Immunomodulatory Activity of Solanum Torvum Ethanolic Extract on Infected Wistar Rats

Syamsudin R., Aldizal M.R.* Barliana Melisa I., Zuhrotun Ade and Moektiwardoyo Moelyono
Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Padjadjaran, Sumedang, INDONESIA
*aldizalmr@gmail.com

Abstract
Immunomodulators are compounds that alter the activites of immune system that had a vital role in preventing diseases. Takokak or Solanum torvum Swartz is empirically known as immunomodulator by its secondary metabollites such as flavonoids and polyphenols. This study was conducted to determine the effect of takokak fruits ethanolic extract against parameters of immunity such as the number of leucocyte with its components in Wistar rat strain using inducted by Shigella dysentriae.

Extract was given orally for 16 days at a dose of 250 mg/kg BW and 500 mg/kg BW. S. dysentriae was inducted on day 7 and 14. Then, blood sampling was conducted on day 0, 7, 9, 14 and 16 and then compared to positive and negative controls. Blood samples were observed using flow cytometry method by flow cytometer instrument. Immunomodulatory activity was observed through changes in numbers of leukocytes components. The result showed an immunomodulatory effect showed by leukocytes, lymphocytes and monocytes alteration in treated group which means that takokak had an immunomodulatory activity in Wistar rats.

Keywords: Solanum torvum, immunomodulatory, flow cytometry.

Introduction

Immunity could be defined as any defense system to protect human body from any invading agents by generating cells and molecules used in recognizing and eliminating foreign agent. Immune system modulation is a term that refers to alteration of immune response being inducted, expressed, or inhibited. Any substance that alters the immune system modulation is called immunomodulator.[1]

Immunomodulators defined as compound that alter immune system activity by regulated messenger cytokines, adhesion molecules, nitric oxide, hormone, neurotransmitter and other peptide.[2]

Solanum torvum Swartz or Takokak in Indonesian is widely used either in traditional Chinese medicine and Indian Ayurveda medicine as medicine for cough, asthma, diabetes, hypertension, liver disease, tuberculosis and anemia.[3] Takokak contains chemical substances such as steroid, saponin, alkaloid and phenol. Previous studies show that *S. torvum* acted as anti-tumor, antibacterial, antiviral and anti-inflammation agent.[3] Further studies are needed to determine if *S. torvum* has an effect as immunomodulator.

This research was conducted by counting amount of leucocyte and its component from whole blood of infected wistar rats fed by ethanolic extract of *S. torvum* by flow cytometry method using *Hematology Analyzer Sysmex XT 1800i.*

Material and Methods

Materials: Takokak fruit was harvested from Manoko Plant, Lembang, Indonesia. Amyl-alcohol, aquadest, chloric acid, ferrous (III) chloride, dimetil sulfoksida (DMSO), ethanol 96% (Bratachem®), etthyl acetate (Bratachem®), methanol (Bratachem®), natrium chloride, n-hexan (Bratachem®), calium hydroxide, chlorophorm (Bratachem®), gelatin solution 1%, natrium sulphate anhydrate, Lieberman-Burchard reagent, sulphate acid reagent 10% in ethanol, Dragendorff reagent (Bismuth subnytrite and calium iodide), Mayer reagent, vanillin reagent 10% in sulphate acid, magnesium powder were purchased and used.

Testing Animal: White wistar rats (2 months old) with weight ± 200 mg were taken Ethical Approval was obtained from Committee of Ethics, Faculty of Medicine University of Padjadjaran.

Methods

Collecting and processing Takokak Fruit: Takokak *(Solanum torvum)* fruits were collected from Manoko, Lembang District, Bandung, Indonesia. Fruits were washed by flowing water, sorted and dried in open air in room temperature for 3 days and covered from direct sun light and then chopped.

Extraction: Dried fruits were extracted by maseration method. It was weighted and rinsed by ethanol 96% as solvent for 3x24 hours. Liquid extract was collected each day and replaced by new ethanol 96%. Liquid extract was evaporated by rotary evaporator (Buchi®) in 350 pressure while temperature was 50ºC and then concentrated on water bath. Yield was determined.

Phytochemical Screening: Phytochemical screening was tested for alcaloid, flavonoid, tannin, polyphenol, saponin, monoterpenoid and sesquiterpenoid, steroid, triterpenoid and also quinon compound by each reagent.

Immunomodulatory Activity: Testing animals used in this research were 30 white Wistar rats were divided into 4 groups:

1. Group I (Positive Control Group): This group was treated by giving rats standard immunomodulator (Stimuno™) for three weeks and inducted by *S. dysentriae* on day 7 and 14.

2. Group II (Negative Control Group): This group was treated by giving rats solvent for three weeks and inducted by *S. dysentriae* on day 7 and 14.

3. Group III (Treated Group Dose 1): Animals were given ethanolic extract of takokak fruit with 250 mg/kg for three weeks and inducted by *S. dysentriae* on day 7 and 14.

4. Group IV (Treated Group Dose 2): Animals were given ethanolic extract of takokak fruit with 500 mg/kg for three weeks and inducted by *S. dysentriae* on day 7 and 14.

On day 1, all of rat's blood were collected and were tested by flow cytometry methods. For 16 days, animals in group III and IV were given takokak Extract Suspension dose 250 and 500 mg/kg while group I was given patent immunomodulator and group II was not given anything. On day 7 and 14 bloods were collected again and then inducted by *S. dysentriae* orally and blood was collected again on day 9 and 16.

Blood Collection and Complete Blood Count-Differential (CBC-Diff) Determination: Blood collection from animal bloods was obtained from rats tail. Rats tail was cleansed by alcohol 70 % swap. Tail then lighted using lamp in 30 cm for 15 minutes to dilate blood vessel. Its tail was then cut and blood was spilled to Eppendorf tube coated by EDTA to prevent coagulation. Whole blood was then tested using instrument Flow Cytometer.

Results and Discussion

Extraction: Takokak fruits collected from Manoko Plant in Lembang were processed and turned then into simplisia. About 500 g takokak fruit was extracted using maceration methods with 20 L ethanol 96% as solvent. After that 55 g brownish black extract was then produced with yield value 11 %.

Phytochemical screening: Chemical constituent tested on ethanolic extract of takokak fruit showed that takokak contains secondary metabolites as flavonoid, polyphenol, monoterpenoid, sesquiterpenoid, steroid and triterpenoid.

Immunomodulatory Effect from Ethanolic Extract of Takokak Fruit: This research was conducted in 16 days. During experiment, animals were observed by physical and behavioral change and blood parameter. Animals given intervention showed more mobility than control group. Animals in treatment group also show feces consistency thicker than control groups. This might be caused by immunomodulatory activity of takokak fruit ethanolic extract in rats against pathogen infection.

1. Leucocyte: Leucocyte or whole white blood cell is the most common immune system parameter. Observation data of leucocyte alteration are shown in table 1.

In this research, animals were inducted by *S. dysentriae* on day 7 and 14 that acted as infectious agent to triggered immune response. Two days after induction, blood was collected again. Every group showed that two days after infection white blood cells are produced in large numbers. Immunomodulator activity could be observed in second induction. Immune system had been sensitized and recognized same antigen that invades body, so in this case large number of leucocyte were not necessary. Animals given takokak fruit in dose 1 and 2 showed great decreasing in leucocyte produced than control group. It could be concluded that Takokak fruit had potential as immunomodulator to stimulating antigen recognition.

Treatment group in dose 1 (250 mg/kg) showed some blood aggregation which caused the leucocyte value could not be analyzed. In previous research it was known that takokak fruit in dose 0.15 mg/kg – 150 mg/kg showed eritropoeitic and hemostatic activity.[4]

2. Lymphocyte: Lymphocyte is part of white blood cells and worked in every part of infection site by producing its cell T and cell B. Data are shown in table 2.

Lymphocites are largest component in white blood cells. In this research it is shown that at second infection in day 14, lymphocyte is still increasing in negative control and dose 1 while it was decreasing in positive control and dose 2. It could be concluded that takokak in dose 2 (500 mg/kg) had a greater potential as immunomodulatory than dose 1.

3. Monocyte: Monocyte known as cell had a role in phagocytosis by its differentiation into macrophage. Monocyte also acted as antigen presenting cells which is the first cell that had been produced in an immune response.

This research showed monocyte in negative control and dose 1 is decreasing as it was not recognized second antigen invasion so that monocytes was produced slowly. In positive control and dose 2 monocytes are produced in large numbers. It produced quicker response immune system than negative control and takokak in dose 1. Takokak in dose 2 again showed greater activity as immunomodulatory agent than takokak in dose 1.

Statistical data showed significances of effect of treatment given by takokak fruit using Anova. Extract of Takokak

fruit had a p value> α which means there is significant differences between treatment of extract and control group.

Takokak fruit seems to have an immunomodulatory activity as immunostimulant by enhancing immune system in rats. Takokak had a potential to be developed as new source of natural immunostimulant. Natural drug works as a substrate of enzyme Cytochrome P 450 (CYP 450) and metabolized in liver [5] and affecting cellular transportation mechanism by drug vehicle which is *P-glycoprotein* by absorption, distribution and excretion in human body. [6]

Immunomodulatory activity of takokak fruit was shown in previous study by Attarde and Mohan[7] in 2010. Methanolic extract of takokak fruit showed good immunomodulatory and adaptogenic activity shown by hypersensitivity test and leucocyte cell counting by induction of antigen. It was stated that monocyte and macrofag acting as antigen presenting cells had important role in phagocytosis process because leucocyte that was observed did not show any significant result. Macrophage held an important role too in our body system against antigen. [7]

Immunomodulatory activity shown by takokak fruit could be caused by phenolic and flavonoid compound that could reduce oxidative stress by inhibiting enzyme Cyp 450 and stimulating free radical inhibitor such as superoxide anion or lipid peroxidase.[8]

Table 1
Leucocyte Value

Group	Day 0	Day 7	Day 9	Day 14	Day 16
Negative Control	17020 ± 6595.605	17140 ± 4567.603	23840 ±3317.077	14600 ± 1324.764	17180 ± 2772.544
Positive Control	9740 ± 3899.102	18175 ± 2884.874	18440 ± 6415.84	18225 ± 4138.74	14940 ± 5498.797
Dose 1	17260 ± 4016.591	17900 ± 3973.663	22560 ± 14294.86	16075 ± 4213.767	18800 ± 282.842
Dose 2	17900 ± 5725.382	14220 ± 3443.399	20320 ± 3774.52	13960 ± 2712.563	17066.67 ± 2138.535

Table 2
Lymphocyte value

Group	Day 0	Day 7	Day 9	Day 14	Day 16
Negative Control	65 ± 11.247	57.6 ± 8.414	66.4 ± 8.173	64.8 ± 8.258	69.8 ± 10.3
Positive Control	71.4 ± 10.644	60 ± 12.463	67.4 ± 9.838	64 ± 6.733	62 ± 13.24
Dose 1	77.4 ± 6.503	60.67 ± 5.85	69.2 ± 18.7	61 ± 7.87	72 ± 4.24
Dose 2	65.4 ± 10.212	63.2 ± 4.816	70 ± 11.853	67.8 ± 7.596	61.67 ± 13.05

Table 3
Monocyte value

Gp	Day 0	Day 7	Day 9	Day 14	Day 16
Negative Control	4 ± 1.58	6.6 ± 2.07	9 ± 4.3	4.8 ± 2.16	5.8 ± 2.58
Positive Control	2.6 ± 3.64	6.75 ± 2.06	8.4 ± 4.33	4.5 3.51	7.2 ± 2.38
Dose 1	3.4 ± 2.07	5 ± 0	7.8 ± 5.97	8 ± 4.69	6.5 ± 2.12
Dose 2	6.2 ± 4.32	6.8 ± 2.77	5 ± 2.44	6 ± 3.39	8.33 ± 0.57

Table 4
Statistical Data

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	249998839525206.400[a]	34	7352907044859.012	1103.081	.000
Intercept	3689576337497.260	1	3689576337497.260	553.509	.000
Extract	116068401642.796	10	11606840164.280	1.741	.066
Error	26829814434330.906	4025	6665792406.045		
Total	296813511641870.100	4060			
Corrected Total	276828653959537.300	4059			

Conclusion

It was concluded that ethanolic extract of takokak fruit (*Solanum torvum* Swartz) contains secondary metabolites as alcaloid, flavonoid, poliphenol, monoterpeneoid and sesquiterpenodid, steroid and triterpenoid, also quinon and saponin. Treatment groups show better mobility and fasces that are more consistent than control group.

Immunomodulatory test shows that ethanolic extract of takokak fruit has positive effect in enhancing immune response shown in second infection as increasing in leucocyte number and decreasing in lymphocyte and monocyte production. Further research is needed to determine specific substances that could possibly cause any immunomodulatory effect.

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