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IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF IVY GOURD [*Coccinia grandis* (L.) Voigt] LEAVES ON BALB/c MICE

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ABSTRACT

Ivy gourd [*Coccinia grandis* (L.) Voigt] is a tropical plant widely distributed throughout Asia, Africa, and Pacific Island. The immunomodulatory effect of this plant has not been proven scientifically. The aim of this work was to study the immunomodulatory effect of ethanolic extract of ivy gourd leaves (EEIL) against the immune response in BALB/c mice. The EEIL was administered orally at the doses of 200 (D1), 100 (D2) and 50 (D3) mg/kg bw of mouse. The assessment of immunomodulatory activity on non-specific immunity was determined by carbon clearance test and organ index, while specific immunity by delayed type hypersensitivity (DTH) test, heamagglutination antibody (HA) titer and cytokines level. The immunomodulatory efficacy of the extract was tested on *Salmonella typhimurium* infected mice. Results showed that D1 and D2 of the extract showed a significant increased ($p<0.05$) in phagocytic index in carbon clearance test. The liver index of D1 increased significantly ($p<0.05$) compared to control. Delayed-type hypersensitivity test showed the increase of mice footpad thickness significantly ($p<0.05$) in D1 and D2. The EEIL also increased in primary and secondary antibody production. The IFN- γ level of D1 increased significantly ($p<0.05$) but IL-2 level of all groups were not affected. *S. typhimurium* infected mice showed improvement in clinical findings. The administration of EEIL showed repairment of damaged liver of *S. typhimurium* infected mice and non – toxic against the liver which can be seen in the increase of albumin and total protein levels and a decrease of SGPT level. It was also non – toxic against the kidney as verified with no change in creatinine level. The study demonstrates that the ethanolic extract of ivy gourd leaves possesses immunomodulatory effect with immunostimulatory activity.

KEY WORDS: Immunomodulatory activity, non – specific immunity, specific immunity, *Coccinia grandis* (L.) Voigt

INTRODUCTION

The immune system is key to human health and is defined as the bodily system that protects the body from foreign substances like microbes as well as to macromolecules, such as proteins and polysaccharides, and small chemicals that are recognized as foreign. Imbalance of the immune system can cause many diseases either autoimmune-related diseases or immunodeficiency-related diseases. There are many ways to regulate or normalize the immune system, one of which uses an immunomodulator either immunostimulant in immunodeficiency patients or immunosuppressant in autoimmune patients.

A large number of the patients with abnormalities of the immune system usually seek alternative treatment which they find more effective with fewer side effects and also less expensive. Traditional medicine has been recognized as an alternative treatment approach either as therapeutic agents or supplements. Actually, the plants as sources of traditional medicine or diseases healing were used since ancient times until now but still remains to be scientifically proven.

Coccinia grandis (L.) Voigt or ivy gourd, it is called papasan, kemarungan, timun padang or timun mungil in Indonesia. This plant abundantly present in tropical countries like India, Indonesia, Malaysia, the Philippines, and Thailand. It is usually consumed as a kind of vegetable or used as traditional medicine in order to heal some diseases such as jaundice disease, cough, antidiabetic and antypyretic. It contains several chemical compounds: alkaloids, phenols, flavonoids, glycoside, saponins, sterols/ triterpenoid, and tannins (Hussain *et al*. 2011). According to Wagner (1987), low molecular weight substances such as terpenoid, quinone, flavonoid and alkaloid could enhance the immune system.

Furtheremore, recent scientific studies revealed that the plants *Momordica charantia* (Deng *et al.,* 2014) and *Luffa acutangulata* Var. amara (Mohan and Saujay, 2014) both belonging to the Cucurbitaceae family possessed significant immunomodulatory activity. *Coccinia grandis* (L.) Voigt also belonging to the family Cucurbitabeae. Therefore, it was suspected that the plants belonging to the same family and possess low molecular weight substances also have the same pharmacological effect.

Currently, there is a few scientific studies to support the traditional claims as evidences scientifically which prove the immunomodulatory activity of *C. grandis* (L.) Voigt leaves. Through this research, the immunomodulatory activity from this plant with different doses has been evaluated in the BALB/c mice.

MATERIALS AND METHODS

Materials

Ethanolic extract of ivy gourd leaves (EEIL), chloral hydrate solution, sterile physiological saline, mice pelleted diet, aquadest, CMC-Na, methylprednisolone (MP), levamisol (LM), ofloxacin (OFL), acetic acid, blank ink Pelican B-17 (Carbon colloid suspension), sheep red blood cells (SRBC), *S. typhimurium* suspension, formalin buffer, Mouse IFN-γ ELISA MAXTM Deluxe Set and Mouse IL-2 ELISA MAXTM Deluxe Set kits (Biolegend®), ALT (SGPT) reagent set (Sclavo), albumin reagent set (PT. Rajawali Nusindo), total protein reagen set (PT. Rajawali Nusindo), and Creatinine reagent set. The experimental animals used were male mice strain BALB/c obtained from PT. Biofarma, 6-8 weeks old, body weight 25-35 g, healthy, no anatomical abnormalities, and had undergone adaptation for a week.

Apparatus

Maceration bottle, microscope, object glass, beaker flask, erlenmeyer flask, beaker glass, glass stirrer and spatula, glass funnel, gloves, filter paper, rotary evaporator, water bath, porcelain dish/ evaporating dish, mortar and pestle, mice cages, needles, disposable sterile syringes 1 mL and 3 mL, oral mouse syringes, dissection instruments, microcentrifuge tube, centrifuge, spectrophotometer, microtiter plate well V shape and 96 well flat bottom plate, ELISA reader (TECAN®), and Microlab 300.

Research Methods

Ivy gourd leaves were collected from Yala, Thailand and determined by Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University (Hat Yai), Thailand, with document number PLSC 05-06/063-01. The research was agreed by the Animal Research Ethics Committee – Bandung Institute of Technology, with the document number 04/KEPHP-ITB/4-2018.

This study was conducted at School of Pharmacy, Bandung Institute of Technology (ITB).

Extraction

The collected plant part (leaves) were separated from undesirable materials or plants part. They were dried for two weeks and ground into a coarse powder with the help of a suitable grinder. The extraction method is maceration in 70% ethanol at room temperature for 24 hours with periodic stirring and the extraction was repeated tree times. The macerated powder has been filtrated using filter paper. The ethanol remnants removed using rotary evaporator at 60 °C.

Characterization and phytochemical screening of ivy gourd leaves powder and its extract

The characterization of ivy gourd leaves powder and its extract include: microscopic examination (plant powder only), total ash content, water and ethanol soluble extractive, water content and density 1% of extract and the phytochemical screening includes the test for alkaloid, steroid, flavonoid, tannin, saponin, and quinone were carried out according to the standard procedures described by Depkes (2000).

Evaluation of Immunomodulatory Activity

There are two types of immunomodulatory effect tests, which are tests against non-specific immune response and tests against specific immune response. Both of these tests each contain 24 mice are divided into 6 groups of 4, which is the control, immunostimulant comparator [Levamisol (LM) 19.5 mg/kg bw], immunosuppressant comparator [methylprednisolone (MP) 5.2 mg/kg bw], and three groups of EEIL in different doses levels 200 (D1), 100 (D2) and 50 (D3)mg/bw.

Test against non-specific immune response

Carbon clearance test: All of test solution which are CMC Na 0.5% b/v, LM, MP, and EEIL with doses of 50, 100 and 200 mg/kg bw was administered orally once a day for seven days as a suspension. On the 8^{th} day, 24 hours after the last oral administration, blood is drawn as the first blood sample; blank (t = 0 min). The mice were then injected with carbon ink suspension intravenously. At intervals of 3, 6, 9, 12 and 15 minutes after the injection of carbon ink suspension, blood samples were drawn from the vein, a 20 µL sample was mixed with 1% acetic acid solution (2 mL) and the solution transmittance was measured at wavelength 675 nm. The rate of carbon clearance rate or elimination rate (K) was calculated from the slope of each time-concentration curve drawn by plotting (100-mean transmittance

value) as the ordinate. The carbon clearance rate or elimination rate and phagocytic index were calculate by using following formula (Hajra *et al*., 2012):

$$\text{Rate of carbon clearance (K)} = \frac{\log OD3 - \log OD15}{T2 - T1}$$

$$\text{Phagocytic index } (\alpha) = \frac{\frac{K}{3} \text{ x Body weight of mice (g)}}{\text{weight of liver (g)} + \text{weight of spleen (g)}}$$

$$\text{Ratio of phagocytic index} = \frac{\text{Phagocytic index of the sample group}}{\text{Phagocytic index of the control group}}$$

Where OD3 is the absorbance of blood at 3min; OD15 is the absorbance of blood at 10min; T2 is the last time point of blood collection; T1 is the first time point of blood collection.

Organ index: The finished mice from blood taking for the carbon clearance test were then sacrificed. The liver, spleen and thymus of mice were isolated and weighed. Then each organs weight were calculated the organ index for each group by using following formula:

$$\text{Organ Index} = \frac{\text{organ weight (g)}}{\text{body weight (g)}} \text{ x100\%}$$

Tests against specific immune response

Determination of Total Antibody Titre: Haemagluttination method titre assay was performed to determine primary antibody titre and secondary antibody titre. On the seventh day, after immunization, blood was collected from individual animals of all the groups from the vein for serum preparation. The 50 µL pooled serum was diluted with 50 µL NaCl 0.9% with 2 fold serial dilution in 96-well micro-titre plates and mixed with 25 µL of 2% SRBC suspension in NaCl 0.9%. Plates were incubated at 37 ºC for 24 hours. The value of primary antibody titre was considered the highest serum dilution showing 50% haemagluttination. This procedure was repeated on the fifth day to determine secondary antibody titre.

On the same day of blood collection for determination of primary antibody titre, the mice were immunized secondly time with 2% SRBC and blood samples were collected on the fifteenth day (five days after second immunization) from the heart of all mice.

Determination of cytokines (IFN-γ and IL-2): The serum which was obtained also used to determine some cytokines (IFN-γ and IL-2). Blood was centrifuged to obtain the serum. Samples are assayed by ELISA using Mouse IFN-γ ELISA MAXTM Deluxe Set and Mouse IL-2 ELISA MAXTM Deluxe Set kits from BiolegendR. The procedures were based on the

guidelines come with the ELISA kit the absorbance has been determined using TECAN – Infinit 200 Nanoquant apparatus. The standard curve was drawn to determine the concentration of Interleukin-2 as well as Interferon-γ.

Evaluation of the Efficacy of EEIL on *Salmonella typhimurium* infected mice

The mice divided to 7 groups, 5 mice in each groups are normal control, positive control (disease group), 1st comparator (LM), 2nd comparator (Ofloxacin) and three groups of ivy gourd leaf extract in different doses (50, 100 and 200 mg/kg bw). Ivy gourd leaves extract and LM groups have been given orally 7 days before infection while Ofloxacin have been given after the infection. At the 7th day, the mice were injected intraperitoneally with 0.1 mL *Salmonella typhimurium* suspension 10^5/mL. The symptoms and the behaviors of the mice as well as the body weight were monitored within 7 days before and 5 days after the infection. On day 5th after the infection, the blood sample have been collected for determination of some chemical profiles include SGPT/ALT, albumin, total protein and creatinine by Semi Automatic Biochemistry Analyser – Microlab 300. The procedure of each parameter is upon each reagent set kits. The mice then have been sacrificed and the liver has been extracted and kept in the formalin buffer for 15 hours, then histology was performed.

Data Analysis

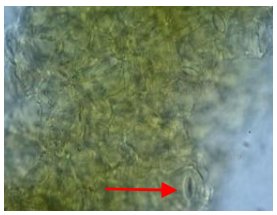
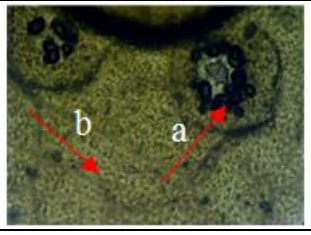
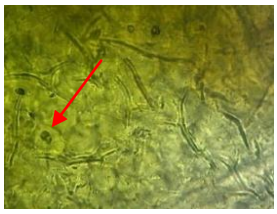
The data have been analyzed statistically that performed using ANOVA test. The significance of difference was accepted at $p < 0.05$. The values are expressed as mean ± S.D

RESULTS AND DISCUSSIONS

The ivy gourd [*Coccinia grandis* (L.) Voigt] leaves were extracted by maceration method in ethanol and got the percentage yield of the ethanolic extract was 14.65%.

Microscopical examination of the ivy gourd leaves powder (Table 1) showed anomocytic stomata, spiral xylem vessel, prismatic calcium oxalate crystals, collenchymatous cells and parenchymatus cells, which showed the same result as was done by Sutar *et al.* (2010) in their study.

Table 1: Powder microscopy of *Coccinia grandis* leaves.

Observed Segment	Sutar *et al.* (2010).	Observed results
Anomocytic stomata		
Spiral xylem vessels		
Collenchyma (a) and parenchymatus (b) cells		
Prismatic crystal of calcium oxalate		

Both plant powder and its extract were determine total ash, water and ethanol soluble matter, water content and density of extract. The value of total ash indicates that the inorganic contents in samples. Water and ethanol soluble matter were done to obtain the solubility level of both plant powder and extract in different solvent. The water content is an inevitable component of crude drugs or its extract, which must be eliminated as far as practicable. The excess water content favors spoilage by molds and bacteria and enzymatic destruction of active principles. the density of extract also was carried out and this value implies the degree of compactness of a substance compared to water. The characteristic result shown in Table 2.

Table 2: The characteristic of ivy gourd leaves powder and extract.

Parameters	Plant powder	Extract
Total ash (%w/w)	12.7	9.55
Water soluble extractive (%w/w)	13.37	35.58
Ethanol soluble extractive (%w/w)	5.18	13.55
water content (%v/w)	8	9
Density 1% of EEIL (g/mL)	-	1.0043

From Table 3, it can be seen that there is absent of tannin in the present study but present in the present study result of Hussain *et al.* (2011). The different results in chemical content due to different sources of the plant collection, where the temperature and soil condition can be causing the chemical substances contained in the plant is also different.

From the results of determination and characterization of both plant powder and its extract, we can conclude that the simplicia is indeed *Coccinia grandis* (L.) Voigt referring to the previous study.

Table 3: The phytochemical screening of ivy gourd leaves powder and extract.

Test	Present study		Ethanolic extract of *Coccinia indica* leaf (Hussain *et al.*, 2011)
	Plant powder	Extract	
Flavonoid	+	+	+
Phenol	+	+	+
Saponin	+	+	+
Tannin	-	-	+
Quinone	-	-	-
Alkaloid	+	+	+
Steroid	+	+	+
Note: + = present/positive; - = absent/negative.			

The immune system is contained of various cells and organs which divided into primary lymphoid organs (bone marrow and thymus gland) – where the immune cells develop and secondary lymphoid organs (such as kupffer cell found on the liver and spleen) – where the immune response is initiated (Owen *et al*., 2013). The immune response is divided into non – specific immune response which is the body's first line of defense against foreign substances that may lead to disease and specific immune response which is a much more specific, delayed response and requires action from the innate system to be initiated. It also have memory cell, so that it will be faster and more specific in the following responses (Coico and Sunshine, 2015).

The carbon clearance test is one of tests against non – specific immune response was used to evaluate the effect of extract on reticuloendothelial cell mediated phagocytosis. When ink containing colloidal carbon is injected intravenously, the macrophages engulf the carbon particles of the ink. Rate of clearance of (carbon particles) ink from blood is known as

phagocytic index. The carbon elimination rate show in Fig. 1 and get the value of the phagocytic index of each group out as shown in Table 4.

Table 4: Phagocytic Index in mice.

Group	Dose (mg/kg bw)	Rate of carbon elimination (*k*)	Phagocytic Index	Phagocytic Index Ratio	Category of immunomodulatory effect (Wagner, 1991)
PC	-	0.028±0.004	0.116±0.015	1	-
LM	19.5	0.038±0.005[c]	0.152±0.029[b]	1.3147	Immunostimulation
MP	5.2	0.018±0.003[b]	0.086±0.011[b]	0.7401	Immunosuppressive
EEIL	200	0.041±0.007[c]	0.151±0.018[b]	1.3064	Immunostimulation
EEIL	100	0.038±0.005[c]	0.149±0.016[b]	1.2852	Immunostimulation
EEIL	50	0.032±0.046	0.132±0.011	1.1382	Immunostimulation
PC: positive control; LM: levamisole; MP: methylprednisolone; EEIL: Ethanolic extract of ivy gourd leaves; MP: methylprednisolone; The values were considered different when $p < 0.1$ (a), significant different when $p < 0.05$ (b), very significant different when $p < 0.01$ (c) compared to control.					

Based on Wagner's criteria (1991), a substance is considered to have immunosuppressive effect if the ratio of phagocytic or clearance index (*k* value) of the test substance group to the control group is less than 1, possess an immunostimulation effect if the ratio of *k* value is in between 1-1.5 and a strong immunostimulation effect if the ratio of *k* value is more than 1.5. From the results showed that EEIL of D1, D2, and D3 stimulated the reticuloendothelial system by 1.3064, 1.2852, and 1.1382 repectively of *k* ratio values when compared to control group. Thereby the obtained result can be categorized that the EEIL possessed immunostimulatory activity.

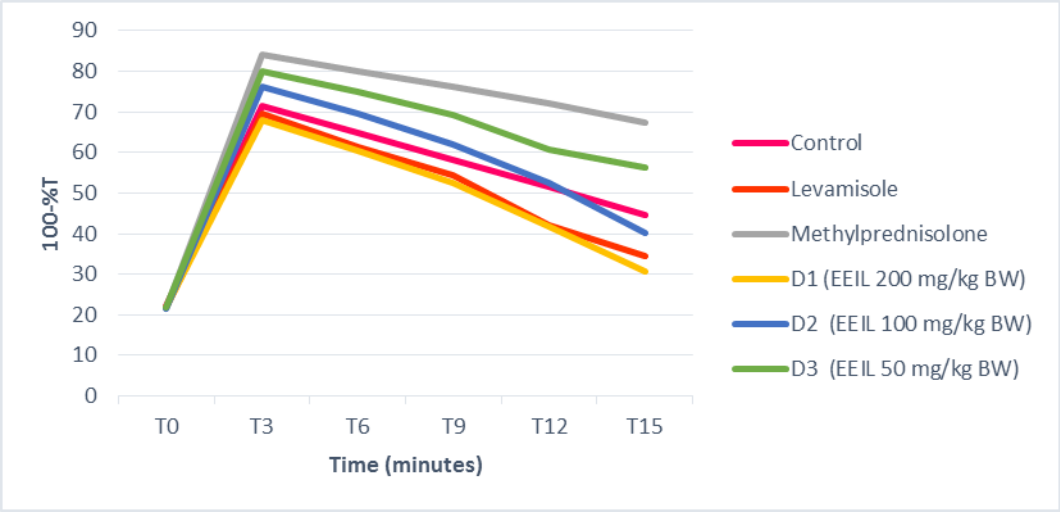


Figure 1: Elimination rates of carbon particles.

The organ index of liver, spleen and thymus was determined to examine the influence of the test substances to humoral immune response. The weight of the liver, spleen and thymus was determined to so as to see the relationship, if any, between the rate of phagocytosis and the weight of organs. An increase organ index values compared to control group represent an increase in proliferation of the immune cells in the respective organ and can be indicated that there were an increase in the immune response. The result of organ index shows in Table 5.

Table 5: Organ Index.

Group	Dose (mg/kg bw)	Ogran index (%)		
		Liver	Spleen	Thymus
Control	-	7.39±0.56	0.54±0.05	0.16±0.03
Levamisole	19.5	7.76±0.74	0.57±0.02	0.19±0.05
Methylprednisolone	5.2	6.60±0.56[a]	0.42±0.09[b]	0.06±0.01[c]
EEIL	200	8.44±0.78[b]	0.59±0.04	0.21±0.03[a]
EEIL	100	7.92±0.61	0.51±0.06	0.16±0.03
EEIL	50	7.54±0.50	0.50±0.07	0.15±0.03
EEIL: Ethanolic extract of ivy gourd leaves; The values were considered different when $p < 0.1$ (a), significant different when $p < 0.05$ (b), very significant different when $p < 0.01$ (c) compared to control.				

Specific immune response was evaluated through delayed type hypersensitivity (DTH) test and total antibody titre. The result of DTH test is shown in Table V.6. Delayed type hypersensitivity test was determined to evaluate the test substances on cellular response. Delayed type hypersensitivity test involves T helper cells 1 (Th1). The increase in Th cell activity in turn increase the activity of macrophages resulting in an increased inflammatory response characterized by the swelling of the footpad of the mice around the site of the injection. From the data in Fig. 2 and Table 6, the percentage of thickness/swelling of footpad in LM and EEIL of 200 and 100 mg/kg bw treated groups showed higher than methylprednisolone treated group by $p<0.05$ at 24 hours after SRBC injection. Methylprednisolone showed the highest activity decrease in footpad swelling compared to the control group showing it has an immunosuppressive effect on the cellular response.

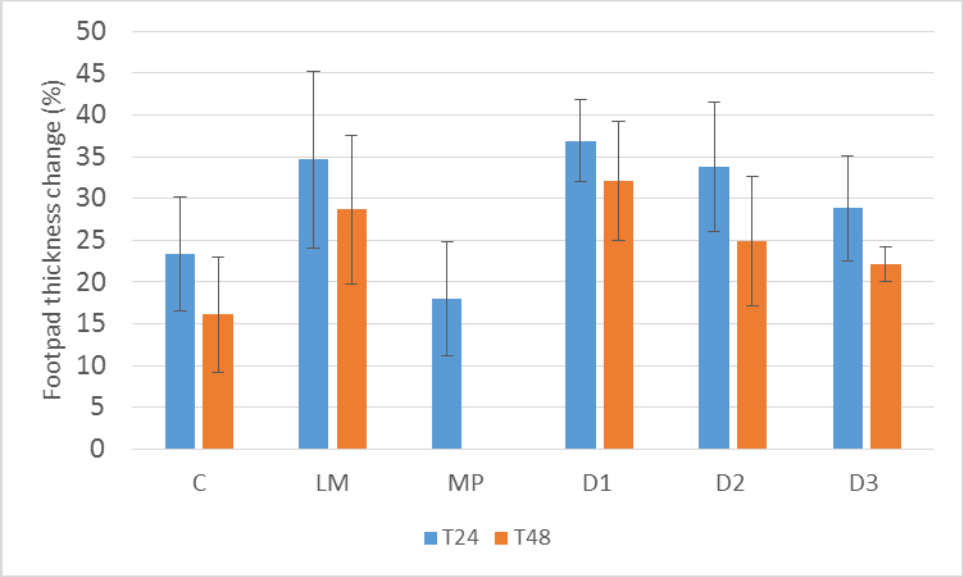


Figure 2: Percentage of footpad thickness change after injection of SRBC. C: control; MP: Methylprednisolone; LM: levamisole; D1: EEIL 200 mg/kg bw; D2: EEIL 100 mg/kg bw; D3: EEIL 50 mg/kg bw.

Table 6: Delayed – type hypersensitivity test.

Group	Dose (mg/kg bw)	Percentage of footpad thickness change after injection of SRBC	
		T24	T48
Positive Control	-	23.31	16.07
Levamisole	19.2	34.64[b]	28.69[b]
Methylprednisolone	5.2	17.95	0[a]
EEIL	200	36.90[b]	32.10[a]
EEIL	100	33.86[b]	24.90[a]
EEIL	50	28.81	22.16
EEIL: Ethanolic extract of ivy gourd leaves; The values were considered different when $p < 0.1$ (a), significant different when $p< 0.05$ (b), very significant different when $p< 0.01$ (c) compared to control.			

Haemagglutination antibody titre was carried out to evaluate the effect of ivy gourd extract against humoral immune response as a representative of specific immune system. The primary antibody titer was determined 7 days after sensitization and the secondary antibody titer was determined 5 days after primary antibody titer determination was carried out. The obtained result shown in Table 7 imply that three groups of ivy gourd dose levels increased in both primary and secondary antibody production so it can be concluded that ivy gourd extract possess an immunostimulatory effect.

Table 7: Haemagglutination Antibody Titre.

Group	Dose (mg/kg bw)	HA titre	
		Primary	Secondary
Positive Control	-	1:64	1:384
Levamisole	19.5	1:256	1:1024
Methylprednisolone	5.2	1:64	1:128
EEIL	200	1:256	1:4096
EEIL	100	1:128	1:1024
EEIL	50	1:96	1:1024

The cytokines were determined by calculation of each absorbance of each sample from its obtained standard curve so got the cytokines level. There are many cytokines that involved in the immune response, but in this study only two types of cytokines which are IL-2 and IFN- γ were determined using the ELISA method. IL-2 is secretd by activated T cells and will stimulate proliferation and differentiation of T and B cells and also activate NK cells. While, IFN- γ is secreted by T_H1, some $CD8^+$ T cells and NK cells and it will support T_H1 differentiation, induces class switching to Ig subclasses, activates macrophages and induces MHC class II expression (Owen *et al.*, 2013). The stimulated immune system will increase the level of these cytokines. The data shown in Table 8 exhibited an efficacy of EEIL on humoral immune response which was increase IFN-γ level, but in this study showed no any influence of extract administration on IL-2 level.

Table 8: Cytokines determination.

Groups	Dose (mg/kg bw)	IL-2 (pg/mL)	IFN- γ (pg/mL)
Control		115.28±29.56	523.81±28.79
Levamisole	19.5	114.81±10.51	$599.57±40.74^b$
Methylprednisolone	5.2	114.12±11.01	$400.81±34.79^c$
EEIL	200	117.63±13.26	$581.57±25.00^b$
EEIL	100	109.99±5.64	556.00±7.68
EEIL	50	115.45±6.64	501.95±37.88
EEIL: Ethanolic extract of ivy gourd leaves; The values were considered different when $p < 0.1$ (a), significant different when $p< 0.05$ (b), very significant different when $p< 0.01$ (c) compared to control.			

In order to evaluate the efficacy of EEIL was designed the immunodeficiency mice model which the mice were infected by *Salmonella typhimurium*. The mice were observed the clinical change in some behaviors before and after infected with *S. typhimurium*. The detail clinical finding of *S. typhimurium* infected mice of each group on day 5^{th} from infection shown in Table 9. From the result, it can be seen that ofloxacin treated group have no any

abnormalities in clinical finding, while in levamisole treated group only have some reduction in some clinical findings. However, the mortality is very important because it can confirm that although the levamisole treated group and the three dose level of test groups did not recover as rapidly as the group treated with antibiotic comparator (ofloxacin), but had a stronger immune system because it could sustain life demonstrated by percentage of mortality that lower than positive control group as shown in Fig. 3 and it can be proven that ivy gourd leaves extract can improve the immune system and increase the survival time of the mice.

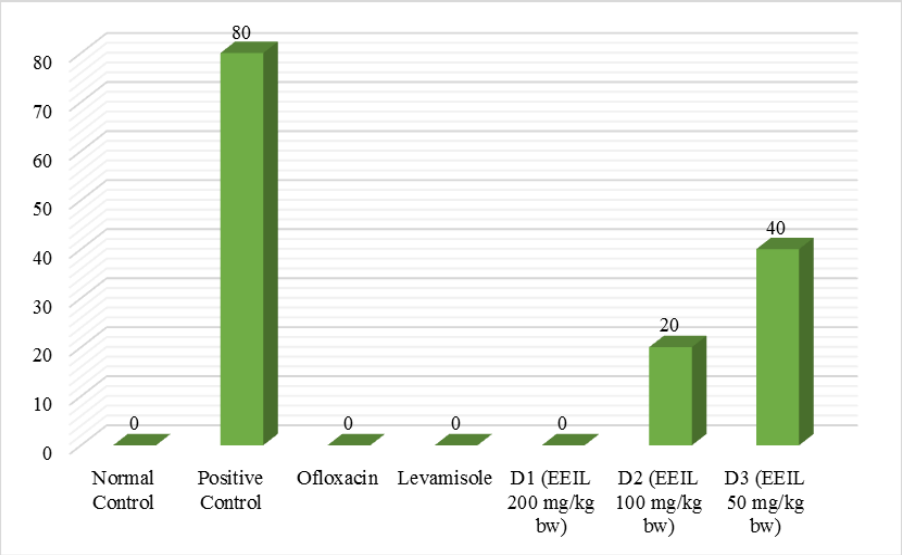


Figure 3: The percentage of Mortality.

Table 9: The percentage of the clinical finding of *Salmonella typhimurium* infected mice on day 5.

Parameter	Healthy	Infected with *Salmonella typhimurium*					
	(NC)	PC	LM	OFL	D1	D2	D3
Motoric activity	100	0	100	100	100	80	60
Hang ability	100	0	100	100	60	60	20
Body posture	100	0	100	100	100	80	60
Loss of hairs	0	100	20	0	0	20	60
Mortality (%)	0	80	0	0	0	20	40
Diarrhea	0	20	0	0	0	0	20
NC: normal control; PC: positive control; LM: levamisole; OFL:ofloxacin; D1: 200 mg/kg bw of ivy gourd extract; D2: 100 mg/kg bw of ivy gourd extract; D3: 50 mg/kg bw of ivy gourd extract; N: normal condition							

Furthermore, besides the clinical finding shown in Table 9 above, the body weight also as a strong parameter in infected mice as shown in Fig. 4.

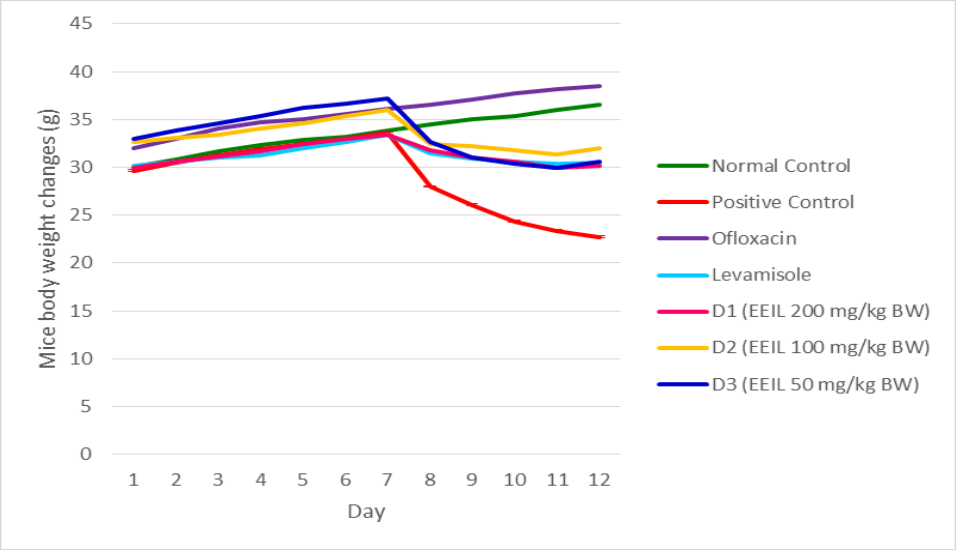


Figure 4: Mice body weight change.

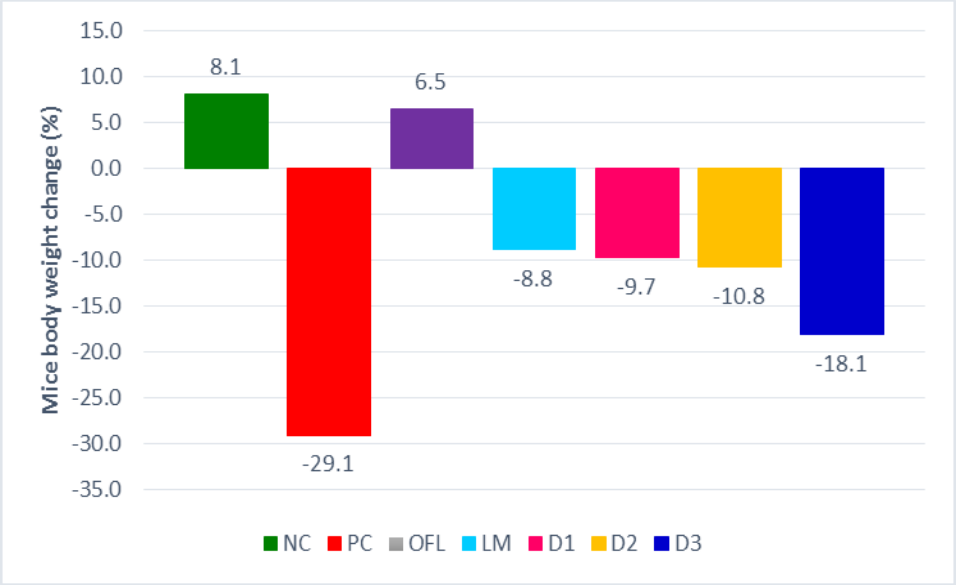


Figure 5: The percentage of mice body weight change.

During the evaluation of an efficacy of EEIL, the body weight of mice have been monitored every day (7 days before and 5 days after injected with *Salmonella typhimurium*. The result showed the percentage of body weight change of mice on day 5th after injected with *S. typhimurium* in positive control group was severe weight loss. It has been decreased by almost 38.39%, while by 13.54%, 19.89%, and 23.56% of 200 (D1), 100 (D2), and 50 (D3) mg/kg bw of doses of ivy gourd leaves extract respectively. We can conclude from the obtained result that the ivy gourd leaves extract can prevent the severe weight loss in the infected mice.

Salmonellosis is caused by *Salmonella* infection. Clinical features of salmonellosis vary in character and in severity in different individuals, one of them is liver disturbances (Stoycheva, 2006). We know that the liver is rich of kupffer cells which is one type of phagocytic cells that involved in immune response. So do, in this study also test against the liver function through some biochemical profiles. Furthermore, the biochemical profiles also were carried out to evaluate the function of liver and kidneys that may be affected by the extent of extract administration.

The liver can be evaluated by determining serum concentration of hepatic-markers. Some tests are associated with functionality (e.g., albumin) and some with cellular integrity (e.g., transaminase) (Sande, 2015). Albumin is synthesized in the liver. This is a sensitive marker of the Liver Function Tests. Decreased serum albumin signifies a decrease in the synthetic ability of the liver in cases of the liver function impairment, which mean a decrease in protein synthesis and can be shown by a decrease in total protein. ALT is found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body. If the liver is injured or damaged, the serum will show an elevation of ALT concentration.

Creatinine is produced from creatine in muscle at a rate of dependent on muscle bulk and is excreted unchanged by the kidneys, mainly by glomerular filtration but to a small extent by active secretion. Serum creatinine measurement is used as a test of renal function. The value of the creatinine level will elevated in cases with impaired renal function.

Table 10: Determination of SGPT/ALT, Albumin, Total Protein, and Creatinine levels.

Group	Dose (mg/kg bw)	SGPT/ALT (U/L)	Albumin (g/L)	Total Protein (g/dL)	Creatinine (mg/dL)
NC	-	57.03±3.15^{c}	34.57±2.64^{c}	7.27±0.72^{c}	0.65±0.17
PC	-	83.74±1.24	19.80±0.49	4.26±0.29	0.67±0.06
OFL	100	59.64±2.20^{c}	31.56±2.62^{c}	7.13±0.40^{c}	0.67±0.08
LM	5.2	62.59±3.61^{b}	29.99±1.90^{c}	6.94±0.56^{c}	0.66±0.19
EEIL	200	62.76±4.75^{b}	32.06±2.09^{c}	6.77±0.55^{c}	0.69±0.17
EEIL	100	70.01±14.31^{b}	30.19±3.10^{c}	6.62±0.66^{c}	0.70±0.10
EEIL	50	69.75±9.94^{b}	27.88±1.82^{c}	6.08±0.21^{b}	0.69±0.08
NC: control; MP: Methylprednisolone; LM: levamisole; EEIL: Ethanolic Extract of Ivy Gourd Leaves; The values were considered different when $p < 0.1$ (a), significant different when $p < 0.05$ (b), very significant different when $p < 0.01$ (c) compared to control.					

The biochemical profiles result shown in Table 10, it can be seen that the administration of EEIL for this long period did not show the toxic effect against the liver and showed a protection and restoration of the damaged liver that caused by *S. typhimurium*. The three test groups of EEIL exhibited very significant ($p<0.01$) increase albumin and total protein levels and also showed decreasing the level of SGPT by significancy $p<0.05$. In accordance with the result of the study of Sunilson *et al.* (2009) have proven that the ethanolic extract of ivy gourd leaves an oral dose of 200 $mgkg^{-1}$ (in rat) exhibited a significant ($p<0.05$) hepatoprotective effect. It also showed no cells spill this enzyme into the blood, raising the ALT enzyme blood levels. The administration of EEIL also showed non-toxic effect against the kidney function which can be seen that no alteration in creatinine level.

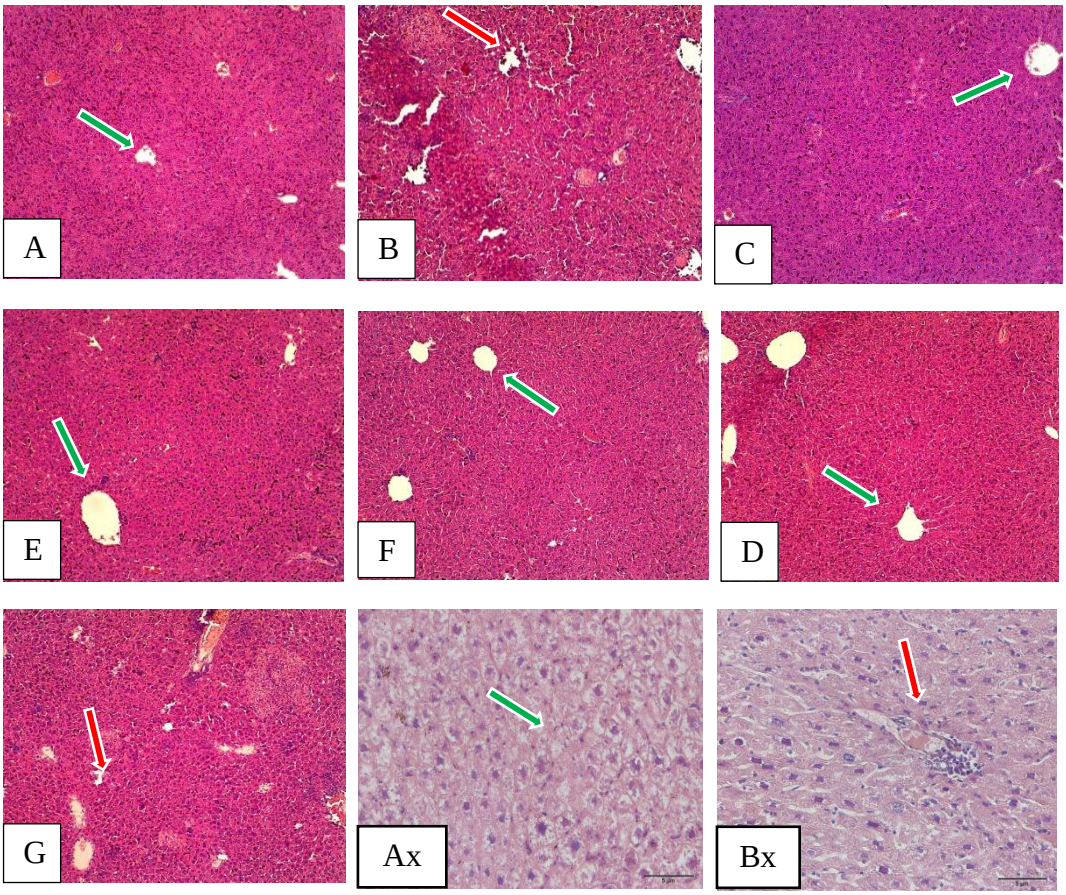


Figure 6: Microscopic examination of liver sections of mice:(A) normal control, (B) positive control, (C) Ofloxacin, (D) Levamisole, (E) D1: 200 mg/kg bw of EEIL, (F) D2: 100 mg/kg bw of EEIL, (G) D3: 50 mg/kg bw of EEIL. The result of the liver histological observation from Mohamed *et al*. (2016), Ax (healthy mice), and Bx (*S. typhimurium* infected mice), green arrow: normal cells (healthy), red arrow: abnormal cells (necrotic cells).

Microscopic examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Fig. 6 – A and Ax). Disarrangement of normal hepatic cells with intense centrilobular necrosis and vacuolization of the periportal vein were observed in the liver of positive control group (Fig. 6– B and Bx). The liver sections of mice treated with EEIL of 200 mg/kg bw (Fig. 6 – E), showed less vacuole formation and absence of necrosis and no visible changes were observed as compared to ofloxacin (Fig. 6 – C) and levamisole (Fig. 6 – D) treated groups, which further corroborated the protective effect of the extract and no toxicity effect of extract administrantion for this long periode when compared to control. Although liver sections of mice treated with EEIL of 100 and 50 mg/kg bw (Fig. 6 – F and G) showed a little bit changes, it was not prominent as that of the positive control group.

CONCLUSION AND SUGGESTION

According to the obtained results, based the tests against non-specific immune response and specific immune response exhibited the EEIL of 200, 100, and 50 mg/kg bw possessed immunostimulatory activity and its activity depends upon the dose (dose-depending) because it showed the highest activity at the highest dose (200 mg/kg bw). The ethanolic extract of ivy gourd leaves also showed the immunostimulatory effect in *Salmonella typhimurium* infected mice through improving symptoms, clinical signs, and percentage of mortality.

Further, the isolation of the extract of ivy gourd leaves to get an active constituent that responsible for its immunomodulatory activity and reviewing its working mechanism in detail is needed.

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