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LIST OF ABBREVIATION

APC	: antigen presenting cells
CAT	: catalase
CMI	: cellular mediated immunity
DNA	: deoxyribonucleic acid
GPX	: glutathione peroxidase
GR	: glutathione reductase
HAI	: histology activity index
HIV	: human immunodeficiency virus
HTLV	: human T cell lymphocyte virus
IFN-γ	: interferon gamma
IL	: interleukin
inos	: inducible nitric oxide synthase
LPS	: lipopolysaccaride
MAC	: membrane attack complex
MHC	: major histocompatibility complex
NK	: natural killer
NO	: nitric oxide
PGE	: prostaglandin E
PMN	: polymorphonuclear
PAMPs	: pathogen associated molecular patterns
PRRs	: pathogen recognition receptors
RES	: reticuloendothelial system
RNI	: reactive nitrogen intermediate
ROI	: reactive oxygen intermediate
ROS	: reactive oxygen species
SARs	: severe acute respiratory syndrome
SOD	: superoxide dismutase
Th1`	: T helper 1
TNF-α	: tumor necrosis factor alpha

CHAPTER I

INTRODUCTION

1.1 Background

Thymus vulgaris possesses a lot of active ingredients including thymol, carvacol and flavonoids. The main chemicals of thyme are essential oils such as borneol, carvacol, linalool and thymol, the bitter flavor, tannin, saponin and triterpenic acids *(Shabnum et al., 2011*). It shows that constituents such as thymol and carvacrol in *Thymus vulgaris* have some effects on the immune response *(F. C. Fachini-Queiroz, et al., 2012).* Medicinal plants and their extracts have been used traditionally worldwide both to treat and prevent some accute diseases such as inflammatory and cardiovascular diseases, arthritis, diabetes, and other diseases *(Juhás et al., 2008).* T. *vulgaris* which is found to be a perennial plant originating from, Africa, central and southern Europe and Asia is commonly used due to those two functions *(Rustaiyan et al., 2000; Braga et al., 2006)*. T. *vulgaris* extracts are famous in alternative medicine because of their activities such as antitussive, antiasthmatic, antiseptic, bronchodilator, antispasmodic, antiviral, antibacterial and antifungal *(Marino et al., 1999; Pina-Vaz et al., 2004)*. In addition, T. *vulgaris* extracts are proved to have anticarcinogenic compounds *(Arcila-Lozano et al., 2004)* and the immunomodulation properties *(Bukovska et al., 2007; Ocaña and Reglero, 2012).* Additionally, T. *vulgaris* is referred by many researchers for its polyphenol and flavonoid, its potential to be antioxidant, scavengers of free radical, antiplatelet, vasorelaxant, antithrombin, antihylperlipidemic, anti-inflammatory and anti-diabetic contents *(Miura et al., 2002; Vigo et al., 2004; Nekeety et al., 2011).*

There are a lot of drugs and chemicals that can improve different immune response aspects. Immunopotentiation can be created by enhancing the level of immune response that develops the response intensity rate and extension or development of response to other non-immunogenic components *(Rofaiil et al., 2007).* Typhoid fever is one of the emerging diseases in developing countries not to mention Indonesia. This disease is caused by *Salmonella* sp. This has been the cause of people being hospitalized. This disease can cause a fatal multisystemic disease as it spurs the intestinal bleeding and perforation. Moreover, it can even lead to complications like hepatitis, meningitis, nephritis, myocarditis, bronchitis, pneumonia, osteomyelitis, and mumps. *Salmonella* sp includes *Salmonela Typhimurium*. There were an estimation of 94 million Salmonella sp cases of gastroenteritis which caused approximately 150 thousands people died every year worldwide by WHO research

from 2001 to 2005. *Salmonella typhimurium* causes the serious problems after S. *Enteritidis*. In Africa, *Salmonella enteritidis* represented just over qquarter and *Salmonella typhimurium* represented quarter from the total isolates. Moreover, in Asia, Europe and Latin America, *Salmonella entiritidis* was often isolated by as much as 38%, 87% and 31% simultabously). As much as 29% of *Salmonella typhimurium* is the most frequent reported cases which then was followed by *Salmonella enteritidis* North America, (WHO et al., 2003).

Research on typhoid fever disease can be done using animal models such as mice, as *Salmonella typhimurium* in mice causes systemic infections and the diseases are similar to those seen in humans who are infected by *S.Typhimurium* (Mittru¨cker *et al*., 2000; Ugrinovic *et al*., 2003).

Chronic gastroenteritis because of S. typhimurium is an essential case that threatens the immune system of people compromised in the West, and is fatal for children and the elderly in developing countries. The estimation of 1.3 billion cases every year causes approximately 3 million people die in the world. Thus, studies of mice infected naturally which then develop symptoms of a disease which has similarity to that examined in human beings are very important (Ozkaya et al., 2011). Moreover, the cause of invasive sickness in rats is closely similar to the case in human suffering from Typhoid fever. There is intravenous inoculation of bacteria residing in the intracellular compartment of spleen and liver. In early stages of infection, salmonella is restricted, and grows mainly in PMN and macrophages (Khana et al., 2009). Additionally, some experiment results showed that microphiles and neutrophils are essential for the host to survive when it gives the initial reaction to Salmonella infections (Alun et al.,2002).

The bacterium ability to attach, attack and persist in the cells of the host becomes important in the Salmonella infection pathogenesis. In infections which is systemic, macrophages are a niche for the proliferation of the bacteria in host organisms. S.Typhimurium is characterized and the frequency at which these bacteria collide is important for infection efficiency. Invasion of salmonella intestinal macrophages, through a mechanism which depends on survival, replication, phagocytosis, and spread in the host organism including, liver (Achouri et al., 2014).

IL12 stimulates TH1 (CD4-specific antigen) cells to release IFN-γ which in turn induce NK cells to produce IFN-γ (Murphy et al., 2012) specifically needed to activate macrophage. Macrophage and natural killer cells are able to produce many

different cytokines and are primarily for the host to survive during the major response to Salmonella infection (Lapaqueet al., 2009).

1.2 Research Problem

The main problem which underlies this study says "Is there any effect of *thymus vulgaris* extract as an anti-inflammatory activity in mice infected with S. *typhimurium*?" Problems in this area can be formulated as the following research questions:

1) Does *Thymus vulgaris* extract increase blood IL-12 in mice infected by *S. typhimurium*?

2) Does *Thymus vulgaris* extract decrease bacterial colonies in the liver of mice infected by *S. typhimurium*?

1.3 Research Objective

1.3.1 General Objective

This research was conducted with the general objective to prove the benefits of *Thymus vulgaris* extract as an anti inflammatory agent in mice infected with *S. typhimurium.*

1.3.2 Specific Objective

According to the research questions, the following specific objectives are drawn as follows:

1) To prove the effect of the *Thymus vulgaris* extract to increase blood IL-12 of mice infected with *S. typhimurium.*

2) To prove the effect of the *Thymus vulgaris* extract to decrease the bacterial colonies in the Liver in mice infected with. *S. typhimurium.*

1.4. Significance of Research

1.4.1 Theoretical benefits

1) This study supports the interests of further research on the benefits of extract of *Thymus vulgaris* especially in infectious diseases.

2) It is also adds knowledge expansion, especially about the benefits of *Thymus vulgaris* as an immunomodulator and antibactrial agent

1.4.2 Practical benefits

1) Practically, the result of *Thymus vulgaris* study is beneficial to be widely used an adjunct to the herbal (phototherapy), immunomodulation and anti bactarial medicine after clinical trial.

CHAPTER 2

LITERATURE REVIEW

2.1 *Salmonella* background.

Salmonella is a gram-negative anaerobic bacterium in the form of a facultative that is generally 2-5 micron in length and 0.5-1.5 micron in width and is driven by peritrichous flagella. Salmonella genome size varies between serovars with a range of 4460 to 4857 kb. Salmonellae belongs to the family Enterobacteriaceae and pathogens are medically important for both human and italic animals (Grimont *et al*., 2007).

Typhoid fever is commonly knows as a worldwide infection caused by *Salmonella typhimurium* bacteria. The disease is transferred from water, food, milk, fruits and vegetables which are contaminated by *Salmonella Typhimurium*. It is also transferred by operators of health and food handlers which are contaminated by *Salmonella Typhimurium* bacteria. *Salmonella Typhimurium* can be mechanically transported from dirt to food by insects like flies, reptiles such as turtles, lizards, and snakes and common pets *(Birgitta et al., 2005*). The World Health Organization predicted the 12-week infection rate of about 12.6 million typhoid fever causing nearly 600,000 people died every year (WHO, 2003).

Typhoid common therapies are antimicrobialagens such as ampicillin, chloramphenicol, and cotrimoxazole or trimethoprim-sulfamethoxazole which is generally used to treat typhoid fever suffered by adults and children who also include carriers, functioned as an alternative medicine to quinolones and ampicillin. Cotrimoxazole restricts the folic acid synthesis which is required by the bacteria to extract nucleic acid. Typhoid fever which is known as enteric fever is one life-threatening chronic illness which is triggered by bacteria of Salmonella Typhimurium (Kotton et al., 2007). This sickness is the most often found fever after malaria, specifically in the tropical regions *(Wilcocks and Manson-Bahr, 1972).* This is possible because *S. Typhimurium* escapes from the cells of macrophage to enter the spleen, liver and other organs in which the bacteria can survive before going back to enter blood *(Jones and Falkow, 1996).*

The rat thypoid model is an experimental model widely used for human typhoid fever. In the previous study experiment, when mice were administered with *Salmonella Typhimurium* at a subletal dose, the infection developed for several weeks

in several clearly identifiable stages. During a natural infection, the early phases of the invasion of bacteria to the intestinal wall were followed by their localization in the cell, perhaps macrophages, first in the local lymph nodes and immediately dominated in the liver and spleen. *(Benjami.et al.1990 and Hohmann et al., 1978).*

2.2 Pathogenesis Of *S.Typhimurium* In mice

Salmonella typhimurium can pass the barrier of epithelial by passively transporting it with the help of dendritic cells extending the pseudopods among the local epithelial cells, or by invading it actively. After the bacteria reach the lower intestine, they stick to the membrane of mucous to attack the cells of epithelials. A site in which this occurs is the Peyer microfold (M) patch cell situated in the small intestine where the bacteria are able to pass through the barrier in the epithelial continuing to the follicle as well as mesenteric lymph nodes that underlie in the lymphoid tissue. During the ongoing bacteremia, secondary infection can happen because of the bacteria spreading to other organs like spleen, gallbladder, and liver. In acute *S. Typhimurium* infection cases, the gallbladder serves as a reservoir *(Hurley et al., 2014).*

After a mouth infection case*, Salmonella Typhimurium* invades epithelial and M cells before going through Peyer's patch, nodes of mesenteric lymph, lymph vessels which then enter the bloodstream. Alternative invasion mechanisms have been described in which Salmonella is covered by mucosal dendritic (DC) cells and after that travelled to the bloodstream from the gastrointestinal tracts by phagocytes of CD18 *(Mastroeni and Me'nager 2003).* The last infection phase is identified by generating immune responses that are able to remove *S. Typhimurium* and then is resistant to reinfection *(Mittru¨cker et al., 2000).*

The clear mechanisms to improve susceptibility of neonatal and dangerous life-threatening illness development are basically still covered. It was hypothesize dthat major protective mediators existing in adults might not be available in newborns because of the their immune system immaturity. On the other hand, other may be the causes like gastrointestinal differences of the population *(Cebra et al., 1999).* Followed by a few-day phase, bacterial intracellular multiplication appears and the bacteria titer in the spleen and liver enhancement. In this case, it is worth noted that *S. Typhimurium* is able to insert and survive in both phagocytic and nonphagocytic cells. Furthermore, the growth intracellularly becomes particularly essential in the time of infection as mutants failing the survival in cells of the host are highly

attenuated in vivo. In the mice, about 108 bacteria appear as a critical burden to survive. If the bacterial titer reaches the threshold, the host organism can no longer resist the infection. As a result, endotoxin shock, secondary bacteremia, and quick death occur. Conversely, during nonfatal infections, rats limit the bacterial titer to some degree. The next infection phase is characterized by splenomegaly, generalized macrophages mediated by immune suppression, and high number of bacteria, relying on the used mice and *S. Typhimurium* strains, which may last one or some weeks. The last infection phase can be identified by the adaptive immune response which is able to remove *S. Typhimurium* and provide long-term immunity to defend from reinfection *(Willi et al., 2000).*

The existence of pathogenic microorganisms is commonly shaped largely by their interactions with allied host species. The initial step of any infection is colonization. As for bacteria which are enteropathogenic, it is a daunting work because the targetted organ hosts have already been colonized by solid microbial communities, microflora, or 'microbiota'. The intestinal colonization of microbiota starts directly just after birth and ends a lifetime. In healthy intestines, microbiota remains fairly stable, and the gross composition of it is the same as other individuals at higher taxonomic levels, even in humans and mice *(ley et al.2006).*

The ecosystem of intestine exists due to the symbiotic that involves interaction between the microbiota and the host organisms. Yet, the composition of microbiota is influenced by the available nutrients, local pH, and it may also be affected by the host immune system *(Suzuki K et al.2004).* In a study, mice were treated with three different antibiotic regimens which had been commonly applied to inhibit microbiota in mice. There were enteric and inflammatory infection models. The effect on colonization of intestinal microbes by some dominant bacterial swamps was evaluated *(Salzman et al., 2001).*

2.3 The Immune Response Of *S. Typhimurium*

Salmonella is the most successful enteric pathogen as it has established a strategy to overcome the imajority of mmune defenses used by the host during different disease phases *(Broz et al., 2012).* Systemic *S. Typhimurium* infection starts on the seventh day after the beginning infection *(Ulhaq et al., 2009).* In addition, the various phases of the infection of *S. Typhimurium* are represented in the various mechanisms of innate and obtanined immunity which cause this response. Bacteria. The innate immune mechanism possesses the foundamental function to identify and combate microbial invaders and notify the system of adaptive immune to their

existence. The innate immune process is carried out by cells that are relatively not limited to the specificity of pathogens, including NK cells, macrophages, and neutrophils in the initial response to *S. typhimurium* as shown in Figure 2

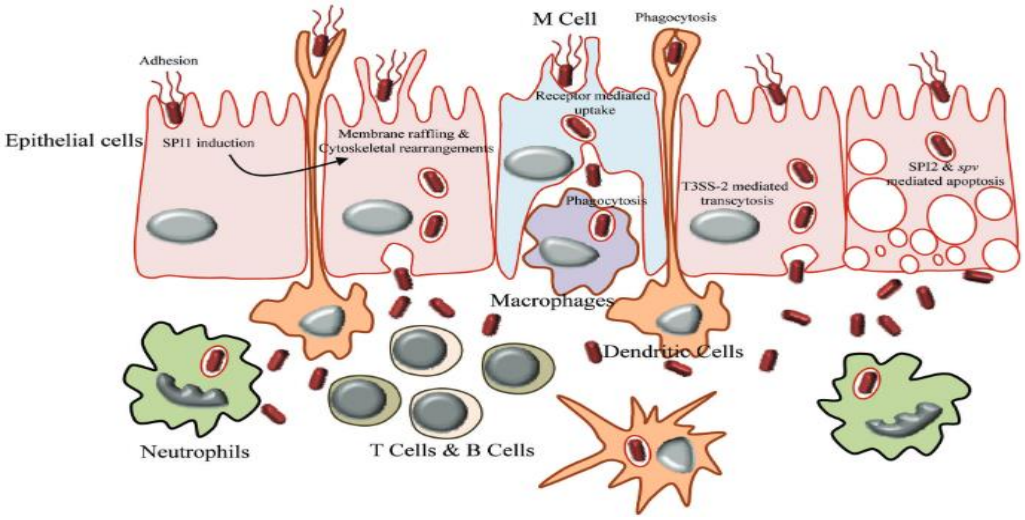

Figure 2.1: The Immune Response of *S. Typhimurium (Garai, et al, 2012).* The immune response of *S.* Typhimurium is noticed by the host's immune system by using TLRs which initiate the innate immune response. The TLRs respond the PAMPS situated on the pathogen surface. This recognition lets the innate immune system to start its response, which cause the macrophage and cytokine activation and recruitment such as IFN-γ as an important cytokine to activate macrophage and to make early resistance of host to *S. Typhimurium* .

The various *S. Typhimurium* infection phases are represented in various innate and immune mechanisms that contribute to the response to these bacteria. Then it differs in its interests during different stages of infection. During the early stages, phagocytes are essential for controlling Salmonella infections, and neutrophil granulocytes and macrophages are essential for the infected mice to survive. Macrophage fagocytize S. typhimurium. Additionally, this process is strengthened by receptor-mediated uptake after opsonizing Salmonella with antibodies or complemnt *(Mittru¨cker et al., 2000),*

Existing data indicate that antibodies or T cells themselves are only able to provide a mediocre protection level from salmonellosis. transfer of immune passive serum or B cells themselves may cause the mice resistant to salmonella or malignant bacteria susceptible to malignant organisms. In addition, immune cell iand mmune transfers are able to protect mice from the infection of low-dose virulent Salmonella

or to infections sufficiently of virulent organisms In contrast, immune serum transfers and T cells can naturally protect susceptible mice from highly malignant *salmonella*, suggesting that humoral and cell-mediated immunities are needed for the animals to be resistant to malignant salmonella. Additionally, besides the production of antibodies, B cells show other various functions in the system of immune, which include presentation of antigen and production of cytokines. The usage of an attenuated bacterium makes it possible for the analysis of immune responses to *S. typhimurium* in susceptible mice *(Mittru¨cker et al., 2000).*

2.4 Pathomechanism of *S.Typhimurium* infection

After absorption and colonization of the small intestine as shown in Figure 2.1, bacteria pass through the intestinal mucosa. *Salmonella* enters macrophages and disseminates through blood to the liver and spleen where it grows intracellularly. The *Salmonella* intracellular niche is an improvised phagolysosome called Salmonago containing vacuoles (SCV). The entry and survival strategies within the target cell correspond to the cell type and depend on the temporal expression of certain genes by Salmonella. Targeted cells include M. cells Epithelial cells, macrophages, neutrophils, monocytes, dendritic cells, granulocytes, B cells, and T cells *(Garai et al., 2012).*

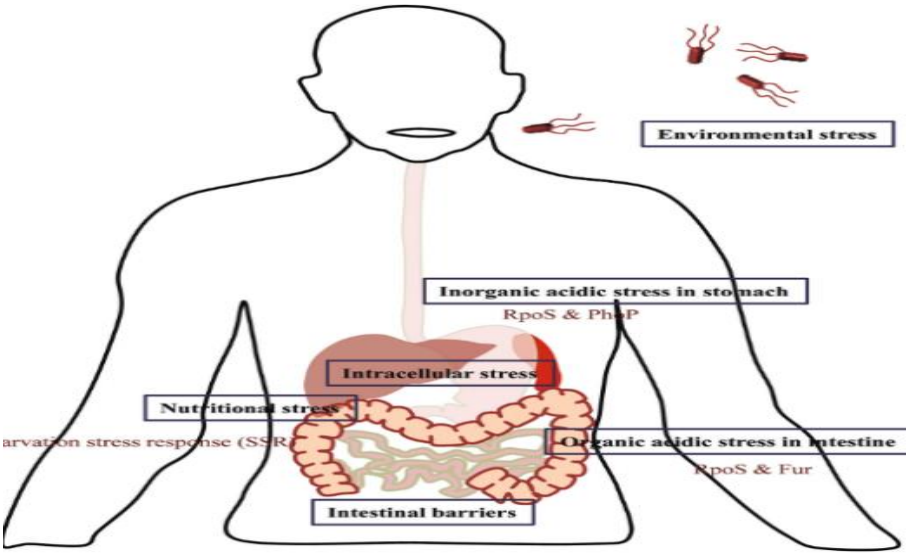

Figure 2.2 Pathogenesis of S.*Typhimurium* (Garai, et al., 2012). After ingestion orally and colonization of the small intestine, *S. Typhimurium* inserts the intestinal epithelium and goes into the pore of Peyer, the lymphoid structure lining the intestine. From the Peyer patch, S. *Typhimurium* enters the nodes of mesenteric lymph, and from that area the bacteria dissiminate through the efferent lymphocytes to the circulatory mechanism, which causes transient bacteremia. Bacteria are quickly excreted from the blood by phagocytes in the spleen and liver, and most bacteria are killed by these cells while bacterial intracellular multiplication happens and bacterial colonies in the spleen and liver enhance.

Salmonella enters M cells and epithelial cells and goes through Peyer patches, mesenteric lymph nodes, lymph vessels into the bloodstream. Alternative invasion mechanisms have been presented in which Salmonella is covered by mucosal dendritic (DC) cells and then moved from the gastrointestinal tract to the blood stream by phagocytes of CD18 (*Mastroeni and Me'nager 2003).* The final stage of infection is characterized by a generation of immune responses that are capable of removing S. *typhimurium*, and is resistant to reinfection *(Mittru¨cker et al., 2000).*

During a natural infection with *S. Typhimurium*, only a small percentage of bacteria pass across the intestinal epithelium, reaching the bloodstream through the gastrointestinal tract. The initial stage of Salmonella infection is usually completed in some hours when the natural response to bacterial challenge involves the introduction of bacterial components, such as LPS and DNA, and cell activation as a result of such encounters. The release of inflammatory mediators results in infiltration of various cell types to the site of infection and strengthening of the response. Uptake and bacterial damage by phagocytic cells also facilitate host protection. The innate immune process is performed by cells that are relatively not limited to the specificity of pathogens, including neutrophils, macrophages, and NK cells *(Alun et al., 2002).*

S. Typhimurium has come as a pathogenic bacterial model o. Using bioluminescent imaging, researchers can estimate that local tissue is influenced by Salmonella concentrations *(Guydish et al., 2005).*

Salmonella enterica serotype *Thyphimurium* (*Salmonella Typhimurium*) is the agent of the cause of typhoid fever murine. After oral retrieval, S. *typhimurium* passes through the intestinal epithelium through the M cell and enters the Peyer's pores. From there, the bacteria spread through the mesenteric lymph nodes to the spleen and liver, where they replicate. The first stage of infection is performed by the manufacturing of inflammatory cytokines and phagocyte *activation (Makela¨et al.,. 2007).*

2.5 Role IL12 Cytokines on Salmonella infection

Salmonellas enter dendritic cells and macrophages, in which the bacteria can reproduce and increase their number. Simultanously, they trigger an immune response that happens in some phases and ultimately control the infection with the assistance of CD4 T cells. Initially, lipoproteins and peptidoglycan on bacterial surfaces ligate receptor in macrophages and dendritic cells. Upon entering the cell, this returns TLRS *(Murphy et al., 2012).*

TLR is the first critical line of defense to combat bacteria attackers and plays an important role in microbial sensing *(Lizard et el.,, 2013).* Especially TLR and mannose receptors, and their ligation help to stimulate the production of nitric oxide with cells, which are hazardous to bacteria. Signals by TLR stimulate the production of IL12, which then encourages NK cells to generate IFN-γ in the early immune response stage. IL12 also stimulates the antigen-specific CD4 cells to produce IFN-γ *(Murphy et al., 2012).* IFN-γ is important during the early bacterial growth phase by limiting the bacterial multiplication rate *(Muotiala et al., 1990).*

In contrast, dendritic cells, macrophages and other cells secrete cytokines that mediate many cellular reactions of innate immunity. In congenital immunity, the main source of cytokines is dendritic cells and macrophages that are activated by the introduction of microbes, the binding of bacterial components such as LPS or viral molecules such as double-stranded RNA to TLR dendritic cells and macrophages that are powerful cytokim stimulative secretions. By cell *(Abbas and Lichtman, 2004).*

At the same time, cytokines are also released from immune-mediated cells. In this adaptive immunity type, the main source of cytokines is helper. T Lymphocytes Cytokines are taken in small amounts in response to external stimuli and bind to high affinity receptors in target cells. IFN γ macrophage activity becomes more effective in killing microbial phagocytes. Thus, NK cells and macrophages function cooperatively to remove the intercellular microbes. Macrophages ingest microbes and produce IL12, IL12 activates NK cells to secrete IFN γ, and IFN γ in turn activates macrophages to kill swallowed microbes *(Abbas and Lichtman, 2004)*.

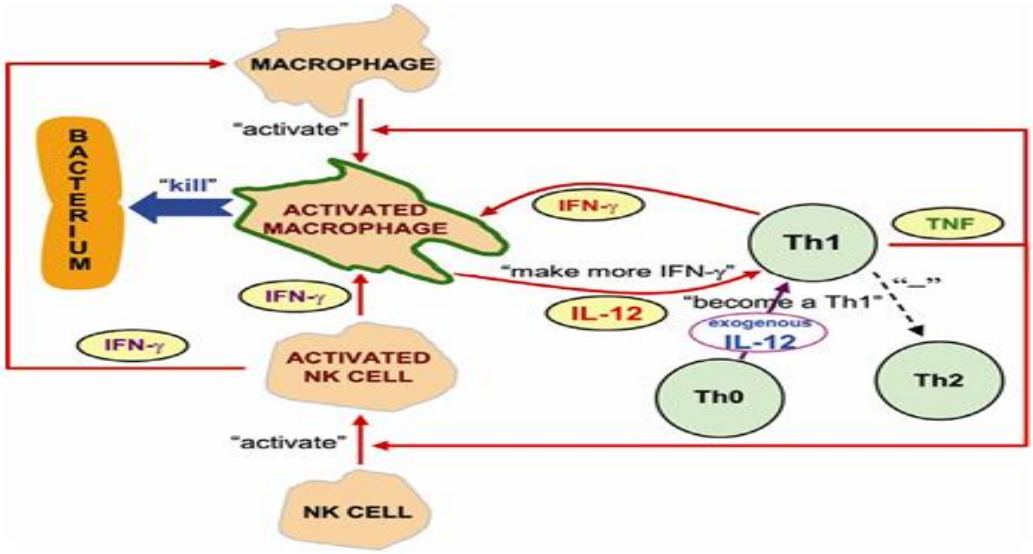

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Figure 2.3 The function of IFN-γ and IL12 to overcome intracellular bacteria *(Hamza et al.,2010)*.

TH1 cells generate IFN-γ cytokine which activates phagocytes to kill ingested microbes and stimulate the production of antibodies that promote the consumption of microbes by phagocytes. ABCs antigen that presents cells finding microbes secretes the IL12 cytokine stimulating naïve CD4 T cells to distintc into IFN -γ secreting Th1 cells and increasing IFN-produksi production. IFN-γ activates macrophages to kill the swallowed ABC, IFN-y, and IL 12 microbes.

IL-12 has many biological activities, and this is a key factor that drives Th1 responses and IFN production. Early application or production of IL-12 during infection can activate macrophages and enhance cell-mediated immunity while forming the main antigen - from specific immune responses. As a result, IL-12 may play a key role in the protection of bacterial and viral infections, and IL-12 immunotherapy may be important in the treatment of diseases where the Th1

response is desirable. While cytokines including IL-12 have short half-lives in vivo and the development of advanced drug delivery systems *(Haynie et al., 2005; Jiang et al., 2009).*

2.6 *Thymus vulgaris*

Thymus vulgaris is an aromatic plant that belongs to the family Lamiaceae, which is used for medicinal purposes and spices almost everywhere in the world *(R. MORALES et al., 2002).* In Romania, the genus of Thymus consists of one species cultivated as an aromatic plant (*Thymus vulgaris* L.) and 18 other wild species *(R. MORALES et al., 2007).*

2.6.1 Botanic description

Thymus vulgaris is known locally as "zaatar" or "zaitra ', a member of the Lamiaceae family, widely used in folk medicine of Morocco for its properties such as expectorants, antispasmodic, antibronkolitik, carminative, anthelmintic, antitussives and diuretic. The nature of aromatic and medicinal of the genus Thymus has made it one of one plant of the most popular around the world. Species thymus is usually used as a herbal tea, flavoring agent (condiments & spices) and medicinal plants *(Stahl-Biskup and Saez, 2002).* The essential oils and plant extracts have been used for thousands of years, especially in the preservation food, medicine, alternative medicine and natural therapy *(Lis-Balchin & Deans, 1997).* it has long been known that some essential oils of plants showed the efficacy of antimicrobial *(Finnemore, 1926).* and it needs to investigate these plants scientifically, which has been used In traditional medicine to improve the quality of health services Essential oil is a potential source of antim compounds New ikroba especially against pathogenic bacteria *(Prabuseenivasan et al., 2006).*

2.6.2 Scientific classification

Kingdom:	Plantae
(unranked):	Angiosperms, Eudicots, Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Thymus*
Species:	*T. vulgaris*

Bionomial name: *Thymus vulgaris*

Figure 2.4: *Thymus vulgaris.(http://sophy.u-3mrs.fr/*).

2.6.3 Major Chemical Constituents

Thymus vulgaris possesses about 2.5% but not less than 1.0% essential oil. The essential oil composition fluctuates depending on the chemotype under consideration. The main components of Thyme are thymol (European pharmacopoeia, 2nd ed. Strasbourg, Council of Europe, 1995). And carvacrol (Materia medika Indonesia, Volume Jakarta, IV Department of Health, Republic of Indonesia., 1980). Up to 64% of oil, along with linalool, p-cymol, cymene, thymene, α-pinene, apigenin, luteolin and 6-hydroxyluteolin glycosides and flavonoids di-, tri and tetrametoksilat, everything was substituted in the 6 - position (for example 5, 4'-dihydroxy-6,7-dimetoksiflavon, 5,4'-dihidroksi- 6,7,3'-trimetoksiflavon and derivatives 8-metoksilasi 5,6,4'-trihydroxy-7, 8,3'-trimethoxyflavone) (European pharmacopoeia, 2nd ed Strasbourg, Council of Europe, 1995). *Thymus vulgaris* essential oil (TEO) is a monoterpene mixture. The main compound of this oil is a natural terpenoids and phenol thymine isomer carvacrol (CVL) (Amiri, 2012; B. Nickavar *et al*., 2005), Terpenoids, aglycone flavonoid, flavonoid glycosides and phenolic acids are also found in the Thymus species.

Thyme (Thymus vulgaris L., Lamiaceae), a small subshrub originating in the western Mediterranean region of Europe, has a long history of use and is a species that varies chemically (A. ZarzueloandE.Crespo *et al.,* 2002).

Thyme leaves (*Thymus vulgaris*) can be used fresh or dried as a spice. Thyme also has various beneficial effects, including antiseptic, carminative, antimicrobial, and antioxidant effects *(R. Baranauskiene, et al., 2003).* Recently, in our laboratory showed that the constituent elements, thymol and carvacrol, *Thymus vulgaris* L. essential oils present on the immune response *(F. C. Fachini-Queiroz, et al ., 2012).*

2.7 Essential oil molecules

1) -**Terpenes**: Terpenes are hydrocarbons formed through a combination of several isoprene units (C5H8). The most common dipes are monoterpenes (C10H16) and sesquiterpenes (C15H24), but longer chains, such as diterpenes (C20H32), triterpenes (C30H40) and so on. Among terpenes, p-Cymene, limonene, terpinene, sabinene and pinene are the most famous. This in vitro test indicates that terpenes exhibit ineffective antimicrobial activity when used as a single compound. *(Filomena et al., 2013).*

2) -**Terpenoid**: Terpenoid terpene with an extra oxygen molecule that has a methyl group or they are transferred or issued by a specific enzyme *(Caballero et al., 2003),* thymol, carvacrol, linalool, menthol, geraniol, linalyl acetate, citronellal and piperitone is terpenoids The most common and famous. The antimicrobial activity of most terpenoids is related to their functional groups, and the phenolic terpenoid hydroxyl groups and the presence of delocalized electrons are important elements for their antimicrobial action. For example, carvacrol is more effective than other EOs, such as p-cymene. (*Dorman et al., 2000; Ultee et al., 2002; Ben Arfa et al., 2006).*

3) -**Phenylpropenes**: Fenilpropen so named because it contains six carbon aromatic phenol group and propene propene three carbon from cinnamic acid, which is produced in the first stage phenylpropanoid biosynthesis. These compounds represent a fraction of EOs. Eugenol, isoeugenol, vanillin, safrole and cinnamaldehyde are the most widely studied phenylpropene. Most of the antimicrobial activity of these molecules is given by the free hydroxyl groups *(Filomena et al., 2013).*

Thyme contains many active ingradients including thymol, carvacol and flavonoids. The main chemicals of thyme are essential oils (borneol, carvacol, linalool

and thymol), tannin principle, saponin and triterpen acid (shabnum et al., 2011). Thymol is part of a class of natural compounds known as biocides, with strong antimicrobial properties when used alone or with other biocides such as carvacrol. In addition, natural biocidal agents such as thymol can reduce bacterial resistance to common drugs such as penicillin *(Palaniappan et al., 2010).*

Numerous studies have demonstrated antimicrobial effects of thymine, ranging from inducing antibiotic susceptibility on drug-resistant pathogens to the potent antioxidant properties (Zarrini G *et al.,* 2010; Ündeğer *et al.,* 2009). Some researchers suggest that natural biocides such as thymol and carvacrol reduce bacterial resistance to antibiotics through synergistic effects *(Palaniappan et al., 2010).*

Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol, isomeric with carvacrol, which is found in thyme extracts. These compounds have shown anti-inflammatory, immunomodulating, antioxidant, antibacterial and antifungal properties *(P. C. Bragae et al., 2006; H. Tian et al., 2006).* Clinical trials were conducted on 12 healthy volunteers. Each subject received a dose of TP Bronchipret tablet, which is equivalent to 1.08 mg of thymol. No thymol is detected in plasma or urine. However, the metabolites of thymol sulfate and timol glucuronide are found in the urine. Thymol sulfate, but not thymol glucuronide, is detectable in plasma. Peak plasma concentration is achieved after 2.0 hours. The average terminal elimination half-life is 10 hours. Thymol sulfate can be detected up to 41 hours after administration. Urinary excretion can be followed for 24 hours. The second amount of thymid sulfate and glucuronide excreted in 24 hour urine was 16% of the dose *(Sahelian et al., 2016).*

Carvacrol and carvacrol bearing essential oils, havCarvacrol occurs in aromatic plants and in many essential oils of the Labiatae family, includine emerged for their wide spectrum antimicrobial activity and have been investigated by a large number of researchers worldwide. However, the susceptible microorganisms are far too many to be dealt with, as they include microorganisms that belong to the Gram-positive bacteria, Gram-negative bacteria, molds and yeasts. The activity of carvacrol is extended to drug-resistant microorganisms, strains with a particular significance for pathogenesis as currently are difficult to treat *(Papalia et al., 2012).* it also have anti biofilm action as mentioned by previous research *(Dalleau et al., 2008).*

Essential oils and plant extracts have been used for thousands of years, especially in the preservation of foods, medicines, alternative medicine and natural

therapies *(Lis-Balchin & Deans, 2011).* It has long been known that some essential oils of plants exhibit antimicrobial properties *(Finnemore et al., 2008).* And it is necessary to investigate these plants scientifically, which have been used in traditional medicine to improve the quality of health care. Essential oils are potential sources of new antimicrobial compounds especially against pathogenic bacteria *(Prabuseenivasan et al., 2006).*

2.8 Mechanism of Action and The target sites of *Thymus vulgaris* against the bacterial cell

Alcohol extracts are more efficient at various pathogenic bacteria and mixed extracts have very high antibacterial activity *(Al-Saimary et al., 2006).* The T. vulgaris extract component has activity against various targets, especially the membranes and cytoplasm, and in some cases, they completely alter cell morphology, some thyme components, such as carvon, thymol and carvacrol, lead to increased intracellular ATP concentrations, an associated event With microbial membrane destruction *(Helander et al., 2014).*

2.9 Immunomodulator

The term " immunomddoulation " means immune change. Responsiveness Increased immune responsiveness is called immunostimulation and decreased immune responsiveness called i2mmunosuppressin *(Mukherjee et al., 2014).* The immune system is part of the body to detect pathogens by using specific receptors to generate an immediate response with activation. From immune component cells. They modulate and define the system *(Kumar et al, 2011).* Immunomodulatory effects can be predicted to there which is stimulation. Emphasis and narrowing of the immune system *(Yeap et al., 2011).* Immunomodulators are used in practice to stimulate and normalize the activity of the immune system. By increasing T cell immunity. Reduce or inhibit suppressant activity. Stimulaling natural killer cells (NKcells) and interferon production as well as inducing the production of specific cytokines by activated target cells *(Gabius et al., 2003; Stanlove et al., 2005; aLam et al., 2010)*

CHAPTER 3

CONCEPTUAL FRAMEWORK AND HYPOTHESIS

Conceptual framework:

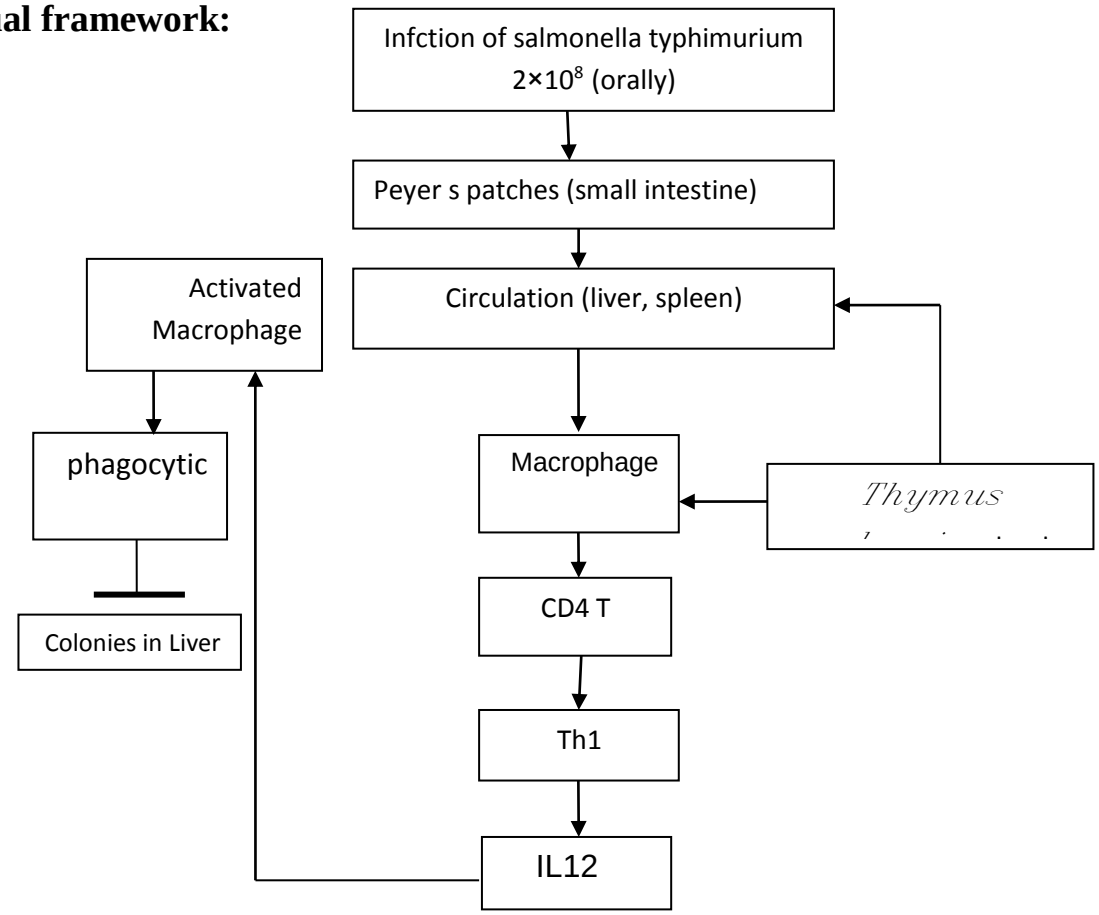

Figure3.1 Conceptual framework

3.1 Explanation of Conceptual Framework

After ingested orally and colonializing the small intestine, *S. Typhimurium* enters the epithelium of intestine and continues to the Peyer patch, the lymphoid structure which lines the intestine.The primary entrance of *S. Typhimurium* to the Peyer patch is the M cell, a special cell lining the Peyer patch which is involved in antigen sampling from the lumen of intestine to the lymphoid follicles. From the Peyer patch, S. *typhimurium* goes to the nodes of mesenteric lymph, from which S. *typhimurium* dissiminate through the lymph into the system of circulatory, causing transient bacteremia. *S. Typhimurium* is quickly removed from blood by phagocytes in the liver and spleen, and these cells kill the majority of bacteria. The first stage of this Salmonella infection, which is usually finished in a several hours, is followed by a multiple-day stage, in which bacterial intracellular multiplication occurs and bacterial titer in the spleen and the liver subsequently increases and causes a second fatal bacteremia *(Mittru¨cker et al., 2000; Salcedo et al., 2001).* After a mouth infection, Salmonella attacks M and epithelial cells and goes through Peyer's patch, nodes of mesenteric lymph, lymph vessels into the bloodstream. Alternative invasion mechanisms have been described in which mucosal dendritic (DC) cells engulfe Salmonella and transport it from the gastrointestinal tract going to the bloodstream by CD8 phagocytes *(Mastroeni and Me'nager et al., 2003).* The last infection stage is performed by the immune generation responses obtained to remove S. *Typhimurium*, and long-term immunity to reinfection *(Mittru¨cker et al., 2000).* TLR is the initial critical form of defense against bacteria attackers and plays an important role in microbial sensing *(Lizard et al., 2013)*. Especially TLR and mannose receptors, and their ligation work as stimuli of the production of nitric oxide with cells, which are toxic to bacteria. Signals by TLR stimulate the produce of IL12, which in turn encourages NK cells in order to produce IFN-γ in the early phases of the immune response.IL12 also stimulates the antigen-specific CD4 cells to produce IFN-ɣ (Murphy *et al*., 2012). Thus, *Thymus vulgaris* extract is expected to be anti-inflammatory and antimicrobial agents because the inflammation is a consequence of infection. Besides, the effect of different doses of thyme as antibacterial and immunomodulator need to be analyzed *(Picone et al., 2013).*

3.2 Research hypothesis

1) the extract of *Thymus vulgaris* increases the blood IL-12 in mice which are infected with *S. Typhimurium*

2) the extract of *Thymus vulgaris* decreases the Bacterial colonies in the Liver in mice infected which are with *S. Typhimurium*

CHAPTER 4

RESEARCH METHODS

4.1 Research Design

This study is an experimental laboratory research that used a completely randomized design. The experiments are performing with simple random sampling. Treatment is used for the provision dose of ethanolic extract of *Thymus vulgaris* in mice with S. Typhimurium.

The experimental design was conducted by post-test control group design consisting of three treatment groups of mice

Control pos(C2): (positive control) of mice infected with. *S. Typhimurium*

Control Negative(C1): mice without infection.

D1 group: mice given ETV+ 250mg/kg body weight (B.Wt)ml mice and infectewith.*S. Typhimurium*

D2 group: mice given ETV + 500mg/kg boody weight mice and infected with. *S. Typhimurium*

D3 group: mice given ETV+ 750 mg/kg boody weight *mg/ml mice and infected with*. *S. Typhimurium*

4.2 Research Subject

4.2.1 Inclusion Criteria

The study population is number of male Balb/c strain mice weighing between 25-30gm.

.4.3 Location and Time of Study.

4.3.1 Place of study

The male mice are take care at the Pharmacology Laboratory in the Medical Faculty of Brawijaya University in Malang.

The measurement of liver were calculacted number colonies in at Microbiology Laboratory

The measurement of IL-12 was at the Physiology Laboratory in Faculty of Medicine of Brawijaya University in Malang.

4.3.2 Time of study

Research take 3 months approximately.

4.4 Variable Identification

4.4.1 Independent Variable

The independent variable in this study was ethanolic extract of *Thymus vulgaris*

4.4.2 Dependent Variable

1) The level serum of IL12.
2) Bacterial coloniesin the liver.

4.5. Operational definitions

1) *Thymus vulgaris* extract Is obtained from Pharmacology Laboratory in the Medical Faculty of Brawijaya University in Malang. In brief, the *Thymus vulgaris* is extracted with 90% ethanolic by maceration and evaporation process

2) The S. Typhimurium is collection of Microbiology Laboratory Faculty of Medicine, Brawijaya University, Malang, Indonesia which is originally from patient. Bacteria were cultured on Bismuth Sulfite Agar (BSA) and checked as *S. Typhimurium* using Gram staining,

3) IL-12 play a key role in protection against bacterial infections and measured by ELISA

4) Bacterial colonies is measured by a culture method using BSA medium.The calculation of the colony in liver samples on each plate dilution contained 30-300 CFU.The calculation of CFU/gram of tissue is based on the formula;

$$\frac{\text{The number of CFU X Dilution x 10}}{\text{Tissue weight (grams)}}$$

4.6 Operational procedures

4.6.1 Preparation of plant extract

Extraction procedure

Identification of *Thymus vulgaris* was done in Biology department,Brawijaya university,Malang,Indonesia.NO.0207/UN10. 4 /03/2017.

Leaves, flowers and stems (whole plants) T*. vulgaris* were bought from local markets (herbalis) in Nalut-Libya. The plant was dried at the temperature of 40° C with air circulation, ground, and extracted using ethanol as much as 70% percolated at room temperature. The extract was then moved to the laboratory for the process of evaporation which involves the ethanol and water removals residu of extraction. The extract was then dried under vacuum condition at 40° C. before frozen dried. Doses are presented as milligram of dry extract per kilogram of mouse body weight. The extract was dissolved in the solvent before each experiment was performed. Furthermore, extracts using alcohol prove to be highly effective to varied pathogenic bacteria, while the mixed extracts show o have very high antibacterial ability (*Komaki et al., 2015)* .OR, Preparation of ethanol extracts from *Thymus vulgaris* performed according to that is described by Nweze et al, which involves fertilizing 200 grams of plants into a liter of absolute ethanol (ethanol 99.8%). The container was sealed using silver paper to avoid volatile solvent loss and lags in a room with normal temperature for a day. At the end of the period, the substances are filtered with a filter paper no.1 and put into a beaker. The solvent was evaporated in a hot air oven at 40 ° C. for 24 hours to concentrate the filtered solution. The extract was then measured, stored in a sterilized bottle, labeled as the name and put in the cooler during the examination. *(Nweze et al., 2010; Eman et al., 2012).*

A. Dried of Herb:

1.Washed the herbs.

2.Cut the herbs approximately 0,2 cm in thick.

3.Dried the sample in oven at 40 – 60 ^{o}C or under the sun until the sample until free water containing in it.

B.Extraction:

1.Dried sample grinded to made powder.

2.Weight the sample 100g.

3.Put the sample into 1L Erlenmeyer.

4.Soak the sample with approximately 900mL the appropriate solvent(until volume reach 1L).

5.Keep it for 8 hour(overnigth) and shaked.

6.Took the solvent(upper layer)that containing active compound and filter edit.

7.Did the maceration 3 times.

C.Evaporation Process:

1.Put the filtrate in to evaporation flask.

2.Set the evaporation flask to th erotary evaporator instrument.

3.Fill the water bath with adequate water.

4.Set the rotary evaporation and water bath heater on 80 ^{o}C or based on the boiling point of the solvent.

5.Turn on the rotary evaporation until the solvent separate/evaporate with the active compound in evaporator flask.

6.Wait until the solvents top drip in to the container flask (±1.5to2hours).

7.The ethanol solution was allowed to separate the existing active substance in a flask

8.The low of ethanol wait until its tops dripping from pump kin container(approximately1.5to 2 hours to1 squash)

9.Results of the extraction of inclusion in glass or plastic bottles

10.The extraction results are stored in a freezer.

(*Remington,2000.The Science and Practice of Pharmacy.20th Edition*).

4.6.2 Dose Determination:

Treatment with ethanol extract of *Thymus vulgaris* at dose level 250-500mg / kg, in oral ingestion has been conducted by Shaban *et al.* (2015). At 750 mg / kg dose, TEO decreased the proportion of migrating cells in the pleurisy model (Fernanda *et al*., 2012). Moreover, carvacol (CVL) and thymol substantially decreased the volumes of exudates of pleural inflammatory as much as 47.3% and 34.2 % with 400 mg / kg dose. besides, CVL also reduced the proportion of migrating cells at 100, 200, and 400 mg / kg doses (Maryam *et al.,* 2016). The dose use in this experiment is D250 mg/kg. 500mg/kg. 750mg/kg.

4.6.3 preparation of bacteria

Using bismuth sulfite agar (BSA), the bacteria were cultured giving the black colonies and examined as salmonella by gram staining(gram negative rods),BSA (uncoloured colonies),Triple sugar iron,acid/base,no gas,H2S positive),IMVIC,VITEK2.it was made in inoculum in 10^8 CFU/ml for 10^7 CFU/100ul/mouse (*Fierer et al.,,.2002*).

$$V1 \times N1 = V2 \times N2$$

$$10^8 \times N1 = 10^7 \times 2500\ \mu L$$

$$N1 = \frac{10^7 \times 2500\ \mu L}{10^8} = 250\ \mu L$$

4.6.4 Infection step

The mice was infected two times with 2-days interval with 300 µL *S. Typhimurium* bacteria (concentration $2x10^8$ cells / mL), and then extract *Thymus vulgaris* was given orally for 7 days. on the seventh day after given treatment, the mice were sacrificed and the whole blood was taken.

4.6.5 Data Collection Procedures

Data collection includes the following steps:

1) The samples are adapted for 1 week in the laboratory with standard feed.

2) Do grouping with simple random, 20 mice were divided in the 5 groups.

3) The group of,D1, D2, D3 will giving ETV administered orally using gavage needle for 7days.

4) The group of D1, D2, D3 was infected with *Salmonella Typhimurium* (2×10^8/100 µL/mice) and in same time will giving ETV for 7 days.

5) The Group C2 positive control were infected with *Salmonella Typhimurium* (2×10^8/100 µL/mice) for 7days.

6) The Group C1 were fed oil for 7 days, and do not infected with *S. Typhimurium*

7) On day 14 all groups' mice were sacrificed by cervical dislocation.

8) The entire ventral surface of mice sprayed with alcohol 70%.

9) In a small incision made in the skin using scissors on the medial abdomen, torn skin using tweezers in the direction of the head and tail so that looks peritoneum. Peritoneal moistened with alcohol 70% for get rid of the hairs.

10) The peritoneum is opened, the liver and whole the blood will taken from heart with aseptic technique.

4.7 Material and Tools

4.7.1 Materials

The required materials were male mice Balb/c strain,animal feed, the bacteria *Salmonella Typhymurium*, extract *of Thymus vulgaris* , blood, alcohol 70%, sterile aquadest, Gram stain, IL12 mouse kit.

4.7.2 Tool / instrument Research

The instruments are animal cage for mice, sonde stomach, 1cc and 10 cc of sterile syringes,glass objects and glass cover, measuring cup, test tube, laminar flow hood, petri ,vortex, and ELISA reader.

4.8 Research Procedure

4.8.1 IL12 by ELISA

Measurement of blood immediately from cardiac puncture of mice before sacrificing. The IL12 level in the serum are analyzed by enzyme-linked immunosorbent assay (ELISA) as follow:

1) 50µLof Standards or samples was added to each other.
2) The place was then covered and incubated at 20-25°C for as long as 2 hours.
3) Without washing, Biotinylated Antibody Reagent is added 50µLof to each other.
4) The plate was covered and incubated at room temperature for 1 hour.
5) The plate was washed three times.
6) 100µLof prepared Streptavidin-HRP Solution was added to each well.
7) The plate was covered and incubated at room temperature for 30 minutes.
8) The plate was washed three times.
9) 100µLTMB Substrate was adde to each well. Then the plate was developed in the dark at room temperature for 30 minutes
10) Stop reaction by adding 100µLof Stop Solution to each well.

11) The absorbance was measured on a plate reader at 450nm or 450 minus 550nm.

12) The results were calculated using graph paper or statistically using curve-fitting software.

4.8.2 Calculate Bacteria Colonies In Liver

1) The liver was be taken using aseptic technique then performed weighing.

2)Network liver is obtained and crushed with a mortar and with 4.5 ml of sterile physiological saline.

3) The test tube is filled with NaCl 4.5 ml.

4)The 0.5 ml of mortar is inserted on the first tube and homogenization using a vortex.

5) 0.5 ml of the first tube is then inserted into the second tube and so do until the tube dilution Vl that has been done 1;10 at each dilution. In the last tube solution taken,

6) Samples of each tube 0.1 ml was inoculated on BSAmediunagar .

7) Calculate the number of colonies on each plate dilution containing 30-300 CFU.

8) Calculate CFU\gram of tissue by the formula;

$$\frac{\text{The number of CFU X Dilution X 10}}{\text{Tissue weight (grams)}}$$

4.9 Data Analysis

The data of this study Using IL12level measurement using ELISA method. This is in vivo experiments well use five mice every group. In the statistical analysis, one- way ANOVA and correlation test were used at level of acceptance 95% ($\alpha = 0.05$).bacterial colonies in the Liver was measured by spectrophotometer.

4.10 Ethics Research

An animal in this study following the animal ethics. The things need to be carried out according to the care ethics, among others in cages, feeding drink (ad libitum).

4.11 Experimental Framework:

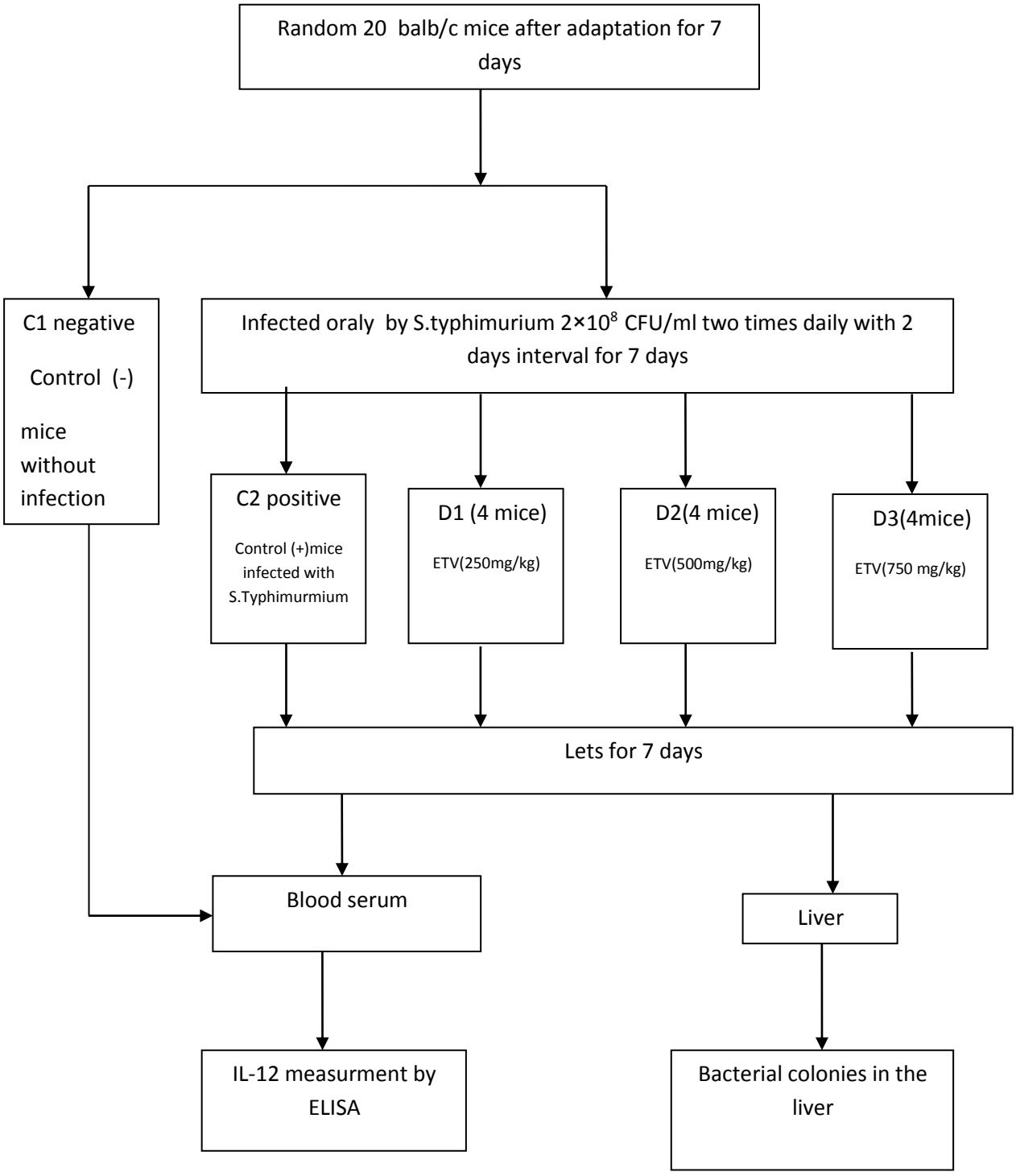

Figure4.1 Experimental Framework

CHAPTER5

TH ERESULT ANDDATA ANALYSIS

In this research,the observation of observing the extract effect of *Thymus vulgaris* on IL-12 in blood used ELISA, and number of bacterial colonies in liver male mice model infected with.*Salmonella Typhimuryum*

This research used 5 groups with 4 mice in each group, with different dose of Thymus vulgaris in D1:250mg/kgB.wt,D2:500mg/kgB.wt,D 3:750mg/kgB.wt of micet here were also positive control(+) *Salmonella Typhimuryum* negative control(-).

5.1.The effect of *Thymus vulgaris* extract on the level of blood IL12

Table 5.1 The results of the study are as shown inas follows:

Groups	Sample				Mean ± Std. dev.
	1	2	3	4	
C-	25.81	1.08	26.06	14.36	16.83±11.83
C+	5.23	4.94	12.32	11.16	8.41±3.88
D1	23.28	25.27	22.99	26.10	24.41±1.52
D2	55.56	39.34	40.91	36.14	42.99±8.61
D3	38.76	45.06	39.92	42.41	41.54±2.80

C-:negative control ,C+:positive control,D1:Treatment group1,D 2:treatment group2,D 3:treatment group3

Based on the table above shows that the difference in dose of *Thymus vulgaris* extract influence or different effects on IL-12. Based on the table above shows that the difference in dose of *Thymus vulgaris* extract influence or different effects on IL-12. The existence of the effect of *Thymus vulgaris* extract is starting to look where the IL-12 in mice induced *Salmonella Typhimurium* bacteria becomes highest, after the treatment was given in the form of *Thymus vulgaris* extract started at a dose of 250 mg/kgETV (D1), compared with IL-12 in the positive control group. Then the IL-12 more increased when given higher doses of 500 mg/kgETV (D2), until the higher doses of 750 mg/kgETV (D3). Thus, based on the assessment descriptively according to the mean IL-12 is, it can be said that the administration of treatment in the form of *Thymus vulgaris* extract at a dose of 250 mg/kgETV (D1), 500 mg/kgETV (D2), and 750 mg/kgETV (D3) showed different influences, where the higher dose of *Thymus vulgaris* extract provided was further increase the IL-12.

As for the overall differences in IL-12 in each treatment above can also be depicted in graphic form as follows.

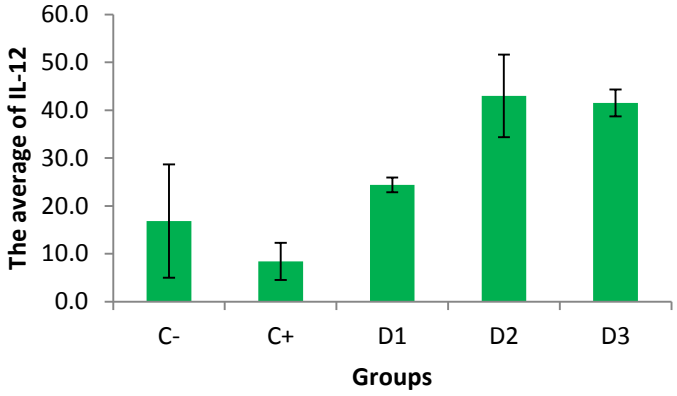

Figure5. 1 Graph of IL-12 at each dose of *Thymus vulgaris* extract

Based on test data normality test using the Kolmogorov - Smirnov test , IL-12 data has p-value of 0.200 (p > 0.05), so it can be concluded that the data IL-12 has a normal distribution. Thus it can be tested by ANOVA, because the assumption of normality of data distribution have been appropriate

5.2 The effect of *Thymus vulgaris* extract on number of bacterial colonies in liver of male mice model infected with.*Salmonella Typhimuryum*

Then the results of the study are as shown in Table as follows

Table5.2 Average of THE COLONY OF *SALMONELLA Typhimuryum*

Groups	Sample				Mean ± Std. dev.
	1	2	3	4	
C-	0	4	0	0	1.0±2.0
C+	223	137	142	238	185.0±52.9
D1	115	32	0	78	56.25±50.6
D2	0	0	0	0	0±0
D3	0	0	0	0	0±0

Based on the table above shows that the difference in dose of *Thymus vulgaris* extract influence or different effects on the colony of *Salmonella Typhimurium*. Based on the table above shows that the difference in dose of *Thymus vulgaris* extract influence or different effects on the colony of *Salmonella Typhimurium*. The existence of the effect of *Thymus vulgaris* extract is starting to look where the colony of

Salmonella typhimurium in mice induced *Salmonella Typhimurium* bacteria in the group D1 becomes lowest than positive control group, after the treatment was given in the form of *Thymus vulgaris* extract started at a dose of 250 mg/ml. Then the colony of *Salmonella Typhimurium* becomes zero when given higher doses of 500 mg/ml (D2), and decrease again when given higher doses of 750 mg/ml (D3).

As for the overall differences in the colony of *Salmonella Typhimurium* in each treatment above can also be depicted in graphic form as follows.

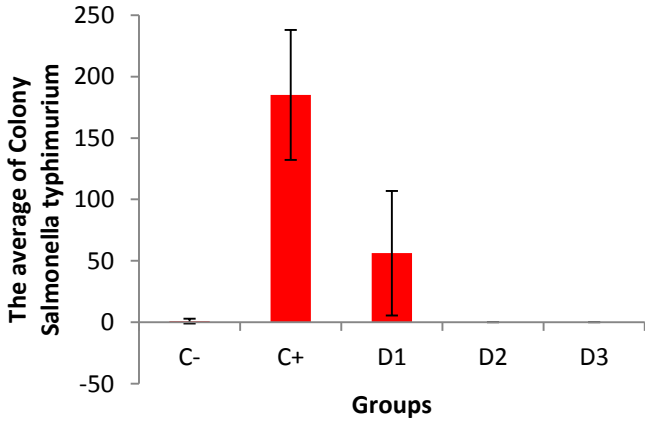

Figure5.2 Graph of the colony of *Salmonella* T*yphimurium* at each dose *Thymus vulgaris* extract

p value 0.005 that is lower than alpha 0.05, so rejected the statistical hypothesis which states there is no relationship between the dose of *Thymus vulgaris* extract with the colony of *Salmonella Typhimurium*. In other words, it shows there's significant relationship between the dose of *Thymus vulgaris* extract with the colony of *Salmonella Typhimurium,* where the higher of the dose *Thymus vulgaris* extract give The colony of *Salmonella Typhimurium* of negative control group, D1 (250 mg/ml), D2 (500 mg/ml), and D3 (750 mg/ml) have different significantly with the colony of *Salmonella Typhimurium* in the positive control group ($p<0.05$). However, The colony of *Salmonella Typhimurium* of negative control group, D1 (250 mg/ml), D2 (500 mg/ml), and D3 (750 mg/ml) did not differ significantly for one each other ($p>0.05$).

Then the test results comparing multiple (Tukey 's Test) in each treatment showed that the colony of *Salmonella Typhimurium* in the positive control group differ significantly with the colony of *Salmonella Typhimurium* in negative control group, D1 (250 mg/ml), D2 (500 mg/ml), and D3 (750 mg/ml) ($p<0.05$).

n, than the colony of *Salmonella Typhimurium* wasl be decrease. And vice vers

the effect of *Thymus vulgaris* extract to the average the colony of *Salmonella Typhimurium* of research results by using One way ANOVA, it is necessary to the

fulfillment of some of the assumptions of data, where's data the colony of *Salmonella Typhimurium* must have a normal distribution and has a homogeneous variance.

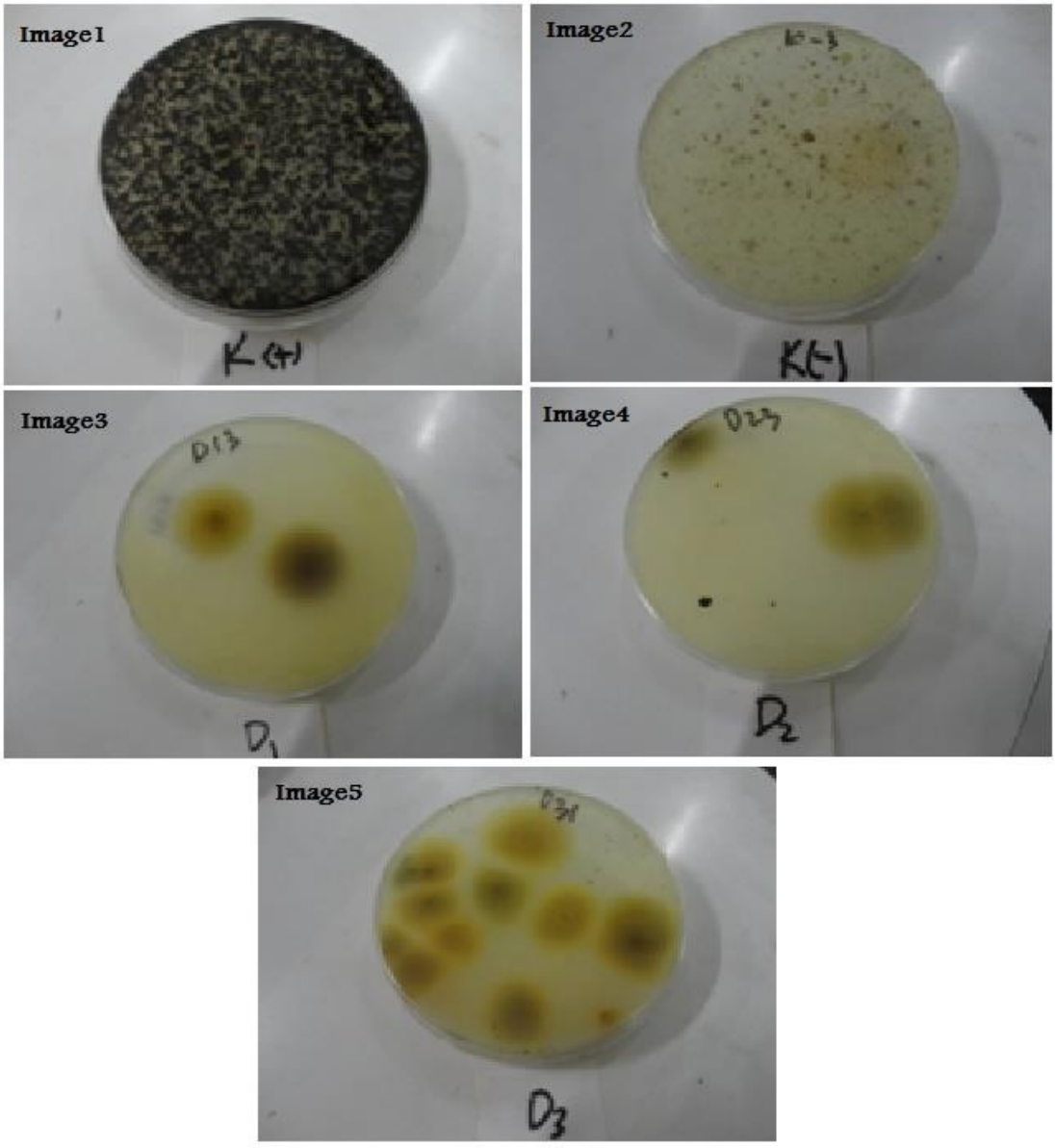

Figure5.3: *Salmonella typhimurium* colonies:

Image1: Positive control (there is over growth of bacteria)

Image2: Negativecontrol (There is no growth of bacteria)
Image3:Treatmentgroup1(there is no growth of bacteria)
Image4:Treatmentgroup2(there is no growth of bacteria)
Image5:Treatmentgroup3(there is no growth of bacteria)

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)*

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* effecton male immunity.

2.The duration of research should be maintained to discover the exact duration for *Thymus vulgaris* to optimizeits effect on immune response agonists

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UNIVERSITAS BRAWIJAYA
FAKULTAS KEDOKTERAN
KOMISI ETIK PENELITIAN KESEHATAN
Jalan Veteran Malang - 65145, Jawa Timur - Indonesia
Telp. (0341) 551611 Ext. 168, 569117, 567192 Fax. (0341) 564755
http://www.fk.ub.ac.id e-mail: kepk@fk.ub.ac.id

KETERANGAN KELAIKAN ETIK
("ETHICAL CLEARANCE")

No. 127 / EC / KEPK – S2 / 03 / 2017

KOMISI ETIK PENELITIAN KESEHATAN FAKULTAS KEDOKTERAN UNIVERSITAS BRAWIJAYA, SETELAH MEMPELAJARI DENGAN SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, DENGAN INI MENYATAKAN BAHWA PENELITIAN DENGAN

JUDUL	:	**Effect of Thymusvulgaris extract on laval of blood INF-Y, IL12 and Bacterial Colonies in the Liver In MIC Infected by Salmonell Typhimuryum.**
PENELITI UTAMA	:	**Emad Khalleefah said abousouh**
UNIT / LEMBAGA	:	**S2 Biomedik – Fakultas Kedokteran – Universitas Brawijaya Malang.**
TEMPAT PENELITIAN	:	**Laboratorium Farmakologi, Laboratorium Fisiologi dan Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Brawijaya Malang.**

DINYATAKAN LAIK ETIK.

30 MAR 2017

Ketua
Komisi Etik Penelitian Kesehatan

Prof. Dr. dr. Moch. Istiadjid FS, SpS, SpBS (K), M.Hum
NIP. 19616 197111 1 001

Catatan :
Keterangan Laik Etik Ini Berlaku 1 (Satu) Tahun Sejak Tanggal Dikeluarkan
Pada Akhir Penelitian, Laporan Pelaksanaan Penelitian Harus Diserahkan Kepada KEPK-FKUB Dalam Bentuk Soft Copy. Jika Ada Perubahan Protokol Dan / Atau Perpanjangan Penelitian, Harus Mengajukan Kembali Permohonan Kajian Etik Penelitian (Amandemen Protokol)

Appendix 1

Parameter IL-12

(1) Normality Test Data Distribution

Tests of Normality

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
IL-12	.133	20	.200*

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

If the value of significance (p) > 0.05 = normal distribution of data

(2) Variance Homogeneity Assumption Test

Test of Homogeneity of Variance

	Levene Statistic	df1	df2	Sig.
IL-12	2.048	4	15	.139

If the value of significance (p) > 0.05 = Data has homogeneous varieties

(3) One way analysis Of Variance test (ANOVA)

Descriptives

IL-12

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C-	4	16.8257	11.83213	5.91607	-2.0018	35.6533	1.08	26.06
C+	4	8.4129	3.87597	1.93798	2.2453	14.5804	4.94	12.32
D1	4	24.4087	1.51642	.75821	21.9958	26.8217	22.99	26.10
D2	4	42.9876	8.61360	4.30680	29.2814	56.6937	36.14	55.56
D3	4	41.5353	2.80157	1.40079	37.0773	45.9932	38.76	45.06
Total	20	26.8340	15.23137	3.40584	19.7055	33.9625	1.08	55.56

ANOVA

IL-12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3689.802	4	922.450	19.269	.000
Within Groups	718.095	15	47.873		
Total	4407.896	19			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: IL-12

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C-	C+	8.41286	4.89249	.452	-6.6948	23.5205
	D1	-7.58299	4.89249	.548	-22.6906	7.5247
	D2	-26.16183*	4.89249	.001	-41.2695	-11.0542
	D3	-24.70954*	4.89249	.001	-39.8172	-9.6019
C+	C-	-8.41286	4.89249	.452	-23.5205	6.6948
	D1	-15.99585*	4.89249	.036	-31.1035	-.8882
	D2	-34.57469*	4.89249	.000	-49.6823	-19.4670
	D3	-33.12241*	4.89249	.000	-48.2301	-18.0148
D1	C-	7.58299	4.89249	.548	-7.5247	22.6906
	C+	15.99585*	4.89249	.036	.8882	31.1035
	D2	-18.57884*	4.89249	.013	-33.6865	-3.4712
	D3	-17.12656*	4.89249	.023	-32.2342	-2.0189
D2	C-	26.16183*	4.89249	.001	11.0542	41.2695
	C+	34.57469*	4.89249	.000	19.4670	49.6823
	D1	18.57884*	4.89249	.013	3.4712	33.6865
	D3	1.45228	4.89249	.998	-13.6554	16.5599
D3	C-	24.70954*	4.89249	.001	9.6019	39.8172
	C+	33.12241*	4.89249	.000	18.0148	48.2301
	D1	17.12656*	4.89249	.023	2.0189	32.2342
	D2	-1.45228	4.89249	.998	-16.5599	13.6554

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

IL-12

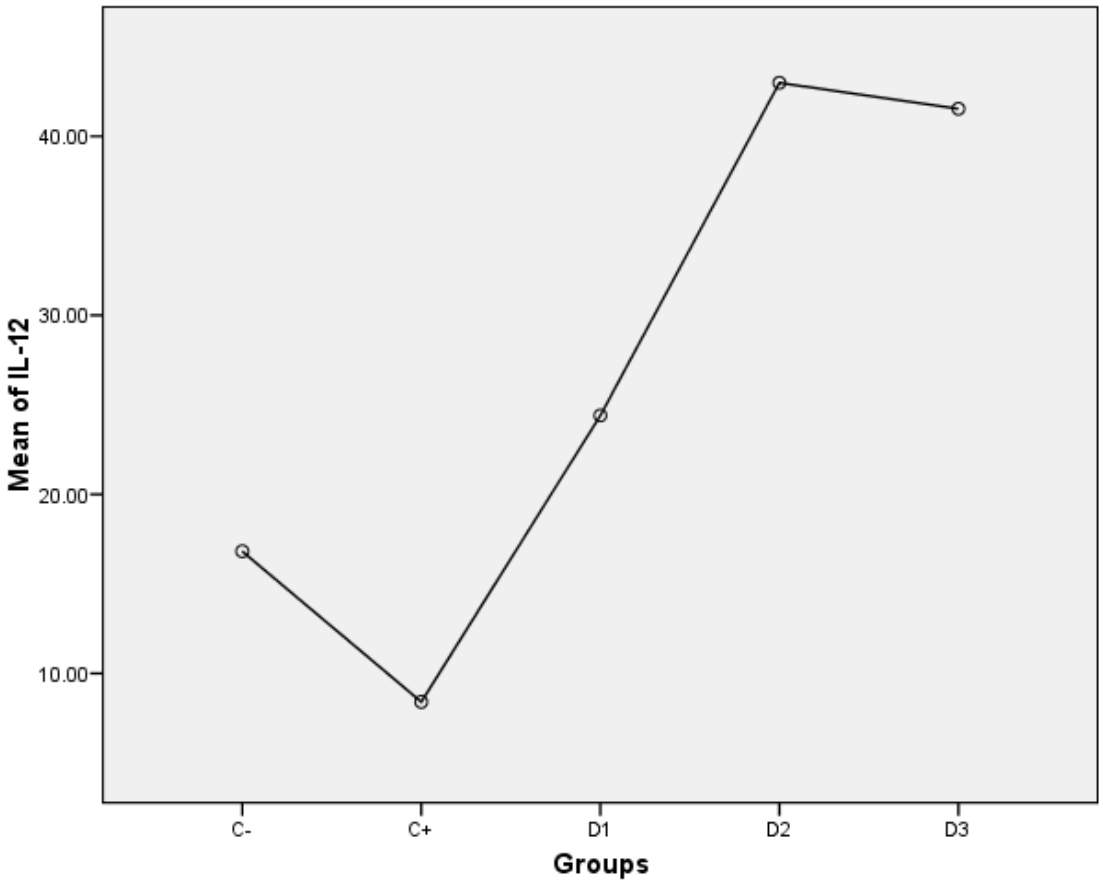
Tukey HSD[a]

Groups	N	Subset for alpha = .05		
		1	2	3
C+	4	8.4129		
C-	4	16.8257	16.8257	
D1	4		24.4087	
D3	4			41.5353
D2	4			42.9876
Sig.		.452	.548	.998

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Means Plots



Interpretation:

Then the results of the study are as shown in Table 1 as follows

Table 1. Average of IL-12

Groups	Sample				Mean ± Std. dev.
	1	2	3	4	
C-	25.81	1.08	26.06	14.36	16.83±11.83
C+	5.23	4.94	12.32	11.16	8.41±3.88
D1	23.28	25.27	22.99	26.10	24.41±1.52
D2	55.56	39.34	40.91	36.14	42.99±8.61
D3	38.76	45.06	39.92	42.41	41.54±2.80

Assumptions Test

a. Normality Test

Tests of Normality

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
IL-12	.133	20	.200*

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

b. Homogeneity of Variances test

Test of Homogeneity of Variance

	Levene Statistic	df1	df2	Sig.
IL-12	2.048	4	15	.139

One – Way ANOVA test

ANOVA

IL-12

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3689.802	4	922.450	19.269	.000
Within Groups	718.095	15	47.873		
Total	4407.896	19			

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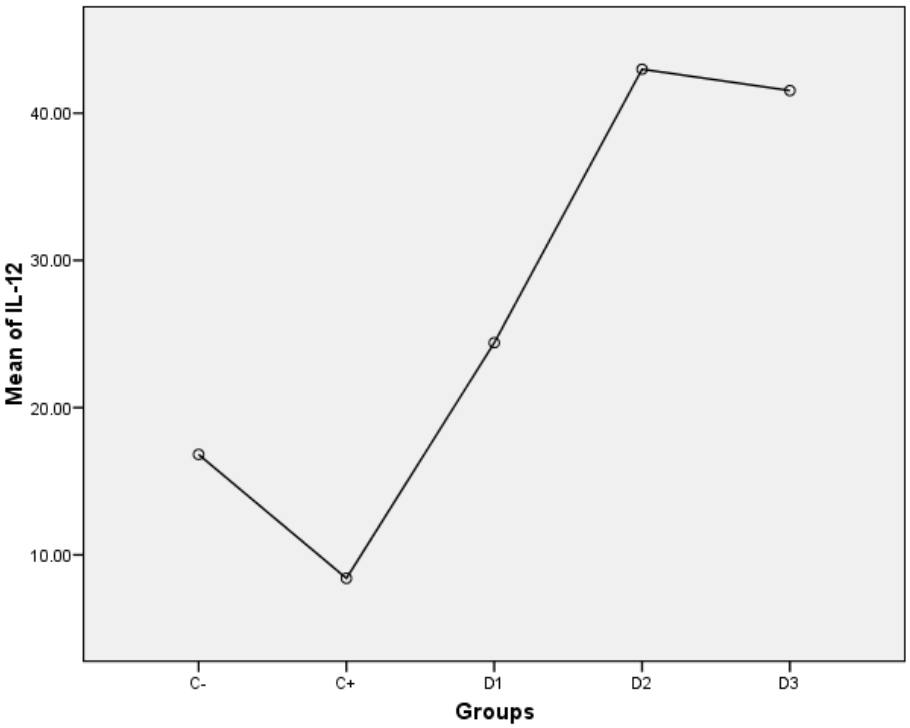
Table 6. Table Multiple Comparison Test of IL-12

Groups		Mean difference	Sig.	Result
C-	C+	8.413	0.452	not significant
	D1	-7.583	0.548	not significant
	D2	-26.162	0.001	significant
	D3	-24.710	0.001	significant
C+	D1	-15.996	0.036	significant
	D2	-34.575	0.000	significant
	D3	-33.122	0.000	significant
D1	D2	-18.579	0.013	significant
	D3	-17.127	0.023	significant
D2	D3	1.452	0.998	not significant

Description:
If the significance (p value) < alpha 0,05 = significant difference



No	Groups	Average of IL-12 (mean±std.dev.)	Notation
1	C+	8.41±3.88	A
2	C-	16.83±11.83	Ab
3	D1	24.41±1.52	B
4	D3	41.54±2.80	C
5	D2	42.99±8.61	C

The same notation, is equal there is no differenc

Case Summaries[a]

	Groups	Colony of Salmonella typhimurium
1	C-	.00
2	C-	4.00
3	C-	.00
4	C-	.00
5	C+	223.00
6	C+	137.00
7	C+	142.00
8	C+	238.00
9	D1	115.00
10	D1	32.00
11	D1	.00
12	D1	78.00
13	D2	.00
14	D2	.00
15	D2	.00
16	D2	.00
17	D3	.00
18	D3	.00
19	D3	.00
20	D3	.00
Total N	20	20

a. Limited to first 100 cases.

(4) Distribution

Normality Test Data

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Colony of Salmonella typhimurium	.363	20	.000	.673	20	.000

a. Lilliefors Significance Correction

If the value of significance (p) > 0.05 = normal distribution of data

(5) Kruskal Wallis Test

NPar Tests

Descriptives

Colony of Salmonella typhimurium

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C-	4	1.0000	2.00000	1.00000	-2.1824	4.1824	.00	4.00
C+	4	185.0000	52.93392	26.46696	100.7703	269.2297	137.00	238.00
D1	4	56.2500	50.58574	25.29287	-24.2432	136.7432	.00	115.00
D2	4	.0000	.00000	.00000	.0000	.0000	.00	.00
D3	4	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	20	48.4500	79.04261	17.67446	11.4569	85.4431	.00	238.00

Kruskal-Wallis Test

Ranks

	Groups	N	Mean Rank
Colony of Salmonella typhimurium	C-	4	8.13
	C+	4	18.50
	D1	4	12.88
	D2	4	6.50
	D3	4	6.50
	Total	20	

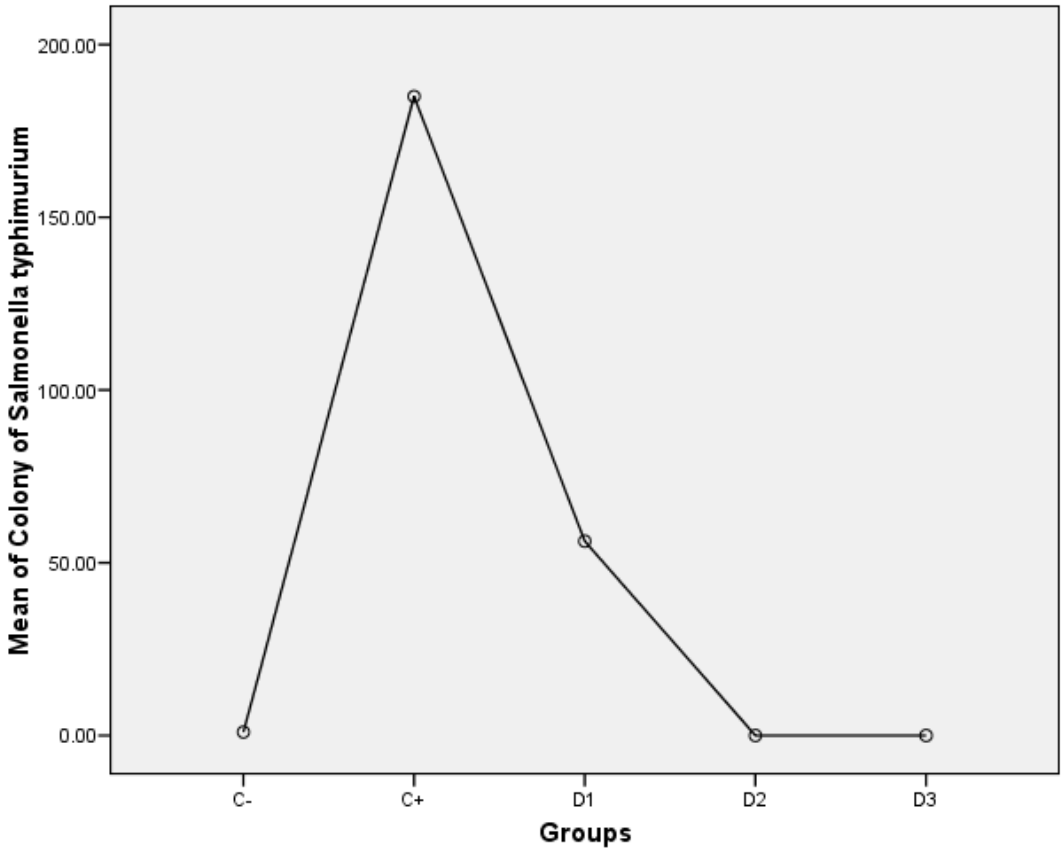
Test Statistics[a,b]

	Colony of Salmonella typhimurium
Chi-Square	15.619
df	4
Asymp. Sig.	.004

a. Kruskal Wallis Test

b. Grouping Variable: Groups

Means Plots



Mann whitney Test

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	C-	4	2.50	10.00
	C+	4	6.50	26.00
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.366
Asymp. Sig. (2-tailed)	.018
Exact Sig. [2*(1-tailed Sig.)]	.029[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	C-	4	3.13	12.50
	D1	4	5.88	23.50
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	2.500
Wilcoxon W	12.500
Z	-1.692
Asymp. Sig. (2-tailed)	.091
Exact Sig. [2*(1-tailed Sig.)]	.114[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	C-	4	5.00	20.00
	D2	4	4.00	16.00
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	6.000
Wilcoxon W	16.000
Z	-1.000
Asymp. Sig. (2-tailed)	.317
Exact Sig. [2*(1-tailed Sig.)]	.686[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	C-	4	5.00	20.00
	D3	4	4.00	16.00
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	6.000
Wilcoxon W	16.000
Z	-1.000
Asymp. Sig. (2-tailed)	.317
Exact Sig. [2*(1-tailed Sig.)]	.686[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	C+	4	6.50	26.00
	D1	4	2.50	10.00
	Total	8		

NPar Tests

Mann-Whitney Test

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.460
Asymp. Sig. (2-tailed)	.014
Exact Sig. [2*(1-tailed Sig.)]	.029[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.460
Asymp. Sig. (2-tailed)	.014
Exact Sig. [2*(1-tailed Sig.)]	.029[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	D1	4	6.00	24.00
	D2	4	3.00	12.00
	Total	8		

NPar Tests

Mann-Whitney Test

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	2.000
Wilcoxon W	12.000
Z	-1.984
Asymp. Sig. (2-tailed)	.047
Exact Sig. [2*(1-tailed Sig.)]	.114[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	D1	4	6.00	24.00
	D3	4	3.00	12.00
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	2.000
Wilcoxon W	12.000
Z	-1.984
Asymp. Sig. (2-tailed)	.047
Exact Sig. [2*(1-tailed Sig.)]	.114[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

NPar Tests

Mann-Whitney Test

Ranks

	Groups	N	Mean Rank	Sum of Ranks
Colony of Salmonella typhimurium	D2	4	4.50	18.00
	D3	4	4.50	18.00
	Total	8		

Test Statistics[b]

	Colony of Salmonella typhimurium
Mann-Whitney U	8.000
Wilcoxon W	18.000
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Groups

Interpretation:

Then the results of the study are as shown in Table 1 as follows

Table 1. Average of THE COLONY OF SALMONELLA TYPHIMURIUM

Groups	Sample				Mean ± Std. dev.
	1	2	3	4	
C-	0	4	0	0	1.0±2.0
C+	223	137	142	238	185.0±52.9
D1	115	32	0	78	56.25±50.6
D2	0	0	0	0	0±0
D3	0	0	0	0	0±0

Assumptions Test

a. Normality Test

Tests of Normality

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Colony of Salmonella typhimurium	.363	20	.000

a. Lilliefors Significance Correction

If the value of significance (p)< 0.05 = not normal distribution of data

c. Kruskal Wallis Test

Kruskal-Wallis Test

Test Statistics[a,b]

	Colony of Salmonella typhimurium
Chi-Square	15.619
df	4
Asymp. Sig.	.004

a. Kruskal Wallis Test

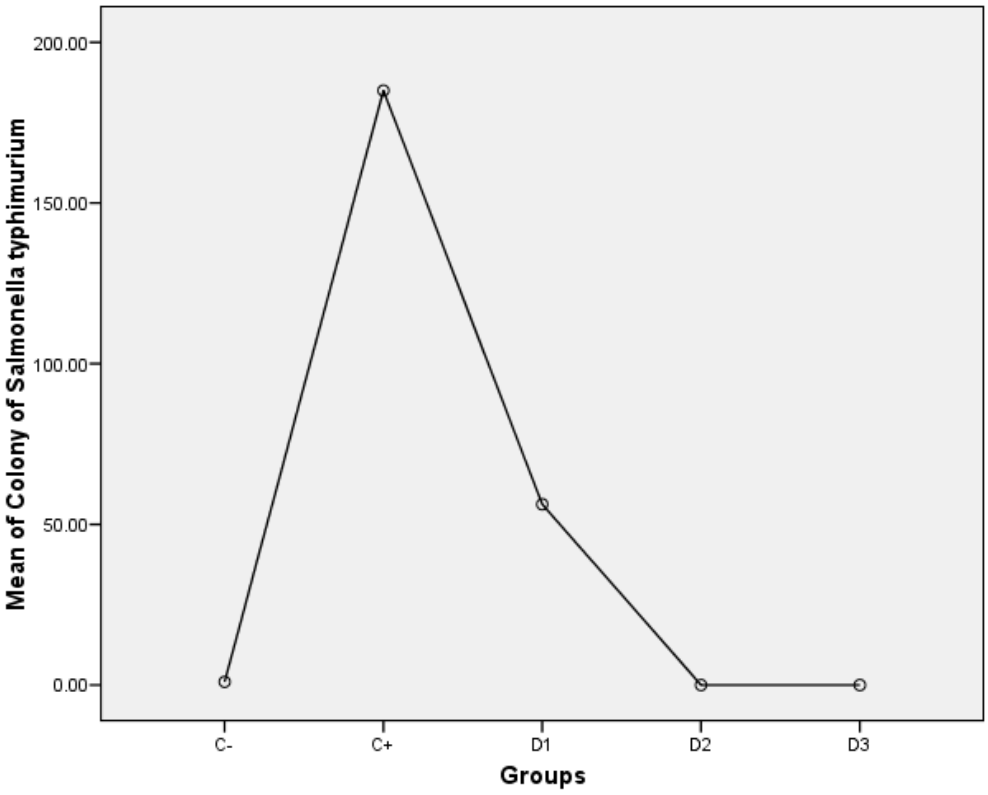
b. Grouping Variable: Groups

Table 6. Table Multiple Comparison Test of the colony of Salmonella typhimurium

Groups		Mann whitney	Sig.	Result
C-	C+	0.0	0.029	significant
	D1	2.50	0.114	not significant
	D2	6.0	0.686	not significant

	D3	6.0	0.686	not significant
C+	D1	0.0	0.029	significant
	D2	0.0	0.029	significant
	D3	0.0	0.029	significant
D1	D2	2.0	0.114	not significant
	D3	2.0	0.114	not significant
D2	D3	8.0	1.0	not significant

Description:
If the significance (p value) < alpha 0,05 = significant difference



No	Groups	Average of the colony of Salmonella typhimurium (mean±std.dev.)	Notation

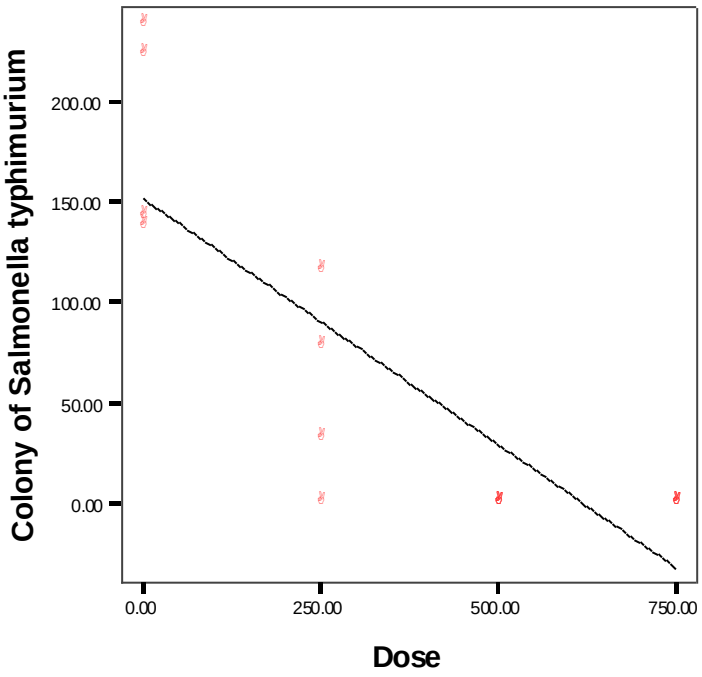
1	C-	0±0	a
2	D2	0±0	a
3	D3	0±0	a
4	D1	56.25±50.59	a
5	C+	185.0±52.93	b

Correlations

		Colony of Salmonella typhimurium	Dose
Colony of Salmonella typhimurium	Pearson Correlation	1	-.834**
	Sig. (2-tailed)		.000
	N	20	16
Dose	Pearson Correlation	-.834**	1
	Sig. (2-tailed)	.000	
	N	16	16

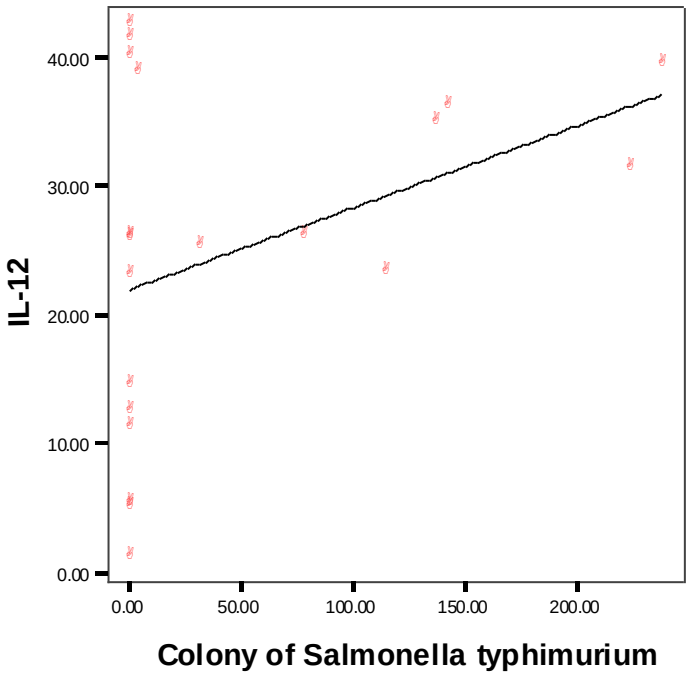
**. Correlation is significant at the 0.01 level (2-tailed).

Correlations



Correlations

		Colony of Salmonella typhimurium	IL-12
Colony of Salmonella typhimurium	Pearson Correlation	1	.383
	Sig. (2-tailed)		.096
	N	20	20
IL-12	Pearson Correlation	.383	1
	Sig. (2-tailed)	.096	
	N	20	20


IL-12
40.00
30.00
20.00
10.00
0.00
0.00
50.00
100.00
150.00
200.00
Colony of Salmonella typhimurium