes highest, after administration is given in the form of extracts of *Thymus vulgaris* started at a dose of 250 mg / kgETV (D1), compared with IL-12 in the positive control group. Then IL-12 increased more when given a higher dose of 500 mg / kgETV (D2), up to a dose of 750 mg / kgETV higher (D3). Thus, based on the descriptive assessment according to the mean IL-12, it can be said that the treatment of *Thymus vulgaris* extract with doses of 250 mg / kgETV (D1), 500 mg / kgETV (D2), and 750 mg / kgETV (D3) Different effects, in which a higher dose of *Thymus vulgaris* extract given will increase IL-12 further.

This study indicates that pre-treatment with carvacrol macrophages significantly inhibited the protein IL-1b and TNFa and gene expression and thymol also significantly decreased the expression of IL-1β β (Nasser Gholijani and Marjan *(Gharagozloo et al., 2015). Thymus vulgaris* extract biological response modifiers, in the culture of alveolar macrophages and blood lymphocytes, the Securities to increase the release of tumor necrosis factor (TNF) and granulocytes colony-stimulating factor-macrophage (GM-CSF) in eo-culture of macrophages with autolo-Lymphocytes gous, and stimulate Production of GM-CSF by lymphocytes *(Valle Balbi, et al, 1992).* Because cytokines modulate inflammatory and immunocompetent cell activity, the ability to stimulate the release of TNF and GM-CSF suggests that it has other effects on macrophage and lymphocyte function. In the lungs, the interaction between macrophages and lymphocytes, which involves the release of cytokines, the expression of surface molecules and cellular proliferation, is the basic step of any immune reaction to antigenic stimulation (*Rossi GA and Toews et al., 2008).*

In human oxidized macrophages, thyme extract induces significant reductions in the expression of proinflammatory mediator genes and enhancement of antiinflammatory cytokines *(Ocaña and Regle-ro, 2012).* In vivo studies have demonstrated a protective effect of thymus against myocardial *infarction (Meeran and Prince, 2012).* Vertgaris extract inhibits the formation of transcription factors p-NF-jBp65 and p-p38, it can be assumed that their anti-inflammatory effect may occur. Due to inhibition of other inflammatory transcription factors, such as SAPK / JNK, STAT3, AP-1 and various NFAT. In this case, the current study shows that the levels of c-fos inducible, NFAT-1 and NFAT-2 significantly decreased by pretreatment with Thymus vulgaris extract c-jun level was significantly lowered *Thymus vulgaris* extract significantly decreased STAT -3 And SAPK / JNK phosphorylation in stimulated macrophages (total cell extract); This suggests to us the possible ability of these

agents to reduce the activity of STAT-3- and JNK-mediated cytokines that are mediated by inflammatory responses *(Kortylewski et al., 2009)*. It should be mentioned that, in a previous study on total cell extracts of mammary epithelial cells stimulated by LPS, *Thymus vulgaris* lower levels of phosphorylation of p65 NF-JB, IjBa, JNK, ERK and p38 MAPK after 1 hour *(Liang et al., 2014)*. In the current study, according to ELISA test results, carvacrol and thymol also inhibited phosphorylation of IjBa, JNK and STAT-3. However, carvacrol promotes the phosphorylation of p38 MAPK. *Thymus vulgaris* significantly increases the phosphorylation of NF-jB p65. The difference between the current yield and Liang et al. Research can be associated with differences in incubation time, techniques and especially the type of cells studied. A number of pharmacological agents have been shown to selectively target key signaling molecules involved in inflammation, including NF-jB. Salicylates and glucocorticoids are the two commonly used anti-inflammatory drugs that have an inhibitory effect on NF-jB activation *(de Bosscher et al., 2014; Park et al., 2013)*.

Thymus vulgaris extract effect on the major transcription factors is included in the regulation of inflammatory processes. This research first studied the impact of this component on the viability of J774.1 macrophage cells in an attempt to rule out the possibility of cytotoxic effects. Furthermore, two high concentrations without cytotoxicity of each of the agents were investigated for the effects of anti-inflammatory by measuring the production of cytokines of proinflammatory TNFa and IL-1β. This cytokine is a major mediator of inflammatory response and its excessive expression can cause severe proinflammatory reactions. Previous studies have reported the effects of *Thymus vulgaris* inhibition on these cytokines *(Guimaraes et al., 2012; Lima et al., 2013; Samara et al., 2014)*.

Extract effects on this type of immune response was researched in the MLR test. T*. vulgaris* extract significantly suppressed T cell proliferation in MLR. In this activity, T*. vulgaris* because it inhibits proliferation at low concentrations. Proliferation of lymphocytes is an important case causing the initial and developing 9oinflammation *(Hosseinzadeh, H., Ramezani et al., 2000)*. Some are testing the effects of thymol and carvacrol on DCs coordinated with T cells to determine their impact on DC function and T-cell response. Both compounds at 10mg / ml significantly reduced the proliferation of T cells in MLR. To determine whether thymol and carvacrol modulate cytokine production by T cells, measured levels of IFN-ɣ and IL-4 as main T helper (h) 1 and Th2 cytokines in a mixed lymphocyte culture supernatant.

This suggests that the extracts of thyme are able to aleviate the presence of polymorphonucleates, lymphocyte total, CD4+ T-cells, CD8+ and NK cells. At the same time, "Oregpig" (such commercially sold feed additive products which cntain 60 g of carvacrol and 55 g of thymol per kilogram) posseses immunostimulatory impacts which are not specific to pig cell immunity. In addition, the number of CD4, CD8, MHC class II and cells such as non-T / non antigen-B in lymphocytes in peripheral blood were found substantally more in pigs receiving Oregpig than in control animals *(Walter and Bilkei 2004).* Recognizing the inhibitory impacts of T. vulgaris extract on allogenic immune reaction, attempting to discover the effects of extracts on IFN-ɣ and IL-4 production, the two major cytokines involved in Th1 and Th2-mediated Th1 and Th2 responses. As the results of this study, although there is a decrease in IFN-ɣ and increased IL-4 secretion in MLR cultures in the presence of T. vulgaris rather than control, the observed differences did not reach significance. Changes in cytokine production in other extracted treatment cells were also insignificant, suggesting that no extracts affected the production pattern of cytokines by CD4Tc against Th1 or Th2 profiles. Further explanations for obtaining nonsignificant results in cytokine secretion may be the presence of various compounds, possibly by various ways of action in the extract. Various phenolic compounds and other chemical compounds have been reported in this plant, including carvacrol, symol, linalool, thymol, tannin, flavonoid, saponin, borneol, and triterpenic acid in T. vulgaris (Riley *et al.,* 2005) and the effects of This extract on MLR, both increase CD40 expression in DC. CD40 expression is important for DC maturation and function. It is possible that DC activation by this extract has resulted in the release of an inhibiting cytokine such as IL-10. This cytokine can inhibit the proliferative response and also the production of IFN-y in immunomodulators *(Allavena et al., 2009).* This suggests T. vulgaris extract also decreases the proliferation of stimulated mouse lymphocytes, whereas. Daenensis increases this activity, demonstrating the ability of the extract to modulate mitogenic activation of T cells. Tcells activation mainly depends on the state and number of adhesion molecules and costs in DC *(MacDonald, H. R., Nabholz et al., 2006)*