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# PROCEEDING INTERNATIONAL SEMINAR

CHALLENGES OF THE DEVELOPMENT  
OF NATURAL COMPOUND AS DRUG FOR INFECTIOUS  
& DEGENERATIVE DISEASES



Faculty of Pharmacy & Sciences  
University Of Muhammadiyah Prof. DR. HAMKA  
(UHAMKA)  
Jakarta, January 10, 2015



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**INTERNATIONAL SEMINAR  
CHALLENGES OF THE DEVELOPMENT OF NATURAL COMPOUND  
AS DRUG FOR INFECTIOUS & DEGENERATIVE DISEASES**

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Editors :

Dr.Priyanto,M.Biomed,Apt; Vera Ladeska M.Farm. Apt. ; Rini Prastiwi, M.Si., Apt.; Rahma Elfiyani, M.Farm., Apt.; Yudi Srifiana, M.Farm.,Apt.; Siska, M.Farm., Apt.; Hariyanti, M.Si., Apt.

Reviewers :

Prof.Dr. Oliver Kayser, Prof. Dr. Ibrahim Jantan, Ph.D, Prof. Dr. Krisana Kraisintu, Ph.D, Prof. Dr.Endang Hanani SU.,M.Si Apt., Dr. Priyanto, M.Biomed.,Apt, Dr. Hadi Sunaryo, M.Si., Apt., Drs. Inding Gusmayadi, M.Si,Apt., Dr. H. Priyo Wahyudi, M.Si.

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website : [www.ffs.uhamka.ac.id](http://www.ffs.uhamka.ac.id)

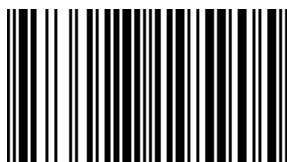
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*International Conference “Challenges Of The Development Of Natural Compound As Drug For Infectious & Degenerative Diseases”*

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## **Remarks of the Dean of Faculty of Pharmacy & Sciences (FFS) UHAMKA**

Assalamu'alaikumWr.Wb.

Distinguished ladies and gentlemen

First of all, on behalf of FFS UHAMKA, I would like to welcome to all of you in FFS UHAMKA Jakarta. Thank you very much for your attention to come and attend the international seminar in FFS UHAMKA. I hope we are all in health condition and in the shadow of God.

The conference is organized by FFS UHAMKA in collaboration with sponsors like PT. Triasindo Jaya , Indolab and UHAMQUA. This event is as part of the routine activities with the purpose are: discuss an update on the challenges of drug development for infectious and degenerative diseases based on natural product and provide a forum for exchange of information on the latest technologies involved in the development of natural compounds as drug.

In this seminar participants from student, lecturer, researchers have been attended and 4 speakers within field of Pharmaceutical sciences will be presented paper with theme “Natural Compound as Therapy for Infectious and Degenerative Diseases”. Besides that, this conference followed by presentation researchers in form of oral and poster presentation. Herewith we would like to express our gratitude to all participants, presenters, and special thanks to plenary speakers for joint us to day to share advance knowledge and expertise in this scientific event in FFS UHAMKA.

The FFS gratefully acknowledges the Rector of UHAMKA University, minister of Health of Indonesia, and sponsors for the nice collaboration in bringing this seminar. Furthermore, personally, I would like to express my deep appreciation to members of the Organizing Committee, for the good teamwork and their great effort to bring success to the seminar.

Finally, I wish all participants could benefit from the seminar and have an enjoyable moment in FFS UHAMKA Jakarta.

I look forward to thank you all for attending this seminar

Wassalamualaikum Warrohamatullahi Wabbarokatuh

**Drs. H. Budi Arman, M. Kes, Apt.**

## Remarks From Rector

*Bismillahirrahmanirrahim,*

***Your Excellency, Minister of Health Republic of Indonesia***

***Respected Resource Persons***

***Respected Participants, Ladies and Gentlemen***

On behalf of University of Muhammadiyah Prof. Dr. HAMKA (UHAMKA), I would like to warmly welcome you all to attend and participate in the International Seminar on “Challenges of the Development of Natural Compound as Drug for Infectious and Degenerative Disease,” on Saturday, January 10, 2015 at Auditorium UHAMKA.

This international seminar is a very prestigious and academic event which has to be appreciated since the topics and sub-topics such as Natural Product Chemistry, Pharmacology, Molecular Biology and Biotechnology and Pharmaceutical Technology & Compound are crucial issues today, particularly in the pharmaceutical discipline.

This academic event becomes more significant as there are some respected experts and resource persons who know how in the field of pharmacology, biotechnology, pharmaceutical technology and compound. Through this seminar, they present their research findings and scientific experiences and share them to the participants. We wish that all participants will get valuable lesson learned from these resource persons and experts.

In addition to the presentation from keynote speaker and experts, there are also poster sessions which display the research findings which, hopefully, inspire other participants to make further research dealing with the current issue in the development of natural compound as Drugs for Infectious and Degenerative Diseases.

To make this international seminar successful, I do hope that all participants are very active to quest and explore the given ideas occurred during this seminar so that this will provide significant contribution to the development of pharmacy in particular and drugs or medicines in general for the sake of humanity health.

At last, I would like to express my sincerely thank all resource persons, Prof. Dr. Nila Djuwita F. Moeloek, Sp.M (K) (Minister of Health Republic of Indonesia) , Prof. Dr. Ibrahim Jantan (UKM), Prof. Dr. Endang Hanani, SU., M.Si (UHAMKA), Prof. Dr. Oliver Kayser ( The Technical Biochemistry, TU Dortmund, Germany), and Prof. Dr. Krisana Kraisintu (Faculty of Oriental Medicine, Rangsit University, Thailand).

Jakarta, January 2015

Rector,

Prof. Dr. H. Suyatno, M.Pd.

## **DRUG AND HERBAL RESEARCH CENTER**

**Ibrahim Jantan**

Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz,  
50300 Kuala Lumpur, Malaysia

### **ABSTRACT**

Drug discovery involves integration of enormous spectrum of research activities, beginning from initial target identification and validation, through assay development, high throughput screening (HTS), hit identification, lead optimization and finally the selection of a candidate molecule for clinical development. The single target or bullet-based approach has been the dominant paradigm to discover natural small organic molecules from natural resources as new leads or models for the development of synthetic molecules for the discovery of drug targets. However, the reductionist approach in finding bioactive natural products from the tropical rainforests at several major pharmaceutical companies had generally declined in the early eighties due to the many major hurdles faced by them such as difficulties in obtaining sufficient supply of high quality natural products screening libraries, ownership issues and research in this field is lengthy, expensive, highly complex and ineffective with low success rate. On the other hand, the process of drug development is often a risky and costly endeavor. Natural products drug discovery has been marginalized in favor of the rational design of synthetic compounds to target specific molecules after the advent of HTS, combinatorial chemistry and advancement in the knowledge of molecular mechanisms, cell biology and genomics. There was a revival of natural products drug development in the later part of the eighties and this was partly due to the advances in chromatographic and spectroscopic techniques which have had a tremendous impact on the isolation and structure elucidation of the constituents of medicinal plants and the development of series of bioassay methodologies which were fast, easy to perform, quantitative and could selectively detect biologically active molecules at very low levels. Recently there is a growing interest to use innovative approaches to drug discovery from natural products by network pharmacology which integrates systems biology and pharmacology. The integrated multidisciplinary concept of multiple targets, multiple effects and complex diseases in network pharmacology have enriched our understanding of complicated pathogenesis and multi-target pathologies of systemic diseases and reduced difficulty in identifying relevant interventions to target such complexities. The ‘-omic’ technologies in system biology have now been widely used to correlate and elucidate multiple targets and network of human diseases and drug actions. The concept of network pharmacology is especially useful in accurately translating and interpreting the therapeutic effects of herbal medicines into modern biochemical and biological meanings. Herbal medicines may serve as valuable resources for network-based multi-target drug discovery. Multi-target drugs could be developed from herbal extracts by first evaluating the efficacy of the extracts, followed by identification of their major bioactive components and redevelopment of a completely new multi-component formulations composed of the major bioactive components in order to reach a synergistic and optimal combination. In this paper, the use of integrated and multi-target



approach to discover new antioxidant and anti-inflammatory drug leads will be illustrated as examples.

Keywords: drug discovery, reductionist approach, network pharmacology, multi-target drugs, antioxidants, anti-inflammatory.

# METABOLIC ENGINEERING STRATEGIES AND CONCEPTS IN GENE TECHNOLOGY FOR MODIFICATION AND OPTIMIZATION OF MEDICINAL AND AROMATIC PLANTS : EXPECTATION AND REALITIES

**Oliver Kayser**

Technical University Dortmund  
Technical Biochemistry  
Emil-Figge-Strasse 66, 44227 Dortmund  
oliver.kayser@bci.tu-dortmund.de

## ABSTRACT

In recent years classic genetic and molecular biology strategies (Bioballistics, *Agrobacterium tumefaciens* transformation, recombinant enzymes) for production of natural compounds or even breeding of medicinal and aromatic plants have expanded and improved productivity of plant-derived fine chemicals. Among those high value natural products with medicinal and cosmetic purpose (e.g. essential oils, paclitaxel, artemisinin, Vinca-Alkaloids) play a major role. Applying genetic and biotechnological techniques like metabolic engineering, site directed mutagenesis, and pathway optimization for plant optimization to reduce costs and increase productivity are in the main focus of academia and industry. Because of some drawbacks with plant cell cultures and isolated enzymes giving no sufficient high production for commercialization, research strategies shifted more and more to metabolic engineering. From the past, engineering a microorganism is proven as a valuable tool and concepts have been transferred to plant science and opened new promising perspectives for improving plants and cell lines. First, engineering crop plants was conducted, but applying these techniques for medicinal plants is rather new and has not yet been explored so well. Today cloning and expression of multiple genes in polycistronic vectors and genomic integration is of high interest and allows the reconstitution of biosynthetic pathways in heterologous organisms either plants or microorganisms. Combining science and engineering in this research field was claimed as *Combinatorial Biosynthesis* and later as *Synthetic Biology*. *Synthetic biology* includes a large number of subareas, including enzymology, protein assembly and interactions, metabolomics, gene regulation, signal transduction and computational biology and is considered as a future approach for biotechnological plant optimization. The possibilities show exciting perspectives for the exploitation of medicinal and aromatic plants to increase the level of wanted natural products, gain insight in metabolic pathways even for new biosimilar chemicals, to improve nutritional and health promoting effects of food (nutraceuticals), and to reduce the amount of unwanted by products with potential toxic or allergic activities.

Dixon, R.A. 2005. Engineering of plant natural product pathways, *CurrOpin Plant Biol.* 8:329-336

Staniak, A., Bouwmeester, H., Fraser, P.D., Kayser O., Martens, S., Tissier, A., van der Krol, S., Wessjohann, L. & Warzecha, H. (2014) Natural products - modifying pathways in plants. *Biotechnol J.* 2013. 8: 1159-1171

Staniak, A., Bouwmeester, H., Fraser, P.D., Kayser, O., Martens, S., Tissier, A., van der Krol, S., Wessjohann, L., Warzecha, H. (2014) Natural products - learning chemistry from plants. *Biotechnol J.* 2014, 9(3): 326-336

# **HERBAL MEDICINE DEVELOPMENT : SCIENTIFIC TRANSFORMATION OF NATURAL COMPOUNDS INTO EFFECTIVE DRUGS**

**Krisana Kraisintu**

Rector of College of Oriental Medicine, Rangsit University, Thailand

## **ABSTRACT**

Indigenous flora of tropical countries are abundant of compounds with pharmacological activities which could be transformed into therapeutic agents and drugs for infectious and degenerative diseases. The normal sequence for development of pharmaceuticals by this approach usually begins with the identification of active lead molecules, detailed biological assays, and formulation of dosage forms in that order, and followed by several phases of clinical studies designed to established safety, efficacy and pharmacokinetic profile of the new drugs. Employing a multidisciplinary approach to drug development from medicinal plants, Thailand is capable of manufacture GMP certified herbal medicinal products in several dosage forms on an industrial scale. The adverse effects of chemical based drugs and the escalating costs of conventional health care, longer life expectancy and life style related problems have brought with them an increased risk of developing chronic, debilitating diseases such as cancer, neurological, infectious, cardiovascular, immunological, inflammatory and genetic diseases. The availability of modern scientific methods for the cultivation, selection, manufacture and clinical evaluation of herbal remedies has made it increasingly feasible for scientists to transform herbal medicinal plants into a modern industrial enterprise capable of making significant contribution to both health care delivery and the economic growth of developing countries.

# INDONESIA TRADITIONAL MEDICINES DEVELOPMENT EVIDENCE BASED APPROACH

**Endang Hanani**

University of Muhammadiyah Prof Dr. HAMKA, Jakarta, Indonesia

## ABSTRACT

Traditional herb (jamu) is a part of culture and national resource in Indonesia, and has been consumed for several hundreds year ago in Java. The existing documents show that the traditional herb medicine has been used by royal families from the Mataram / Surakarta and Madjapahit palace in form of herb preparation as Jamu and cosmetics. More than 7500 species are known as medicinal plants around 400 have been registered, so they are used by the community as Indonesian traditional medicines. Traditional medicine/Jamu has been past down from generation to generation to maintain body fitness, health care, cosmetic and physiological body function and even to treat specific illness. Although formally not acceptable by the medical doctor, jamu are continuously being development used by the community. Based on the medicinal basic research carried out by Ministry of health (2010) showed that the Indonesian more than 50% use Jamu. On the other side the medical doctor still not accept, the reason is have no scientific research / evidence based data. Researcher start to search evidence based of medicinal herb for supporting scientific data.

The used of *Andrographis paniculata* for diabetes mellitus patients. The *A. paniculata* capsule showed that administration of APC for 14 days fasting blood glucose levels lower compared to PC (placebo capsule) but not significantly. The APC significantly reduced blood glucose 2 hours after meal. The reducing effect of *Morinda citrifolia* capsule was investigated on total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in hypercholesterolemia patients. Sixty subjects divided into 2 groups; for experimental (*M. citrifolia* capsule) and placebo (P capsule). Both groups received 2 capsules, 3 times daily for 14 days. The result suggested that *M. citrifolia* capsule significantly reduced TC and LDL-C levels. The mixture of essential oils of *Pogostemon cablin*, *Jasminum sambac* and *Zingiber officinale* significantly reduce the thigh circumference measurements, skin roughness and photographic data was an improvement in skin surface appearance in women cellulite. *Carica papaya* capsule (2 capsules 3 times daily) had significantly increased the platelet count, maintained stability of hematocrit in the normal level, shorten hospitalization in dengue fever patients, and accelerates the increased in platelet count compared with the control.

We suggest those evidence based research will be make the Indonesia herbal medicine more develop, and will be accepted by the health society.

Keywords : *Andrographis paniculata*, *Carica papaya*, *Morinda citrifolia*, *Jasminum sambac*, *Pogostemon cablin*, *Zingiber officinale*

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# IMUNOMODULATORY ASSAY OF $\beta$ -GLUCAN OF WHITE-OYSTER MUSHROOM (*Pleurotus ostreatus*(Jacq.) P. Kumm) ON THE MURINE INNATE IMMUNE SYSTEM

**Priyo Wahyudi**

Faculty of Pharmacy and Sciences, UHAMKA – Jakarta

Email: [priyowhd@gmail.com](mailto:priyowhd@gmail.com)

## ABSTRACT

Oyster mushroom (*Pleurotus ostreatus*) contains  $\beta$ -glucan which build its cell-wall, that could be extracted from its mycelial or fruit body. Beta-glucan is a polysaccharide compound that has immunomodulatory activity. This study used mycelial  $\beta$ -glucan produced through liquid fermentation and  $\beta$ -glucan extracted from fruit body. Immunomodulatory assay of  $\beta$ -glucan on innate immune system has been conducted based on the phagocytic activity and capacity of murine peritoneal macrophages, carbon clearance, and on the classical complement pathway. *In vitro* phagocytosis assay on murine peritoneal macrophage against *Staphylococcus epidermidis* showed that water-soluble and alkali-soluble  $\beta$ -glucan of mycelial and fruit body of *P. Ostreatus* have immunomodulatory activity equivalent to the positive control at the concentration of 1,000 ppm. Carbon clearance test conducted using BALB/c mice showed that  $\beta$ -glucan extracts of *P. ostreatus* have strong immunomodulatory activity equivalent to the positive control at a dosage of 100 mg/kg. CH<sub>50</sub> assay showed that  $\beta$ -glucan extracts of *P. ostreatus* have immunomodulatory activity equivalent to the positive control at a dosage of 100 mg/kg. Results of a series of assay concluded that the  $\beta$ -glucan extracts of *P. ostreatus* have immunomodulatory activity on the murine innate immune system at a dosage of 100 mg/kg.

Keywords :  $\beta$ -glucan, *P. ostreatus*, phagocytosis, carbon clearance, classical complement pathway

## INTRODUCTION

Immunity is the ability of human body to resist diseases, especially microbial infection. A combination of cells, molecules, and tissues that play a role in resistance to infection is called the immune system. Regulated reaction of cells, molecules and other materials against microbial infection called immune response (Nafrialdi, 2007). The activity of the immune system plays an important role in recognizing and destroying or neutralizing the objects in the body that are considered foreign (*non self*) (Sherwood, 2001).

Beta-glucan is a polysaccharide of fungal cell-wall component that has a wide pharmacological activity. White-oyster mushrooms (WOM) contain  $\beta$ -glucan called pleuran, acts as Biological Response Modifier (Karacksonyi & Puniak, 1994; Bohn & BeMiller, 1995; Smith *et al.*, 2002). Beta-glucan of WOM produced by mycelia or fruit body extraction. Mycelial biomass produced through liquid fermentation, while fruit body biomass produced by solid-state fermentation for several months. Mycelial and fruit body  $\beta$ -glucan of *Pleurotus ostreatus* were studied their immunomodulatory effect on the murine innate immune system. Immunomodulatory activity is an immune response parameter induced by a foreign substance enter to the body. In this experiment, studied the immunomodulatory activity of *P. ostreatus*  $\beta$ -glucan on the murine innate immune system, including phagocytic and the complement system.

Immunomodulatory activity assay conducted by determining the *in vitro* phagocytic activity and capacity of peritoneal macrophages, followed by *in vivo* carbon clearance in mice. Peritoneal macrophage phagocytic activity was defined as the number of macrophages that engulf bacteria per 100 macrophages. Phagocytic capacity is defined as the bacteria removed by 50 activated macrophages (Cannon & Swanson, 1992). Carbon clearance is defined as the rate of carbon elimination from the blood. The calculation performed by a linear regression equation of the decrease of the carbon content in the blood of each tested animal (absorbance vs. time). The rate of carbon clearance expressed by phagocytic index (PI) (Gokani *et al.*, 2007).

Carbon clearance assay might also evaluate the effect of drug on the reticulo-endothelial system (RES) includes macrophages, the most differentiated cells of the mononuclear phagocyte system (MPS). RES and MPS cells are very important to clean particles from the blood stream. When the carbon ink injected into the blood circulation system, the removal mechanism (clearance) of carbon by macrophages will run exponentially (Ismail & Asad, 2009).

Complement is a complex system consists of a number of proteins that involve in the non-specific and in the specific immune system (Bratawidjaja & Rengganis, 2009). Activation complement stimulated by various substances and takes place through three pathways, namely: an alternative pathway or properdin pathway, the lectin pathway, and the classical pathway (Kresno, 2010). Complement assay of all three pathways, conducted by haemolytic test that gives overall picture of cascading reaction

of complement pathways. Haemolytic test observed the occurrence of 50% lysis of sheep red blood cells called as haemolytic complement assay 50 (CH<sub>50</sub>) (Kirschfink & Mollnes, 2003).

The aim of this experiment was to study the immunomodulatory activity of mycelial and fruit body  $\beta$ -glucan of *P. ostreatus* on the murine innate immune system, based on the *in vitro* and *in vivo* phagocytic activity, and on the classical complement pathway.

## **MATERIALS AND METHODS**

### **Mushroom And $\beta$ -glucan Extract.**

White-oyster mushroom (*Pleurotus ostreatus* BPPTCC 6017) was supplied by BPPT Culture Collection, Serpong. White-oyster mushroom  $\beta$ -glucan was extracted from fruit body biomass obtained from solid-state fermentation, and mycelial biomass obtained from liquid fermentation (according to Wahyudi, 2014). There were four tested  $\beta$ -glucan samples: water-soluble  $\beta$ -glucan of fruit body of WOM (GWF), alkali-soluble  $\beta$ -glucan of fruit body of WOM (GAF), water-soluble  $\beta$ -glucan of mycelial of WOM (GWM), and alkali-soluble  $\beta$ -glucan of mycelial of WOM (GAM).

### ***In vitro* Phagocytic Activity and Capacity of Murine Peritoneal Macrophages Assay**

#### Preparation of intraperitoneal macrophages

Male white mouse (*Mus musculus*) BALB/c strain, 2 – 3 months old, 25 – 35 g supplied by Universitas Gajah Mada, Yogyakarta. Mice were euthanized with ether and dissected abdomen using sterile surgical instruments. If the peritoneal fluid is small amounts, added 1-2 ml phosphate buffer saline (PBS), homogenized carefully and then taken out. The number of cells was counted in Neubauer chamber, set the number of 10<sup>7</sup> macrophages/ml.

#### Preparation of bacterial suspension of *Staphylococcus epidermidis*

Bacterial suspension of *S. epidermidis* (supplied by Dept. of Microbiology, Faculty of Medicine, Universitas Indonesia) were prepared on the Mueller-Hinton broth

(MHB) incubated at 37°C for 24 hours, then centrifuged on 4,000 rpm for 15 minutes. Supernatant was discarded, the precipitate resuspended in 10 ml of PBS pH = 7.8 and determined its 10% transmittance at 620 nm, equivalent to 10<sup>9</sup> cells / ml.

*In vitro* assay

Prepare a 96 wells microplate, then placed 100 µl macrophages suspension, 100 µl of β-glucan extracts to get the concentration assay of 0.1, 1, 10, 100, 1,000, 10,000, and 100,000 ppm. Bacterial suspension (100 µl) added to the microplate, then incubated at 37°C for 30 minutes. EDTA 0,2 M then added to the microplate, made the Giemsa micropreparation. Negative control was made by prepared macrophages suspension and bacterial suspensions only. Positive control was also made using commercial immunomodulatory drugs as a comparative at concentration of 5 mg/ml. All treated and control was made in triplicate. Activity and capacity peritoneal macrophage phagocytic counted under microscopic observation.

$$\text{Phagocytic activity} = \frac{\text{number of active macrophages}}{\text{number of total macrophages}} \times 100\% \dots\dots\dots(1)$$

***In vivo* Carbon Clearance Test**

The male BALB/c mice were divided into fourteen groups of six animals each. The treated group orally received 1 ml of tested β-glucan extracts: group II, III, and IV were administered GWF extract, group V, VI, and VII were administered GAF extract, group VIII, IX, and X were administered GWM extract, and group XI, XII, and XIII were administered GAM extract at a dosage of 50, 100, and 200 mg/kg/day, p.o., respectively for five days. Group I was normal control that administered water only, while group XIV was positive control administered commercial immunomodulatory drugs at a doses adjusted to the mice. Carbon ink (Rotring art. 591017) diluted 1.6 ml in 8.4 ml of 1% gelatin, then injected via tail vein to each mouse at 48 hours after the five day treatment. Blood samples (25 µl) were then withdrawn from tail vein at 0, 3, 6, 9, 12, and 15 minutes after injection of colloidal carbon ink and lysed in 3 ml of 0.1% sodium carbonate solution. The optical density was measured spectrophotometrically at 660 nm (Ismail & Assad, 2009). The slope and the value of the carbon clearance in the

blood then determined. Phagocytic index (PI) value determined by comparing carbon clearance value of treated group and the normal control group. PI values criteria:  $1.0 < PI < 1.2$  (have no activity);  $1.3 < PI < 1.5$  (weak activity) , and  $PI > 1.5$  (strong activity) (Gokani *et al.*, 2007).

### **Classical Complement Pathway Assay**

The male BALB/c mice were divided to fifteen groups of six animal each. Group I was normal control administered water only. Group II, III, and IV were administered GWF extract, group V, VI, and VII were administered GAF extract, group VIII, IX, and X were administered GWM extract, and group XI, XII, and XIII were administered GAM extract at a dosage of 100, 200, and 400 mg/kg/day, p.o., respectively for twelve days. Group XIV was positive control administered commercial immunomodulatory drugs at an adjusted dosage to the mice, while group XV was negative control administered water only. Group II - XV were induced 0.2 ml/ 20 g BW of 1% sheep red blood cells (SRBC)(supplied by Dept. Microbiology, Faculty of Medicine, Universitas Indonesia) intraperitoneally at eight day. After 24 hours of the last administration (at thirteenth day) blood withdrawn (25  $\mu$ l) retro-orbitally for CH<sub>50</sub> assay. CH<sub>50</sub> assay conducted according to the protocol of mouse CH<sub>50</sub> ELISA Kit supplied by Novateinbio (2011).

## **RESULT AND DISCUSSION**

### **Phagocytic Activity and Capacity of Murine Peritoneal Macrophages**

White-oyster mushroom (WOM) is widely known edible mushroom that has many benefits to human health. One of the benefit is the immunomodulatory activity that has been studied in this experiment. Phagocytic activity and capacity of murine peritoneal macrophages against bacterial cells of *S. epidermidis* induced by four  $\beta$ -glucan extracts of *P. ostreatus* were presented on Table 1 and 2.

**Table 1. Average value of phagocytic activity of murine peritoneal macrophage against *S. epidermidis* induced by  $\beta$ -glucan extracts of *P. ostreatus*.**

Concentration (ppm)	Phagocytic Activity (%)			
	GWF	GAF	GWM	GAM
0.1	45,00	45,67	46,00	45,67
1	50,33	51,00	50,00	53,67
10	54,00	53,67	53,00	54,67
100	57,67	56,67	58,67	57,00
1,000	66,33	66,00	66,00	65,33
10,000	67,67	69,33	67,67	67,00
100,000	72,67	73,00	72,33	73,00
Normal Control				50,00
Negative Control				42,67
Positive Control				67,33

**Note:** **GWF:** water-soluble  $\beta$ -glucan of fruit body of white-oyster mushroom; **GAF:** alkali-soluble  $\beta$ -glucan of fruit body of white-oyster mushroom; **GWM:** water-soluble  $\beta$ -glucan of mycelial of white-oyster mushroom; **GAM:** alkali-soluble  $\beta$ -glucan of mycelial of white-oyster mushroom (GAM).

**Table 2. Average value of phagocytic capacity of murine peritoneal macrophage against *S. epidermidis* induced by  $\beta$ -glucan extracts of *P. ostreatus*.**

Concentration (ppm)	Average Number of Phagocytosed Bacteria			
	GWF	GAF	GWM	GAM
0.1	242,00	243,00	252,67	256,00
1	247,33	256,33	265,67	249,00
10	268,67	267,33	271,67	264,00
100	270,67	277,67	276,67	276,67
1,000	286,00	285,33	288,33	283,33

<b>10,000</b>	303,67	299,00	311,00	298,00
<b>100,000</b>	318,67	320,33	318,00	315,00
<b>Normal Control</b>			265,67	
<b>Negative Control</b>			251,67	
<b>Positive Control</b>			281,67	

**Note:** **GWF:** water-soluble  $\beta$ -glucan of fruit body of white-oyster mushroom; **GAF:** alkali-soluble  $\beta$ -glucan of fruit body of white-oyster mushroom; **GWM:** water-soluble  $\beta$ -glucan of mycelial of white-oyster mushroom; **GAM:** alkali-soluble  $\beta$ -glucan of mycelial of white-oyster mushroom (GAM).

Macrophages are effector cells that play an important role in the natural immune system against bacterial, viral, parasitic, or tumor cell proliferation (Novak & Vetvicka, 2008). Phagocytic enhancement of mouse peritoneal macrophage by treatment of the WOM  $\beta$ -glucan extracts, proving that polysaccharides in the fungal cell walls could increase phagocytic and cytokine production (Savelkoul *et al.*, 2007; Yin *et al.*, 2007). These results indicated that both water and alkali-soluble  $\beta$ -glucan extracted from fruit body and mycelial biomass of WOM have immunomodulatory activity, and potentially developed as an immunomodulatory drugs.

#### ***In vivo* Carbon Clearance Test**

Water and alkali-soluble  $\beta$ -glucan extracted from fruit body and mycelial biomass of WOM showed a strong immunomodulatory effectivity (PI > 1.5) at a dosage of 100 mg/kg. Beta-glucan extracts of WOM administered orally for seven days, gave a phagocytic index equivalent to the positive control at a dosage of 100 mg/kg, proved the immunomodulatory activity of  $\beta$ -glucan extracts of WOM (GWF, GAF, GWM, and GAM) in mechanism of carbon clearance. This data was in line with Laroche & Michaud (2007) reported that  $\beta$ -glucan was able to delay apoptosis, increase the proliferation and differentiation of macrophages. The presence of fungal  $\beta$ -glucan can increase the number, size, and function of macrophages.

**Table 3. Carbon clearance value of mouse blood, Phagocytic Index, and Immunomodulatory effectivity of  $\beta$ -glucan extracts of *P. ostreatus*.**

No	Group	Dosage (mg/kg)	K value	Phagocytic Index (PI)	Immunomodulatory Effectivity
1	Normal Control		0,02398	-	
2	Positive Control		0,05045	2,10	Strong
3	GWF	50	0,02614	1,09	No effect
		100	0,04519	1,88	Strong
		200	0,04065	1,70	Strong
4	GAF	50	0,02697	1,12	No effect
		100	0,04403	1,84	Strong
		200	0,03689	1,54	Strong
5	GWM	50	0,02763	1,15	No effect
		100	0,0423	1,76	Strong
		200	0,03724	1,55	Strong
6	GAM	50	0,02789	1,16	No effect
		100	0,04266	1,78	Strong
		200	0,04091	1,71	Strong

Mechanism of action of  $\beta$ -glucan in enhancing phagocytic was by stimulates T lymphocytes and induces production of interferon. Beta-glucan will affect the body's immune system including by improving humoral immunity, macrophage phagocytic and chemotaxis activity. Large molecular weight of polysaccharide compound acts as a pseudo-antigen to activate Th1 that will stimulate T lymphocytes to secrete interleukin, and further stimulate NK cells to destroy neoplastic cells (Novaes *et al.*, 2007).

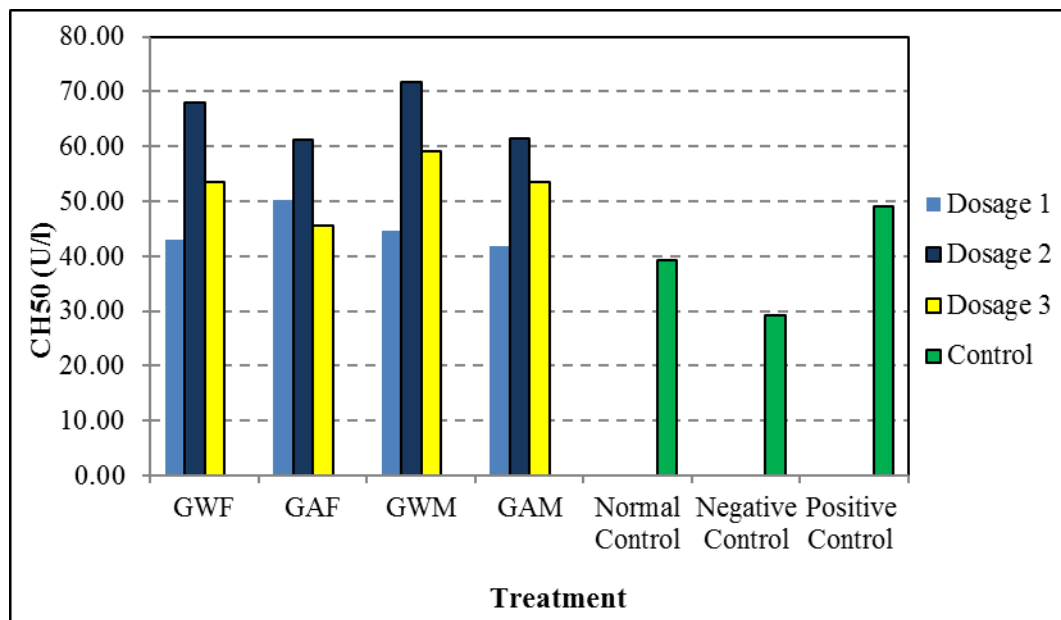
Phagocytosis is one of the mechanisms of non-specific innate immunity. In this process involves phagocytic cells against foreign objects that enter the body, analogous



to carbon in this study as extracellular bacteria enter the blood. When the carbon reaches into the bloodstream, a swallowing process conducted by phagocytic cells that activated through the process of phagocytosis as a form of self defense. First, phagocytes identify, move into the target (chemotaxis), and then attach the the target. Continued by the phagosome formation (phagocytic bubbles), lysosomes (a place intracellular digestion), then the phagosome and lysosome combine to form phagolysosome. This phagolysosome will destroy foreign bodies (Roitt & Delves, 2001).

### Classical Complement Pathway

The result of the average  $CH_{50}$  value of tested mice showed that the highest levels found in the group of test animals administered WOM  $\beta$ -glucan extracts at a dosage of 200 mg/kg. Increasing of mouse  $CH_{50}$  due to  $\beta$ -glucans were known as biological response modifier (BRM) activates the classical pathway of the complement system (Lull *et al.*, 2005).  $CH_{50}$  values were decreased in administration of extract greater than 200 mg/kg due to the immune depression resulting from the use of large dosage immunostimulant for 12 consecutive days. Immunosuppressive effects of administration immunostimulant may occur if given in a long time (Khan, 2008).



Picture 1. Graph of average of  $CH_{50}$  value of mouse treated by *P. ostreatus*  $\beta$ -glucan extracts, normal, negative, and positive control.

CH<sub>50</sub> haemolytic assay results demonstrate the ability of the complement system in a sample to activate a cascading reaction in lysing foreign cells (Shevach, 2003). The higher the complement activity, the higher the value of CH<sub>50</sub>. CH<sub>50</sub> values categorized lower if the value <100 units, normal if between 100-300 units, and high if > 300 units (Siggins *et al.*, 2011).

Results of CH<sub>50</sub> test proved that the WOM  $\beta$ -glucan extracts even water-soluble or alkali-soluble extracted from fruit body and mycelial biomass, had immunomodulatory activity on the classical pathway of the complement system. The immunostimulatory activity has been shown in the lowest dosage of the study, i.e 100 mg/kg, and equivalent to the positive control ( $\alpha = 0.05$ ). In a greater dosage than 100 mg/kg gave a higher immunostimulatory activity (Figure 1). The increase of the immune response is too high, not necessarily give better effect, because can lead to hypersensitivity reactions that harm the body (Kindt *et al.*, 2007). The use of high dosage of immunostimulant in a long-term less recommended, as it can be an immunosuppressant that suppress the immune system and induce susceptibility to malignancy (Ramberg *et al.*, 2010).

## CONCLUSION

Water and alkali-soluble  $\beta$ -glucan extracted from fruit body and mycelial biomass of white-oyster mushroom (*P. ostreatus*) have a strong immunomodulatory activity on the innate immune system, based on the phagocytosis activity, and the classical pathway of the complement system at a dosage of 100 mg/kg.

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# **EFFECT OF INCREASING HPMC CONCENTRATION ON PARTICULATE DISSOLUTION OF SOLID DISPERSION SYSTEM KETOPROFEN-HPMC**

**Ani Pahriyani, Inding Gusmayadi, Onny Indriyani**

Faculty of Pharmacy and Science, Prof. Dr. HAMKA Muhammadiyah University

## **ABSTRACT**

Ketoprofen is one of practically water insoluble drug compounds, the factor causing dissolution and bioavailability the drugs is very low. To dissolve this problem, a research was conducted by making solid dispersion system of ketoprofen using HPMC as hydrophilic polymer. The ketoprofen solid dispersion was made in 5 combination i.e. 0%, 5%, 10%, 15%, and 20% of HPMC. Method of production of solid dispersion using dissolving method, with ethanol 70% as solvent. The solid dispersions were characterized for its crystal using thermal analyses by DSC (Differential Scanning Calorimetry), and followed for dissolution using paddle-type method in HCl pH 1.2 buffer as medium. The results showed that increasing of HPMC increased  $ED_{60}$  of ketoprofen, while the maximum value at 10% HPMC concentration with  $ED_{60}$  40.42%. By one-way ANOVA analyses with 95% of significance level, among all combinations showed a significant differences. On the other hand, thermal analyses results showed that increasing of HPMC gave a slight decreasing melting point on solid dispersion system ketoprofen-HPMC below pure ketoprofen. It could be concluded that HPMC is less effective when used as a hydrophilic combination in solid dispersion.

Keywords: Ketoprofen, HPMC, solid dispersion

## **INTRODUCTION**

Medicine can be defined as a substance which is intended to be used in diagnosis, reduce pain, treat or prevent disease in humans or animals. Medicine preparations or pharmaceutical dosage forms were manifold. It is a challenge for pharmaceutical experts in the pharmaceutical technology to choose the best in formulating and determining the route of administration. In various drug administration route, oral administration is the most important route to obtain a good systemic effect, even up to more than 90% (Lachman 1994). Solid dosage forms (tablet and capsule) is a dosage form that more favored, due to its comfort and stability and easy on handling, and use by patients (Ansel 1989).

One of the oral drug administration is ketoprofen. This is a propionic acid derivative which is effective as Anti-inflammatory nonsteroid drug (NSAID) slightly

stronger than the other types of NSAIDs (Tan, et al. 2002). Ketoprofen is widely used to reduce pain and inflammation caused by other conditions such as osteoarthritis and rheumatoid arthritis (Alatas, et al. 2006). However, characteristics of ketoprofen are practically insoluble in water,therefor the problems arise in terms of absorption and bioavailability.

There are many studies have been conducted to improve the dissolution rate of drugs like soluble in water. One way to improve the dissolution rate of the drug substance is the formation of solid dispersion. Solid dispersion is a dispersion system consisting of one or more drugs in an inert carrier or matrix in the solid state (Halim, et al. 1997). A series of poorly soluble drug substance (e.g. digitoksin, benzocaine) showed an increase in dissolution rate significantly with hydrophilic solid carrier (Lachman, et al 1994). One of the hydrophilic carrier is hydroxypropyl methylcellulose (HPMC) (Swarbrick et al. 1990).

In this study the effect of HPMC 2208 type as carrier on the dissolution efficiency of ketoprofen will be try. The combination of ketoprofen-HPMC created in the dispersion solid system bydilution method

## METHOD

### 1. Preparation of Solid Dispersion of ketoprofen - HPMC 2208

The solid dispersions of ketoprofen - HPMC 2208 made by dissolving method. Ketoprofen and HPMC 2208 was weighed and dissolved with ethanol in a porcelain cup, stirred until got homogeneous. The solution was evaporated in an oven at 30°C of temperature to form a solid mass and dry. The solid mass, then crushed and sieved by 60 mesh sieve and stored in an airtight glass container (desiccator).

**Table I. Solid dispersionscombinationof ketoprofen-HPMC2208**

<b>Material</b>	<b>F 1</b>	<b>F 2</b>	<b>F 3</b>	<b>F 4</b>	<b>F 5</b>
HPMC 2208	0 mg	10 mg	20 mg	30 mg	40 mg
Ketoprofen	ad 200 mg	ad 200 mg	ad 200 mg	ad 200 mg	ad 200 mg

## **2. Preparation of a physical mixture powder ketoprofen - HPMC 2208**

A number of ketoprofen powder - HPMC 2208 with a specific weight ratio is placed in a mortar, stirred with a spatula and then sieved by 60 mesh sieve and stored in an airtight glass container (desiccator).

The combination which was made was similar combination with solid dispersion that has the best dissolution efficiency of four formulas.

## **3. Characterization of Solid Dispersions and Physical Mixtures ketoprofen - HPMC 2208**

### **a. Dissolution test**

The dissolution test carried out on pure ketoprofen, combination of ketoprofen - HPMC 2208, and the solid dispersion of ketoprofen - HPMC 2208. Dissolution test was performed by using paddle-type in HCl pH 1.2 buffer medium at  $37^{\circ} \pm 0.5^{\circ}\text{C}$  of temperature and 50 rpm of speed. Weighed powder equivalent to 100 mg of ketoprofen, then put into a container containing 900 ml of HCl pH 1.2 buffer solution. Aliquot taken after 5, 15, 30, 45, and 60 minutes respectively 10 ml and immediately replaced with 10 ml of the same dissolution medium. Measured its absorbance UV-Vis spectrophotometry at maximum wavelength, then determined the assay of the sample.

### **b. Thermal Analysis by the Differential Scanning Calorimetry (DSC) Method**

A total of 5-10 mg of sample, is inserted into the disposable container of aluminum. Used as the disposable container samples of aluminum without content. Both are inserted into the sample cell contained in the thermocouple. Furthermore, a comparison sample and heated at a constant rate of  $10^{\circ}\text{C}$  per minute. Endothermic or exothermic process that occurred was recorded on a recorder.

## **RESULTS AND DISCUSSION**

Solid dispersion system is a dispersion of one or more active ingredients in an inert carrier or matrix in the solid state. This system can be used to improve the solubility of the drug. Examples of drugs that have been tested with this technique are digitoxin, hydrocortisone, chloramphenicol, and others. The carrier used are polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and urea (Abdou 1989).

Characterization of solid dispersion begins with dissolution test, then test the thermal analysis using DSC to determine the decrease in the melting point. For the

calculation of the levels of the previous dissolution test should be made of the spectrum and the calibration curve of ketoprofen. All tests were performed in a buffer solution of HCl pH 1.2 due to ketoprofen dissolution test using this buffer. This is according to tests performed by Iskandarsyah and colleagues (2000).

### 1. Particle dissolution of ketoprofen in HCl pH 1.2 buffer solution

The dissolution done by placing each solid dispersion formula into the chamber (dissolution apparatus) with 50 rpm of speed and  $37^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$  of temperature for 60 minutes in HCl buffer as much as 900ml. Sampling is done on the 5<sup>th</sup>; 15<sup>th</sup>; 30<sup>th</sup>; 45<sup>th</sup>; and 60<sup>th</sup> minute. Dissolution test results were obtained particulates contained in Table II. The results of dissolution test showed that the third combination (F3) got the best percent dissolution, so it followed by dissolution test for physical mixture of 10%. The dissolution results are shown in Table III.

**Table II. The fraction of drug dissolution on average each solid dispersion formula**

Times (minutes)	The fraction of drug dissolution (%)				
	F1	F2	F3	F4	F5
5	3.9044	6.0126	7.1135	6.1066	5.5094
15	14.6668	22.5960	28.3604	19.0076	18.6500
30	31.7069	38.8461	46.5441	36.4291	35.9535
45	44.3043	48.9545	56.4631	47.6033	45.5862
60	52.4591	56.1603	63.0429	55.1215	53.8016

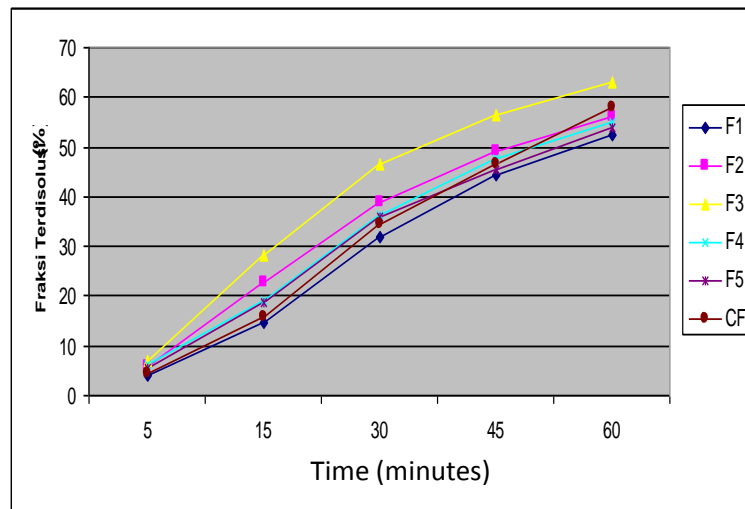
**Table III. The fraction of drug dissolution average formula physical mixture of 10%**

Times (minutes)	The fraction of drug dissolution (%)
5	4.2902
15	15.6206
30	34.3828
45	46.5123
60	58.0706

The dissolution test is an important parameter not only in the pre-formulation studies but also in studies of a drug formulation. The increasing in the dissolution rate of solid dispersion occurs due to particle size reduction, polymorphic or amorphous formation, formation of complexion and formation of solid solutions. The interaction between the molecules of ketoprofen and HPMC 2208 may occurred during the process of formation of solid dispersions. Ketoprofen molecules will be dispersed and entrapped in the polymer network HPMC 2208. The dissolution test showed an increase in ED<sub>60</sub>



values in solid dispersion system compared with pure ketoprofen. Likewise, when compared to the dissolution test in the physical mixture.



**Figure 1. The dissolution profile of ketoprofen in HCl pH 1.2 buffer solution**

## 2. Efficiency Dissolution ( $ED_{60}$ ) the average dispersion of ketoprofen in HCl buffer pH 1.2

The results of statistical analysis on various formulas  $ED_{60}$  using one-way ANOVA test showed that the data were normally distributed homogeneously and significantly more than the value of 0.05. Followed by LSD test at level of 95% to see where the different formulas significantly. From the analysis of the results obtained in formula 3 (10%) had a significant difference to the overall formula compared to other formulas. In addition, the ANOVA results also show the calculated F value of 9.201 and F table 3.48 with a probability value of less than 0.05.

**Table IV. Efficiency Dissolution ( $ED_{60}$ ) the average dispersion of ketoprofen in HCl buffer pH 1.2**

Formula	Efficiency Dissolution (%)
1	29.43
2	34.43
3	40.42
4	32.62
5	31.75
CF	31.28

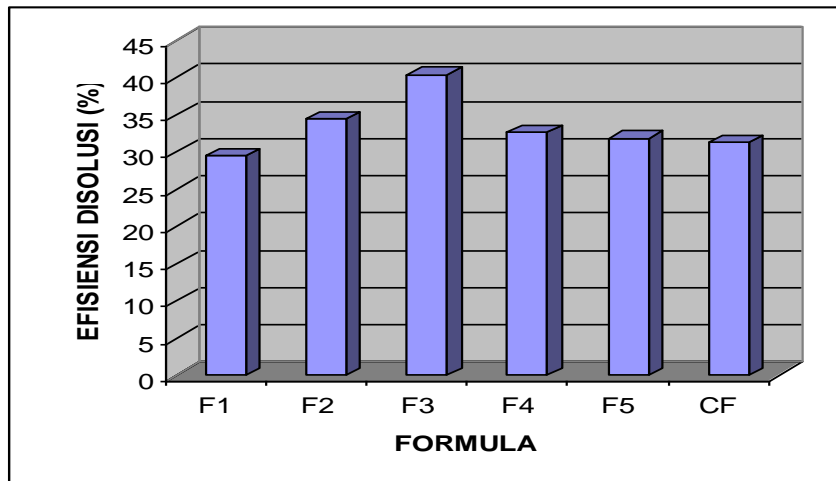


Figure 2. ED<sub>60</sub>Graph Average of 3 Replicas

Table IV. Efficiency Dissolution (ED<sub>60</sub>) ketoprofen dispersion in Buffer HCl pH 1.2 Rates

Formula	EfficiencyDissolution (%)
1	29.43
2	34.43
3	40.42
4	32.62
5	31.75
CF	31.28

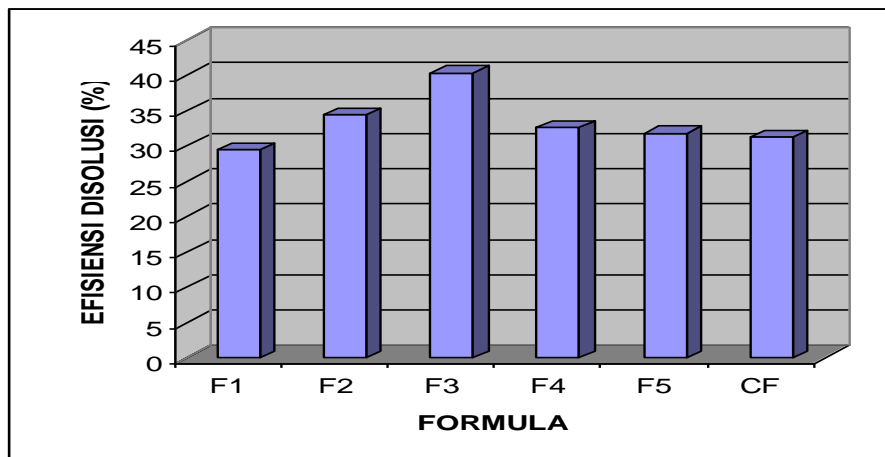


Figure 3. ED<sub>60</sub>Graphic,the rate of 3 replicas

In the pure state of ketoprofen has characteristics that is practically insoluble in water so it has low ED<sub>60</sub> value. In the physical mixture, ED<sub>60</sub> tend to be low value related to the mechanism of drug release from the matrix to form a layer of cellulose gel

in the presence of water around the particle, so that the rate of drug release is reduced. While in the solid dispersion system  $ED_{60}$  increase due to the ability of the gel layer formation cellulose derivatives will be reduced cause hydrated first (with dissolution of ethanol - water). From the data dissolution there is decreased ED at a concentration of 15% and 20%. It is alleged in the concentration already oversaturated and HPMC 2208 which does not form a dispersion system to form a gel layer back, so it make the rate of drug dissolution slow. Hestiar's research using HPMC this type as a coating on enteric coated preparations. The results in high concentrations as over 50% which drug rapidly dissolves in the small intestine medium and less soluble in acid medium (Hatakayema 2000). It is also the underlying decline in  $ED_{60}$  of 4<sup>th</sup> and 5<sup>th</sup> formula, respectively.

### **3. Test results Differential Scanning Calorimetry ( DSC )**

DSC test results at the operating temperature  $T = 300 - 150^{\circ}\text{C}$  and rate of  $10^{\circ}\text{C}/\text{min}$ . Obtained results melting point of ketoprofen  $96.1^{\circ}\text{C}$  and solid dispersions 10%  $95,8^{\circ}\text{C}$ .

Next characterization of solid dispersion is a thermal analysis using Differential Scanning Calorimetry (DSC). DSC aims at showing the difference profile between pure ketoprofen and solid dispersions of ketoprofen. DSC results showed decreasing in the endothermic point of solid dispersions compared with pure ketoprofen. Ketoprofen curves sharper than solid dispersion shows a perfect crystalline form of pure ketoprofen. Decreased melting point and a shorter curve allegedly due to changes in the form of ketoprofen be amorphous. However, the small difference between the shape of the curve of pure ketoprofen and solid dispersions of ketoprofen - HPMC 2208 show in this system there have been few changes in the form of ketoprofen. To analyze the formation of bonding between ketoprofen with HPMC 2208 can use the diffraction - X devices. It supports the dissolution test results that showed least improvement, even tended to fall back as the 4<sup>th</sup> and 5<sup>th</sup> formula.

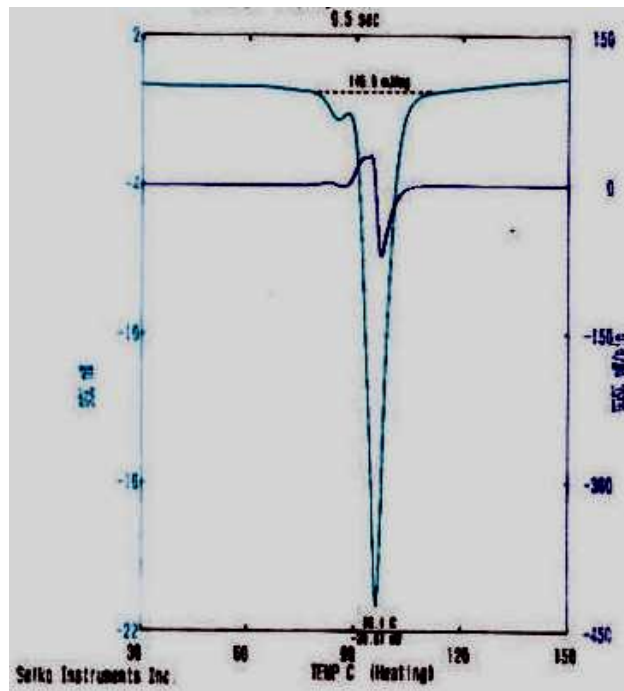


Figure 3. DSC Ketoprofen Profile

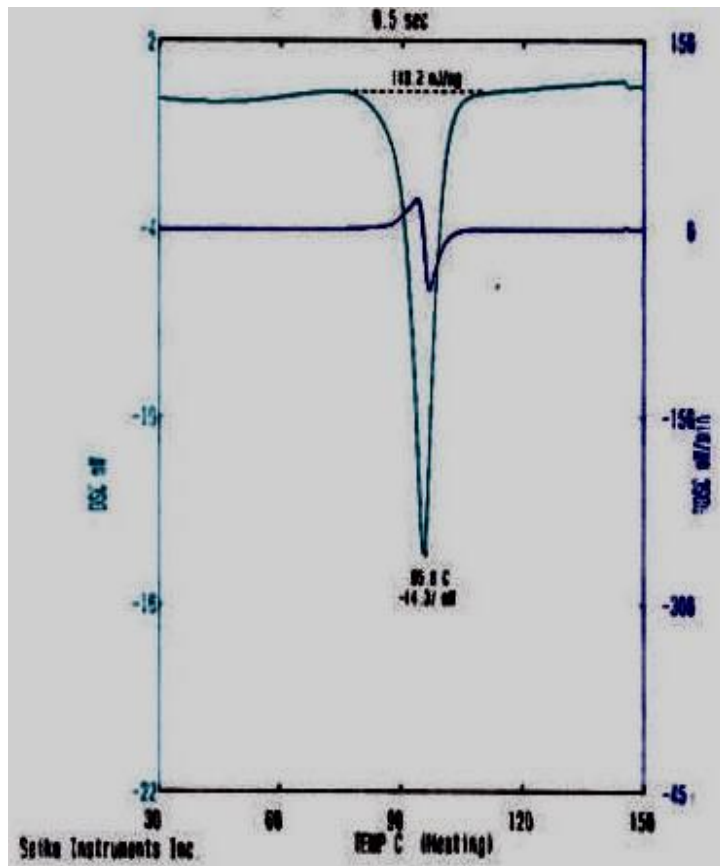


Figure 4. DSC dispersion profile combination of 10%

The result of all above test results indicate that the formation of solid dispersions cause ED<sub>60</sub> ketoprofen increases although the increasing did not reach optimum value. DSC results showed there was a slight decrease in the melting point of the solid dispersion as compared with pure ketoprofen. Both of the above results indicate that HPMC type 2208 is less effective when used as a hydrophilic carrier for solid dispersion system. Testing with various hydrophilic carrier is required to determine carrier effective to increase the solubility of ketoprofen.

## CONCLUSION

Increasing concentrations of HPMC 2208 as a carrier material in solid dispersions of ketoprofen may affect the dissolution efficiency of ketoprofen in HCl buffer pH 1.2. Increasing HPMC 2208 up to 10% showed increasing in the ED<sub>60</sub> value, but HPMC 2208 concentration more than 10% decreased the ED<sub>60</sub> value. Low ED<sub>60</sub> value of each formula shows that HPMC 2208 is less effective when used as a hydrophilic carrier for solid dispersion of ketoprofen.

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# **SYNTHESIS AND SOME PROPERTIES OF CARBOXYLMETHYL-CHITOSAN AFTER IRRADIATED**

**Gatot Trimulyadi Rekso**

Center for Application of Isotopes and Radiation- National Nuclear Energy Agency  
Corresponding author; e-mail; gatot2811@yahoo.com

Fax: +62-21-.7513270, HP ; 08129419442

## **ABSTRACT**

An investigation of synthesis and some properties of carboxymethyl-chitosan after irradiated were undertaken. A simple method for synthesis of carboxymethyl-chitosan has been developed at various temperature reaction by addition of sodium monochloroacetic for a reaction time of 2 hr. Irradiation of carboxymethyl-chitosan are done at solid and paste-like condition with variation doses of 10, 20, 30, 40, 50 kGy. The result shows that irradiation of carboxymethyl-chitosan in solid condition the solubility is increase by increasing of irradiation dose due to degradation of carboxymethyl-chitosan. On the other hand, carboxymethyl-chitosan was irradiated at paste-like condition the fraction gel increase and the swelling decrease by increasing of irradiation dose it means cross-linked structure is occur.

Keywords : carboxymethyl-chitosan, synthesis, properties after irradiated

## **INTRODUCTION**

Chitin is the most abundant polysacharides and available largely in the exoskeleton of shellfish and insects. Deacetylation of chitin readily afford chitosan , poly $\beta$ (1- 4)-D-glucosamine (Goosen, 1997). Chitosan is a biodraradable, biocompatible and non toxic polymer finds widely applications in food, pharmaceutical and fungicide (Sabharwal, 2000). Chitosan has been considered for pharmaceutical formulation and drug delivery applications in which attention has been focused on its absorption-enhancing, controlled release and bio-adhesive properties (Khroscwitz, 1992)

Chitosan is a semi-crystalline polymer, a weak base, which is insoluble in water, alkali or aqueous solution above pH 7, and common organic solvents due to its stable and rigid crystalline structure. Chitosan is normally poly dispersed and has the ability to dissolve in certain inorganic and organic acids such as hydrochloric acid, phosphoric acid, lactic acid, propionic acid, succinic acid, acetic acid, tartaric acid, citric acid and formic acid at certain pH values after prolonged stirring (Binh Doan, 2001). One of the

most popular ways to convert this polysaccharide into a water soluble form is carboxyl methyl-chitosan (Cm-chitosan).

Radiation processing can modify the molecules weight, hydrophilic and mechanical properties of chitosan resulting in enhanced properties (Rekso et. al., 2002). Radiation processing also provides a simple and fast method for cross linking and degradation of Cm-chitosan for a specific application. Radiation-degraded chitosan can induce various kinds of bioactivities such as anti microbial activities (Fumio Yoshii et. al., 2003)

The aim of the research was to synthesis a carboxyl methyl-chitosan (Cm-chitosan) in aqueous medium in order to obtain a water soluble chitosan and to know the some properties of irradiated Cm-chitosan in different conditions.

## **MATERIALS AND METHODS**

### **Preparation of Chitin**

Chitin extracted from prawn shell (*Penaeus monodon*), it was got from Muara Karang, North Jakarta. To deproteination of the shell, aqueous of 1 N sodium hydroxide was used to remove protein from a known weight of a particular fraction. The deproteinated shell were then demineralized by means of 1, 0 N hydrochloric acid in order to remove inorganic salt.

### **Preparation of Chitosan**

Chitosan can be obtained by treating chitin with 50 percent sodium hydroxide with liquid solid ratio of 20 : 1, at 100<sup>0</sup>C for 120 minutes.

### **Radiation Source**

Gamma radiation source of Co-60, IRKA batch irradiator, with irradiation dose rate about 8,5 kGy/hr was employed in these experiments. This radiation source is located at Pasar Jumat, Center for Research and Development of Isotopes and Radiation Technology, Jakarta, Indonesia.

### **Preparation of Cm-chitosan**

Cm-chitosan is formed by alkoxidation of the hydroxyl groups in the presence of concentrated sodium hydroxide (NaOH). Chitosan powder was stirred in isopropanol while sodium hydroxide was added drop wise during 10 min and continues stirring. Sodium monochloroacetate was added for esterification. The



mixture was placed in water bath for 2hr with stirring. The mixture was filtered and suspended in 200 mL of aqueous methanol and washing with ethanol. The solid obtained was dried in oven at 60°C. A paste- like condition from Cm chitosan solution of 25 % and 15 % were prepared by dissolving in the deionizer water

### **Solubility**

To estimate the solubility of Cm-chitosan, 100.0 mg of sample were suspended in 10.0 mL of distilled water and the suspension was stirred at 25°C for 5 hr. Then the mixture was filtered through filter paper to retain the undisclosed portion, which was then washed with acetone and dried at 50°C overnight. The total weight of sample was subtracted by the weight of the insoluble portion to obtain the weight of the soluble portion. The solubility of samples was expressed as g/100 mL. Subtracted by the weight of the insoluble portion to obtain the weight of the soluble portion. The solubility of samples was expressed as g/100 mL.

## **RESULTS AND DISCUSSION**

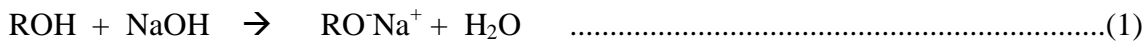
### **The Synthesis of Cm-chitosan**

The synthesis of Cm-chitosan was carried out using various temperature and time of reaction. The yield of Cm-chitosan shows in table 1.

**Table 1. The yield of Cm-chitosan**

<b>Number of Experiment</b>	<b>Reaction condition</b>			<b>Yield of Cm-chitosan</b>
	<b>Stirring Speed (RPM)</b>	<b>Temperature of Reaction (°C)</b>	<b>Time of Reaction (Hour)</b>	
1	150	55	2	72,4
2	100	55	2	73,5
3	100	50	2	55,8
4	100	40	2	40,2
5	150	Room	24	30,5

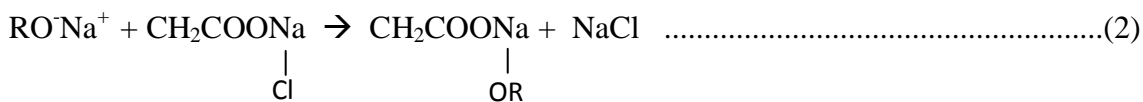
As can be seen in Table 1, the yield of Cm-chitosan the best result using stirring speed of 100 rpm, reaction temperature of 55°C for 2 hour. The reaction of Cm-chitosan is obtained through two steps, step number one is chitosan reacts with concentrated sodium hydroxide solution to form alkoxides as follows:



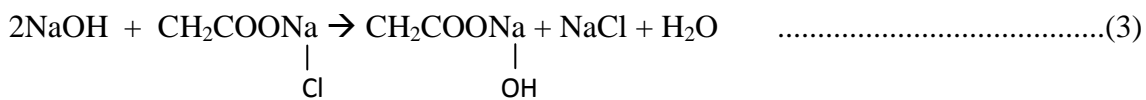
where ROH ; chitosan molekul

Due to hydroxyl group that is weakly acidic in chitosan structure therefor concentratrated NaOH wit the higher reaction temperatur the production of alkoxides higher and the reaction yield of Cm-chitosan increase.

The second step substitution of chlorida from sodium monochloroacetic on alkoxides to form Cm-chitosan .



In addition of the above reaction , there is a competitive substitution reaction of OH on sodium monochloroacetic



### FTIR Analysis

The FTIR spectra of chitosan, are shown in Figure. 1 shows the basic characteristic of chitosan at 3429 cm<sup>-1</sup> (O-H stretch) and N-H stretch, 2923 cm<sup>-1</sup> (C-H stretch), 1642 cm<sup>-1</sup> N-H bend), 1148 cm<sup>-1</sup> (bridge-O-stretch), and 1078 cm<sup>-1</sup> (C-O stretch). IR spectrum of sodium carboxymethyl chitosan, in Fig.1 also shows peak at 1603 cm<sup>-1</sup> indicating of appearence of -COO<sup>-</sup> group. H-form carboxymethyl chitosan spectrum, also shown appearence of peak at 1725 cm<sup>-1</sup> representing the carboxylate C=O asymmetric stretching. The signal at 1394 cm<sup>-1</sup> could be assigned to the symmetric stretching vibration of carboxylate C=O (Sonntag, 1980; Sonntag & Schuchmann, 2001).

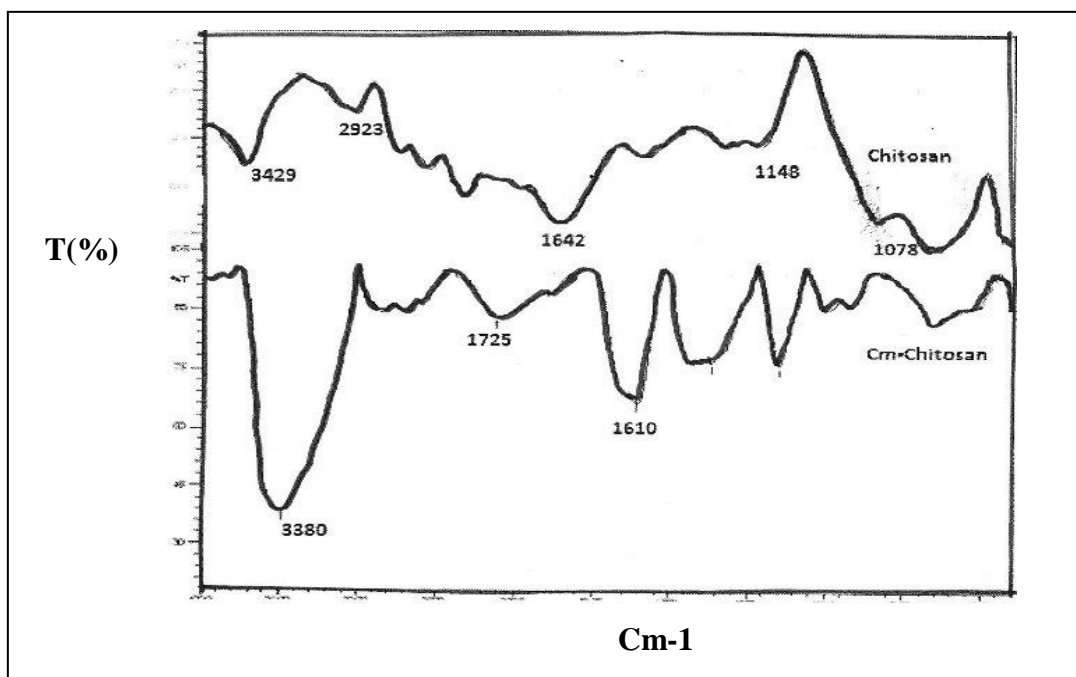


Figure 1. The FTIR spectra of chitosan and Cm-chitosan

### Solubility of Cm-chitosan Powder

As shown in Figure 2, all Cm-chitosan showed sufficient solubility in all irradiation doses, Interestingly, the difference in solubility of all Cm-chitosan in water was ranging between 74 -99 %, by increasing the irradiation dose the solubility of Cm-chitosan powder is increases, due to degradation of carboxymethyl-chitosan.

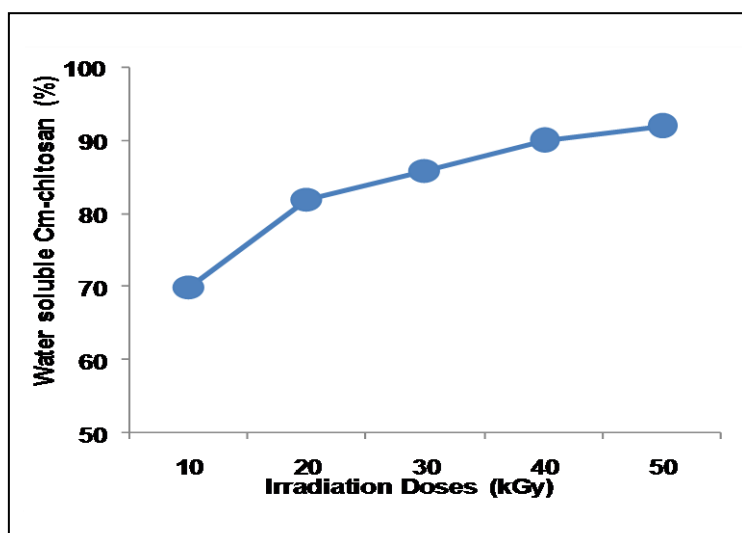


Figure 2. Solubility of Cm-chitosan powder

### A Paste-like Condition From Cm-chitosan

It was found that paste-like condition was effective for cross linking of Cm-chitosan under gamma irradiation. Figure 3 shows the relationship irradiation doses and the swelling of a paste- like condition Cm-chitosan.

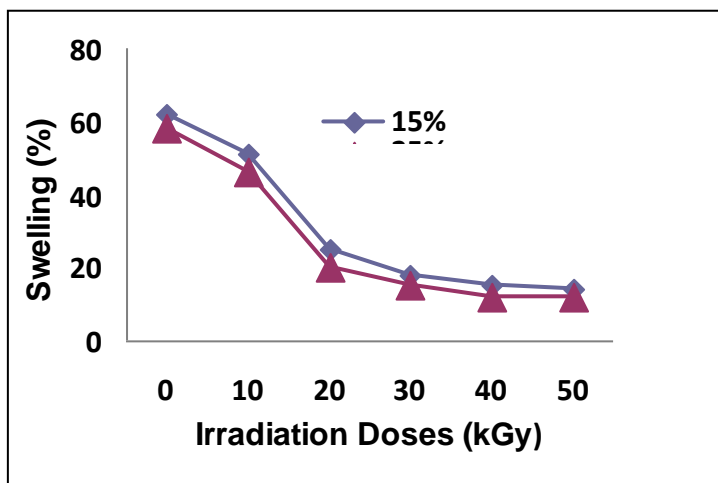


Figure 3. Relation between irradiation dose and swelling (%) of Cm-chitosan

It can be seen that the swelling is higher at low dose and it decreased as the dose increased. The Cm-chitosan sample with the concentration of 15 % gave a slightly high swelling than that of 25 %.

In Figure 4, shows the correlation of irradiation doses and gel fraction (%) of paste-like condition Cm-chitosan.

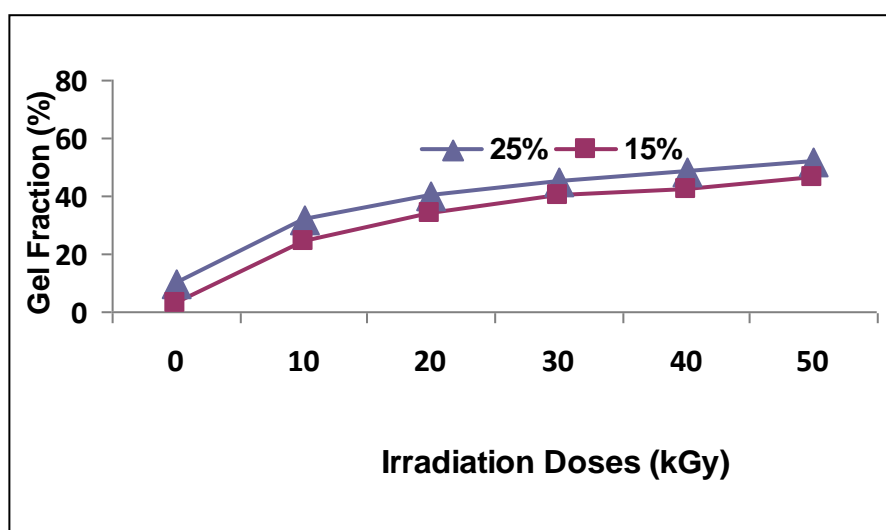


Figure 4. Relation between irradiation dose and swelling (%) of Cm-chitosan

It can be seen that the gel fraction increased as the dose increased. The Cm-chitosan sample of 25 % concentration gave a gel fraction up to 50 % with higher than of 15 % concentration. From Figure 3 and 4, it can be seen that the irradiation in paste-like condition the fraction gel of Cm-chitosan increase and the swelling is decrease by increasing of irradiation dose, it means cross-linked structure is occur.

## **CONCLUSION**

The carboxyl methyl-chitosan irradiated in the solid (powder) state the water solubility increases by increasing the irradiation dose due the degradation process. The other hand irradiation in paste-like condition the fraction gel increase and the swelling decrease by increasing of irradiation dose it means cross-linked structure is occur.

## **ACKNOWLEDGEMENT**

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# COMPARISON OF SODIUM LAURYL SULPHATE, SODIUM BENZOATE, POLYAETHYLENE GLYCOLUM 6000 AS LUBRICANT ON DISSOLVING TIME OF EXTRACT CIPLUKAN (*Physalis angulata* L.) EFFERVESCENT TABLET

Ari Widayanti, Hadi Sunaryo, Nurita Sari

Faculty of Pharmacy and Sciences  
University of Muhammadiyah Prof.DR.HAMKA, Jakarta  
Email: [ariwidayanti@yahoo.com](mailto:ariwidayanti@yahoo.com)

## ABSTRACT

Ciplukan is a plant that widely used as a traditional medicine e.g : anti-inflammatory, bronchitis, ulcers, cancer, tumors, leukemia and diabetes mellitus due to chemical compounds like saponin , terpenoids, and alkaloids. This research was conducted by making extract ciplukan effervescent tablet using sodium lauryl sulfat, PEG 6000 and sodium benzoate as lubricant. This tablet was made in 3 formula i.e 2% of sodium lauryl sulfat (F1), 3% of PEG 6000 (F2) and 4% of sodium benzoate. The tablet was evaluated for weight uniformity, size uniformity, hardness, friability, dissolve time, and pH's test. The result shows that the comparison of lubricant can give different in dissolving time. Formula II shows the dissolving time quicker than formula I and formula III. By one way ANOVA analyses with 95% of significance level , among all combinations showed a significant differences.

Keywords: Sodium Lauryl Sulphate, PEG 6000, Sodium Benzoate, Extract Ciplukan Effervescent Tablet

## INTRODUCTION

Ciplukan is a plant that widely used as a traditional medicine e.g : anti-inflammatory, bronchitis, ulcers, cancer, tumors, leukemia and diabetes mellitus due to chemical compounds like saponin , terpenoids, and alkaloids. Based research, herbal water extract ciplukan (*Physalis angulata* L.) at a dose of 10 mg / kg body weight can lower blood glucose levels alloxan-induced mice (Sutjiatmo et al. 2011). In this study the yield of the extract obtained 36.26%. The bioavailability of diabetes tablet dosage forms of requires a long time to be absorbed Therefore, the leaf used ciplukan (*Physallis angulata* L.) as antidiabetic drugs in effervescent tablet dosage form.

Effervescent tablets are used to make beverages practical. In the manufacture of effervescent tablets used material soluble lubricant to get the best time of the third comparison of this lubricant. Lubricant materials used in effervescent tablets are sodium lauryl sulfate, sodium benzoate, and PEG 6000. Efficient lubricants are generally

insoluble in water and give turbid solution after disintegration. Magnesium stearate as a lubricant does not cause the release of the drug particles from the unit (Stewart, 1981). Magnesium stearate coating will stick and granule. Magnesium stearate will give a negative effect on disintegration time and dissolution rate of tablets (Bossert, Stamm, 1980). Plus magnesium stearate is hydrophobic so that a layer of magnesium stearate that happens will impede penetration of liquid medium to crush the tablet and the dissolution of the drug (Soebagyo 1994).

Has conducted research that the presence of polyethyleneglycol 6000 as a lubricant material will accelerate the disintegration time of tablets, the higher its level of polyethyleneglycol 6000, faster time destruction. Because polyethyleneglycol 6000 hydrophilic and soluble in water, the contact time with water tablets, polyethyleneglycol 6000 will cause the tablet dissolves easily and quickly destroyed as conducted research that the addition of surfactant sodium lauryl sulfate as a lubricant effective in accelerating disintegration and dissolution of the active substance.

Sodium lauryl sulfate may improve wetting and penetration of the solvent into the tablet as a result of the decline in surface tension between the surface of the tablet particles and solvent (Alatas et al. 2006). Sodium benzoate can be used as a water-soluble lubricant. Has conducted research on sodium benzoate in effervescent tablet tamarind fruit pulp extract with the lowest tablet hardness and friability of tablets were high but longer tablet dissolution (Annisa 2011). Supposedly if you have a tablet hardness and friability of tablets least high then the time should be faster dissolution. For the used lubricants sodium benzoate on this ciplukan extract which is expected to produce a rapid dissolve. So that the resulting effervescent formula can produce a soluble effervescent tablets faster and attract consumers to be used as an alternative dosage form of diabetes mellitus in a more enjoyable. Based on the above background, it is necessary to research on the comparison of the use of sodium lauryl sulfate, sodium benzoate, and PEG 6000 as a lubricant to time ciplukan extract soluble effervescent tablets (*Physalis angulata L.*)

## **MATERIAL AND METHODS**

Ciplukan dry extract powder as made at IPB ( Institute Pertanian Bogor) . Ciplukan extract powder was made spray dry methods. Sodium benzoat, PEG 6000 ,



Sodium Lauril Sulfat , citric acid , tartaric acid, PVP,Sorbitol as a gift sample from Kimia Farma PT. All other chemicals and reagents used were analytical grade and were used as gift.

## **METHODS**

### **Preparation of ciplukan dry extract powder**

Ciplukan extract was made dry powder extract ciplukan in IPB ( Institut Pertanian Bogor). Ciplukan extracts diluted with 3 liters of water, then add 35% maltodextrin as filler after it is dried by spray drying at 175<sup>0</sup>C inlet and outlet temperature of 75<sup>0</sup>C for 2 hours until it becomes dry powder

### **Evaluation of ciplukan dry extract powder**

Organoleptic test include color, smell, taste and the water content test : enter the 1.7 to 2 grams of dry powder into the tool let moisture balance until the temperature rises up to 105<sup>0</sup>C for 5 minutes after it will get the percent moisture content of the sample.

### **Preparation of tablet**

All tablet formulations with different drug to lubricant ratio were prepared by wet granulation. ( Table. I ) All powdered were weighed accurately in electronic balance then passed separately. A number of citric acid, tartaric acid, cipluka powder, part of sorbiton and part of PVP ( diluted at ethanolum) with a specific weight ratio is place, grind in mortar until homogenous and then sieved by 14 mesh sieve and storage in oven at 50<sup>0</sup>C for 7 hour . After than sieved granul with 16 mesh sieve .( mass 1). A number of sodium bicarbonat, a part of sorbitol and a part of PVP ( diluted at ethanolum) with a specific weight ratio is place, grind in mortar until homogenous and then sieved by 14 mesh sieve and storage in oven at 50<sup>0</sup>C for 7 hour ( mass 2). After than sieved granul with 16 mesh sieve Mix the acid ( mass 1) and alkaline ( mass 2) granules then add lubricant. Evaluated the granules. Rotary eight station punch tablet machine was used to press tablets of 4 g weight.

**Table I. Formula of Effervescens tablet**

<b>Material</b>	<b>F1(mg)</b>	<b>F2 (mg)</b>	<b>F3 (mg)</b>
Ciplukan extract	562,7	562,7	562,7
Citric Acid	630,39	630,39	630,39
Tartaric acid	450,27	450,27	450,27
Sodium bicarbonate	1260,15	1260,15	1260,15
PVP	120	120	120
Sodium Lauril Sulfat	80	-	-
PEG 6000	-	120	-
Sodium Benzoat	-	-	160
Apple Flavour	50	50	50
Sorbitol ad	4000	4000	4000

#### **Evaluation of effervescens granules**

Water contents test ( Fausett et.al.,2000), fluidity test, angle of rest , compressibility test and granul size distribution.

#### **Evaluation of effervescens tablet**

There effervescens tablets of each formulation were examined for their diameter, thickness and height of tablet by using micrometer gauge ( MOH, 1979).

**Weight variation** : to study weight variation, 20 tablets of each formulation were weighed individually using four digital elektronik balance ( Sartorius Pioneer).

**Determination of tablet hardness.** The crushing strength of the tablet was measure by YD-2 Tablet Hardness Tester. Tablet hardness tester which applies compression force diametrically to the tablet. The force required to crush the tablet was recorded as hardness of the tablet in kg/cm<sup>2</sup>.

**Determination of tablet friability.** The friability was determined by weighing 10 tablets and placing them in a Guoming CS-2 type friability apparatus and rotating it at 25 rpm for 4 minutes ( i.e 100 drops). After dusting tablets were weighing for the final weight and the % friability was calculated as follows :

$$\% \text{ friability} : \left\{ \frac{\text{weight initial} - \text{weight final}}{\text{Weight initial}} \right\} \times 100$$

**Dissolving time.** This test was used for guidance to monitor the development of physical changes in tablets morphology when placed in the dissolution medium ( Siregar 2010). One of tablet placed in the 200 ml water until the tablet dissolved. Recording the result time.

**pH test.** Take the effervescent tablet dissolved in 200 ml of water, then measured by using a pH meter pH values obtained are recorded.

## RESULTS AND DISCUSSION

### Physical properties of Ciplukan powder

Base on phytochemical test result that the ciplukan extract powder contained terpenoids and alkaloid. Organoleptic test is a test that is performed to determine the taste and smell of a material. The resulting dry powder showed that the dry powder form of fine powder, brownish green, bitter taste and distinctive smell. Result of water content are average 3,94%.

### Evaluation of granules effervescens

Materials lubricant additives is one important also in the manufacture of effervescent tablets, lubricants used in this research consisted of sodium lauryl sulfate, polyethyleneglycol 6000, and sodium benzoate. The concentration of lubricant F1, F2, and F3 are 2%, 3%, and 4%. The concentration and type of lubricant in this effervescent tablets each different. It is intended as a lubricant want to know which one has the fastest time of the late F1, F2, and F3 and see the impact that would be caused by increasing concentrations of soluble lubricant to time. Evaluation includes water content test, flow velocity, angle of poise, particle size distribution, and tapped bulk density . (Table II)

**Table II. Result of granules evaluation**

<b>Evaluation</b>	<b>F1</b>	<b>F2</b>	<b>F3</b>
Water content (%)	1,24 ± 0,015	1,20±0,025	1,18±0,030
Flow velocity (g/sec)	9,77 ± 0,332	9,18±0,036	9,38±0,032
Angle of rest (°)	30,16±0,592	29,96±0,648	29,54±0,313
Compresibility (%)	2,671±0,578	3,665±0,578	2,996±0,008
Particle size distribution (µm)	716	729	726

### **Evaluation of effervescent tablet**

Effervescent tablets evaluation included : organoleptic, uniformity size and weight, friability, hardness , pH and solubility time. The results of organoleptic test effervescent tablet has a brown color white and that has been diluted with water has a clear green solution color, slightly sour taste. The comparison of physical properties of the effervecsen tablet (Table III). The weight and thickness of the tablets range from 4.027 to 4.029 and 2.31 respectively.

Tablet hardness is a parameter that affects the solubility time. A tablet must have a certain hardness to resist interference or mechanical shocks. In the persent study , the percentage friability for all the formulation was below 1%, indicating that the friability is within the pharmakopeia limits. The hardness of the tablet was found to be 9,58-9,82 kg/cm<sup>2</sup> which show sufficient mechanical strength. All the tablet formulation showed acceptable pharmachotechnicals properties and acceptable acording to pharmacopeia speciffication. Test the pH of the tablet effervescet conducted to determine the acidity of a solution of preparation. Measurements were made by using a pH meter, the results of the study showed that the F1-F3 has pH is 6.83; 6.83; and 6,87. It is proved that the solution is safe to use effervescent tablets orally. At test time effervescent tablets dissolve a process of acid and alkaline reaction which will generate CO<sub>2</sub> gas. Time dissolves with the type and concentration of different lubricants, have different solubility time. The results of the formula 1 of 10:15 minutes, the formula 2 at 3:48 minutes, and formula 3 for 4:12 minutes. It shows that the effervescent tablets dissolve meet the timing requirements are less than 5 minutes unless the formula 1. In formula 1 using the lubricant sodium lauryl sulfate. Basically lubricant sodium lauryl sulfate is soluble in water and solubility was fast but because these lubricants include surfactants making the solution generates foam that inhibit this effervescent tablet within a period of dissolution. At the time of effervescent tablets of formula 1 was added to water, the tablet should react more effervescent tablets. CO<sub>2</sub> gas produced will produce a layer of foam on top and make effervescent tablets for long pushed to the top, when the effervescent tablet dissolves pushed up process begins to slow down because of the foam covering and inhibiting this tablet to dissolve freely and quickly. Unlike the formula 2 and 3, namely lubricants PEG 6000 and sodium benzoate which produces a rapid dissolve and meet the requirements. Although with different concentrations of

PEG 6000 3% and 4% sodium benzoate but these lubricants are both hydrophilic lubricant which makes time ciplukan extract soluble effervescent tablet is fast. Plus sodium benzoate and PEG 6000 which has the form of powder so that the finer the particle size, and it can improve the wetting which makes the tablet will quickly dissolve. The big difference in the results of a late time in each formula due to differences in the type of lubricant used.

**Table III. Result of tablet effervescent evaluation**

Evaluation	F1	F2	F3
Uniformity of weight (g)	4,029±0,051	4,027±0,034	4,027±0,046
Uniformity of size (mm)	T/D: 0,57/2,31	T/D: 0,57/2,31	T/D: 0,57/2,31
Hardness tes (kg)	9,58±0,385	9,91±0,183	9,82±0,319
Friability test (%)	0,46±0,040	0,59±0,027	0,55±0,058
Dissolving time (min)	10:15	3:48	4:12
pH of solution	6,83	6,83	6,87

The test results were analyzed with the late time statistical calculations. Begins with a normality test to determine the resulting data were normally distributed or not. The results obtained show the data are normally distributed. Furthermore, followed by a one-way ANOVA (One Way ANOVA), to test whether a late third formulas are significantly different or not. Results of one-way ANOVA statistical calculations obtained sig = 0.000 The results showed significantly smaller than 0.05, then the third formula results show significant difference. To see more clearly the existence of significant differences in each formula Tukey HSD test results showed an average difference significant at the formula 1 of the formula 2 and 3, the formula 2 of the formula 1 and formula 3 of the formula 1.

## CONCLUSIONS

From the results of this research concluded that the time-soluble lubricant formula PEG 6000 is faster than the lubricant sodium benzoate and sodium lauryl sulfate.

In this research, sodium lauryl sulfate as a lubricant slow time ciplukan extract soluble effervescent tablets, it is necessary to do more research on other additives that may affect the timing soluble tablets of other natural materials.

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**EFFECT OF TAMARIND (*Tamarindus indica* Linn.) IN DECREASING  
CONTENTS OF MERCURY (Hg) AND PLUMBUM (Pb) IN WATER SPINACH  
(*Ipomoea aquatica* Forssk.)**

**Yusnidar Yusuf, Vera Ladeska, Atiyyatul Maula**

Faculty of Pharmacy and Sciences  
University of Muhammadiyah Prof.DR.HAMKA, Jakarta

**ABSTRACT**

Water spinach is one of vegetables that often consumed by people, however it dangerous if it is contaminated by heavy metal which exceeds high maximum level metal limit of contaminated vegetables by Indonesian National Standard 7378:2009 Hg 0,03 mg/kg and Pb 0,5 mg/kg. This research is aimed to figure out the contents of mercury and plumbum in water spinach, study the effect of tamarind solution in variety concentration, and also to acquaint the finest concentration of tamarind solution which can reduce the Hg and Pb levels in 30 minutes of soaking. The content of Hg was in level  $0,0134 \pm 0,0018$  mg/kg and Pb  $0,0700 \pm 0,0055$  mg/kg in water spinach before the experiment. However, the water spinach was cultivated to be not contained by any metal levels which can be harmful for society that consume it in large number and repeatedly so that the metal will be accumulated in body. The soaking analysis with tamarind solution variety concentration of 5, 10, 15, 20, and 25% has resulted in metal level decreased. 25% concentration showed the best metal level decreased; Hg  $0,0074 \pm 0,0009$  mg/kg (45%) and Pb  $0,0379 \pm 0,0046$  mg/kg (46%).

Keywords: Tamarind, water spinach, mercury, plumbum, and atomic absorption spectrophotometer (AAS)

**INTRODUCTION**

One of industrial waste that can cause pollution is heavy metal waste. Plants are one of mediators of heavy metal disseminator on living thing. Heavy metal can enter plant through root and stoma.

Industrial area in Jakarta, such as Cakung Industrial Area, has automotive, logistic, and transportation rental industries which involve heavy metal in the production process. Many people plant variety of vegetable, one of it is water spinach. Related to many of people's agriculture at Cakung Industrial Area, the vegetable planted was worried to be polluted by Mercury (Hg) and Plumbum (Pb). This was due to water source for the water spinach land came from rain and sewer, which was part of industrial and domestic wastes disposal, located around the land.



Heavy metal contained in the vegetable could be dangerous for human body. Therefore, an effort was needed to reduce Hg and Pb waste in vegetable consumed by human. Degradation content of heavy metal could be conducted by adding ligand or sequestran. Sequestran most often used on food was citric acid (Winarno 1997). According to research conducted by Napitupulu (2008), tamarind contained 15% of citric acid so that its fruit could be used to reduce metal content.

Based on the explanation above, we had interest to check Hg and Pb contents in water spinach and then conducted research to reduce Hg and Pb contents by tamarind solution with concentration of 5, 10, 15, 20, and 25% for 30 minutes using Atomic Absorption Spectrophotometer. This research was expected to find the best treatment in order to reduce Hg and Pb contents in water spinach using tamarind in easy, simple, and effective way so that it could be socialized to society as general, and particularly to housewives and food seller. Moreover, this research was expected to avoid exposure effect of Hg and Pb.

## **MATERIAL AND METHOD**

### **Material**

Water spinach (*Ipomoea aquatic* Frossk.), fresh tamarind (*Tamarindus Indica* Linn.), Hg(NO<sub>3</sub>)<sub>2</sub> 1000 mg/l, Pb(NO<sub>3</sub>)<sub>2</sub> 1000 mg/l, HCl 16%, HCl 3%, HCl 6M, H<sub>2</sub>O<sub>2(p)</sub>, HNO<sub>3(p)</sub>, HNO<sub>3</sub> 0,1M, HNO<sub>3</sub> 5N, NaBH<sub>4</sub> 0,2%, NaOH 0,05% from Merck dan aquabidest.

### **Method**

Water spinach was obtained from farm near Cakung Industrial Area and tamarind (separated from its seed) was obtained from Ijem herbal. Making tamarind solution concentration, fresh seedless tamarind was taken for the amount of 5, 10, 15, 20, and 25 gram to be mashed and dissolved with 100 ml of aquabidest. 15 gram of chopped water spinach was soaked into tamarind solution for 30 minutes within each concentration. After that, water spinach was seeped and re-washed by aquabidest, meanwhile tamarind solution was kept inside glass bottle.

Destruction conducted was wet and dry destructions. Calculation of mercury (Hg) and Plumbum (Pb) contents in water spinach and tamarind solution used wet destruction (Badan Standardisasi Indonesia 1998). Measurement of mercury (Hg) content in sample solution that has been destructed was reacted with NaBH<sub>4</sub> 0,2%,

NaOH 0,05%, and HCl 3% by the assistance from FIAS perkin-100 equipment alongwith absorbent reading using atomic absorption spectrophotometer with 253.7 nm wave-length. The absorbent level obtained was still in range of mercury solution standard calibration curve. Measurement of Plumbum (Pb) content in sample solution that has been destructed was measured by absorbent using atomic absorption spectrophotometer with 283.3 nm wave-length. The absorbent level obtained was still in range of plumbum solution standard calibration curve.

Data of metal concentration obtained from atomic absorption spectrophotometer was calculated using formula according to Association Of Analytical Communities 999.11/9.1.09.2005 and Indonesian National Standard 19-2896-1998.

Formula to calculate metal content on sample (recovery):

$$c = \frac{a \times v}{m}$$

Note :

c is metal content (mg/kg)

a is metal concentration ( $\mu\text{g/L}$ )

v is solvent volume (L)

m is sample weight (g)

## **RESULT AND DISCUSSION**

Water spinach was plant that absorbed heavy metal contained within its growth media. The heavy metal came from domestic, agricultural, or industrial waste. Plant mechanism or adaptation towards metal pollution was by forming phytochelatin which would tie heavy metal its environment. Phytochelatin was small peptide which was rich of amino acid where there was carboxyl functional group. Carboxyl group in amino acid could tie the metal (Prasetyawati 2007).

Before reading by Atomic Absorption Spectrophotometer (AAS), sample must be destructed first in acid room. There were two procedures used; wet and dry desructions. Function of destruction was to cut bond between organic compound with the analyzed metal so that only the metals would be left. Dry destruction process had weakness; temperature used for dusting was relatively very high and needed long time. Wet destruction process used mix of strong acid (which most of it was dangerous) so that there was potential of acid contamination. However, both procedures were used

within this research according to Association Of Analytical Communities 999.11/9.1.09.2005 and Indonesian National Standard 19-2896-1998.. In order to determine mercury (Hg) content on water spinach and tamarind solution, wet destruction was used because wet destruction generally could be used to determine metal element that was weak to heat, so that Hg would not be lost. The same procedure also conducted to determine plumbum (Pb) content because it was not possible to start dusting process in electrical furnace used in dry destruction process. To determine Pb content in water spinach, dry destruction could be used to avoid polluter.

Analysis of Hg needed *hybrid vapour generator* which was a *Flow Injection Analysis System* (FIAS) Perkin-100. This was because mercury was easy to vaporize so that Hg atomic vapour would be formed after reacted in acid condition with NaBH<sub>4</sub>, NaOH, and HCl with FIAS Perkin-100. Atomization process of mercury was as follow, Hg atom within sample as positive ion was reduced until it became neutral and vapoured as free atom in normal temperature. As reductor, NaBH<sub>4</sub> and NaOH inside HCl could be used. After that, vapour of Hg atom together was flown through gas cell which was followed by absorbent reading using atomic absorption spectrophotometer (Pangabean dkk 2010).

The mercury (Hg) and Plumbum (Pb) contents in water spinach before submersion with tamarind solution was analyzed using atomic absorption spectrophotometer. The result was shown on table as follow:

**Table 1. Analysis Result of Hg and Pb metal in Sample before Treatment Compared to Indonesian National Standard 7378:2009**

Metal	Metal Content (mg/kg)		Mean	Maximum Limit of Metal Content on Vegetable According to SNI (mg/kg)
	I	II		
Hg	0.0146	0.0121	0.0134	0.03
Pb	0.0661	0.0739	0.0700	0.5

Based on the analysis, Hg and Pb metal content from agriculture land near Cakung Industrial Area did not exceed maximum limit of metal contamination on vegetable. This was possible because industries in Cakung had managed their waste before it was discarded. As result, metal contamination was not high and fulfill standard of edible consumption. However, Hg and Pb content in water spinach could be caused by air and water pollution. Air pollution was caused by industrial and vehicle

smoke, whereas water pollution was caused by rainpolluted by industrial smoke and industrial waste that was not perfectly manage. These pollutions could enter soil and then sediment as poisonous chemical substance.

Although the heavy metal content in water spinach did not exceed the limit, however, it would be wise if the metal content could be reduced so that it would not be harmful for people who often consumed it in large number repeatedly. Toxicity of Hg and Pb metal was high level toxic. Metal content that was absorbed into the body could not be destroyed, but it would stay inside and wasted later through excretion.

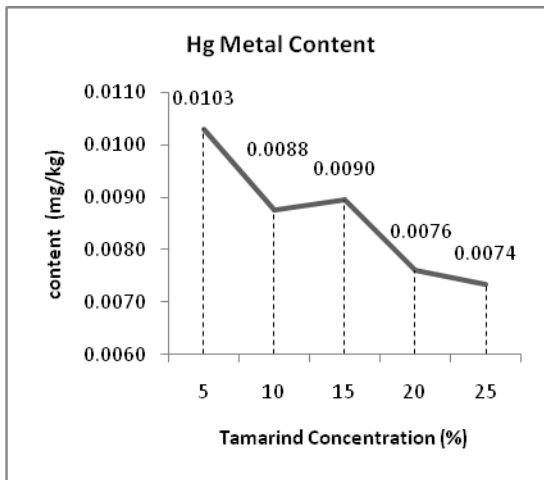
Therefore, this research was an effort to reduce Hg and Pb metal content in water spinach with submersion of various concentration of tamarind solution for 30 minutes. The result was shown in table below:

**Table 2. Percentage Hg and Pb Content Degradation after Treatment**

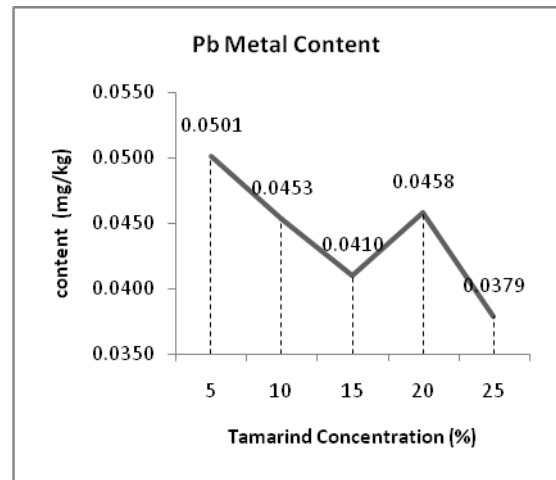
Tamarind Solution Concentration	Metal Content (mg/kg)									
	Mercury (Hg)					Plumbum (Pb)				
	I	II	Mean	SD	%	I	II	Mean	SD	%
Control negatif	0,0146	0,0121	0,0134	0.0018	-	0,0661	0,0739	0,0700	0.0055	-
5 %	0.0111	0.0095	0,0103	0.0011	23	0.0527	0.0475	0.0501	0.0037	28
10 %	0.0093	0.0082	0,0088	0.0008	34	0.0485	0.0421	0.0453	0.0045	35
15 %	0.0090	0.0089	0,0090	0.0001	33	0.0448	0.0372	0.0410	0.0054	41
20 %	0.0094	0.0058	0,0076	0.0025	43	0.0474	0.0442	0.0458	0.0023	35
25 %	0.0080	0.0067	0,0074	0.0009	45	0.0411	0.0346	0.0379	0.0046	46

From the analysis result of Hg and Pb metal content in water spinach after submersion, we could see that there was reduction of metal content in each concentration of tamarind solution. The highest reduction occurred in 25% concentration of tamarind solution with 45% reduction of Hg content  $0.0074 \pm 0.0009$  mg/kg and 46% for Pb  $0.0379 \pm 0.0046$  mg/kg. Tamarind contained 15% of citric acid. Citric acid was a tricarboxylic acid which was naturally available in fruits. Citric acid was very effective as metal binder. Carboxylic ion was a good electron donor so that it could bind metal in form of complex electron bond (Indasah 2002).

**Graphic 1. Graphic of Hg Metal Content Content in Water Spinach**



**Graphic 2. Graphic of Pb Metal Content in Water Spinach**



From the graphic above, we could see metal reduction on water spinach was not linear in each concentration. Only 5% concentration difference so that it looked the same. The analysis result showed that there was Hg and Pb metal content on water spinach and that there was degradation of heavy metal after submersion of tamarind solution for 30 minutes. The solution later was analyzed with atomic absorption spectrophotometer and proved to be contained by heavy metal. It meant that citric acid contained in tamarind solution could bind heavy metal on sample. From the research result and discussion above, reduction of heavy metal content in water spinach or in other food can use tamarind fruit.

## CONCLUSION

Based on research result, it was acknowledged that water spinach taken from Cakung Industrial Area contained Hg  $0.0134 \pm 0.0018$  mg/kg and Pb  $0.0700 \pm 0.0055$  mg/kg. There was degradation of Hg and Pb metal in water spinach after submersion of various concentration of tamarind solution; 5, 10, 15, 20, and 25% for 30 minutes. Highest reduction occurred on 25% concentration of tamarind solution with 45% reduction of Hg content  $0.0074 \pm 0.0009$  mg/kg and 46% for Pb  $0.0379 \pm 0.0046$  mg/kg.

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## ANDROGRAPHOLIDE EFFECTS AGAINST INHIBITION OF HEME DETOXIFICATION *IN VITRO*

Risdawati Djohan<sup>1</sup>, Mohamad Sadikin<sup>2</sup>, Sri Widia K. Jusman<sup>2</sup>, Din Syafruddin<sup>3,4</sup>

<sup>1</sup> Department of Pharmacology - Medical school of Yarsi University, Jakarta, Indonesia. <sup>2</sup> Department of Biochemistry and Molecular Biology-Faculty of Medicine University of Indonesia, Jakarta, Indonesia. <sup>3</sup> Eijkman Institute for Molecular Biology, Ministry of Research and Technology of the Republic of Indonesia, Jakarta, Indonesia. <sup>4</sup> Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

\*Corresponding author phone: 08129884287, e-mail: risdawati@yarsi.ac.id

### ABSTRACT

Andrographolide exhibited antimalarial activity both *in vitro* and *in vivo*, but its mechanism of action had not been known yet. Heme detoxification has been validated as antimalarial drug target. To study andrographolide effects on inhibition of heme polymerization and glutathione (GSH)-dependent heme degradation *in vitro*. Inhibition of heme polymerization assay was determined spectrophotometrically. Hemin solution was incubated with acetate buffer and Tween-20 at 37°C for 250 minutes and treated with andrographolide. Heme degradation inhibition assay was conducted spectrophotometrically at 360 nm after 1 and 30 minutes. Heme degradation inhibition activity of andrographolide was evaluated as percentage decrease compared with control absorbance. Both of the assays were compared to chloroquine. Andrographolide has inhibited heme polymerization with the IC<sub>50</sub> values of 367±171 μM. This figure was higher than chloroquine. While the activity of heme degradation inhibition of andrographolide was better than chloroquine at any level of concentrations (p < 0.05), indeed the highest activity has been shown at 15 μM. Andrographolide has the heme detoxification activity that was less than chloroquine through heme polymerization inhibition. However it was better than chloroquine to inhibit GSH-dependent heme degradation.

**Keywords:** Andrographolide, hemepolymerization, GSH-dependent heme degradation, *in vitro*.

### INTRODUCTION

The attempts to find new antimalarial drugs has become a priority to manage the increasing burden of malaria, caused by drug-resistant parasites. Antimalarial properties of *Andrographis paniculata* (sambiloto) have been studied in detail by Mishra et al. (Mishra et al., 2009). As well as its active ingredient, andrographolide, which is responsible for antimalarial properties, this plant has also been reported (Mishra et al.,

2011). However, the antimalarial target of the andrographolide is unknown. Andrographolide, a diterpenelactone compound, is one of the major phyto constituents of *A.paniculata* and it has been reported to have diverse pharmacological activities including antiviral, anti-inflammatory, and anticancer properties (Chao and Lin, 2010; Jarukamjorn and Nemoto, 2008). Andrographolide also has pro-oxidant activity because it can be depleting GSH content and producing reactive oxygen species (ROS) in cancer cells (Ji et al., 2009; Li et al., 2007).

Malaria parasites require a lot of nutrients for its growth during intra-erythrocytic asexual stage. They digest hemoglobin within the digestive vacuole through a consecutive metabolic process involving proteases. Degradation of hemoglobin produces toxic free heme (Tekwani and Walker, 2005). To protect themselves, the parasites detoxify free heme via neutralization with histidin-rich protein, degradation with reduced glutathione (GSH), and crystallization into hemozoin (Wiser, 2008). The effective process of free heme detoxification is essential for the parasite in order to survive. Chloroquine and other antimalarial drugs have been shown to inhibit the formation of a synthetic heme crystal,  $\beta$ -hematin, which is structurally identical to hemozoin (O'Neill et al., 2012; Huy et al., 2007) and are believed to inhibit hemozoin formation in food vacuole of the malaria parasite. Recent reports indicate that inhibiting the  $\beta$ -hematin formation is ideal target for antimalarial screening (Tekwani and Walker, 2005).

We have evaluated the effects of andrographolide on heme detoxification *in vitro*. In this paper, we report that andrographolide has heme detoxification activity both through inhibition of heme polymerization and inhibition of GSH-dependent heme degradation.

## **MATERIALS AND METHODS**

Pure grade andrographolide (ANDRO) (Cat no. 365645), chloroquine (CQ) (Cat no. C6628), hemin chloride, diethylenetriamine-penta-acetic acid (DETEPAC), glutathione (GSH), dimethyl sulfoxide (DMSO), acetic acid galical, sodium acetate, Sodium dodecyl sulfate (SDS), and Tween-20 were from Sigma-Aldrich, St. Louis, USA.



## Heme Polymerization Inhibition Assay

The heme crystallization/polymerization inhibition assay were measured spectrophotometrically as described by Huy et al (Huy et al., 2007) with a little modification. A stock solution of hemin was prepared by dissolving hemin chloride (16.3 mg) in 1 mL of DMSO and then was passed through a 0.2 µm-pore membrane filter to remove insoluble particles. The concentration of hemin in the stock solution was determined by measuring the absorbance of the diluted hemin with 2.5% sodium dodecyl sulfate in 0.1 M NaOH at 400 nm. The stock solution can be kept at 4°C up to 1 month until used. The stock solution was diluted to 111,1 µM of hemin with 1 M acetate buffer, pH 4.8, just before being used. Andrographolide was dissolved in DMSO and chloroquine and Tween-20 (1,26 g/100mL) was dissolved in distilled water.

In order to know the linearity of relationship between absorbance at 415/630 nm and heme concentration, standard curve was made. 700 µL of serial dilution (1:2 dilutions, final concentrations ranged from 2.4 to 76 µM) of hemin buffered by 1 M acetate buffer, pH 4.8, was loaded into a cuvette. The absorbance was read at 415 and 630 nm (absorbance at 630 nm was subtracted from absorbance at 415 nm) using a Shimadzu spectrophotometric. To measure the effect of drug on β-hematin formation, andrographolide and chloroquine (as reference drug) were prepared in various concentration. 315 µL of 50 µM hemin solution, freshly buffered by 1 M acetate buffer (pH 4.8) from stock solution, was pipetted into a Eppendorf tube and followed by 10 µL of Tween-20 (0,84 g/100 mL) and 200 µL of 1 M acetate buffer (pH 4.8). The tube was incubated at 37°C for 4 hours. After incubating time, the reaction mix was moved into a cuvette and samples were added and were mixed by being pipetted three times. The absorbance was read at 415/630 nm. Percentage of inhibition of β-hematin formation by drugs was calculated as followed:

$$\% \text{ inhibition} = \frac{A_{\text{sample}} - A_{\text{min}}}{A_{\text{control}} - A_{\text{min}}} \times 100 \quad \text{Formula 1}$$

Where  $A_{\text{sample}}$  is the absorbance of the heme the presence of both Tween 20 and drugs at 415/630 nm, while  $A_{\text{control}}$  represents the absorbance of the heme without Tween 20 or an antimalarial at 415/630 nm and  $A_{\text{min}}$  is the absorbance of the heme with Tween 20 in the absence of an antimalarial at 415/630 nm

### **GSH-dependent heme degradation Inhibition Assay.**

The heme degradation inhibition assay were measured spectrophotometrically as described by Steele et al (Steele et al.,2002) with a bit of modification. The stock solution are 1mM DETEPAC in 10 mM Na phosphate pH 7.0; 2 mM hemin in DMSO (prepared fresh daily); and 100 mM GSH in distilled water (prepared fresh daily). Three working solution were prepared as followed; Solution A: mix 4 volume of the 1mM DETEPAC in 10 mM Na phosphate pH 7.0 stock solution with 1 volume of ethanol. Solution B: mix 5 µl of 2 mM hemin in DMSO stock solution with 1 mL of solution A. Solution C: mix 0,15 mL of GSH stock solution with 1 mL of solution A. Sample was prepared in various concentration, which andrographolide was dissolved in DMSO, while chloroquine (as reference drug) was dissolved in distilled water. Assay were performed by mixing of 200 µl of solution A with 4 µl of sample or solvent (control), 400µl of solution B and 100 µl of solution C in a cuvette. The absorbance at 360 nm ( $A_{360}$ ) was read after 1 and 30 min with Shimadzu spectrophotometric to determine the  $\Delta A_{360}$ . The effect of drug on inhibition of heme degradation was calculated as followed:

$$\% \text{ inhibition} = \frac{(\Delta A_{360})_{\text{sample}}}{(\Delta A_{360})_{\text{control}}} \times 100 \quad \text{Formula 2}$$

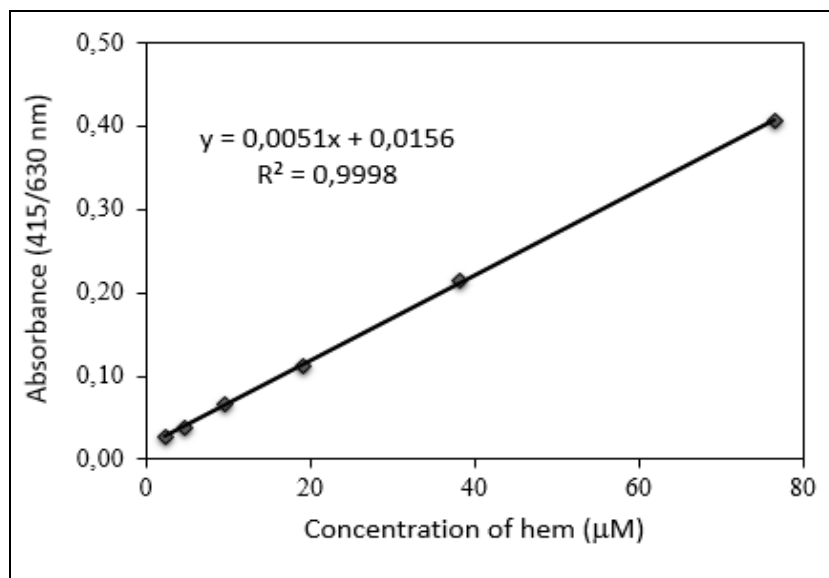
### **Statistical analysis**

Differences between groups were assessed by Kruskal-Walis test and were continued by Mann-Whitney U test using the SPSS v.17.0.  $P < 0.05$  was considered a statistically significant difference.

## **RESULTS**

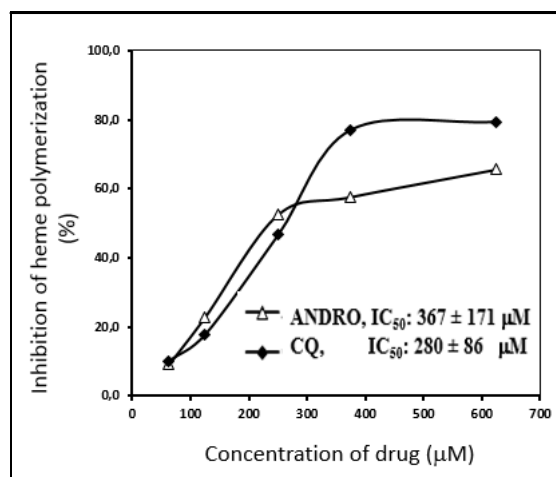
### **Inhibition of heme polymerization**

Several experimental approaches have been described for determination of  $\beta$ -hemin *in vitro* formation and different antimalarials evaluation. In this study, we estimated the effects of andrographolide against heme polymerization inhibition spectrophotometrically. The standard curve showed linear relationship between absorbance at 415/630 and heme concentration ranges of 2,4 to 76,6 µM at 415/630 nm (Figure.1). Hence, the assay was performed by using 50 µM of heme.



**Figure1.** Linear relationship between absorbance at 415/630 nm and heme concentration.

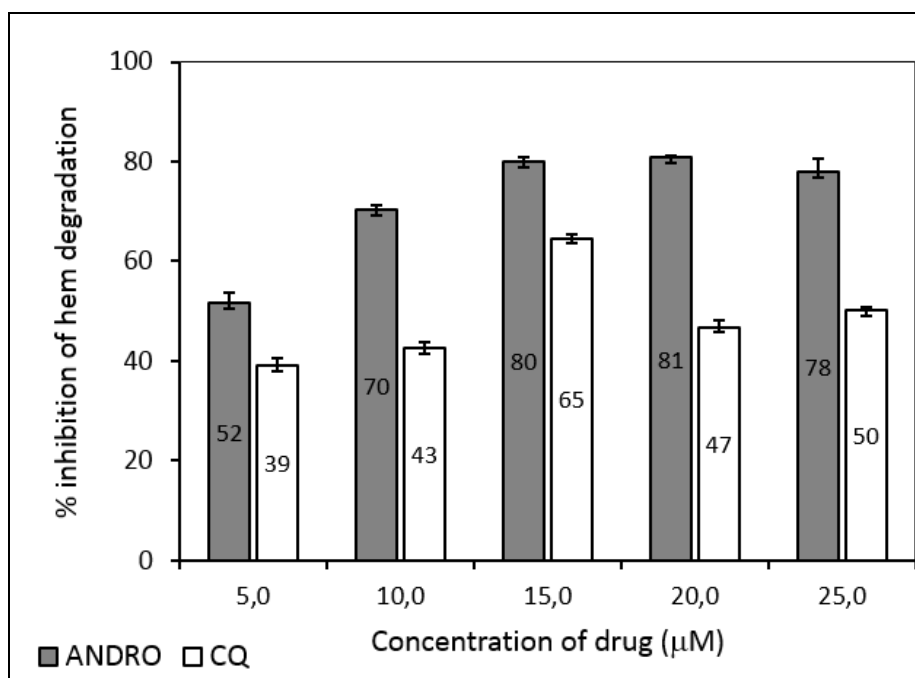
Inhibition of heme polymerization assay was performed by using five concentration level of andrographolide that is 62,5 µM, 125 µM, 250 µM, 375µM dan 625 µM, and was compared to chloroquine at the same concentration level. The results showed that IC<sub>50</sub> value of andrographolidewas greater than chloroquine, that was 367 ± 171 µM vs 280 ± 86 µM (Figure 2).



**Figure2.** Inhibition of heme polymerization by andrographolide and chloroquine. Heme was incubated with 62,5 µM, 125 µM, 250 µM, 375µM dan 625 µM of ANDRO or CQ in duplicate, the absorbance was read at 415/630 nm. The results are collected from three independent experiments and are presented as mean ± SD. IC<sub>50</sub> value was calculated by probit analysis (Statistical Package for Social Sciences, SPSS, v.17). ANDRO: andrographolide; CQ: chloroquine

### Inhibition of GSH-dependent heme degradation

The reaction of hemin with glutathion at pH 7.0 and the interference of antimalarials can be monitored spectrophotometrically. In this experiment, we measured the *in vitro* of GSH-dependent heme degradation inhibition activity of andrographolide at five concentration level namely 5  $\mu$ M, 10  $\mu$ M, 15  $\mu$ M, 20  $\mu$ M and 25  $\mu$ M that were compared to chloroquine as a reference drug. The results showed that inhibition of heme degradation by andrographolide better than chloroquine at all concentration levels ( $p < 0.05$ ). The best activity was seen at 15  $\mu$ M either by andrographolide or chloroquine. At higher concentrations (20  $\mu$ M and 25  $\mu$ M), the effect of andrographolide looks no difference compared to 15  $\mu$ M (15  $\mu$ M vs. 20  $\mu$ M,  $p = 0,261$ ; 15  $\mu$ M vs. 25  $\mu$ M,  $p = 0.275$ ), but the effects of chloroquine at those concentration shows lower than 15  $\mu$ M ( $p = 0.046$ ) (Figure 3).



**Figure 3.** Inhibition of GSH-dependent heme degradation by andrographolide and chloroquine. Heme was reacted with various concentration of andrographolide or chloroquine in duplicate. After addition of GSH, the absorbance at 360 nm was recorded at 1 min and 30 min. Percentage of hem degradation inhibition was calculated as described in text. The results are collected from three independent experiments and are presented as mean  $\pm$  SD. ANDRO: andrographolide; CQ: chloroquine

## DISCUSSION

*Andrographis paniculata* and its major constituent, andrographolide, have become intensive research subject due to its diverse pharmacological activities and proven biosafety (Coon and Ernst, 2004). Identification antiplasmodium activity of this compound through *in vitro* ((Mishra et al., 2011; Mishra et al., 2009). And *in-vivo* (Kusumawardhani, 2006) experiments indicate that it has a potent antimalarial properties so that it is necessary to develop as a new antimalarial. Alam et al (Alam et al., 2009) have reviewed the potential antimalarial drug targets such as nucleic acid regulation, oxidative stress, folate biosynthesis, DNA biosynthesis, merozoite invasion, merozoit release, hemoglobin degradation, heme detoxification, ect.

Inhibition of heme detoxification function of the malaria parasite would lead to accumulation of toxic heme which would kill the parasite due to its ability to destabilize and lyse membranes, as well as inhibiting the activity of several enzymes (Wiser, 2008). Quinoline antimalarials have been reported to be the most consistent inhibitors of hemozoin synthesis. Several novel antimalarials have also been found to disrupt the process of hemozoin formation. Further, some inhibitors of hemozoin synthesis are being investigated as novel antimalarial structures (Tekwani and Walker, 2005). Here we demonstrated heme detoxification activity of andrographolide through both inhibition of heme polymerization ( $\beta$ -hematin formation) and inhibition of heme degradation with role of GSH. The inhibitory effect of andrographolide was lower than chloroquine, through  $\beta$ -hematin formation, where the  $IC_{50}$  value of andrographolide and chloroquine is  $367 \pm 171 \mu\text{M}$  and  $280 \pm 86 \mu\text{M}$  respectively. However, its effects was equal to other quinoline compound, quinine ( $IC_{50}$  value  $365 \pm 103 \mu\text{M}$ ) (Huy et al., 2007). Heme polymerization assay was conducted by determining of heme absorption reduction at 415/630 nm and using Tween-20 as an initiator/inducer of  $\beta$ -hematin formation. The difference of  $IC_{50}$  values, between andrographolide and chloroquine, could be due to difference in the ability of the drug to bind to heme in the presence of inducer and the difference in mechanism initiation of heme polymerization between Tween-20 and other inducers (Huy et al., 2007). In addition, incubation time of assay also affects hem polymerization process *in vitro* (Huy et al., 2007).

Another mechanism proposed for detoxification heme is through its glutathione mediated degradation which takes place outside the parasite food vacuole (Tekwani and Walker, 2005). The reaction of heme with glutathione will induce the breaking heme structure and produce less toxic compounds. *In vitro* study shows the broad absorption of heme is immediately changed to a peak at 360 nm upon addition of GSH and then is decreased to approximately half of previous value without any absorption decrease during 30 min (Steele et al, 2002). Our experiments showed that the effects of andrographolide against GSH-dependent heme degradation inhibition was better than chloroquine. The optimal inhibitory effect occurs in 15  $\mu$ M either by andrographolide or chloroquine. There are two reasons to explain this situation. Firstly, addition of andrographolide to heme can cause an alteration of heme spectrum due to immediate formation of an alkaloid-heme complex and then lead to altered effects of glutathione. The second reason could be due to formation of andrographolide-GSH complex and depletion of GSH to fail heme degradation. There are some reports that suggest that andrographolide may directly react with the thiol of GSH *in vitro*, and the assumed hypothesis is that the  $\alpha$ - $\beta$ -unsaturated lactone moiety of andrographolide can react with GSH through the Michael addition reaction (Woo et al, 2008; Zhang et al., 2008). Our previous result also indicates that andrographolide depletes GSH intracellular content of *P. berghei* in *ex vivo* study (data not shown).

Further research is needed in order to verify the ability of andrographolide to inhibit heme detoxification by using animal (*in vivo* study). The conclusion of these experiments is that andrographolide has heme detoxification activity that was less than chloroquine through heme polymerization inhibition. However, it was better than chloroquine to inhibit GSH-dependent heme degradation.

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# HEPATOPROTECTIVE ACTIVITY TEST OF ETHANOL FRACTION OF *Ocimum americanum* L LEAFS AGAINST CARBON TETRACHLORIDE INDUCED IN RATS

**Hayati. Sediarmo. Rangga**

Pharmacy and Science Faculty of Muhammadiyah Prof. DR. Hamka University

## ABSTRACT

The liver diseases beside caused by viruses. it is also caused of exposing on to toxic substances such as carbon tetrachloride (CCl<sub>4</sub>) . This study aimed to determine the hepatoprotective activity of 70% ethanol fraction Kemangi leafs on white male rats were induced by CCl<sub>4</sub>. This study used 24 male rats Sparague Dawley strain were divided into 6 groups. K1 is a negative control which was given CCl<sub>4</sub>. K2, K3, and K4 are the treatment group which were given a dose of 7.8 mg / 200 g BW, 15.6 mg / 200g BW, 31.2 mg / 200 g BW. K5 is a positive control which was given Cursil® and K6 is a normal control. The treatment was done for 8 days. On the day of 8, all groups were induced by CCl<sub>4</sub>, except K 6. On day of 9 all rats were taken their blood to be measured and their liver were taken to make preparations histopathology. Having obtained the results in the form of SGOT, SGPT, and the liver histopathology, the data were analyzed by one-way ANOVA and Tuckey test. The results indicated that the dose of 15.6 mg / 200 g BW have hepatoprotective effects and the dosage of 31.2 mg / 200g BW have hepatoprotective effect that is comparable to the positive control.

**Keyword :** Ethanol fraction of *Ocimum americanum* L. leafs. Hepatoprotective. CCl<sub>4</sub>

## INTRODUCTION

Hepatitis is an inflammation of the liver tissue where almost all cases of hepatitis are caused by viruses that undergo liver cells damage .The other liver cells damage was caused by a virus, can also be caused by exposure to toxic substances such as carbon tetrachloride (CCl<sub>4</sub>) (Solomon et al. 2007 ). Carbon tetrachloride hepatotoxicity due biontransformasinya in the liver by the cytochrome P450 into

trichloromethyl (CCl<sub>3</sub> \*) are highly reactive. CCl<sub>3</sub> \* This is a reactive free radicals that can initiate damage to liver cells by peroxidated lipid membranes (Sen et al. 2007).

The liver is the largest organ that is very important for the defense of life and play a role in almost every metabolic function of the body. One of its functions in the body's defense is in the form of detoxification. Detoxification is done with a variety of processes carried out by enzymes in the liver against toxic substances (Rachmawati et al. 2013). In the liver cells contain an enzyme transaminase in large numbers that SGOT and SGPT (Price and Wilson 2005). When a cell or tissue that contains transminase necrosis or destroyed, then the enzyme will be separated and enter into the bloodstream so that levels in the serum increased. To determine the presence of impaired liver function, it is necessary to biochemical and histopathological examination of liver (Sudoyo et al. 2009).

Drugs to treat liver dysfunction during this that has been circulating is hepatoprotective drug classes that aim to maintain the function of liver cells and helps the healing process (Hadi 2000). Currently being developed various kinds of drugs that have hepatoprotective properties to prevent or reduce damage to the liver.

Kemangi is a plant that is common to the community. These plants contain essential oils, saponins, flavonoids and tannins (MOH 2001). Several studies have shown that this plant has antioxidant activity and anti-bacterial (Sarma and Venkata 2011), immunomodulator (Sunitha and Nasreen 2013), antidiabetic and anti-fungal (and Preeti Verma 2012).

Results of previous studies showed that water extract of leaves of Kemangi has a hepatoprotective activity. It is proved that the water extract of basil leaves in a dose of 200 mg / kg BW can decrease the rat SGOT and SGPT levels after administration of paracetamol (Aluko et al. 2013). In this study will be conducted using a solvent fractionation with ethanol 70%. Fractionation is a process of separation of compounds based on the degree of polarity. Expected fraction of 70% ethanol Kemangi leaves can draw polar compounds and then tested as a hepatoprotective activity by measuring the levels of SGOT, SGPT and liver histopathology male rats induced carbon tetrachloride.

## **METHODS**

### **Tools**

Analytical balance. container maceration. rotary evaporator. rat cage. a test animal drinking. weight scales mice. the sonde. disposable syringes. centrifuge. eppendorf tube. clinical spectrophotometer (VARTA 506). micropipette. glass tools. gloves. cotton sterile. refrigerator. oven. eksikator. pipette. and surgical instruments.

### **Materials**

Kemangi (*Ocimum americanum* L.) were obtained from the Research Institute for Spices and Medicinal Plants (Balitro) Bogor. distilled water. phenobarbital injection. carbon tetrachloride. ethanol 70%. FeCl<sub>3</sub>. solution Boin. olive oil. ethyl acetate. n-hexane. Na CMC. reagent kits SGOT and SGPT. 0.9% NaCl solution. hematoxylin-eosin stain. Drug comparator used is Coursil® and male rats Sprague Dawley strain aged 3-4 months and  $\pm$  200 g body weight as much as 24 tails

### **Making of Ethanol Extract 70% of Kemangi Leafs**

Fresh Kemangi leafs as much as  $\pm$  8 kg were cleaned. then dried with aerated. Then after drying. Kemangi leafs were crushed by a blender. The powder obtained was sieved with 60 mesh sieve and then stored in a clean container and sealed. Dried powder obtained is then extracted using 70% ethanol and then soaked for 3 days accompanied by stirring. After 3 days is filtered. and the waste is macerated back with 70% ethanol. Perform using the same procedure as much as 3 times. Maserat obtained is then mixed. then evaporated using a rotary evaporator to obtain a thick extract. Once evaporated. dried in an oven at a temperature of 500C (MOH. 2008).

### **Making of Ethanol Fraction 70% of Kemangi Leafs**

Viscous extract ethanol 70% entered into a separation funnel. Then fractionated with n-hexane (1: 1). shake for  $\pm$  15 minutes. After settling some long-established fraction of n-hexane and ethanol fraction. N-hexane fraction was separated. while the fraction of ethanol in the solvent fractionation with ethyl acetate (1: 1) and the addition of warm water. shake for  $\pm$  15 minutes. After settling some long-established two layers.

the layer of ethyl acetate and 70% ethanol layer. Ethyl acetate layer (top) separated by opening the tap funnel until the ethanol layer depleted. Taken ethanol layer was then separated as a fraction of ethanol. Fraction of 70% ethanol was evaporated with a rotary evaporator to obtain a thick fraction. Then the fraction is dried in an oven at a temperature of 50 °C.

### **Characteristics And Phytochemical Screening Test**

Test characteristics of the 70% ethanol fraction basil leaves covering organoleptic and drying shrinkage. Phytochemical screening of 70% ethanol fraction basil leaves covering test flavonoids, saponins, tannins, and terpenoids.

### **Research Design and Preparation of Test Animals**

Test animals used were male Sprague Dawley strain rats weighing  $\pm$  200 g, aged approximately 3-4 months, divided into 6 groups, the test animals prior to the study acclimatized for 7 days in space research.

Group I: negative control group. Rats were given 0.5% CMC Na for 7 days, then on the day of 8 were given CCl<sub>4</sub>, after the mice were fasted, on the day of 9 the blood drawn to be measured and dissected to take his heart.

Group II: Group test. Rats were given 70% ethanol fraction suspension Kemangi low doses for 7 days, then on the day of 8 was given CCl<sub>4</sub>, after the mice were fasted, on the day of 9 the blood drawn to be measured and dissected to take his heart.

Group III: Group test. Rats were given 70% ethanol fraction suspension Kemangi medium dose for 7 days, then on the day of 8 were given CCl<sub>4</sub>, after the mice were fasted, on the day of 9 the blood drawn to be measured and dissected to take his heart.

Group IV: Group test. Rats were given 70% ethanol fraction suspension Kemangi high doses for 7 days, then on the day of 8 were given CCl<sub>4</sub>, after the mice were fasted, on the day of 9 the blood drawn to be measured and dissected to take his heart.

Group V: positive control group. Rats were given Cursil® suspension for 7 days, then on the of day 8 were given CCl<sub>4</sub>, after the mice were fasted, on the of day 9 the blood drawn to be measured and dissected to take his heart.

Group VI: normal group. Rats were given 0.5% CMC Na for 7 days. then on the day of 8 were given olive oil. after the mice were fasted. on the day of 9 the blood drawn to be measured and dissected to take his heart.

### **Determination of dose**

#### **a. Dose Cursil®**

In this study. a comparison of drug use Cursil®. Cursil® dose used in humans (70 kg) is  $3 \times 1 \text{ capsule / day} = 3 \times 250 \text{ mg} = 750 \text{ mg / day}$ . Dose first converted to dose rats. Human-to-rat conversion factor (0.018). obtained Cursil® dose for rats  $13.5 \text{ mg / } 200 \text{ g BW rat}$ .

#### **b. Dose of carbon tetrachloride**

The dose of carbon tetrachloride were used in this study was  $0.4 \text{ mg / g BW}$  (Sari et al. 2008). Then converted to a body weight of rats of  $200 \text{ g} = 0.4 \text{ mg / g BW} \times 200 \text{ g BW} = 80 \text{ mg / g}$ .

#### **c. Fraction Dose Ethanol 70% Kemangi Leaves**

This study was used a 3-dose variation of 70% ethanol fraction basil leaves. which is a low dose ( $7.8 \text{ mg / } 200 \text{ g BW}$ ). medium dose ( $15.6 \text{ mg / } 200 \text{ g BW}$ ) and high dose ( $31.2 \text{ mg / } 200 \text{ g BW}$ ).

#### **d. Making the test solution**

Solution of the test substance and drug comparison is made using a certain concentration with Na CMC suspension solvent. While carbon tetrachloride were used as inducers made using a certain concentration with olive oil solvent.

### **Intake of Animal Blood Serum Test**

After induced by carbon tetrachloride. blood serum samples were taken rats. This stage is done by taking blood from the orbital sinus eyes. Blood was placed in a tube and then centrifuged eppendorf with a speed of 3000 rpm for 10 minutes. Separate serum taken with caution. and then placed in a clean and dry container.

### **Measurement of serum AST and ALT Animal Blood Test**

Serum samples were taken of 100 mL using a micropipette. add the reagent kit (R1) of 1000 mL. then add the reagent kit (R2) 100 mL. shake until homogeneous with

a vortex. and incubated for 1 min at 37°C. Measure AST and ALT activity with clinical spectrophotometer (VARTA-506) at a wavelength of 340 nm.

### Harvesting of the heart organ

Rat liver was taken to surgery. Before surgery, the rats anesthetized with phenobarbital injection advance. After the rat is placed on a surgical board, then all four legs tied mice. chest and abdomen moistened with 70% alcohol. Then chest surgery using surgical scissors. Furthermore, the liver is cleaned in a solution of 0.9% NaCl. Then liver weighed, and made preparations with paraffin method and hematoxylin-eosin staining

### Making of histology preparations (Suntoro 1983)

Stages of making preparations histology: Fixation. Dehydration. Purification. infiltration. planting (embedding). Penyanyatan (sectioning). attachment of the glass object (mounting). staining with hematoxylin-eosin.

### Data Analysis

The data obtained are SGOT and SGPT levels of blood serum as well as the diameter of the central vein of the various groups are then analyzed by one-way ANOVA, and then see whether there is a significant difference. if there is a significant difference then followed by Tukey test (Santoso 2011).

## THE RESULTS

### a. Measurement results SGOT activity

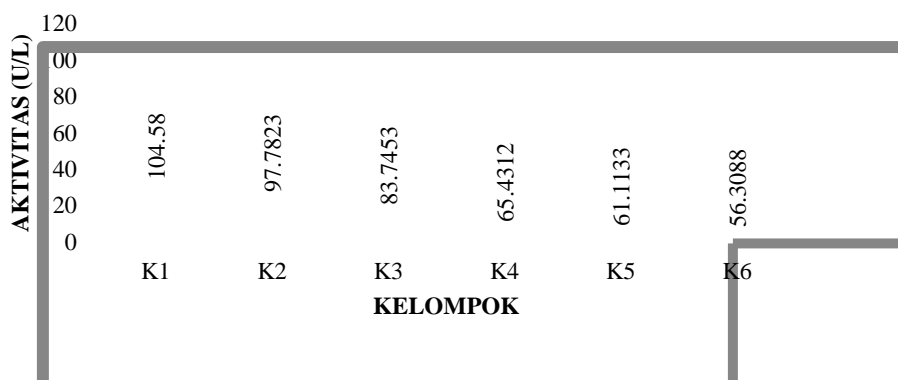


Figure 1. Graph average SGOT Activity Measurement Result

b. Measurement results SGPT activity

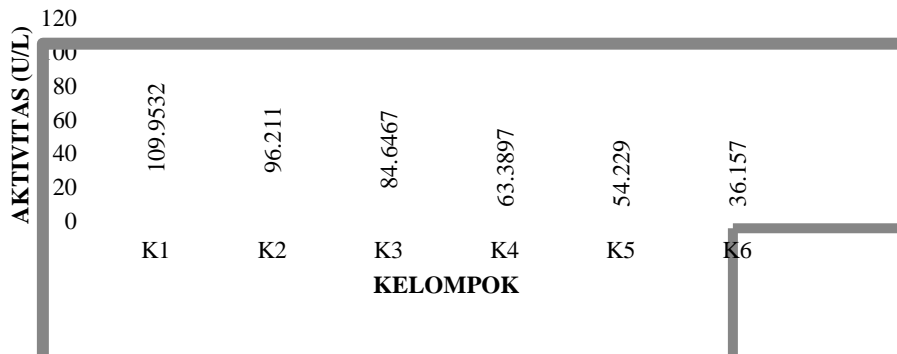


Figure 2. Graph average Measurement Results SGPT activity

e. Measurement results diameter central vein

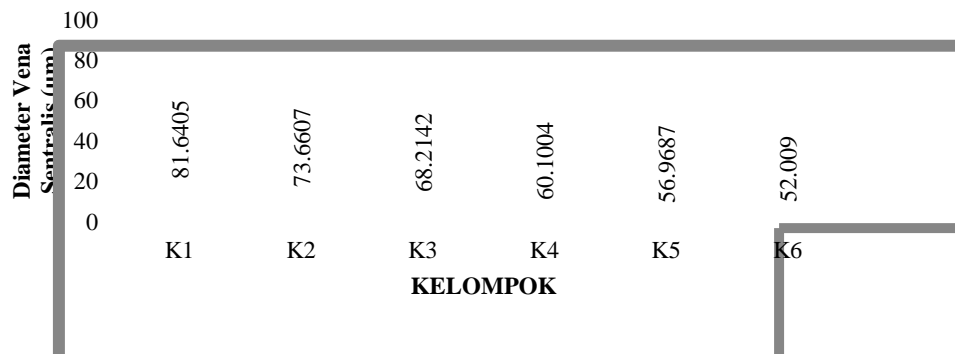


Figure 3. Graph average Measurement Results of the central vein diameter (m)

Specification:

- K1 = negative control
- K2 = low dose group (7.8 mg / 200 g BW)
- K3 = medium dose group (15.60 mg / 200 g BW)
- K4 = high dose group (31.2 mg / 200 g BW)
- K5 = positive control
- K6 = normal control

d. Results Percentage Damage Liver Cells

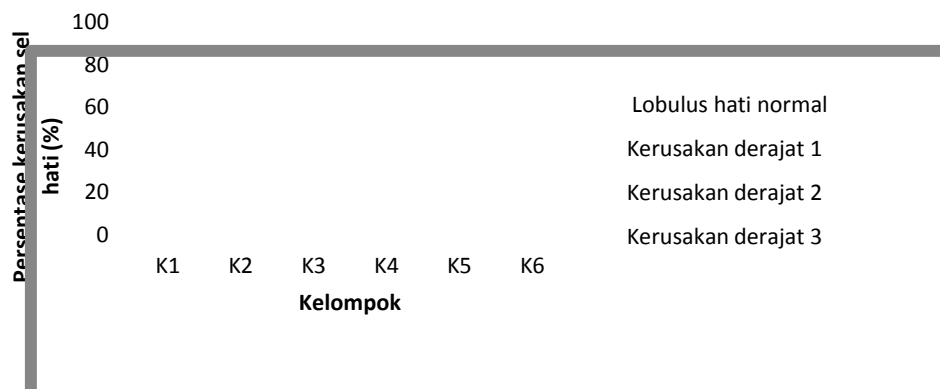


Figure 4. Percentage of Normal liver lobules and Degrees Lobules Liver Damage (%)

e. Results Overview Histopathology

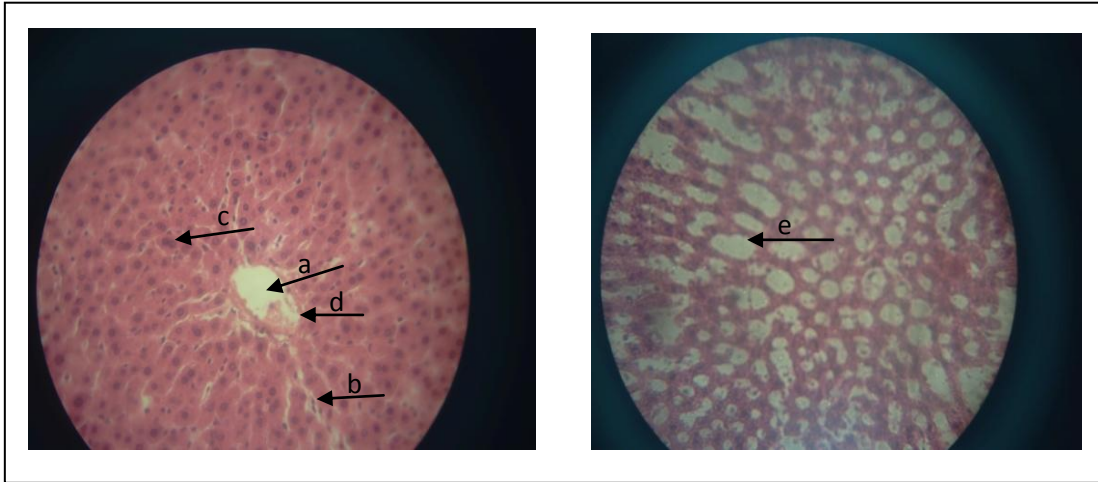


Figure 5. Cells negative control hearts hearts      Figure 6. Cells negative control

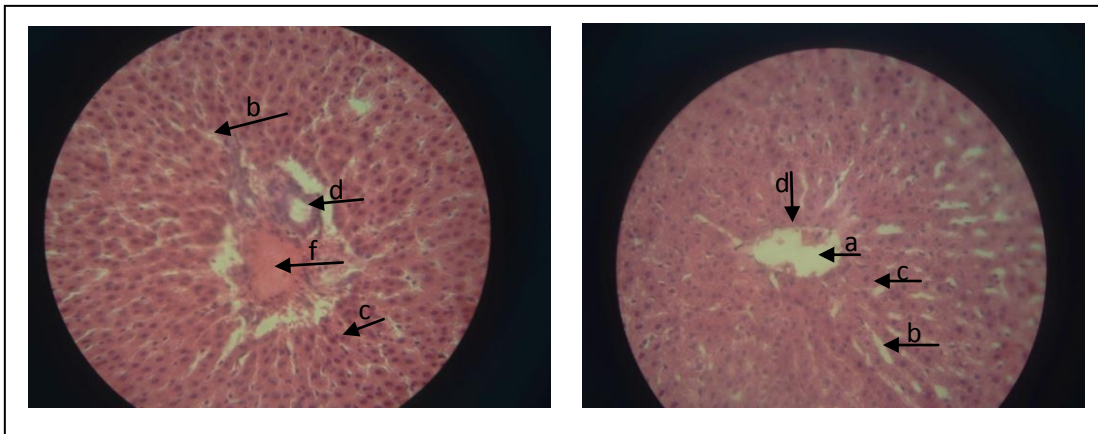


Figure 7. Cells liver low dose      Figure 8. A liver cell medium dose

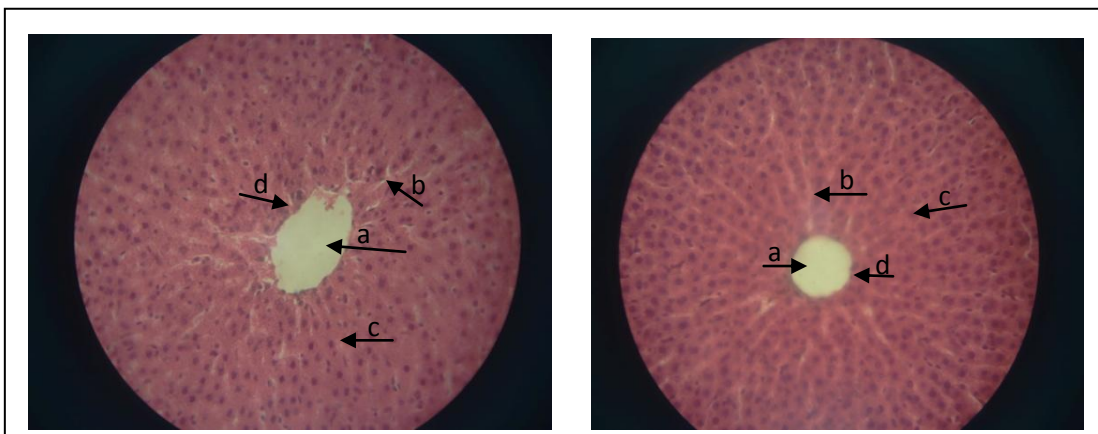


Figure 9. Liver cells high dose      Figure 10. Liver cells positive control





Figure 11. Liver cells of normal control

Description: a = central venous ; b = sinusoid. ; c = hepatocytes ; d = endothelial cells.  
e = fatty liver. liver ; f = damming

## DISCUSSION

Kolmogorov-Smirnov test results indicate that the data SGOT, SGPT, and central venous diameter normally distributed and homogeneous ( $p > 0.05$ ). Then proceed with the one-way ANOVA test and the results showed a difference between groups ( $p < 0.05$ ). This was followed by the Tukey test.

Tukey test results indicate that the data AST and ALT showed significant differences between K1 are given CCl<sub>4</sub> dose of 0.4 mg / g with all treatment groups (K3, K4, K5, K6) unless the group K2. In the K1 group showed an average value of SGOT and SGPT is 104.58 IU / L and 109.953 IU / L. In this group, an increase in SGOT and SGPT. This is caused by carbon tetrachloride in which these compounds are often used to induce liver cell damage in experimental animals. In liver cells contain enzymes transaminase in large numbers that SGOT and SGPT. When these cells are damaged, then the enzyme is knocked into the blood so that the levels in the serum increased.

Results of liver histology showed lyse in liver cells (Figure 5). This causes the levels of AST and ALT in serum increases. In addition, the group K1 also occur fatty liver (Figure 6). Fat deposition occurs due to disruption of the process of fat metabolism in the liver. Mekanime most common is damage to the liver triglyceride plasma discharge. Because the liver triglyceride secretion only when in a state associated with lipoprotein forming very low density lipoproteins (VLDL). Conjugation triglycerides

and lipoprotein can be disrupted due to the presence of carbon tetrachloride. so it will accumulate triglycerides in liver tissue (Frank Lu 1995).

Tukey test results indicate the data SGOT and SGPT in the K2 group given 70% ethanol fraction basil leaves dose of 7.8 mg / 200 g BW did not differ significantly between groups K1. This shows the group has not been able to protect the cells of the liver caused by exposure to carbon tetrachloride. Histological results showed lyse the central vein endothelial cell area. The result is a damming of blood flow in the liver (Figure 7). Damming the flow of blood in the heart always starts from the central vein. This can occur because the central vein is a reservoir of blood from the hepatic artery and portal vein (Rusmiati 2004).

Tukey test results indicate the data SGOT and SGPT in the K3 group given 70% ethanol fraction basil leaves dose of 15.6 mg / 200 g BW was significantly different to the group K1 and K6 group. It shows the group has had influence in protecting liver cells caused by exposure to carbon tetrachloride. Flavonoid contained in the leaves of basil has been able to protect the cells of the liver caused by exposure to carbon tetrachloride. Histological results showed no damage in the central vein and liver cells. damage only occurs in the sinusoids (Figure 8).

Tukey test results indicate the data SGOT and SGPT in K4 group given 70% ethanol fraction basil leaves dose of 31.2 mg / 200 g BW significantly different with K2 group. but not significantly different to the group K6. This suggests these groups have different hepatoprotective activity of the group K3. This is due to the presence of flavonoids contained in the content of the basil leaves. Flavonoids are primary antioxidants that have proven beneficial in preventing cell damage caused by oxidative stress. Mechanism of action of flavonoids as antioxidants can be directly or indirectly. Flavonoids as antioxidants directly is to donate hydrogen ions so as to neutralize the toxic effects of free radicals. Flavonoids as antioxidants indirectly ie by increasing endogenous antioxidant gene expression through several mechanisms. One is through the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) resulting in an increase in genes involved in the synthesis of endogenous antioxidant enzymes such as SOD gene (superoxide dismutase) (Sumardika and Jawi 2012). Histological results showed the central vein. liver cells. and normal sinusoid in this group (Figure 9).

Tukey test results indicate the data AST and ALT in K5 group given cursil® for comparison showed significant differences with K2 group. but not significantly different with K6 group. It shows the group has hepatoprotective activity which is comparable with the group K4. Coursil® used as a comparison because the drug had been shown to improve liver function and disorders of the liver inflammation. Histological results also show the central vein, liver cells, and normal sinusoids (Figure 10).

The results of measuring the diameter of the central vein. K1 group had an average diameter of the central vein, most notably 81.6405  $\mu\text{m}$ . It is proved that carbon tetrachloride is also an impact on central venous dilation. The wider the central vein indicates that there is damage to the veins. Damage to the central vein showed that liver degeneration in the central vein, starting at the very sensitive to toxins that endothelial cells. Lysis of endothelial cells which cause dilation of central venous diameter (Geneser 1994).

In normal control, the central vein is a vein that is surrounded by endothelial cells are compact and lies at the center of the lobules. Histological damage such as inflammation of the liver caused by damage to the central vein endothelial cells are very sensitive to carbon tetrachloride. Inflammation of the liver starts from the central venous blood as a shelter from the hepatic artery and portal vein. As a result of this inflammation and impaired blood circulation can lead to liver cell degeneration necrosis due to lack of nutrients and oxygen (Rusmiati 2004).

Based on the results of the percentage of liver cell damage, it can be said that the higher dose of 70% ethanol fraction basil leaves is given, then the percentage of damage to the liver cells decreases. So it can be said that the 70% ethanol fraction basil leaves can protect liver cells from exposure to harmful chemical compounds, namely carbon tetrachloride ( $\text{CCl}_4$ ).

Liver protection from damage that occurs due to the presence of flavonoids contained in the leaves of basil. Flavonoids have scavenging activity (arrests) high against free radicals and can increase antioxidant enzyme in the body, such as the enzyme superoxide dismutase (SOD) (Sumardika and Jawi 2012).

## CONCLUSION

Results of studies have shown that 70% ethanol fraction of Kemangi leaf as a hepatoprotective activity. Based on the measurement results of SGOT, SGPT, and histopathologic observation at a dose of 7.8 mg / 200 g BW do not have hepatoprotective effects. Dose of 15.6 mg / 200 g BW already have a hepatoprotective activity but not optimal. Dose of 31.2 mg / 200 g BW has hepatoprotective activity which is comparable with the positive group.

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# **IDENTIFICATION DRPs IN WRONG DRUG CATEGORY AND DRUG INTERACTION TO TYPE-2 DIABETES MELLITUS INPATIENTS IN GATOT SOEBROTO ARMY CENTRAL HOSPITAL JAKARTA**

**Zainul Islam. Hadi Sunaryo**

Faculty of Pharmacy and Science  
University of Muhammadiyah Prof. Dr HAMKA Jakarta

## **ABSTRACT**

Drug Related Problems (DRPs) are unwanted incidence that happen to patients associated with drug therapy. Among the categories of DRPs are incorrect drug selection and drug interactions, which are often found in common patients who have the disease, such as type 2 diabetes mellitus. This study aimed to determine the incidence of DRPs with category of incorrect drug selection and drug interactions in patients with type 2 diabetes mellitus in Gatot Subroto Army Central Hospital Jakarta. This research was conducted with non-experimental descriptive design and data acquisition retrospectively for medical record data.

The patient's criteria as subject in this study were inpatient in Gatot Subroto Army Central Hospital in Jakarta, diagnosed with type-2 diabetes mellitus, have no pregnancy during the study, receive diabetes mellitus medications, and have complete medical record data. The data were analyzed descriptively. The results showed there were 42 patients diagnosed with diabetes mellitus type 2 in which 50% of men and 50% of women, with aged 40-60 years 64.29% and  $\geq 61$  years 35.71%. Single hypoglycemic drugs most widely used class of short-acting insulin as much as 83.3%. hypoglycemic drug combination is the most widely used short-acting insulin + long-acting insulin as much as 54.76%. From 42 patients, 12 patients (28.57%) experienced DRPs with category of incorrect drug selection including the used of unsafe drugs 19.05%, incorrect drugs 7.14%, and drug combinations that are not necessary 2.38 %. On DRPs with category of drug interactions, patients can receive more than one drug interactions. From 42 patients observed 112 drug interactions, there were 47 (41.96%) cases were interaction between sulfonylurea with other drugs, cause increasing effect of sulfonylurea drugs that pose an increased risk of hypoglycemic, 29 (69.05%) cases were interaction between sulfonylurea with other drugs caused decreased effect sulfonylurea and 36 (32.14%) cases were interaction between metformin with other drugs caused increasing the risk of hypoglycemic effect.

**Keywords:** Drug Related Problems (DRPs), incorrect drug selection, drug interactions and type-2 diabetes mellitus.

## **INTRODUCTION**

Type 2 diabetes mellitus is a disease that is concern of public, because this is a chronic disease which is found in many people, patients who suffer type 2 diabetes

mellitus could have complications. this disease is a degenerative disease and it is not curable but can be controlled in order to avoid complications (ADA. 2003).

Incorrect drug selection is DRPs events that need to be considered. because the drug is one of the most important elements in health care efforts. Treatment and prevention of various diseases can not be separated from drug therapy. Various of drug selection are currently available. it needs careful consideration in choosing a drug for a disease. if there are wrong drug selection. it will result in therapeutic failure (Cipole. 98).

Research has been done in the General Hospital of Dr. Soetomo on Drug Related Problems study in patients with type 2 diabetes mellitus in Dr Soetomo Hospital. The results of the analysis in patients with type 2 diabetes mellitus which is also accompanied by other diseases. but the patient did not receive all the drugs needed to treat diseases suffered by 25%. The percentage of patients with diabetes mellitus type 2 is receive diabetes mellitus medications are also receive other drugs that are not necessary. because there is no indication for receive the drug by 5.56%. Percentage wrong drug selection in patients with type 2 diabetes mellitus caused by the condition of the patient. with the presence of complications suffered by 12.04%. This research was conducted to determine the incidence of DRPs with category of incorrect drug selection and drug interactions in patients with type 2 diabetes mellitus in Gatot Subroto Army Central Hospital Jakarta.

## **RESEARCH METHODS**

### **Design Research**

This research was conducted with non-experimental descriptive design and data acquisition retrospectively for medical record data. Data were retrieved from the medical records of patients diagnosed with diabetes mellitus type 2 in Gatot Subroto Army Central Hospital Jakarta.

### **Materials and Devices Research**

The tool used was data sheet collection. While the materials used were the medical records of patients with type 2 diabetes mellitus.

### **Sample Research (Inclusion Criteria)**

- a. Male and female patients  $\geq 40$  years were diagnosed with type 2 diabetes mellitus.
- b. Patients hospitalized at Gatot Subroto Army Hospital from June-August 15, 2010.
- c. Medical records include the diagnosis of disease. anamnesa. fasting plasma glucose  $\geq 126$  mg / dl. plasma glucose  $\geq 200$  mg / dl.
- d. Patients have no pregnancy during the study
- e. Patients who received diabetes mellitus medications

### **Data Source and Data**

- a. Data Source : data source from medical record of patients diagnosed with diabetes mellitus type 2. then observed the use of the medicine from June 15 to August 15, 2010.
- b. Data : The data that will be taken include the patient's name. age. gender. diagnosis of disease. anamnesa. fasting plasma glucose. plasma glucose. drugs regimen. adverse effects and minimum laboratory data containe creatinine data. The results of discussions with doctors and nurses.

### **1. Data Analysis**

Results were analyzed with descriptive method to determine :

- a. Patients characteristics include gender and age.
- b. Characteristics of hypoglycemic drugs.
- c. DRPs identification with category of incorrect drug selection and drug interactions.

## **RESULTS AND DISCUSSION**

### **1. Patients Characteristics include Sex and Age.**

Patients with type 2 diabetes mellitus by gender were found 42 patients in which 21 (50%) mens and 21 (50%) women. The majority of patients with type 2 diabetes mellitus were patients with aged under 60 years. There were 27 Patients



(64.29%) with aged 40-60 years and 15 patients (35.71%) with aged  $\geq 61$  years. Data can be seen in Table 1.

**Table 1.** Characteristics of Patients by Sex and Age

Characteristics of Patients		Number of Patients	Percent
Gender	Men	21	50
	Women	21	50
	<b>Total</b>	<b>42</b>	<b>100</b>
Age (years)	40 – 60 (adult)	27	64.29
	$\geq 61$ (elderly)	15	35.71
	<b>Total</b>	<b>42</b>	<b>100</b>

## 2. Type of Hypoglycemic Drugs

### a. Single hypoglycemic drugs

Hypoglycemic drugs were used in patients with type 2 diabetes mellitus include sulfonylurea 12 cases (28.56%). biguanide 8 cases (19.05%). short-acting insulin 35 cases (83.33%). 23 cases with long-acting insulin (54.76%). The data presented in Table 2.

**Table 2.** The use of single Hypoglycemic Drug in Patients Diabetes Mellitus Type 2

Group	Name of Drug	Number of Patients	Percent
Sulfonylurea	Glibenclamide	5	11.90
	Gliquidone	4	9.52
	Glimepiride	2	4.76
	Glicazid	1	2.38
Biguanide	Metformin	8	19.05
	InsulinHM Recombinant	26	61.90

Short Acting Insulin	DNAoriginal		
	Humalog insulin lispro	8	19.05
	Insulin Glulisin	1	2.38
Long Acting Insulin	Glargine insulin	23	54.76
	<b>Total</b>	<b>77</b>	

**b. Hypoglycemic drugs with combination**

Hypoglycemic drug combination were used in patients with type 2 diabetes mellitus include a combination of two drugs known as sulfonylureas 1 case (2.38%). sulfonylureas with biguanide 4 cases (9.52%). Combination sulfonylurea with biguanide + short-acting insulin + long-acting insulin 1 case (2.38%). biguanide with short-acting insulin 1 case (2.38%). short-acting insulin with a long-acting insulin 23 cases (54.76%). The data presented in Table 3.

**Table 3.** Combination Hypoglycemic Drug In Patients Diabetes Mellitus Type 2

<b>GroupDrugs</b>	<b>Type of Drug</b>	<b>Number</b>	<b>Percent</b>
Sulfonylurea + Sulfonylurea	Glimepiride + Glibenclamide	1	2.38
	Metformin + Glibenclamide	2	4.76
Sulfonylurea + Biguanide	Metformin + Glicazid	1	2.38
	Metformin + Glimepiride	1	2.38
Sulfonylurea + Biguanide + Short Acting Insulin + Long Acting Insulin	Glikazid + Metformin + Insulin HM + Glargine Insulin	1	2.38
Biguanide + Short Acting Insulin	Metformin + Insulin HM	1	2.38
Short Acting Insulin + Long	Insulin HM + Glargine Insulin	22	52.38

Acting Insulin	Insulin Glulisin + Glargine Insulin	1	2.38
	<b>Total</b>	<b>30</b>	<b>71.42</b>

### 3. DRPs Identifications

#### a. Incorrect Drug Selection

The results showed the presence DRPs with category of incorrect drug selection 12 (28.57%) patients with type 2 diabetes mellitus. DRPs with highest incidence was incorrect drug selection including 8 patients (19.04%) the use of unsafe drugs. 3 patients (7.14%) the use of incorrect drugs. 1 patient (2.38%) the use of drug combination that are not necessary. The use of drugs in patients with type 2 diabetes mellitus lead to incorrect drug selection can be seen in Table 4.

**Table 4.** Types of Drugs and DRPs with Category of Incorrect Drug Selection in patients with Type-2 Diabetes Mellitus

Causes of DRPs	Type of Drug	Drug Effects	Number of patients	Percent (%)
	Dexamethasone	Effect on increasing diabetes mellitus and hypertension	1	2.38
The use of unsafe drugs	Cilostazol	Having side effects on diabetes mellitus and hypertension	2	4.76
	Siprofloxacin	Having side effects on diabetes mellitus and hypertension	5	11.90
The use of incorrect drugs	Spironolacton	Incorrect drug selection in diabetes mellitus patients with hypertensive (Chobanion. et al. 2004)	2	4.76
	Clonidin	Incorrect drug selection in diabetes	1	2.38

		mellitus patients with hypertensive (Triplit. et al. 2005)		
Incorrect combination	Glimepiride + Glibenclamide	Incorrect combination because both sulfonylurea class	1	2.38

**b. Drug Interactions**

The results observed from 42 patients showed 112 cases were presence DRPs with category of drug interactions. where patients may receive more than one drug interactions. Drug interaction between sulfonylurea with other drugs as many as 47 cases (111.9%) increasing sulfonylurea effect cause increased risk of hypoglycaemic and 29 cases (69.05%) were decreased the sulfonylurea effects cause sulfonylurea effect become inadequate. Drug interaction metformin with other drug as many as 36 cases (32.14%) were increased metformin effect cause risk of hypoglycaemic.

**Table 5.** Types of Drug and DRPs Categories of Drug Interactions In patients with diabetes mellitus type 2

Type of drug		Effects	Number of case	Percent
Drug A	Drug B			
Sulfonylurea	Clopidogrel	Increased sulfonylurea effects: increased hypoglycemic effect due to various mechanisms such as a decrease in hepatic metabolic. renal excretion barriers. expulsion of protein binding. decreased plasma glucose. changes in carbohydrate metabolism	5	11.90
	Cilostazol		1	2.38
	Acetosal		14	33.33
	Naenoxaparin		2	4.76
	Gemfibrozil		3	7.14
	Ranitidine		15	35.71
Sulfonylurea	Bisoprolol	Reduction in the effects of sulfonylureas: hypoglycemic effect decreased. due to a variety of mechanisms that increased hepatic metabolism. decreased insulin	4	9.52
	Carvediol		1	2.38
	Amlodipin		13	30.95
	Nifedipin		4	9.52

	Dexametasone	release. increased urinary excretion	1	2.38
	Phenitoin		1	2.38
	Isoniazide		1	2.38
	Rifampicin		1	2.38
	CaCO <sub>3</sub>		1	2.38
	Na-bikarbonate		2	4.76
Sulfonylurea	Ciprofloxasin	Increased effect of sulfonylureas: hypoglycemic effect occurs potentiation	5	11.90
Sulfonylurea	Glicosyde digitalis (digoxin)	Increased effect of sulfonylureas: increased serum levels of digitalis glycosides	2	4.76
Metformin	Nifedipine	Nifedipine increases the absorption of metformin: metformin C <sub>max</sub> and AUC increased by respectively 20 and 9% of the metformin is excreted in the urine increased	4	9.52
Metformin	Furosemide	Furosemide increases plasma levels of metformin. the C <sub>max</sub> and AUC increased by 22% to 15%. Renal excretion is not a significant change. C <sub>max</sub> and AUC of furosemide lower 31 and 12%. t <sub>1/2</sub> terminals down 32% without a significant change in furosemide renal clearance.	15	35.71
Metformin	Digoxin	Drug cationic (digoxin. ranitidine) which eliminated any potential kidney interact with metformin and compete on secretion system / tubular transport. metformin levels may increase.	2	4.76
	Ranitidine		15	35.71

## CONCLUSION

1. The results showed that from 42 patients diagnosed with type 2 diabetes mellitus were found 50% men and 50% women. There were 27 Patients (64.29%) with aged 40-60 years and 15 patients (35.71%) with aged  $\geq$  61 years.
2. Single hypoglycemic drugs most widely used class of short-acting insulin is as much as 83.3%. while the hypoglycemic drug combination is the most widely used short-acting insulin + long-acting insulin as much as 54.76%.
3. DRPs Identification :
  - a. From the 42 patients. 12 patients (28.57%) experienced DRP with category of incorrect drug selection include 19.05% the use of unsafe drugs. 7.14% the use of incorrect drugs. and 2.38 % drug combinations that are not necessary.
  - b. From 42 patients observed 112 cases drug interactions. There were 47 (41.96%) cases interaction between sulfonylurea with other drugs cause increased sulfonylurea effect so that increased risk of hypoglycaemia. and 29 (25.89%) cases were decreased sulfonylurea effect. While interaction Metformin with other drugs 36 (32.14%) cases were increased metformin effect and risk of hypoglycaemia.

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# POTENTIAL ROLES LEAVES EXTRACT OF BABADOTAN (*AGERATUM CONYZOIDES* L.) AND BINAHONG (*ANREDERACORDIFOLIA* (TEN) STEENIS) AND ITS COMBINATION AS ANTI HYPERGLYCEMIA IN INSULIN RESISTANCE ANIMAL MODEL

Patonah<sup>1</sup>. Ari Yuniarto<sup>1</sup>. ZahniahNur Putri<sup>1</sup>

Sekolah Tinggi Farmasi Bandung  
[Patonah.stfb@gmail.com](mailto:Patonah.stfb@gmail.com)

## ABSTRACT

Babadotan (*Ageratum conyzoides*L) and binahong (*Anrederacordifolia* (Ten) Steenis) are plant that easy to find in the community and empiricalliy used to treated various diseases including diabetes mellitus. This study aimed to determine the activity of babadotan. binahong leaves extracts and its combination in insulin resistance animal models induced by fat emulsion (Ivelip®) 3 mL/kg bw intravenously. 10 mg/kg bw propylthiouracil orally and 0.01 IU insulin intraperitonally for 10 days. The animals were randomly divided into 7 groups(each 5 mice) that received metformin 1.3 mg/kg bw (group 1). normal control group received the vehicle (group 2). positif control received the vehicle (group 3). 100 mg/ kg bw babadotan (group 4). 100 mg/ kg bwbinahong (group 5) and the combination of babadotan and binahong (50:50mg/kgbw and 100:100mg/kgbw as group 6 and 7 respectively). Each group induced insulin resistance except group 2. Test drugs were given for 10 days. Glucose levels were measured at day 1 dan day 11. The study showed that leaves extract of babadotan. binahong and its combination decreased blood glucose level ssignificantly different ( $p < 0.05$ ) to control group and increased insulin sensitivity. Leaves extract of babadotan. binahong and its combination same lio rate insulin resistance in diabetes mellitus type 2.

**Keywords:** *Ageratum conyzoides*L. *Anrederacordifolia* (Ten) Steenis. Insulin resistance. diabetesmellitus.

## INTRODUCTION

Insulin resistance is a condition in which the body produces insulin but does not use effectively. When people have insulin resistance. glucose builds up in the blood instead of being absorped by the cells. leading to type 2 diabetes or prediabetes (KoheiOkita. 2014).Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia (elevated blood glucose levels) were due to the decreased secretion of insulin. or insulin sensitivity or both (ADA. 2004).

The prevalence of diabetes mellitus (DM) in the world is increasing from year to year. According to the World Health Organization (WHO). the number of people with



diabetes could reach 366 million by 2030. Indonesia ranks fourth as a country of people with diabetes in the world after India, China, and the United States (Sarah Wild et al. 2004). According to the data of Basic Health Research (Riskesdas) reported an increase in the prevalence of diabetes of 5.7% in 2007 to 6.8% in 2013 in Indonesia.

Indonesia is known for its natural resources have the potential to develop drugs from nature. One of the plants that can be used for anti-diabetes mellitus is babadotan (*Ageratum conyzoides* L.) and binahong (*Anrederacordifolia* (TEN) Steenis). Babadotan (*Ageratum conyzoides* L.) is a medicinal plant that has been used in some countries to treat various diseases including diabetes mellitus (Duke, 2005). Binahong (*Anrederacordifolia* (TEN) Steenis) is known as a multipurpose plant because almost all parts of the plant from the roots to the leaves, beneficial to humans. Empirically, people use binahong to help the healing process of various diseases (Dalimartha, 2009). Binahong leaves are used as an alternative treatment for Diabetes Mellitus (Indri, 2013).

Based on the description above, this study aimed to test the anti-diabetic activity of extract babadotan leaves (*Ageratum conyzoides* L.) and binahong leaves (*Anrederacordifolia* (TEN) Steenis) in animal models of insulin resistance.

## **MATERIALS AND METHODS**

### **Materials**

Babadotan (*Ageratum conyzoides* L.) leaves (BaL) and binahong (*Anrederacordifolia* (TEN) STEENIS) leaves (BiL) were collected from Manoko, Lembang, Bandung, West Java, Indonesia and botanically identified at ITB, Bandung. The fresh leaves were cut into small pieces, dried, and powdered. The powdered leaves were macerated by ethanol 96% for three days. The extracts were filtered and concentrated by rotary evaporator. Phytochemical analysis carried out on the dry extract.

### **Animals**

Forty nine male Swiss Webster mice of 2 months age and weighed about 25-30 g were used in this study. Before treatment, animals were adapted in a cage room temperature ( $\pm 25^{\circ}\text{C}$ ) and were given access to food and drink for a week.

### **Experimental design**

Animals were divided into seven groups of seven animals in each group. Group 1 as normal group received the vehicle drug, group 2 as control received the vehicle

drug. group 3 received metformin 1.3 mg/kg bw. group 4 received BaL 100 mg/kg bw. group 5 received BiL 100 mg/kg bw. group 6 received BaL and BiL combination 1 (50 and 50 mg/kg bw). group 7 received BaL and BiL combination 2 (100 and 100 mg/kg bw). All group (except normal group) were induced insulin resistance by lipid emulsion (Ivelip®) (3 mL/kg bw) and propylthiouracil 0.01% for 10 days. Glucose levels in serum were measured at day 1 (as baseline) and day 11. At day 11, glucose level were measured every 30 minutes for 150 minutes after injected insulin intraperitoneally (0.05 U/kg bw) using glukometer®. Metformin were used as standard drug.

The data obtained were analysed using ANOVA ( $p < 0.05$ ). The plasma glucose disappearance rate (KITT) correlated to insulin sensitivity (H. Grulet *et al.* 1993). Data blood glucose levels during 150 minutes graphed against time as absciss and blood glucose levels as ordinate. Regression coefficient ( $r$ ) or slope determined from the linear regression and constant Insulin Tolerance Test (KITT) was calculated by multiplying  $r$  by 100. The value KITT showed insulin sensitivity. lower K values showed lower sensitivity and higher K values showed higher sensitivity (SoviaEvi *et al.* 2011).

## **RESULT AND DISCUSSION**

The result of phytochemical screening showed that the extract babadotan with 9.5% moisture content containing compounds alkaloids, steroids, saponins and flavonoids, while extracts binahong with 7.8% moisture content containing compounds alkaloids, steroids, saponins, flavonoids, quinones and tannin.

Provision of lipid emulsion (Ivelip®) and propyl tiourasil for 10 days causes hypertriglyceridemia (patonah, 2011). High-fat feeding for 10 days and followed by intravenous injection of insulin on Day 11 resulted in an animal model of diabetes with insulin resistance (SoviaEvi, 2011). Modified method showed that administration of lipid emulsion Ivelip® and propyl tiourasil for 10 days and the intravenous injection of insulin on day 11 resulted in an animal model of insulin resistance.

The results of measurements of blood glucose levels for each treatment group is shown in Table 1. Blood glucose levels on day 1 is expressed by  $t_0$ . Blood glucose levels in group 2 (receiving metformin 1.3 mg / kg bw) start at minute 30 have shown that blood sugar levels comparable to normal. The change in blood glucose levels

during 150 minutes observation showed no significant difference compared to the normal group.

Metformin is a drug classes of insulin sensitizer for treating diabetes mellitus type 2. Patients with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) that received metformin showed improvement in glucose tolerance and reversion of prediabetes to normal glucose tolerance(Armato John *et al.* 2012).

Measurement of blood glucose levels for each treatment group at minute 30. 60. 90. 120. 150 showed that the administration ofBaL and BiL extract. and its combination lowered blood glucose levels that were statistically significantly different compared to the control group (p <0.05).Blood glucose levels (the group that received the extract BaL. BaL and its combinations) at 120 minutes showed no significant difference compared to the normal group. It showed that the blood glucose level had returned to normal level start at 120<sup>th</sup> minutes.

**Table 1.**glucose levels for all group treatment

Glucose levels of treatment groups for 150 minutes (mean±sd) (mg/dL)						
Group	Time (Minutes)					
	t0	t30	t60	t90	t120	t150
1		(96.3 ±1.3)	(98±0.8)	(98.7±1.3)	(99±0.8) <sup>o</sup>	(98.3±1.3) <sup>o</sup>
2		(179.8 ±6.8)	(188.7±3.5)	(189.7±2.5)	(184.7±0.6)	(177±2.7)
3		(70.3±7.5)	(73.7±6.0)	(84.7±4.5)	(91±5.3)*	(90.7±4.0)*
4	(92 ±1.6)	(129.7±2.5)	(122±2.0)	(115±1.0)	(104.7±4.7)*	(98±6.3) <sup>o*</sup>
5		(122.4±5.0)	(111±8.2)	(103.3±8.0)*	(91.3±5.9) <sup>o*</sup>	(80±1.0)
6		(133.3±12.0)	(120.7±12.0)	(109.3±8.1)	(95±1.0) <sup>o*</sup>	(88.3±2.1) <sup>o*</sup>
7		(107.3±6.7)	(100±9.0)*	(93±9.0) <sup>o*</sup>	(88±9.0) <sup>o</sup>	(85±9.0) <sup>o</sup>

Data are expressed as means ± standard deviation. 1.normal group. 2. control group. 3. group received metformin. 4. group received BaL.5. group received BiL.6. group received combination 1 (BaL and BiL 50/50 mg/kg bw). 7. received combination 1 (BaL and BiL 100/100 mg/kg bw).

Blood glucose levels in group 7 minutes to 30. 60. 90. and 150 conveniently indicates no significant difference (p> 0.05) compared to the normal group. It is proved that the administration of a combination of extracts BaL and BiL (100/100 mg / kg bw) can normalize blood glucose levels from the 30<sup>th</sup> minute. These results demonstrate that administration of a combination of extract dose of 100/100 mg / kg bw better than the dose of 50 / 50 mg / kg bw.

KTTI (konstanta test tolerance insulin) value was used to determine insulin sensitivity. Lower K value indicates a low insulin sensitivity. Vice versa, a higher K value indicates a higher insulin sensitivity. KTTI value sequence from the lowest to the highest. control group (7.2) <group 3 (9.1) <group 7 (9.7) <group 6 (9.9) <group 5 g (9.9) <group 4 (9.9). Value KTTI 4-7 groups comparable with group 3. It is clear that the control group (not given drug or test extract) showed the lowest insulin sensitivity.

## CONCLUSION

BaL extract (*Ageratum conyzoides* L). BiL extract (*Anredaracordifolia*(Ten Steenis). and their combination (50/50 and 100/100 mg/kg bw) showed antihyperglycemia activity and increased insulin sensitivity. Combination of BaL and BiL (100/100 mg/kg bw) showed the best of antihyperglycemia activity and improved insulin sensitivity.

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**CONSTRUCTION YEAST SHUTTLE VECTOR CONTAINING DENGUE  
VIRUS 3 NON-STRUCTURAL NS1 ENCODING GENE EXPRESSION  
CASSETTES FOR SUBUNIT DENGUE VACCINE CANDIDATE**

**Vanny Narita<sup>1</sup>. Wahyu Hidayati<sup>2</sup>. Tjahjani Mirawati Sudiro<sup>3</sup>**

1. Center for Pharmaceutical and Medical Technology. Agency for the Assessment and Application of Technology. Jakarta 10340. Indonesia
2. Faculty of Pharmacy and Science. University of Muhammadiyah Prof. DR. Hamka. Jl. Delima II/IV. Klender. East Jakarta. Indonesia.
3. Department of Microbiology. Faculty of Medicine. Universitas Indonesia. Jakarta 10320. Indonesia

**ABSTRACT**

Non-structural 1 (NS1) protein is one of ten proteins coding by dengue virus genome. The protein has two forms in mammalian infected cells. secreted and membrane-associated. The protein is potentially developed as a subunit dengue vaccine. In this study, we aimed to construct yeast shuttle vector containing DENV 3 strain Indonesia NS1 encoding gene expression cassette for expression in yeast. The gene fragment was amplified from the cDNA with some necessary changing by Polymerase Chain Reaction (PCR). Finally, the fragment gene inserted to yeast vector pYES2/CT containing regulatory sequences for expressing the polypeptide product. Final construct were confirmed by PCR of desired fragment and sequencing. One yeast shuttle vector containing NS1 DENV 3 strain Indonesia was constructed. The yeast shuttle vector containing DENV-3 strain Indonesia NS1 pYES-NS1D3 can be further transferred into yeast in the next stage to produce protein for subunit dengue vaccine development.

Keywords : Non-structural protein. Dengue. subunit vaccine. yeast shuttle vector

**INTRODUCTION**

Dengue virus (DV) infection is mostly asymptomatic or produces a mild self-limiting acute febrile illness, dengue fever (DF), and a life threatening severe illness, dengue haemorrhagic fever (DHF) with minor or major bleeding from different sites (Agarwal *et al.*, 1999). DHF has emerged as the most important arbovirus disease in man in the last three decades. It has been estimated that about 50 to 100 million cases of DF occur

every year with about 250,000 to 500,000 cases of DHF in the Southeast Asia and the Western Pacific Region (Rigau-Perez *et al.*, 1998). During dengue outbreaks, 40 to 90 per cent among hospitalized patients is children (Halstead, 2002; Chaturvedi and Shrivastava, 2004).

Today DHF affects most Asian countries and has become a leading cause of hospitalization and death among children in several of them. The risk factors for DHF are infestation with *Aedes* mosquito, hot and humid climate enhancing mosquito breeding, mosquito density, and presence of all the four serotype of the dengue virus with the secondary infection in the host, the water storage pattern in the houses, population density and large movement of people towards urban areas. At present, there is no specific therapy available for DHF. Appropriate symptomatic treatment has been successful in reducing the mortality of DHF. Mosquito control has been the only method of preventing DHF but is costly and often ineffective (Ooi *et al.*, 2008).

Flaviviruses are enveloped, single-stranded, positive-sense RNA viruses formed by three structural proteins. The genome is approximately 11 kb long and contains a single open reading frame encoding a polyprotein precursor of about 3,400 amino acid residues (Beasley and Barret, 2008). The three structural proteins derive from the N-terminal part of the polyprotein and are followed by seven nonstructural proteins: NS1, NS2A/2B, NS3, NS4A/4B, and NS5. NS1 is a 46-50 kDa glycoprotein expressed in infected mammalian cells in both membrane-associated (mNS1) and secreted (sNS1) forms (Winkler *et al.*, 1989; Chang, 1997; Flamand *et al.*, 1999). Furthermore, circulating sNS1 proteins have been detected in patients' plasma with DENV infections. sNS1 could therefore be a target of humoral immunity in DENV infection. In addition, several groups have reported that immunization with NS1 provide protection to mice against lethal DENV, Japanese Encephalitis virus (JEV), and West Nile Virus (WNV) infections, thus suggesting that NS1 could be a target for therapy a subunit vaccine against flavivirus (Henchal *et al.*, 1988; Chen *et al.*, 2009).

The first dengue vaccines were evaluated in 1929 (Thisyakorn and Thisyakorn, 2014). Development of safe and effective dengue vaccines faces many challenges. Although no licensed dengue vaccine is yet available, several vaccine candidates are under development. These include live attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines, and live recombinant DNA and subunit vaccines

(Murrel, 2011). Recent advances in molecular biology have spurred dengue vaccine effort were using live recombinant, DNA and subunit vaccines. There are several subunit dengue vaccine under develop : envelope, domain III of envelope protein (EDIII), and NS1 protein. In this study DENV-3 NS1 encoding gene were cloned and recombinant plasmids analyzed by restriction enzyme and sequencing.

## **MATERIALS AND METHODS**

### ***Strain and plasmid***

Dengue virus strain used in this study was Indonesian isolate supplied by Microbiology Department, Faculty of Medicine, University of Indonesia. pYES2/CT plasmid (5963 bp, Invitrogen Corporation, CA, USA) was used as a cloning vector. Bacterial strain used as host cells was *E. coli* DH5 $\alpha$  (wild type; Invitrogen Corporation, CA, USA).

### ***Amplification DENV-3 NS1 gene fragment***

The DENV-3 strain Indonesia gene fragment was amplified by PCR using total cDNA. The cDNA was synthesized by previous researchers (Sudiro et al. 2010). The PCR amplification was using a pair of NS1 specific primer, d3-sbam (forward primer) and d3-1056c (reverse primer). These primers were designed by using PRIMER software with required cut sites which do not cut the NS1 gene. Amplification using PCR Core System (Promega). At the end of the amplification, the size of the PCR product was determined to compare with DNA marker, using 0.8% agarose gel electrophoresis followed by staining ethidium bromide. These PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega) and then used for cloning.

### ***Cloning steps***

pYES2/CT vector was digested with *Bam*HI and *Xho*I restriction enzymes (Promega). DENV-3 NS1 fragment was flanked by artificial 5' *Bam*HI and 3' *Xho*I sites, respectively. Then, DENV-3 NS1 PCR products were digested with *Bam*HI and *Xho*I and co-ligated into *Bam*HI - *Xho*I digested pYES2/CT, which led to the production of pYES2/CT construct containing the DENV-3 NS1 gene. Plasmids were transformed into the *E. coli* DH5 $\alpha$  strain to proliferate. Bacteria were cultured in LB-Broth medium containing 100  $\mu$ g/ml ampicillin.



### Confirmation

All clones and subclones were confirmed by colony PCR and sequencing. The constructs were directly sequenced by the dideoxy chain termination method (Biotechnology Laboratory, BPPT, Indonesia) using vector Primers. T7 and CYC1 primers.

## RESULT AND DISCUSSION

Visualization of PCR product in agarose gel after staining with ethidium bromide shown the DENV-3 NS1 amplification process from cDNA was done successfully (Figure 2). The amplification resulted a fragment with 1.160 bp size. In these process we used a pair of specific primer which added by some restriction site on both ends. 5' end and 3' end. Those primers amplified along DENV-3 NS1 amplification process. It resulted NS1 fragment flanked by restriction sites. *Bam* HI and *Xho*I.

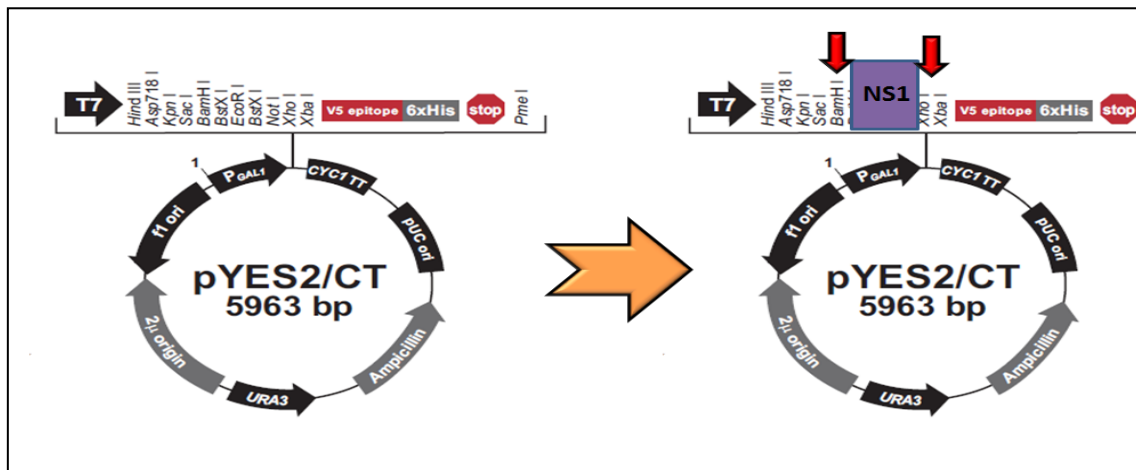
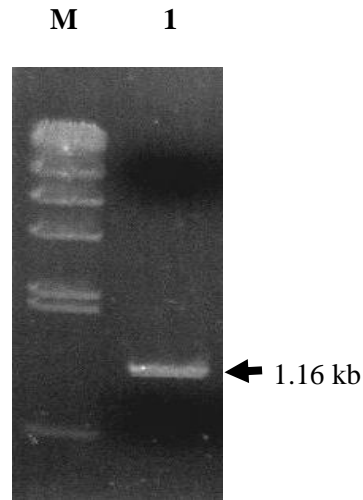


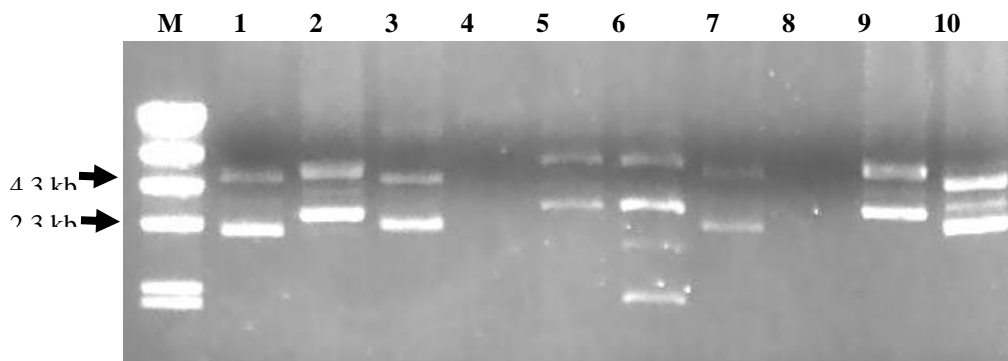
Figure 1. Schematic of vector restriction and ligation

For cloning process, the NS1 fragment and vector, were cut by specific restriction enzymes. *Bam* HI and *Xho*I. This process would open the vector and became linear with sticky end. The NS1 fragment would have sticky end also after cutting by those enzymes. Restriction sites were created based on multiple-cloning site (MCS) information on inside vector but do not cut inside the gene fragment. Both restriction enzymes do not cut inside NS1 fragment. After ligation the NS1 fragment replaced MCS and the vector becomes circular (Figure 1).



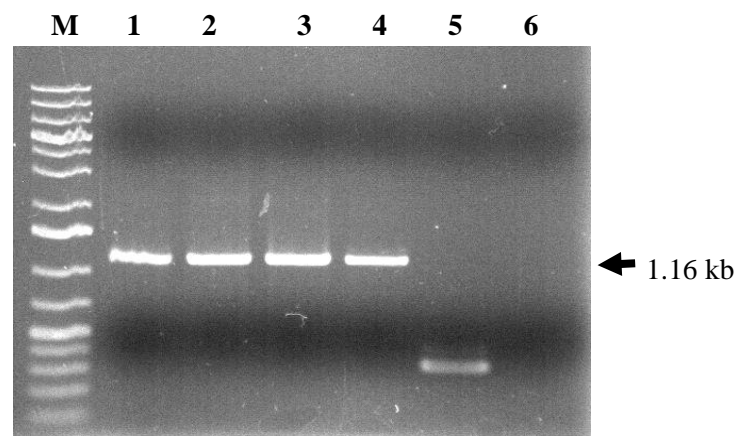
**Figure 2.** PCR Amplification of NS1 Gene Fragments from cDNA of Dengue Virus 3 isolated by Dept. of Microbiology, Faculty of Medicine, University of Indonesia . M:λ/Hind III Markers; Line 1. NS1 Gene Fragment Amplified with the Annealing Temperature of 56°C. 0.8% Agarose Gel (w/v). 50 Volt. EtBr 1 ug/mL

The plasmid were transformed to *Escherichia coli* strain DH5α by heat-shock transformation method. Two hundred bacterial colonies were grown in ampicillin selective agar medium indicated transformation process was succeed. Those colonies picked randomly to analyzed recombinant cells by plasmid isolation. PCR and sequencing. Bacterial strain used in genetic engineering designed lack of antibiotic resistance ability. This ability brought by circular chromosome which called as extra chromosomal DNA or plasmid. By growing transformed cells onto medium containing antibiotic will trigger the antibiotic resistance gene to work or calling by operon system. This process resulted bacterial containing plasmid will grow on selective medium (Brown, 1996). Further analysis, such as PCR, restriction analyze and sequencing, are needed to differ bacterial containing recombinant plasmid and empty vector.



**Figure 3.** Recombinant plasmids electrophoresis from plasmid isolation. M:λ/Hind III Markers; Line 1-9: Plasmids from bacterial colonies; Lane 10: negative control. pYES2/CT. 0.8% Agarose Gel (w/v). 50 Volt. EtBr 1 ug/mL

Plasmid isolation produced four colonies with different size bands compare to empty vector as negative control (Figure 3). Those plasmids shown higher position than the negative control. Those four plasmids got further analysis by PCR using DEN-3 NS1 specific primers. The data shown all plasmids are recombinant plasmids (Figure 4). One fragment analyzed by sequencing with pair of vector primers. T7 and CYC1 primers. Both data from sequencing give resulted 1.157 bp then analyzed by GENETIX and GENEIUS software and data alignment to GenBank by *blastn* analysis. The alignment resulted 100 sequences were similar to our sequence. all of which are dengue virus serotype 3. The highest similarity value (99% similarity) is shown by gb|AY858041.1| Dengue virus type 3 strain FW06. The recombinant NS1 gene in this study will be expressed in yeast *Saccharomyces cerevisiae* and used as antigen for active immunization.



**Figure 4.** PCR Verification of pYES2/CT Recombinant. M: 1 kb DNA Ladder Marker; Line 1.2.3.4: pYES2/CT Recombinant; Line 5: Positive Control PCR; Line 6: Negative Control PCR. 0.8% Agarose Gel (w/v). 50Volt. EtBr 1  $\mu$ g/mL

Some literature have reported the production of NS1 protein by several expression systems. such as *E. coli*. insect cells. and mice. Wan *et al.* (2013) reported that several studies indicated that passive immunization with anti-NS1 Abs. DNA vaccine against NS1 proteins. or recombinant vaccinia virus expressing NS1 and active immunization with NS1 proteins could provide protection in mice against DENV challenge.

## CONCLUSION

One yeast shuttle vector containing NS1 DENV-3 strain Indonesia were constructed. named pYES-NS1D3. The pYES-NS1D3 can be transferred into yeast in the next stages to produce protein for subunit dengue vaccine development.

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# MOLECULAR DOCKING STUDIES OF FLAVONOIDS OF GUAVA LEAVES (*Psidium guajava* L.) TO $\alpha$ -GLUKOSIDASE RECEPTOR

Supandi<sup>1,2</sup>. Rizky Arcintha<sup>2</sup> and Ulfa Nurhamidah<sup>2</sup>

<sup>1</sup>Pharmacy Department. State Islamic University Syarif Hidayatullah. . Tangerang Selatan 15412. Indonesia <sup>2</sup>Faculty of Pharmacy. University of Muhammadiyah Prof.DR.Hamka. Jakarta 12130. Indonesia

## ABSTRACT

Type 2 of diabetes is a major life-threatening disease which number of case increase every year . Leaves of guava ( *Psidium guajava* L. ) contain flavonoids compounds such as apygenin . avicularin . guaijaverin . hiperin . isoquercetin . kaempferol . leukocyanidin . miricetin . quercetin and quercitrin. These compounds have antidiabetic activity by inhibiting  $\alpha$  - glucosidase enzyme but less study showed which compound had the best effect. Molecular docking was conducted to determine and visualize the interaction of guava leaves flavonoids to  $\alpha$  - glucosidase enzyme. The study was conducted to search structure of  $\alpha$  - glucosidase enzyme in Protein Data Bank and designing 10 flavonoids ligand and acarbose as a comparison. Docking file was conducted by using Vina Autodock software. Results showed that molecular docking of guaijaverin and quercitrin have better interaction to  $\alpha$  - glucosidase enzymes compared to acarbose compound with a free energy value of -9.7 kcal / mol and guaijeverin residu contact His 645 (3.2Å); Arg 520 (3.2); Lys 776 (3.2); Phe 535 (2.7); Thr 778 (2.8 ) and quercitrin Ser 521 (2.1); Val 779 (3.2); His 645 (3.4); Lys 776 (3.0); Phe 535 (2.5).

Keywords : Molecular Docking . antidiabetic . *Psidium guajava* Flavonoids

## INTRODUCTION

Diabetes mellitus ( DM ) is a chronic disease that occurs either when the pancreas does not produce sufficient amounts of insulin or when the body does not effectively use the insulin. Previous research stated that the leave of guava (*Psidium guajava* L.) can be used for the treatment of diabetes mellitus ( Khan *et al* .. 2012; Mukhtar *et al*. 2005). Mukhtar *et al* (2004) reposted that guava leaf extract showed hypoglycemic activity in mice that had been induced alloxan and streptozotocin. Guava leaf contains flavonoids such as ; kaempferol. guaijaverin. miricetin. hyperin and apygenin ( Singh and Marar 2011). Guava leaf extract inhibits  $\alpha$  - glucosidase activity in the small intestine of diabetic rats (Wang *et al* . 2007) and inhibits 39.5% of  $\alpha$  – glucosidase ( Ram'irez *et al* . 2012). Alpha - glucosidase is an enzyme that degrade starch and disaccharides to glucose by a competitive inhibition of  $\alpha$ -glucosidase activity. it will reduce the absorbtion of glucose decrease (Rupp 2004).

Based on these studies . it is necessary to conduct further research on the interaction between the flavonoid compounds in guava leaves as a ligand and a target receptor of  $\alpha$  - glucosidase. This research was conducted out by simulating molecular docking file molecular between the ligand and the receptor conformation that would predict the receptor - ligand complex. Prediction conformation bond between the ligand and the receptor protein is a very important stage in further drug development ( Trott and Olson 2010). This study used the drug acarbose as a comparison. since flavonoids from leaves of guava has the same activity with acarbose inhibits  $\alpha$  - glucosidase enzyme.

## **RESEARCH METHODS**

### **Preparation of Three Dimensional Structure of $\alpha$ – glucosidase.**

Three-dimensional structure of the enzyme  $\alpha$  - glucosidase in .pdb format downloaded from the RCSB PDB accessed through

<http://www.rcsb.org/pdb/home/home.do>. Based on previous research conducted Roy ( 2013 ) obtained  $\alpha$  - glucosidase enzyme macromolecules with PDB ID 2QMJ. 2QMJ macromolecules is the crystal structure of the N - terminal maltase - glucoamylase human subunit and an enzyme - ligand complex structure so that the removal of ligands proceed by using Discovery Studio Visualizer 4.0 program. Alpha glucosidase separation results that have been clean of residue stored in pdb format .

### **Determination of Pocket Cavity $\alpha$ - glucosidase**

Pocket Cavity of the enzyme was visualized using online software Pocket – Cavity Search Application ( POCASA ) 1.0

( [http://altair.sci.hokudai.ac.jp/g6/Research/POCASA\\_e.htm](http://altair.sci.hokudai.ac.jp/g6/Research/POCASA_e.htm) ) by entering the code PDB ID of macromolecular enzyme obtained from RCSB PDB database .

### **Preparation of Three Dimensional Structure of Ligands**

The structure of the ligand in a format designed using software .mol ACDLabs and refers to PubChem compound databases was (<http://pubchem.ncbi.nlm.nih.gov/>). The format of the file was then converted into .pdb using software Vega ZZ . The ligands used were derived flavonoid compound leaves of guava ( *Psidium guajava* L. ) that apigenin . avikularin . guaijaverin . hiperin . isokuersetin . kaempferol .

leukosianidin . mirisetin . quercetin and kuersitrin ( Singh and marar 2011; Kumari *et al* . 2013 ) and acarbose.

### **Preparation of tethering ( Docking File )**

Preparation docking file was conducted with Autodock software tools . In the preparation of the ligand carried torque settings . while the  $\alpha$  - glucosidase enzyme preparation by adding a polar hydrogen atoms and set the grid box by looking at the coordination of the active site on the target receptor . Setting the grid box in macromolecular receptor to regulate an area or location will interact and bind ligands with residues the enzyme. Determination of the grid box notice two parameters: the size of the grid box and the center of the ligand which will be docking . In the study conducted by Roy *et al.* ( 2013 ) noted that the center coordinates X = -17 151 ; Y = -4154 ; Z = -22 157 with size X = 60 ; Y = 60 ; Z = 60 and spacing of 0.375 Å . Ligands and macromolecules reseptor files stored in pdbqt format and stored in the same folder in c drive windows .

### **Docking Process**

Docking parameters set in the configuration file was created in notepad . ligands containing the file name . file receptors . size and center of the grid box . the output file and exhaustiveness . In this study docking process completed by using software Autodock Vina run the command prompt .

### **Docking Analysis**

Analysis of docking results through notepad with a view free energy and RMSD values were displayed from the log file output results docking .

### **Visualization of Complex Enzyme - Ligand Interaction**

Visualization of complex interactions  $\alpha$  - glucosidase enzyme - ligand performed using PyMOL software . Previous results ligand output format .pdbqt converted into .pdb format Open Babel 2.3.2 software that read by PyMOL .

### **Drug Scan**

Drug Scan using online software ACD I- Labs that can be accessed through <https://ilab.acdlabs.com/iLab2/>

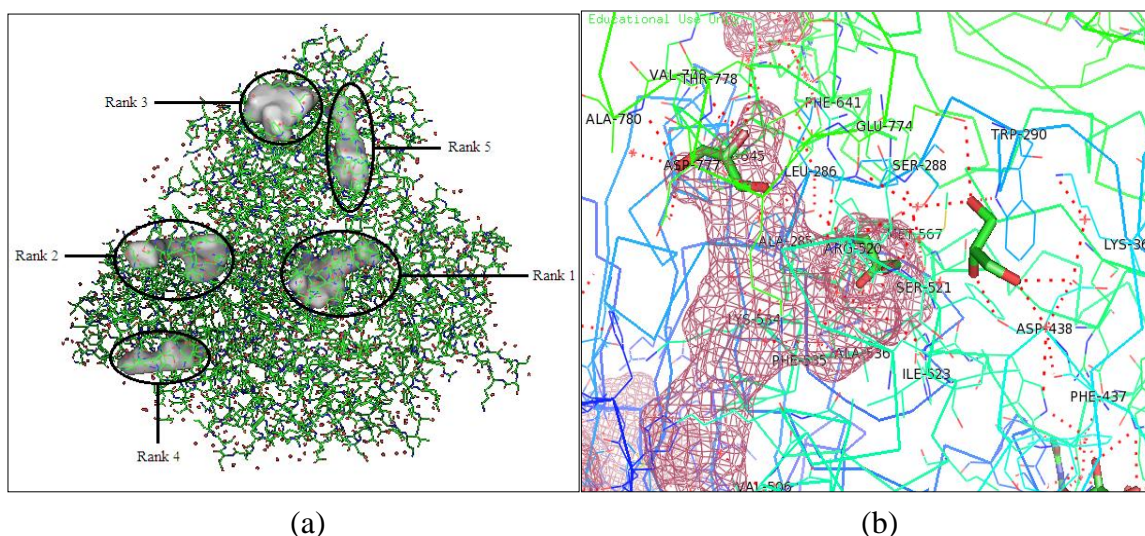


## RESULTS AND DISCUSSION

### Pocket Cavity enzyme $\alpha$ - glucosidase

Enzymes as protein showed biological responses through interaction with the inhibitor. Generally, this interaction occurs in area of surface protein commonly called pocket ( sac ) or the cavity was covered with a protein commonly called cavities ( Yu et al . . 2010). Therefore . the determination of the pocket and  $\alpha$  - glucosidase enzyme cavity finished before the docking process of ligand - receptor predict the binding site on the enzyme  $\alpha$  - glucosidase as a receptor .

There were 27 pockets and 3 cavities in 2QMJ but only 5 pockets that have a higher probability as the binding site at residues 693 . 267 . 593 . 49 and 1009 as shown in Figure 1a . Pocket with the highest possible role as a binding site was a pocket residues 693 to 207 volumes . VD value of 945 and had the amino acid residues Trp 290. Glu 774. Leu 286. Phe 641. Val 506. Ile 523. Ser 288. Asp 438. Asp 777. Thr 778. His 645. Ala 536. Ser 521. Phe 535. Ala 285. Val 779. Ala 780. Met 567. Lys 534. Arg 520. Lys 360 and Phe 437 as shown in Figure 1.b. Residue position 693 which was the first rank that used as a reference for comparison of the results of docking .



**Figure 1. Visualization ( a ) Top 5 Pocket Cavity . ( b ) Amino Acid Residues Pocket Rank1 Molecular Docking**

Docking process is divided into two phases, the scoring function and the use of algorithms process. Scoring function estimate the binding affinity between macromolecules and ligands, and algorithms process will be determine the most stable conformation of complex formation. The value of the free energy generated by the

enzyme - ligand complexes represented by the affinity of the ligand to the receptor. The highest affinity of a ligand to the enzyme . the smallest the value of the free energy. If the value of the free energy was greater the affinity become smaller. High affinity of a ligand to the enzyme produced from large intermolecular force between the ligand and the enzyme. Affinity is the strength of a bond or two molecules tendency to form a bond . which will produce a stable conformation.

**Table 1. Value of Free Energy Enzyme - Ligand Complexes**

No.	Ligan	$\Delta G^{\circ}$ (Kcal/mol)
1	Acarbose (Standart of ligan)	-7.8
2	Guajaverin	-9.7
3	Kuersitrin	-9.7
4	Avikularin	-9.5
5	Hiperin	-9.5
6	Isokuersetin	-9.5
7	Mirisetin	-9.1
8	Kuersetin	-9.0
9	Apigenin	-8.7
10	Leukosianidin	-8.7
11	Kaempferol	-8.6

Results showed that there were two ligands with the smallest value of the free energy it is guajaverin and kuersitrin with free energy value -9.7 kcal / mol. These two ligands had a great affinity to the enzyme  $\alpha$  - glucosidase and gave the most stable conformation. Acarbose only had the free energy of -7.8 kcal / mol .Acoriding to their results.  $\alpha$ -glucosidase prefer to bind to ligands of guava leaves flavonoids compared to acarbose. Flavonoids of guava leaves can be further developed to be an antidiabetic drug candidate because it has good affinity . even better than acarbose compounds.

#### **Visualization of Complex Enzyme - Ligand Interactions**

Visualization process showed the form of hydrogen bonds between the enzyme and its ligand showed the amino acid residues with the ligand -bound enzyme (so it can be compared with the catalytic enzyme known from pocket cavity search). More interaction of the ligand with the amino acid residues in the active site . it is

expected that inhibition of ligand function be better .Here is the contact residues and their respective ligand hydrogen bond distance .

**Table 2. Amino Acid Ligand - Enzyme Residues Contact**

No.	Ligan	Residues (Hydrogen binding spacing (Å))
1	Acarbose (Standart of ligan)	Asn 207 (2.9) ; Thr 544 (3.1) ; Arg 526 (2.7; 3.3) ; Asp 203 (2.2) ; Asp 542 (2.0) ; Thr 205 (2.8)
2	Apigenin	<b>Val 779</b> (2.8) ; <b>Thr 778</b> (2.8; 3.1) ; <b>Arg 520</b> (2.4)
3	Avikularin	<b>His 645</b> (3.3) ; <b>Val 779</b> (3.0) ; Lys 776 (3.3)
4	Guaijaverin	<b>His 645</b> (3.2) ; <b>Arg 520</b> (3.2) ; Lys 776 (3.2) ; <b>Phe 535</b> (2.7) ; <b>Thr 778</b> (2.8; 3.3)
5	Hiperin	Arg 283 (2.7) ; <b>His 645</b> (2.9) ; <b>Asp 777</b> (2.7) ; <b>Thr 778</b> (2.9; 3.2) ; <b>Val 779</b> (3.0) ; <b>Ala 780</b> (3.6)
6	Isokuersetin	<b>Phe 535</b> (2.3) ; <b>Thr 778</b> (2.8; 3.2) ; <b>Val 779</b> (2.9; 3.5) ; <b>Ala 780</b> (3.5) ; Arg 283 (2.3) ; <b>Asp 777</b> (2.6) ; <b>His 645</b> (3.0)
7	Kaempferol	<b>Phe 535</b> (2.0) ; <b>Thr 778</b> (2.8; 3.2) ; <b>Val 779</b> (3.0)
8	Leukosianidin	<b>Lys 534</b> (2.7) ; <b>Thr 778</b> (2.8; 3.2) ; <b>Val 779</b> (2.8; 3.4) ; <b>Ala 780</b> (3.3) ; <b>His 645</b> (3.1)
9	Mirisetin	<b>Phe 535</b> (2.1) ; Thr 775 (2.6) ; <b>Thr 778</b> (2.8; 3.3) ; <b>Arg 520</b> (3.2) ; <b>His 645</b> (3.3)
10	Kuersetin	<b>Thr 778</b> (2.7; 3.2) ; <b>Val 779</b> (2.9; 3.3) ; <b>Ala 780</b> (3.3) ; <b>His 645</b> (3.1)
11	Kuersitritin	<b>Ser 521</b> (2.1) ; <b>Val 779</b> (3.2) ; <b>His 645</b> (3.4; 3.4) ; Lys 776 (3.0) ; <b>Phe 535</b> (2.5)

Description : Bold text is the target residue

Table 2 showed that the average contact occurs 3-7 amino acid residues . Ligands that have little contact residues that apigenin . kaempferol with 3 avikularin and contact residues . These three amino acid residues apigenin and kaempferol owned is the target residue . whereas in avikularin only 2 that is the target residue . Ligands that have the most contact residues are isokuersetin with 7 contact residues but only 6 that is the target residue . Mirisetin and quercetin have 5 contacts with only four residues that

are the target residue . While hiperin has 6 contact residues with residues and leukosianidin 5 targets have 5 contacts residues that are all a target residue . Both ligands with the best value of free energy that is guaijaverin and kuersitrin has 5 contacts residues but only 4 which is the target residue ( Figure 2.a and 2.b ) . Acarbose as standard of ligands having amino acid residues that are very different from other ligands as shown in Figure 2.c and not a target residue .

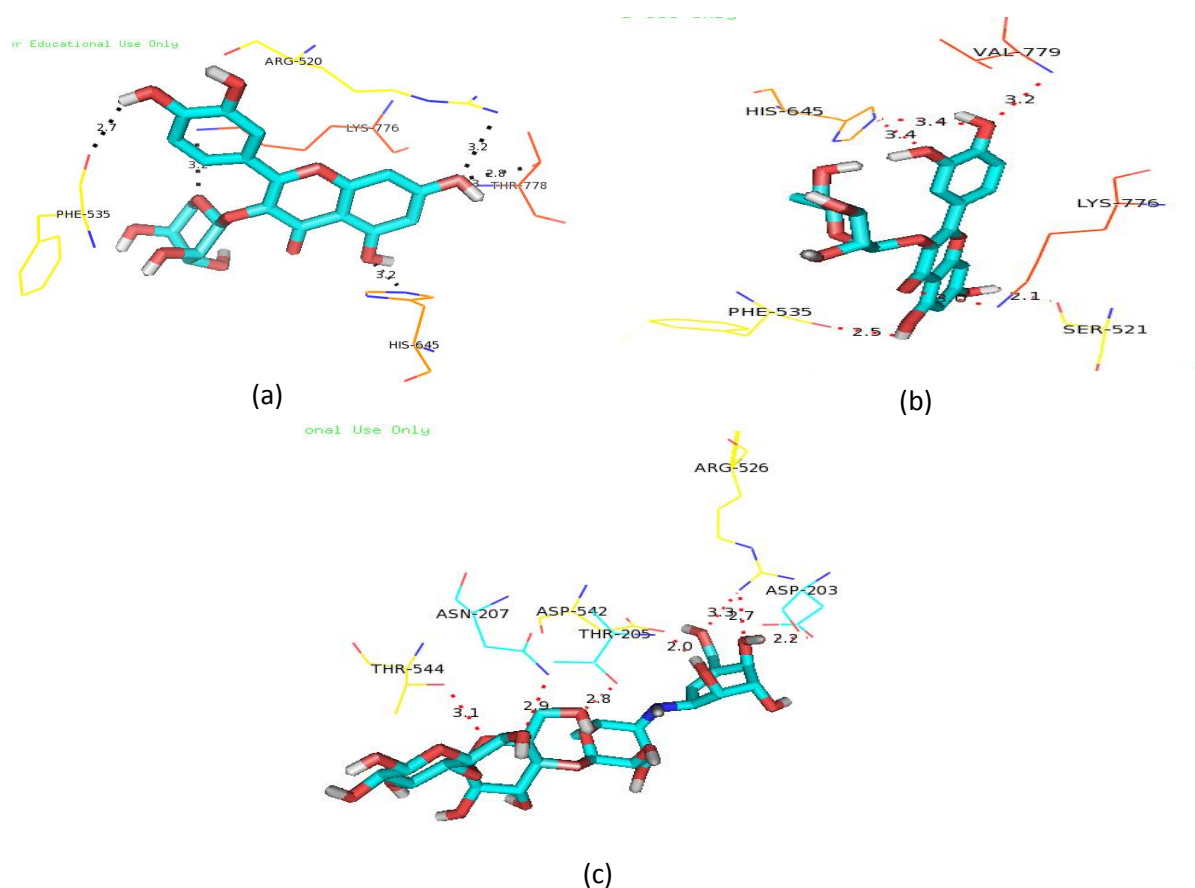


Figure 2. Visualization Interaction ( a ) Guaijaverin . ( b ) Kuersitrin . ( c ) Acarbose on  $\alpha$  - glucosidase

## Drug Scan

Drug scan was performed on the results of docking to observe the similarities between the properties of the ligands tested and existing drugs ( Drug Likeness ) . Drug Likeness viewed using Lipinsk 's rules ( Lipinski 's Rule of Five ) . This rule help to distinguish between drug molecules and non- drug like like having regard to the extent of absorption or permeability of the lipid bilayer that is contained in the human body.

The criteria Lipinski 's Rule of Five are less than 500 g / mol . logP less than 5 . the hydrogen bond donor and acceptor is less than 5 hydrogen bond is less than 10 ( Lipinski 2001). Results of the scan drug ligand docking results with online software ACD I- Labs prepared in table 3

**Table 3. Drug Scan Result**

No.	Ligan	LogP	BM (g/mol)	H-Bond Donor	H-Bond Acceptor
1	Acarbose (Standart of ligan)	<b>0</b>	645.6	14	19
2	Apigenin	<b>2.56</b>	<b>270.24</b>	<b>3</b>	<b>4</b>
3	Avikularin	<b>0.34</b>	<b>434.35</b>	7	11
4	Guaijaverin	<b>-0.04</b>	<b>434.35</b>	7	11
5	Hiperin	<b>-0.17</b>	<b>464.38</b>	8	12
6	Isokuersetin	<b>-0.17</b>	<b>464.38</b>	8	12
7	Kaempferol	<b>1.98</b>	<b>286.24</b>	<b>4</b>	<b>6</b>
8	Leukosianidin	<b>-0.41</b>	<b>306.27</b>	6	7
9	Mirisetin	<b>1.4</b>	<b>318.23</b>	6	8
10	Kuersetin	<b>1.82</b>	<b>302.24</b>	<b>5</b>	<b>7</b>
11	Kuersitrin	<b>0.29</b>	<b>448.38</b>	7	11

Description : Bold text is fulfilling the criteria

There were only 3 ligands that met all four criteria Lipinski 's Rule of Five: apigenin . kaempferol and quercetin . Other ligands only met the criteria for logP and BM have logP value of less than 5 and less than 500 BM . while the hydrogen bond donor and acceptor did not met the criteria . While acarbose as ligands only met one of 4 criteria BM logP because it has more than 500 g / mol is 645.6 g / mol . hydrogen donor and acceptor hydrogen 14 19.

LogP value indicates the nature of polarity. that when the value was  $> 5$  . the non -polar . so that all ligands have tended polar properties because it has a logP value below 5 and will be more easily excreted by the body and does not cause toxic . Molecular weight of a drug candidate compounds should not be too large ( $< 500$  g / mol). because it will be difficult to penetrate the membrane that can affect the

bioavailability of drugs in the body . The amount of hydrogen donor and acceptor also affect the permeability of the membrane to penetrate because it can increase the area of the polar surface . All ligands flavonoids have BM below 500 g / mol and logP no more than 5 . while the BM acarbose has more than 500 g / mol . so theoretically ligand guava leaf flavonoids have better bioavailability than acarbose .

## CONCLUSION

From the results of the three-dimensional structure of the molecule tethering ligands flavonoids guava leaves ( *Psidium guajava* L. ) against  $\alpha$  - glucosidase enzyme receptor . it is known that the enzyme - ligand complexes with  $\alpha$  - glucosidase acarbose has a free energy value of 7.8 kcal / mol . whereas flavonoid ligands of guava leaves ( *Psidium guajava* L. ) has a lower free energy than acarbose and there are 2 ligand which has the lowest free energy is guaijaverin and kuersitrin the value of the free energy of - 9.7 kcal / mol .

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# THE EFFECT OF $\beta$ -LACTAM ADMINISTRATION ON NEW INFECTION BY ESBL PRODUCTION BACTERIA AT PERSAHABATAN HOSPITAL

Numlil Khaira Rusdi, Tri Kusumaeni\*, Erna Pratiwi

Faculty of Pharmacy UHAMKA, Persahabatan Hospital

## ABSTRACT

The excessive administration of antibiotics betalactame, especially third generation cephalosporin widely for the treatment of infections in hospital was thought to be one factor contributing to infection by bacteria Extended-Spectrum-Betalactamase (ESBL). In hospital Persahabatan known that many third-generation cephalosporin antibiotic used for the treatment of hospitalized patients. This study aimed to determine the effect of beta-lactam antibiotics against infection by the emergence of ESBL-producing bacteria at Persahabatan hospital Jakarta period January to March, 2013. This study was case control study.involving patients with ESBL positive as case group and ESBL negative as control group. Historical use of antibiotics during hospitalization in Persahabatan traced in medical records retrospectively. Data were analyzed using chi-square test or fisher exact test followed by odd ratio calculation and calculate the DDD (Defined Daily Dose) to determine the quantity administration of antibiotic betalactame. A total of 100 patients.50 patients are included into the case group and 50 into the control group. Bivariate analysis showed that a history of antibiotic Betalactame use in general ( $p = 0.357$ ) was not a factor influencing the emergence of infections by ESBL-producing bacteria. Historical administration of ceftriaxone (third-generation cephalosporin) ( $p = 0.016$ , OR = 2.705, 95% CI = 1.197 to 6.113) were factors that influence the emergence of infections by ESBL producing bacteria.

**Keywords:** ESBL, antibiotics, Betalactame, ceftriaxone, cephalosporins

## BACKGROUND

ESBL (*Extended Spectrum Beta Lactamase*) originally appeared in Western Europe allegedly due to inappropriate use of expanded-spectrum  $\beta$ -lactam antibiotics. In a short time ESBL was detected in the United States and Asia. ESBL prevalence varied among countries and institutions. In the United States ESBL in *Enterobacteriaceae* 0-25%. Among the isolates resistant to *ceftazidime* percentage of 5% in non-intensive care unit (non-ICU) and 10% in the ICU. The prevalence of ESBL *Enterobacteriaceae* in Europe varied widely. In Netherlands, survey in 11 hospital laboratories found that ESBL <1% of and *K. Pneumoniae*. in France 40% *K. pneumoniae* were resistant to ceftazidime. For Europe the incidence of ceftazidime resistance to *K. Pneumoniae*



reached 20% (non-ICU) and 42% (ICU). In Japan, the percentage of ESBL by *E. coli* only <0.1% and 0.3% by *K pneumoniae*. Percentage of ESBL *E. coli* and *K. Pneumoniae* 4.8% in Korea, 8.5% in Taiwan and 12% in Hong Kong (Emery et al., 1997, Jain et al A. 2008).

ESBL-producing bacteria that produced enzymes which could destroy beta-lactam ring and raised resistance to beta-lactam class of antibiotics. ESBL was an enzyme that could hydrolyze penicillins, cephalosporins generation I, II, III and aztreonam (except Cephameycin and carbapenem) (Winarto 2009). ESBL enzymes derived from  $\beta$ -lactamase mutant. This mutation caused an increase in the enzymatic activity of  $\beta$ -lactamase which could hydrolyze the third generation cephalosporins and aztreonam (Paterson and Bonomo 2005).

The use of third-generation cephalosporins antibiotics widely for the treatment of infections in hospitals was thought to be one of the causes of infection by ESBL-producing bacteria (Paterson and Bonomo 2005). In addition to overuse of antibiotics, patients with severe disease, long LOS (*Length of Stay*) and treated with medical devices which were invasive (urinary catheters, venous catheters and endotracheal tube) were also other factors to be infected by ESBL-producing bacteria (Paterson and Bonomo, 2005, Nathisuwan et al. 2001).

Data from research conducted in *Persahabatan* Hospital was found that the third-generation cephalosporin antibiotic used for the treatment of ICU patients (Liskandra R 2012). Data from Clinical Microbiology Laboratory also showed an increase in patients with positive (+) infected with ESBL-producing bacteria. However, research data on the effects of beta-lactam antibiotics against infection by the emergence of ESBL-producing bacteria in the RSUP *Persahabatan* Jakarta was still unknown. Therefore, it required further study.

## **OBJECTIVES**

This study aimed to determine the effect of beta-lactam antibiotics against infection by the emergence of ESBL-producing bacteria at *Persahabatan* hospital Jakarta period January to March, 2013.

## METHODOLOGY

This study used a case-control study. the sample was divided into two groups: case group (positive ESBL) and control groups (negative ESBL). Data were analyzed by chi-square test or Fisher test using SPSS 17 and then calculated odds ratios. and calculate the quantity of beta-lactam antibiotics using ATC/DDD method.

## THE RESULTS

### A. Characteristics of Sample

In this study. the research subjects were 100 patients consisted of 50 cases and 50 controls.

#### 1. Characteristics of patients by Gender and Age

Of the total sample were included in this study. in terms of gender in the case group of men obtained a total of 37 patients (74%) and women were 13 patients (26%). while the control group 40 male patients (80 %) and women by 10 patients (20%). Based on the age. the average age of the study sample was 50 years old. The average age of the case group was 53 years and the control group was 52 years.

**Table 1. Characteristics of patients by Gender and Age**

Characteristics	Case group	Control group (ESBL -)
	(ESBL +) n = 50	n = 50
<b>Gender</b>		
Male	37 (74%)	40 (80%)
Female	13 (26%)	10 (20%)
<b>Average age</b>	53 years old	52 years old

#### 2. Typical of Bacterial

Of the total sample were included in this study. the *Enterobacteriaceae* bacteria cause most infections are *Klebsiella sp.* (82%) and *Escherichia coli* (18%).

**Table 2. Typical of Bacterial *Enterobacteriaceae* In Study Sample**

Type of	Case group	Control group	Total n = 100
	(ESBL +) n = 50	(ESBL -) n = 50	
<i>Klebsiella. sp</i>	36	46	82 (82%)
<i>Escherichia coli</i>	14	4	18 (18%)

**B. Influence of Antibiotics *Beta-lactams* on the incidence of infection by ESBL-producing bacteria**

Data were collected in this study found that 79% of patients receiving the third-generation cephalosporins. 3% of patients receiving fourth generation cephalosporins. 17% of patients receiving carbapenem and 6% received a combination of penicillin.

**Table 3. Influence of Antibiotics *Beta-lactams* on the incidence of infection by ESBL-producing bacteria**

Typical of Antibiotics	ESBL (Total of patient)		P Value	OR	CI 95% (lower-upper)
	ESBL +	ESBL -			
<b><i>Beta-lactams</i></b>	47 (51.1%)	45 (48.9%)	0.357*	1.741	0.393-7.713
Cephalosporin third generation	41 (51.9%)	38 (48.1%)	0.461	1.439	0.545-3.797
<b>Ceftriaxone</b>	28 (63.6%)	16 (36.4%)	<b>0.016</b>	2.705	1.197-6.113
Ceftazidime	13 (39.4%)	20 (60.6%)	0.137	0.527	0.226-1.231
Cefotaxime	3 (50.0%)	3 (50.0%)	0.661*	1.000	0.192-5.210
Cefixime	6 (60.0%)	4 (40.0%)	0.505	1.568	0.414-5.935
Ceftizoxim	1 (33.3%)	2 (66.7%)	0.500*	0.490	0.430-5.582
Cefoperazone	1 (50.0%)	1 (50.0%)	0.753*	1.000	0.610-16.444
Cefoperazone + sulbactam	3 (100%)	0 (0%)	0.121*	0	
Cephalosporin fourth generation	3 (100%)	0 (0%)	0.121*	0	

Cefepime	3 (100%)	0 (0%)	0.121*	0	
Carbapenem	10 (58.8%)	7 (41.2%)	0.424	1.536	0.533-4.422
Meropenem	10 (58.8%)	7 (41.2%)	0.424	1.536	0.533-4.422
Combination of Penicillin	2 (33.3%)	4 (66.7%)	0.339*	0.479	0.084-2.743
Ampicillin + sulbactam	2 (40.0%)	3 (60.0%)	0.500*	0.653	0.104-4.085
Amoxicillin + Clavulanat	0 (0%)	1 (100%)	0.500*	0	

OR= odd ratio. CI= confidence interval

\*p value derived from fisher test where the expected value <5 more than 20%

Based on bivariate analysis with chi-square test on the use of antibiotics on the incidence of infection by ESBL-producing bacteria from the case group and the control group showed that the use of beta-lactam antibiotics in general. the probability value (p) is greater than 0.05 (p = 0.357). Results obtained from this study indicate that the use of beta-lactam antibiotics in general in hospitalized patients was not a causative factor affecting the incidence of infection by ESBL-producing bacteria or did not have a significant relationship to the occurrence of ESBL-producing bacteria injection. However, if viewed from all variables tested, history of the use of ceftriaxone (third generation cephalosporin) probability value (p) less than 0.05 (p = 0.016). It showed that ceftriaxone were factors that influence the emergence of ESBL-producing bacterial infection.

Data statistical that included the use of ceftriaxone (one third generation cephalosporin antibiotic) with using Chi-square, the value of the probability (p) on the Sig (2-tailed) is smaller than 0.05 (p = 0.016), meaning that there was a significant influence between the use of ceftriaxone with the emergence of ESBL-producing bacterial infection. Value Odd Ratio (OR) = 2.705 (95% CI = 1.197 to 6.113), indicating that the patient were hospitalized or ICU that using ceftriaxone have 2.705 times greater risk of infection of ESBL-producing bacteria than who did not use any ceftriaxone. The results of the bivariate analysis of the use of ceftriaxone using Chi-square test.

The results were consistent with the theory proposed by Paterson and Bonomo (2005) stated that the use of antibiotics third generation cephalosporins widely for the treatment of infections in hospitals was one of the causes of the infection or colonization of ESBL-producing bacteria.

Agno Pajariu (2010) at DR Kariadi hospital found that generally using antibiotics. cephalosporins and fluoroquinolones or combination therapy was not causes of infection by ESBL-producing bacteria. However. the use of ampicillin and gentamicin are the factors that cause infections by ESBL-producing bacteria.

The result was different probably due to a history of the use of ceftriaxone (third generation cephalosporin) widely used as empiric therapy in almost all inpatients and *Persahabatan* Hospital ICU in order to see any significant differences would influence the use of ceftriaxone with the incidence of infection infections by ESBL-producing bacteria. Ceftriaxone was widely used because it has advantages such as ceftriaxone which was a broad-spectrum antibiotic that can be used for infections by gram positive and negative bacteria and the price was relatively low leading to high use of ceftriaxone in hospitalized patients and ICU. Another thing that caused the results of this study differed from previous studies used of antibiotics prior to the patient being treated was not known and may be a confounding variable that could not be controlled because of the limited information that was only obtained from the medical records of patients.

### C. Quantity Use of Antibiotics Betalactam (DDD /day)

**Table 4. The use of antibiotics Beta-lactams by top five use.**

No	ATC	Antibiotics	Case	Control	Totality
			group	Group	DDD/day
			DDD/day	DDD/day	
1	J01DD04	Ceftriaxone	26.58	17.59	44.17
2	J01DD02	Ceftazidime	11.82	12.54	24.36
3	J01CR01	Ampicillin+sulbactam	7.26	12.27	19.53
4	J01DH02	Meropenem	10.48	8.33	18.81
5	J01DD08	Cefixime	2.57	2.08	4.65

Type of beta-lactam antibiotics used were of the highest ceftriaxone which was equal to 44.17 DDD /day (cases of 26.58 DDD / *day* and control groups at 17.59 DDD / *day*). Ceftriaxone was a third generation cephalosporin class of antibiotics that had a broad-spectrum antibiotic that was useful in the fight against infections due to gram-positive and negative bacteria (Katzung 2004). Ceftriaxone was mainly used for the treatment of tract infections. respiratory tract. biliary tract. abdominal infections. skin. bones. joints. soft tissue. as well as for the prevention of infection before and after surgery (Ganiswara S 2008).

Advantages of ceftriaxone had a half-life of 7-8 hours could be injected or given every 24 hours (once per day). Ceftriaxone was given by infusion or bolus once daily showed no significant difference in the concentration of drug in the blood. both in patients with normal renal conditions. as well as in patients with renal impairment conditions (Liskandra R 2012). Ceftriaxone ability to penetrate throughout the network and across the brain barrier into consideration in the selection of antibiotics. so it could be used as a therapeutic treatment of severe infections including infections. In addition to the brain. these drugs also penetrate well into the bone. Drug was excreted through the gallbladder and no dose adjustment was necessary in a state of decline in kidney state so that it could be used in psien renal insufficiency (Katzung 2004). In addition. the relatively low price of ceftriaxone could also cause high possibility of use in hospitalized patients. Based on the formulary of this health insurance. ceftriaxone and cefotaxime were the first line of infectious diseases.

Disadvantages of cephalosporins were often caused hypersensitivity reactions to penicillins identical. fever. nephritic. skin rash. hemolytic anemia and even anaphylaxis. Local irritation may occur after intramuscular injection and tromboplebitis after intravenous injection (Katzung 2004).

### **1. Use of Antibiotics *Beta-lactams* In Case Group and Control Based on Therapeutic Class**

The use of beta-lactam antibiotics based therapy group obtained the result that the use of third-generation cephalosporins in the case group (ESBL +) was greater than the control group (ESBL-).

**Table 5. Use of Antibiotics *Beta-lactams* In Case Group and Control Based Therapeutic Class**

No.	ATC	Therapeutic Class	Cases Group (DDD/day)	Control group (DDD/day)
1	J01DD	Cephalosporin third generation	43.95	36.72
2	J01CR	Combination of Penicillin	7.26	14.58
3	J01DH	Carbapenem	10.48	8.33
4	J01DE	Cephalosporin fourth generation	2.18	0

## CONCLUSION

Based on the results of research on the effect of beta-lactam antibiotics against infection by the emergence of ESBL-producing bacteria in the department of *Persahabatan* Jakarta. January-March 2013 can be summarized as follows:

1. The use of beta-lactam antibiotics was generally not a factor for the emergence of infection by ESBL-producing bacteria at *Persahabatan* hospital period January to March. 2013.
2. The use of ceftriaxone (third generation cephalosporin) ( $p = 0.016$ . OR = 2.705. 95% CI = 1.197 to 6.113) were the factors that most influence in the emergence of infections by ESBL-producing bacteria at *Persahabatan* hospital period January to March. 2013.

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**DETERMINATION OF TOTAL FLAVONOID CONTENT AND  
ANTIOXIDANT ACTIVITY ASSAY IN SEVERALLY PART OF ETHANOL  
FRACTION OF CASSIAVERA (*Cinnamomum burmanii* [Nees] Bl)**

**Dira. Wida Ningsih and Vinni Rahayu Ningsih**

*Sekolah Tinggi Farmasi Indonesia Yayasan Perintis Padang*

*Email : [dira06139060@yahoo.com](mailto:dira06139060@yahoo.com)*

**ABSTRACT**

A research have been performed concerning determination of total flavonoid content and antioxidant activity assay in severally part of cassiavera (*Cinnamomum burmanii* [Nees] Bl) by spectrophotometry UV-Visible. Ethanol fraction obtained from soaking residue that has been done soaking with hexane. Quersetin was used as standart compound for determination of total flavonoid and antioxidant activity. Where total flavonoid content in ethanol fraction of cassiavera part of root. stick. branch and twigs to accompany are equivalent to quersetin 2.75 mg/g. 3.681 mg/g. 3.328 mg/g and 2.037 mg/g respectively. Antioxidant activity in received of IC<sub>50</sub> values cassiavera of part of root. stick. branch and twigs to accompany are 25.731 µg/mL. 6.019 µg/mL. 1.517 µg/mL and 2.498 µg/mL respectively. The result of statistical test using ANOVA one way program given a significant different (p<0.05) for total flavonoid on severally part of cassiavera.

Keyword: total flavonoid. antioxidant. severally part of cassiavera. *Cinnamomum burmanii* [Nees] Bl

**INTRODUCTION**

*Cinnamomum burmanii* [Nees] Bl is a multipurpose plant producing cinnamon (cassiavera) located in Kerinci district. West Sumatra and Tapanuli. Cassiavera can be obtained from the bark. bark of branches. twigs of the plant *C. burmanii* [Nees] Bl which has been processed further (Rismunandar. 1998). In trade. cinnamon sold based on the specified quality of the leather part of what was taken. Where cinnamon bark plants sold divided into bark. bark of branches. twigs and bark of the roots (Tjahjadarmawan. 2011). It is based on the thickness of the bark of cinnamon plants. The thickness will affect the sales price. But no discussion of the distribution of chemical compounds in plants evenly and an amount equal to the difference in the thickness of the bark of cinnamon and an explanation of the likelihood of the thickness

will affect the composition and activity of contents chemical compounds in plant bark cinnamon. Cinnamon bark contains compounds that are very useful. Fraction of cinnamon bark contains sinamaldehyda, eugenol, Anethole, cinnamic acid, ethyl cinnamate, flavonoids, saponins, tannins, and terpenoids (Tjahjadarmawan, 2011).

From the research Azima, ethanol extract of cinnamon contains tannin, alkaloids, steroids, flavonoids and saponins. Where tannin and flavonid been reported to function as an antioxidant (Azimaa, et al. 2004). Flavonoids are secondary metabolites which includes one of the largest natural phenols in plants. Flavonoids are used as an antioxidant, which has a molecular structure that can provide electrons to the free radical molecules that break the chain reaction of free radicals (Pourmorad, et al. 2006). The last few years have seen considerable interest to obtain a natural antioxidant. Studies show that phenolic compounds such as flavonoids have antioxidant activity of superoxide radical catcher (Gulcin, et al; 2002). Based on this, the researchers wanted to make the determination of total flavonoid content of some parts of bark cinnamon by using spectrophotometric method and antioxidant activity by using DPPH (2,2-diphenyl-1-picryl hidrazyl) as free radical.

## **METHODS**

### **Materials**

The materials are used some parts of bark cinnamon (*C. burmanii* [Nees] Bl), distilled water, aluminum chloride, potassium acetate, ethanol 70 %, ethanol pa, quercetin and DPPH (2,2-diphenyl-1-picryl hidrazyl).

### **Preparation of Samples**

Samples were taken in the area Siulak, Kerinci district. Parts of the bark cinnamon which is processed into a fine powder form. Some parts were taken that bark of root, stick, branch and twigs.

### **Extraction of Samples**

1 kg of powder each part of bark macerated with n-hexane, performed for 5 days with 2 repetitions in maceration bottle while stirring occasionally, then filtered. While the residual obtained, dried and then macerated with 70% ethanol for 5 days with repetition 2 times in a bottle maceration with occasional stirring, and then filtered. All

the obtained filtrate were combined and then concentrated using a rotary evaporator. Condensed fraction obtained sample was then weighed.

#### **Determination of Total Flavonoids Content**

The total content of flavonoids in each part of cinnamon is determined based on the regression equation obtained in the calibration curve of quercetin.

#### **Determination Of The Wavelength Of Maximum Absorption And Establishment Of Calibration Curve Of Quercetin**

A series of Quercetin concentrations by ethanol and distilled water (1:1) by concentrations of 25. 50. 75. 100 and 125 mg /mL. This solvents then pipette approximately 0.5 ml and then were mix with 1.5 mL of ethanol and were added by 0.1 mL 10% aluminum chloride solution. 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water. This mixture then being vortex and incubated for 30 minutes in room temperature. Measurement of standard solution absorption were done in 200-800 nm wavelength using visible spectrofotometer to obtained the maximum absorption of it's wavelengths. The calibration curve was made by measuring absorbance of each series of concentrations at maximum wavelength of quercetin so its linear regression can be calculated.

#### **Measurement Of The Amount Of Total Flavonoids Content Of The Sample**

Measurement of Flavonoids concentration of a plant were determined by aluminum chloride reagent. The obtained Flavonoids concentration equivalent with quercetin each gram of weight of dry sample.

#### **Measurement Of Antioxidant Activities Using DPPH as Free Radical**

A series of reference solution of quercetin by concentrations of 1. 2. 3. 4 and 5 mg / mL and a series of sample solution in aquadest. An amount of 2 ml were pipette and inserted into the vials. and add 4 ml of DPPH solution into the vials. Let stand for 30 minute in dark location. The absorbance of a solutions were measured by UV-visible spectrophotometer in maximum wavelength of DPPH.

The absorbance value of ascorbic acids and references were measured as % inhibition and were counts by this formula :

$$\% \text{ Inhibition} = 1 - \left[ \frac{\text{sample absorbance and DPPH} - \text{sample absorbance without DPPH}}{\text{absorbance of DPPH control (without sample)}} \right]$$

Afterward we made a curved between quercetin reference solution concentration or sample with % inhibition so that the linear regression equation can be obtained. The activities were expressed by the value of  $IC_{50}$ .

## **RESULTS AND DISCUSSION**

In this study, the sample used is cinnamon bark taken from the Regional Siulak, Kerinci, Jambi. The purpose of this research is to investigate the levels of flavonoids in each part of plant and antioxidant activity. The sections were selected based on the parts of plants traded on the market. Fractionation was done by maceration method using ethanol 70%. Determination of total flavonoid content using a calibration curve of quercetin. Quercetin is used as a standard in the determination of total flavonoids, because it has a structure that represents the structures of other flavonoid compounds found in cinnamon bark. Quercetin has two benzene rings attached to the chain connected by bridges propane and oxygen (Arini, et al. 2003).

The results of the assay of flavonoid compounds in the bark of root; 2.75 mg / g. stick; 3.681 mg / g. branch; 3.328 mg / g. and twigs; 2.037 mg / g. Where the highest total flavonoid content was obtained from bark of cinnamon stick, this may be influenced by the thickness of cinnamon bark, which accumulates in tissues that thick which will serve as defense compounds. The thickness of the bark will affect the distribution of chemical compounds, in which the distribution is not equal to any parts of a plant. Where the composition of the compound will vary with the stem section that is at the branches, twigs or the root section, although the compounds contained the same but the numbers will be different. Flavonoids are secondary metabolites in young bark accumulates still low and then increases with increasing his bark. But the best quality when the flavonoids contained in plant metabolic processes running at maximum, which is when the bark is not too old and not too young (Yulianis, 2010). Based on statistical analysis by the method of one-way ANOVA followed by Duncan test using SPSS 17:00 shows that the flavonoid content of each fraction have significant differences at  $p < 0.05$ .

Test the antioxidant activity of each part of cinnamon bark done with DPPH. DPPH is a stable free radical at room temperature and rapidly oxidized due to air and light. DPPH blackish violet. Using DPPH for antioxidant activity chosen because it is simple, easy, fast, and sensitive and requires only a small sample. Antioxidant

compounds will react with DPPH radical by hydrogen atom donor mechanisms and causes decay DPPH color from purple to yellow color (Arjuna. 2004).

The antioxidant activity of the sample solution is expressed in percent inhibition. Percent inhibition is obtained from the difference between the absorbance of DPPH absorption with and without sample sample measured by UV-Visibel. The amount of antioxidant activity characterized by IC<sub>50</sub> values. ie the sample solution is needed to inhibit 50% of the free radical DPPH (Arjuna. 2004).

Test of antioxidant activity using DPPH obtained IC<sub>50</sub> values are: bark of root; 25.731 mg / mL. stick; 6.019 mg / mL. branch; 1.517 mg / mL. and the twigs; 2.498 mg / mL. Where this value indicates that almost all samples of ethanol fractions had IC<sub>50</sub> values greater than quercetin (comparator) which is worth 4.158 mg / mL. The smaller the IC<sub>50</sub> value. the stronger antioxidant activity.

From the research that has been done shows a pattern of antioxidant activity in all samples from low to high: the roots. stick. twigs and branches. Where allegedly obtained aktivitas antioxidants influenced by thick and age of cinnamon bark. thus affecting the content of substances that act as antioxidants (Yulianis. 2010). The ethanol extract was also better than the BHT with the protection factor of 1.08 (Azima<sup>b</sup>. et al; 2004). Whereas in other studies derived from cinnamon bark contains 263 mg equivalent of catechins / g spices. where it is proved that cinnamon contains proantosianin (Son. et al; 2009). From the data above proves that the ethanol extract of cinnamon bark contains flavonoids and has antioxidant activity. The big difference in the levels of flavonoids and antioxidant activity IC<sub>50</sub> of each piece of cinnamon bark of this plant may be due to differences in skin thickness which is influenced by the age of the plant cinnamon. thus affecting the distribution and accumulation of these compounds in cinnamon bark.

## **CONCLUSION**

Levels of total flavonoids found in cinnamon bark which highest in ethanol fractination is bark of stick. From the calculation. lowest IC<sub>50</sub> values obtained is from the bark of branch. where parts of this plant has the highest antioxidant activity than the other parts in each fraction.

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# THE CAPSULE AND TABLET FORMULATION OF ETHANOL EXTRACT OF RED GINGER (*Zingiber officinale*. Roxb.)

Fifi Harmely. Dira. Lilis Kurniawati. Chris Deviarny

Sekolah Tinggi Farmasi Indonesia Yayasan Perintis Padang  
Jl. Adinegoro / Simpang Kalumpang KM. 17 Lubuk Buaya Padang  
Email : [harmelyfifi@yahoo.co.id](mailto:harmelyfifi@yahoo.co.id)

## ABSTRACT

Red ginger is an efficacious plant that were widely used as a traditional medicine because it contains some chemical compounds e.g zingiberen. curcumin. farnesen. gingerol. etc. That has efficacy as an antioxidant particularly as an antihyperurisemia. This research was conducted to formulate the aethanol extract of red ginger in capsule dosage forms as an antihyperurisemia medicine. The formulation was made as capsule and tablete dosage forms by the dose of 300 mg. Capsule evaluation includes organoleptic. weight uniformity. disintegration time and hygroscopicity test. Tablete evaluation includes organoleptic. uniformity of weight and size. friability. hardness and disintegration time test. The result of these research showed that the aethanol extract of the red ginger can be formulated in capsule and has been fulfil the requirement test and can be formulated into tablet dosage form. but has not fulfil the requirement test of uniformity test of size. hardness and disintegration of time.

Keywords : Capsule. tablete. extract of red ginger. antihyperurisemia.

## INTRODUCTION

Red Ginger is an efficacious plant and widely used as a traditional medicine e.g stimulant. carminative. sore throat because it contains chemical compounds like *zingiberen* . *curcumin* . *farnesen* . *gingerol* . and *others* . which are efficacious as antioksidan particularly as anti hyperuricemia (Ramadhan. 2013). In the previous research. it had already been conducted an antihyperurisemia activity test of ethanol extract of red ginger (*Zingiber officinale* Roxb.) *in vivo and in vitro* (Mulyadi. 2013).

Based on the above. then in this research. try to formulate ethanol extract of red ginger (*Zingiber officinale* Roxb.) in the solid dosage forms. they are capsules and tablets. Capsules are solid dosage forms are encased in a hard or soft shell that can dissolve in the gastrointestinal tract (Syamsuni. 2006). The advantages of capsule is that. it has an interesting shape. easily swallowed. the capsule shell can cover the

unpleasant taste of the active substance. Tablets are solid dosage forms containing one or more active substances with or with no various excipients and made by clamping the powder mixture in a tablet machine (Siregar. 2007). The advantages of the tablets are. they may contain active ingredients in small or large quantities. can cover the unpleasant taste of the active substance. a practical oral dosage in storage.

## RESEARCH METHODS

The tools used in this research are: maceration bottle. rotary evaporator. furnes. digital scales. mortars. stamfer. measuring cup. beaker glass. test tube. spatula. tweezers. pipette. drip plate. vaporizer cup. crucible porcelain. sieve. desiccator. oven. calipers. capsule board. tablet printing machine. disintegrator tester. friabilator tester. infrared moisture balance.

The materials used in this research are: Ethanol extract of red ginger (*Zingiber officinale* Roxb.). Aerosil. gelatin. lactose. talc. disintegrator. Avicel PH 102. stearic acid. empty capsules 00 (Brataco). filter paper. HCl 1N. 70% ethanol. chloroform. ammonia. sulfuric acid. Mayer reagent. Mg powder. anhydrous acetic acid. FeCl<sub>3</sub>. norit. distilled water.

This research was conducted through several phases as follows:

1. Sampling and identification were performed in the Herbarium of Andalas (ANDA). Department of Biology. Faculty of Science of the University of Andalas. Padang.
2. Preparation of ethanol extract of red ginger rhizome by maceration using 70% ethanol. Maserat obtained is then concentrated with a rotary evaporator at a temperature of 30° - 40° C to obtain a thick extract.
3. Examination of the extract include organoleptic examination. determination of yield. phytochemical test. drying shrinkage and ash content (Harbone. 1987)

**Table 1. Capsule formula**

Composition	F 1	F 2
Extract of red ginger	300 mg	300 mg
Aerosil	-	22.8 mg
Lactose	329.6 mg	306.8 mg



Amprotab	100 mg	100 mg
Talcum	22.8 mg	22.8 mg
Stearic acid	7.6 mg	7.6 mg

The methods of making: All ingredients were weighed according to the formula. red ginger extract was dried with lactose portion (F1) and aerosil (F2). Disintegrator then added little by little in order to obtain a dry extract. Furthermore. the rest of the lactose was added little by little. talc and stearic acid. mixed until homogeneous. capsule mass was dried using an oven at 30 ° -40 ° C for 48 hours. after that the evaluation of capsule mass was inserted into the capsule shell no. 00 capsules weighing 0.760 g mass.

4. Evaluation of the capsule include organoleptic examination. disintegration time test. weight uniformity and hygroscopicity test

**Table 2. Tablet formula**

Composition	F 1	F 2
Extract of red ginger	300 mg	300 mg
Aerosil	12 mg	12 mg
Mucilago amili 20%	6 mg	-
Gelatin solution 10%	-	48 mg
Stearic acid	18 mg	18 mg
Avicel PH 102	237.6 mg	199.8 mg
Lactose	26.4 mg	22.2 mg

The methods of making: All ingredients were weighed according to the formula. Created mucilo amili 20% (F1) and a 10% gelatin solution (F2) as a binder in the manufacture of the granules. Thick red ginger extract was dried with aerosil. then added Avicel PH 102 and lactose little by little. Then added a binder to form a mass that was ready to be granulated. Granule mass sieved with a sieve with no. 16. the

result was dried in an oven at a temperature of 30° - 40°C for 48 hours. Once dried, sieved granules back with the sieve no. 18. then the evaluation of granul. afterwards stearic acid was added and molded into tablets.

7. Evaluation of the tablet include organoleptic examination. weight and size uniformity. disintegration time test. tablet friability and hardness test.

## RESULTS AND DISCUSSION

### *Examination of red ginger extract*

Extract examination aimed to determine the quality of the bulbs. The yield of red ginger extract was 12.79%. 9.68% and drying shrinkage ash content of 0.79%. Based on phytochemical test result that the red ginger extract contained flavonoids. terpenoids and phenolic.

### *The evaluation of capsule mass*

The evaluation of capsule mass covers. angle of rest. flow rate. compressibility and water content (Lachman et al. 1994; Voigt. 1995; Aulton. 1988). Table 3 shows the mass of the capsule of the two formulas had compressibility. good flow rate was > 10 g / sec. the resulted angle of rest also illustrated that the mass of the capsule had a good flow and water content of the two formulas met the requirement. that was <5%.

**Table 3. Results of the the capsule mass evaluation**

Evaluation	F1	F2
Bulk density (g/mL)	0.5263	0.4763
Tapped density (g/mL)	0.6250	0.5683
Compressibility (%)	15.67	16.19
flow velocity (g/detik)	10.35	11.11
the angle of rest (°)	25.2	25.15
Water content (%)	3.01	2.95

### *The evaluation of the capsule*

The evaluation of the capsule aimed to determine whether the capsules produced met the stipulated requirements. Capsule evaluation included organoleptic . weight

uniformity, disintegration time and hygroscopicity (Ministry of Health of the Republic of Indonesia, 1979; Augsburger, 2000).

The results evaluation of both capsule formulas met the requirements of uniformity of weight was not more than 2 capsules in defiance of 7.5% and none capsule that deviate more than 15%. The disintegration time test of both formulas also meet the requirements, that was <15 min. Hygroscopicity test to see the capsule ability to absorb moisture from the air after standing under the certain conditions (Hadisoewignyo, 2013). Hygroscopicity test on both formulas performed for 7 days, it appeared that the relative humidity of 70% with a temperature of 28 ° C did not change the weight of the capsule.



**Picture 1. Capsule mass F1 and F2**

**Table 4. Results of the evaluation of the capsule**

Evaluation	F1	F2	The requirement
Uniformity of weight (g)	0.7679 ± 0.00632	0.7632 ± 0.00447	not more than 2 capsules in defiance of 7.5% and none capsule that deviate more than 15%
Disintegration time test (min.)	5.33 ± 0.8165	6.83 ± 0.7528	<15 min
Hygroscopicity test (%)	0.18± 0.0652	0.17± 0.1127	Not hygroscopicity



**Picture 2. Capsule F1 and F2**

### ***The granules evaluation***

The purpose of the granules evaluation was to predict properties of the tablet characteristics which would be produced in order to obtain tablets that met the requirements. Evaluation of the granules include organoleptic examination, measure of a real density, incompressible density, the granules density, porosity, hausner factor, compressibility, flow velocity, angle of rest, water content and levels of fines (Lachman, 1994; Siregar, 2007; Hadisoewignyo, 2013).

**Table 5. Results of the granules evaluation**

Evaluation	F1	F2
Bulk density (g/mL)	0.5211	0.4903
Tapped density (g/mL)	0.5817	0.5210
Granul density (g/mL)	1.3866	1.4224
Porosity (%)	30.17	33.67
Hausner factor (g/mL)	0.42	0.37
Compresibility (%)	10.42	5.89
Flow velocity (g/detik)	10.89	10.42
Angel of rest(°)	24.9	28.8
Water content (%)	3.21	3.16
Level of fines (%)	3.68	4.22

### ***The tablet evaluation***

The tablet evaluation aimed to determine whether the tablet produced met the stipulated requirements. Tablet evaluation included organoleptic, uniformity of size and weight, hardness, friability and disintegration time of the tablets (Lachman, 1994; Siregar, 2007; Hadisoewignyo, 2013).

The tablet weight uniformity of both formulas, no more than two tablets that deviated more than 5% and none that deviated more than 10%, so it could be found to comply with the requirements of uniformity of weight. tablet friability test is useful to

determine the tablet resistance toward the shocks that occur during process of manufacturing, packaging, and distribution. Both formulas meet the requirements of having a tablet friability <0.8% (Hadisoewignyo, 2013).

Based on the evaluation results, the size uniformity of the tablet did not meet the requirements, because the diameter of the tablet was more three times thicker than the original tablet. This was due to the type of mold devices used only for a single tablet type, so the size of the thickness of the tablet was not in accordance with the diameter of the tablet, the volume of the mold / die on the tablet mold devices was not in accordance with the weight of the tablet to be molded and the lack of pressure at the time of tablets molding.

The hardness is a parameter that describes the tablet resistance against mechanical stress such as shock, bumps and cracks during packaging, storage, transportation, and to the user's hand (Hadisoewignyo, 2013). Tablet hardness evaluation results of both formulas did not meet the requirements for <4-8 kg.

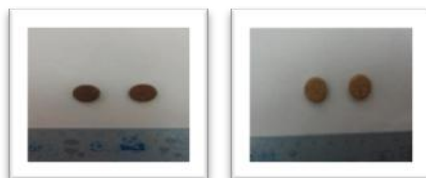
Tablet disintegration time is the time needed a sum of tablets to disintegrate into granules / constituent particles. The results of the evaluation of both tablet formulas did not meet the requirements for tablet disintegration time was more than 15 minutes.

**Table 6. Results of the evaluation of the tablet**

Evaluation	F1	F2	The requirement
Uniformity of weight (g)	0.6061 ± 0.00510	0.6142 ± 0.00245	no more than two tablets that deviated more than 5% and none that deviated more than 10%
Uniformity of size (mm)	D = 13.276 ±0.02655 T = 4.259 ±0.04134	D = 13.281 ±0.02449 T = 4.327 ±0.04529	the diameter of the tablet was more three times thicker than the original tablet
Friability test (%)	0.01	0.09	0.5% - 1%
Hardness test (Kg)	3.3±0.258 2	3.05±0.2838	4-8 Kg

Disintegratin test (min.)	23±2.6077	15.67±0.816 5	< 15 min.
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**Picture 3. Tablets F1 and F2**

## CONCLUSION

Based on research conducted, it can be concluded that:

1. The ethanol extract of red ginger (*Zingiber officinale* Roxb.) could be formulated in the form of capsules and tablets dosage.
2. The preparation of the capsule based on the evaluation met the requirements and tablets based on the evaluation had not met the requirements of uniformity of size, hardness and disintegration time.

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**THE COMBINATIONS ETHANOL EXTRACT OF GINGER (*ZINGIBER OFFICINALE* ROSCOE) AND ZINC AS ANTI ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC NEW ZEALAND RABBITS**

**Priyanto MSc<sup>1</sup>. Prof. Agik Suprayogi. PhD<sup>2</sup>. Nastiti K. PhD<sup>2</sup>. Dewi Ratih A. PhD<sup>3</sup>**

1. UHAMKA

2. Department of Anatomy and Physiology.

3. Pathology Department Faculty of Veterinary Bogor Agricultural Institute

**ABSTRACT**

Atherosclerosis is a disease affecting arterial blood vessels due to the accumulation of macrophage white blood cells and low density lipoprotein (LDL), and is triggered by hypercholesterolemia and oxidized LDL. To prevent plaque atherosclerosis, hypercholesterolemic rabbits were treated by the combination ethanol extract of ginger (EEG) and Zinc, which hypothesized to work as cholesterol reduce and antioxidant. In the present study, 24 male New Zealand white hypercholesterolemic rabbits were divided into 6 groups. For six weeks, group 1 was fed with a standard diet. In succession group 2, 3, 4, 5, and 6 were treated by Zinc at dose 6.67 mg/kg, EEG 200 mg/kg, combination EEG 50 mg/kg and Zinc, combination EEG 100 mg/kg and Zinc, and atorvastatin at dose 1.9 mg/kg BW per day, as supplements to the standard diet. Zinc treatment did not effectively reduce total cholesterol, LDL, and plaque atherosclerosis because the effect did not show different to diet standard ( $p > 0.05$ ). EEG 200 mg alone and combination EEG 100 mg and Zinc treatment effectively reduce total cholesterol, LDL, and plaque atherosclerosis because the effect equal to atorvastatin (positive control) ( $p < 0.05$ ). Based on reduced LDL and plaque atherosclerosis, combination EEG 50 mg and Zinc treatment resulted higher than positive control ( $p > 0.05$ ). Comparative study with EEG 200 mg/kg and positive control treatment have shown that combination EEG 50 mg and Zinc has the best antioxidant effect ( $p < 0.05$ ) and resulted higher than combination EEG 100 mg and Zinc based on MDA levels ( $p > 0.05$ ). This research concluded that combination EEG 50 mg and Zinc treatment showed the highest atherosclerosis prevention effect compared the other treatments.

Key word: Atherosclerosis, hypercholesterolemic, antioxidant, oxidized LDL, ethanol extract of the ginger (EEG), and plaque atherosclerosis

**INTRODUCTION**

Both clinical and epidemiologic study were shown that hypercholesterolemia and oxidized LDL are perhaps the major risk factor of atherosclerosis and its complications, such as stroke and ischemic heart disease (IHD), which leads to



myocardial infarction (Sanghal et al. 2012). A wide variety of therapeutic agents in modern medicine are available against atherosclerosis like statins, fibrates, and resins (Kimble et al. 2007).

Modern medicines are costly and with potentially serious side effects. Moreover, if the therapy is not regularly monitored, this can lead to fatal toxicity and noncompliance. Despite widespread availability of lipid-lowering drugs with proven beneficial outcome of treatment, dyslipidaemia remains unsatisfactorily managed in routine clinical practice. The patients undergoing pharmacological lipid-lowering treatment fail to attain target levels of LDL and other lipids recommended guideline. Studies in Europe and Asia concluded that only 55.3% and 48% of patients on lipid-lowering drug treatment achieved target levels recommended (Chan et al. 2012, Park 2012). Besides that, most of these drugs are not suitable to be used as a preventive measure against atherosclerosis and antioxidant. Lipid-lowering drugs have not shown effectiveness for hypercholesterolemia familial (type II) and hypercholesterolemia familial combinations (type III) (Stapleton et al. 2010)

Many studies reveal that both the extract ethanol of ginger (EEG) and zinc possess positive effect in reducing cholesterol, as well as being antioxidant (Ajith et al. 2012, Agoreyo et al. 2008, Fuhrman et al. 2000, Nammi 2009). The extract ethanol of ginger (EEG) dose for atherosclerosis in hypercholesterolemic rabbits is 200-400 mg/kg BW per day (Nammi 2009) and if converted to human (70 kg), the dose will be about 4260-8520 mg (Paget & Barnes 1971). This dose is not applicable and economic for routine clinical practice.

One among many mechanisms of EEG in reducing cholesterol is by increasing the activity of enzyme cholesterol 7- $\alpha$ -hydroxylase (CYP7A1) hepatic. Rate limiting enzyme for biosynthesis of bile acid (Fuhrman et al. 2000). However, the increase of cholesterol 7- $\alpha$ -hydroxylase enzyme could stimulate the formation of free radical and oxidative stress (Murray et al. 2003). Oxidative stress conditions lowering the effect of EEG in reducing cholesterol level and atherosclerosis. So, in order to enhance the effect of EEG to reduce cholesterol and/or atherosclerosis, the EEG needs to be supplemented by substances that have effects as antioxidant or both antioxidant and cholesterol reductant.

To enhance the effect of EEG in reducing cholesterol and atherosclerosis, we chose zinc as supplement. Zinc was chosen because it has effect like antioxidant, lowering cholesterol, anti inflammation, and safe for long term application. Zinc and active compound of EEG have different chemical structure but have similar effects as cholesterol reductant and antioxidant, when combined, they may have synergic effect to impede atherosclerosis. The present study aimed to reveal the effects of the combination of EEG and zinc to impede atherosclerosis on hypercholesterolemic rabbits.

## **MATERIALS AND METHODS**

### **Animals**

Adult healthy male New Zealand rabbits, weighing 2.5-3.5 kg were used in the present study. Animals were procured from Indonesian Center for Animal Research and Development Ministry of Agriculture (Balitnak Ciawi Bogor), certified animal house. To make hypercholesterolemia rabbits, they were given atherogenic diet food that content 0.5% cholesterol and 5% palm oil and water ad libitum during 6 weeks or more. Atherogenic diet food was prepared by Indonesian Center for Animal Research and Development Ministry of Agriculture. The animals were kept in institutional animal house under temperature, humidity and light and dark cycle controlled environment.

### **Test Drugs**

Ginger was procured from Indonesian Research Center Spices and Medicinal plants Ministry of Agriculture (Balitro Bogor). EEG was administered in a dose of 200 mg/kg, but if combined with zinc, a dose become a half (100 mg/kg) and a quarter (50 mg/kg). Zinc and atorvastatin were procured from registered Pharmaceutical Industry in Indonesia (Kalbe Farma). The dose of zinc was 6.67 mg/kg and the dose of atorvastatin was 1.9 mg/kg (account from pharmacological therapy and clinical study). In the present study, we used atorvastatin as a reference drug (positive control) as it is most effective tested drug against hypercholesterolemia, antioxidant, and also prescribed for longer time usage.

## **Experimental Protocol**

Total numbers of 24 male New Zealand rabbits were included in the study. The weight of all rabbits was taken before starting the research. All rabbits were fed with atherogenic diet for 6 weeks or more to induce hypercholesterolemia. Initial number of total cholesterol (TC) and LDL was recorded before feeding atherogenic diet and initial hypercholesterolemia was recorded after 6 weeks or more atherogenic diet.

Twenty four hypercholesterolemic rabbits were divided into 6 groups. Group 1 were given only standard diet for 6 weeks period. Group 2 was given standard diet and zinc. group 3 was given standard diet and EEG 200 mg/kg. group 4 was given standard diet and combination EEG 100 mg/kg and zinc. group 5 was given standard diet and combination EEG 50 mg/kg and zinc. and group 6 was given standard diet and atorvastatin for period of 6 weeks. After 6 weeks. TC. LDL. malonaldehyde (MDA). plaque atherosclerosis/atheroma were measured to see the curative effect of EEG alone. combination EEG and zinc compared with atorvastatin on impede atherosclerosis.

## **Biochemical Analysis in Blood**

Blood sample of 2 ml volume was taken from ear vein for chemical analysis. Blood plasma and serum were separated by centrifuge at a rate of 3000 rpm for 15 min.

### Estimation of TC and LDL in Serum

Cholesterol reagent (Boehringer Mannheim) was used for the determination of TC and LDL base on enzymatic method using cholesterol esterase. cholesterol oxidase. and peroxidase.

### Estimation of MDA in Plasma

Thiobarbituric acid (TBA) test used for the determination of MDA. Under acidconditions. 2 molecules TBA bind with one molecule MDA to formation a pink color measured at about 532 nm in spectrophotometer. For the standard curve. tetraethoxypropane (TEP) was used.

## **Atherosclerosis Study**

The degree of atherosclerosis in aorta was evaluated by the quantification of lesion area and foam cells or plaque formation on tunica intima.

### Quantification of Lesion Area

After fixation in 10% formalin solution, the aorta was opened longitudinally and briefly rinsed in 60% isopropanol, then stained with Oil red o 0.3% b/v on isopropanol (made up fresh from the stock solution each time) for 10 minutes. Samples were washed afterwards with 10% v/v isopropanol three times. To analyze the tissue distribution of lesion area (accumulation of foam cells), photographs were taken. Accumulation of foam cells were determined as light red region and detected by image analysis using digital image Image J Bioimaging Macbiophotonic software (NIH 2009).

### Histopathology

Plaques were detected by histopathology techniques. Small tissue pieces of aortic arch were collected in neutral buffer formalin for routine histoprocessing by paraffin embedding technique for frozen microtomy. Paraffin sections of aorta were stained with Haematoxylin and Eosin (H and E).

### **Statistical Analysis**

The biochemical values and lesion area were expressed as mean  $\pm$  SD and analyzed statistically using 1-way analysis of variance (ANOVA) followed by the multiple comparison test Tukeys. The minimal level of significance was fixed of  $p < 0.05$ . The analysis was performed using Minitab software version 15.

## **RESULT AND DISCUSSION**

The decrease of total cholesterol and LDL as the result of normal diet or treatment are shown in table 1 and 2. In hypercholesterolemic rabbits receiving a normal diet for 6 weeks, the total cholesterol (TC) and LDL decreased by 67.13% to 72.53%. Declining levels of total cholesterol or LDL in hypercholesterolemic rabbits receiving a normal diet showed smallest drop in comparison with other treatments. This proves that the

treatment of ethanol extracts of ginger rhizome (EEG) and EEG combined with zinc has a cholesterol-lowering effect.

The ability of EEG and combination of EEG and zinc in reducing TC and LDL did not differ significantly with the positive control (atorvastatin). This proves that the EEG and combination of EEG and zinc were as effective as cholesterol-lowering effect in atorvastatin. Atorvastatin effect was quantitatively higher than EEG or EEG combined with zinc in reducing TC. but based on the ability to reduce LDL, the combination of EEG 50 mg and zinc was better than the others, including positive control (Table 2).

LDL is a better predictor compared with TC and total fat to estimate the possibility of atherosclerosis (Kimble et al. 2007). Based on this, the combination of EEG 50 mg and zinc as anti atherosclerosis provide the best results. Further more the combination of EEG 50 mg and zinc can reduce levels of LDL to below normal levels, i.e. 35.1 mg/dl (Table 2), while normal levels were 57.4 mg/dl.

Based on the value of accumulation of lipids in the tunica intima, the potential of zinc, EEG, EEG combination with zinc were significantly different from those with standard diet, and insignificantly different from the positive control (Table 3). But quantitatively, the accumulation of fat in the tunica intima group of animals with treatment EEG 50 mg and zinc is the smallest, i.e. 39.73%. From fat accumulation data in tunica intima, the group receiving zinc was not different from other treatments, however, histology result on those receiving zinc still showed the formation of plaque at about 1 in 4 experimental animals with a thickness of 11%. For the group with standard diet, the plaques were found on 2 among 4 experimental animals with a thickness of 9 and 52% (Figure 2)

The proposed mechanism in various studies done so far for this effect of ginger as follows (Sanghal et al. 2012):

1. Inhibits the hydroxymethylglutaryl CoA (HMG-CoA) reductase which is a rate limiting enzyme for cholesterol biosynthesis (like that of statins).
2. Promotes the excretion and impairs absorption of cholesterol.

3. Increases the activity of 7-alpha-hydroxylase, the rate limiting enzyme in the catabolic conversion of cholesterol to bile acids in liver.

Malondialdehyde (MDA) level was chosen as the parameter of oxidative stress because the formation of MDA is proportional to the free radical reactions with cellular component or parts of the cell (Halliwell 1991). Based on the table 3, EEG and EEG combined with zinc, and atorvastatin has the effect as an antioxidant because it can decrease the MDA better than a normal diet. Among the three treatments, i.e. EEG 200 mg/kg, the combination of EEG 50 mg/kg and zinc, and atorvastatin, the combination of EEG 50 mg and zinc gave the best results.

Higher MDA levels were potential to cause injury to the endothelium that can lead to atherosclerosis (Matfin and Port 2008). In addition, high MDA also showed a high oxidized LDL. Oxidized LDL are pro-inflammatory, inhibiting synthase endothelial nitric oxide (eNOS), inducing vasoconstriction and adhesion, increase deregulation of vascular endothelial growth factor (VEGF), and cytotoxic. Oxidized LDL is not recognized anymore by the receptor in the liver but is recognized by the macrophage scavenger receptor that is not down regulated. As a result, oxidized LDL to enter and ingested continuously macrophages and foam cells are formed (Singh and Jialal 2006).

The antioxidant properties of the EEG because they contain compounds 6-gingerol which serves as a scavenger of oxygen radicals (Misra 2012), while the antioxidant properties of zinc that has been confirmed are the protection of sulfhydryl groups against oxidation and the inhibition of the production of reactive oxygen by transition metal (Powell 2000). Zinc competes with Fe and Cu for binding to cell membranes and some proteins, displacing these redox-active metals and making them more available for binding to ferritin and metallothionein, respectively (Bettger 1993).

**Table 1: The Average of TC Levels(mg /dl) Initial. after Treatment. and the Percentage Decrease**

Treatment Groups	TC Initial	TC after Treatment	% Decrease
Standard Diet	614.8 ± 341.7	182.87 ± 144.3	67.13 ± 14.64 <sup>a</sup>
Zinc	603.3 ± 367.6	91.44 ± 26.4	81.16 ± 8.24 <sup>ca</sup>
EEG 200	741.2 ± 140.3	74.77 ± 38.8	90.07 ± 4.02 <sup>bc</sup>
EEG 50 + Zinc	747.8 ± 146.2	92.83 ± 56.0	87.73 ± 6.52 <sup>bc</sup>
EEG 100 + Zinc	744.1 ± 170.2	75.00 ± 28.7	89.86 ± 3.31 <sup>bc</sup>
Atorvastatin	734.6 ± 204.1	58.33 ± 8.4	91.43 ± 3.19 <sup>bc</sup>

Explanation: Value followed by similar alphabet and in same columns were not significantly different based on Tukey's at the  $\alpha$  Of 5%

**Table 2: The Average of LDL Levels (mg /dl) Initial. after Treatment. and the Percentage Decrease**

Treatment Groups	LDL Initial	LDL After Treatment	% Decrease
Standard Diet	405.1 ± 225.5	110.3 ± 56.8	72.55 ± 7.96 <sup>a</sup>
Zinc	453.8 ± 314.3	86.2 ± 61.0	80.66 ± 1.68 <sup>ca</sup>
EEG 200	539.0 ± 158.0	50.0 ± 16.9	90.79 ±

			1.31 <sup>bc</sup>
EEG 50 + Zinc	591.2 ± 138.5	35.1 ± 15.8	93.89 ± 3.07 <sup>bc</sup>
EEG 100 + Zinc	448.1 ± 82.3	47.4 ± 16.0	89.20 ± 4.25 <sup>bc</sup>
Atorvastatin	569.0 ± 187.6	75.8 ± 49.9	86.21 ± 8.56 <sup>bc</sup>

Explanation: Value followed by similar alphabet and in same columns were not significantly different based on Tukey's at the  $\alpha$  Of 5%

**Table 3. The Average Percentage of Atherosclerotic Plaque Area (Lesion) in Aorta and MDA Levels in the Various Groups**

Treatment Groups	MDA Levels (nmol/ml)	Lesion Area (%)
Standard Diet	0.28663 ± 0.02006 <sup>a</sup>	56.03±2.67 <sup>a</sup>
Zinc	0.24025 ± 0.00262 <sup>b</sup>	44.18±5.13 <sup>b</sup>
EEG 200	0.20888 ± 0.01755 <sup>c</sup>	43.00±6.29 <sup>b</sup>
EEG 50 + Zinc	0.16423 ± 0.00703 <sup>de</sup>	39.73±4.32 <sup>b</sup>
EEG 100 + Zinc	0.18513 ± 0.00903 <sup>ed</sup>	42.50±1.34 <sup>b</sup>



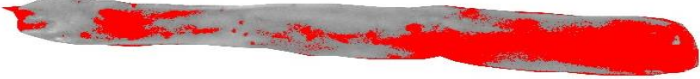

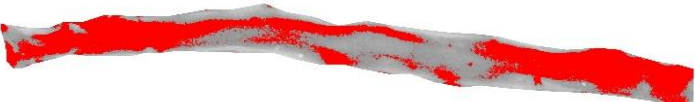



Atorvastatin       $0.20848 \pm 0.01325^{fce}$        $44.15 \pm 5.22^b$

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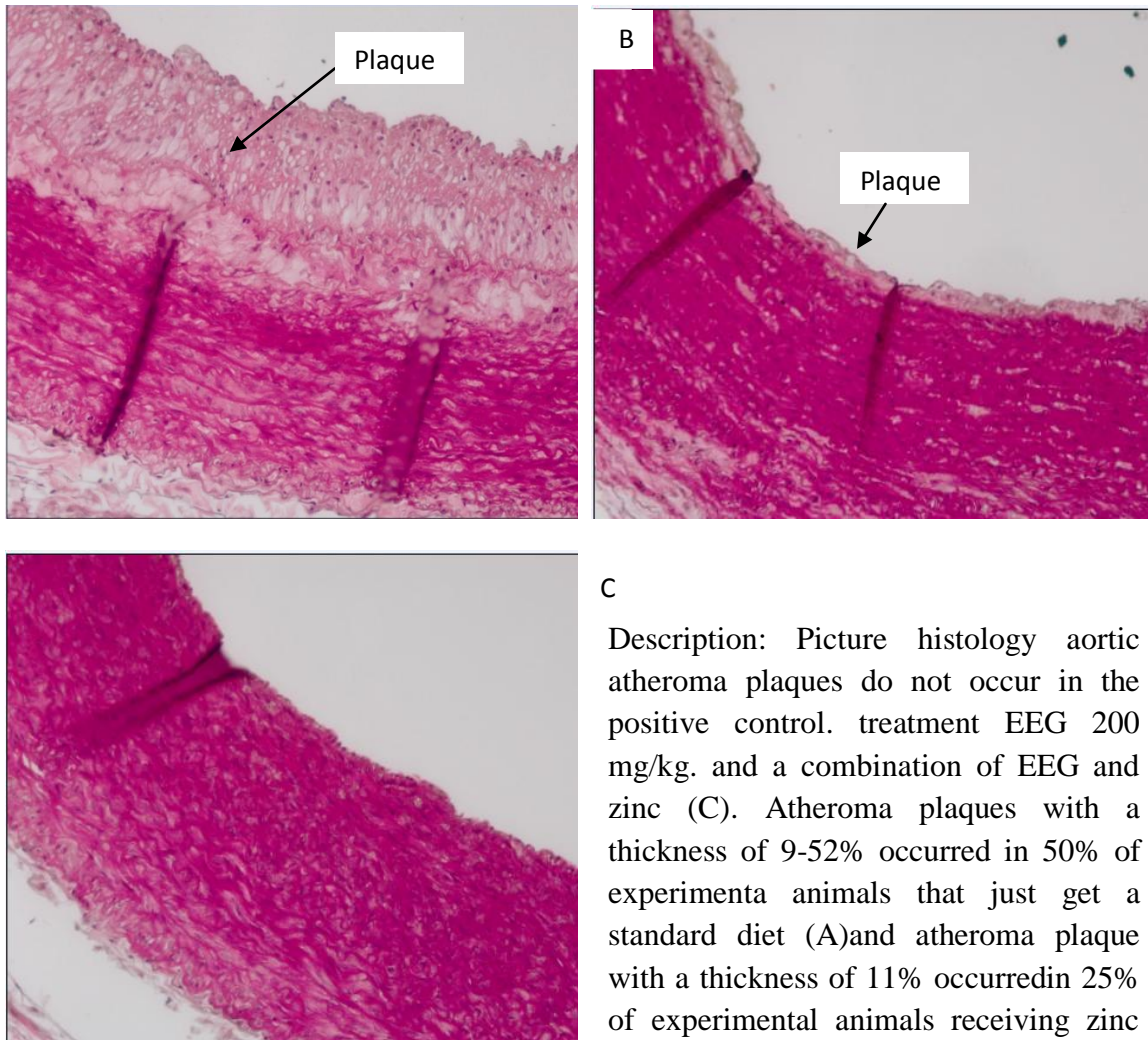
Explanation: Value followed by similar alphabet and in same columns were not significantly different based on Tukey's at the  $\alpha$  Of 5%

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Treatment (mg/kg)	Example Image from Each Group
Standard Diet	
Zinc	
EEG 200	
EEG 50 + Zinc	
EEG 100 + Zinc	
Atorvastatin	

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**Figure 1. Representative Images from Each Group Treatments**



Description: Picture histology aortic atheroma plaques do not occur in the positive control. treatment EEG 200 mg/kg. and a combination of EEG and zinc (C). Atheroma plaques with a thickness of 9-52% occurred in 50% of experimental animals that just get a standard diet (A) and atheroma plaque with a thickness of 11% occurred in 25% of experimental animals receiving zinc (B).

**Figure 2. A. Band Care Aorta Histology (HE Staining) with 200 Times Magnification. Figure A and B formed atheroma plaque**

### **Conclusion**

1. Compared to the EEG 200 mg/kg. combination of EEG 100 mg/kg and zinc or positive control. the combination of EEG 50 mg/kg and Zinc showed the best effect although not significantly different based on value of LDL and the accumulation of fat in the tunica intima (lesion area) in hypercholesterolemic rabbits.

2. Compared to other treatments, the combination of EEG 50 mg/kg and zinc showed significant effect as antioxidant, based on the inhibition of MDA formation in hypercholesterolemic rabbits.

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## **SUBCHRONIC TOXICITY TEST OF COMBINATION OF GINGER (*Zingiber officinale* Rosc.) EXTRACT AND ZINC ON SWISS WEBSTER MICE**

**Hadi Sunaryo. Siska. Dwitiyanti. Rizky Arcintha Rachmania. Roja Fathul Mubdy**

Faculty of Pharmacy and Science University of Muhammadiyah Prof. Dr. Hamka

Corresponding author : [hadi\\_itb@yahoo.com](mailto:hadi_itb@yahoo.com)

### **ABSTRACT**

The combination of ginger (*Zingiber officinale* Rosc.) extract and zinc has the effect of lowering levels of glucose, triglycerides, cholesterol and anti-oxidants. This study aimed to determine the safety administration of combination ginger extract and zinc, through subchronic toxicity test. Parameters measured were body weight, liver and kidney histopathology, SGOT, and SGPT. The study used a sample of 20 male mice, divided into one control group and one treatment group. The extract was given orally through a sonde for 75 days. The treatment group were given a combination of ginger extract and zinc (ginger 30mg / 20g BW + zinc 0.4mg / 20g BW ) and control group was given a water. After 75 days of treatment sample blood the heart and then checked SGOT and SGPT. Collected statistical from result with the method of independent samples T test showed no significant difference between the levels of SGOT and SGPT control group to the treatment group. It can be concluded combination ginger extract and zinc did not show any toxic effects in subchronic and did not cause toxicity and damage to vital organs, such as liver and kidney.

**Keywords:** subchronic toxicity , ginger extract and zinc, SGOT, SGPT

### **INTRODUCTION**

Studies on medicinal plants need to be done so that it can be used safely and effectively. Pre-clinical trials is one of step drug testing.

Subchronic toxicity test is one of preclinical trials for test the toxicity and safety of a given compound With repeated doses in animal specific, for less than three months. This test is intended to reveal the toxic effects of test compounds as well as to show whether the toxic effects related to measure of the dose (Donatus, 2005).

Diabetes mellitus is a chronic disorder of the metabolism of carbohydrates, fats, and proteins (Robbins 2007). Lipids in diabetics caused by insulin deficiency. It occurs due to disruption of the function of insulin because of the complications of high blood

lipid levels. especially cholesterol and triglycerides (Widyastuti 2001). DM is a degenerative disease. so patients need treatment special diet. lifestyle regularly and heavily dependent on drugs hypoglycemic (Baines 1999).

Ginger (*Zingiber officinale* Rosc.) is one of medicinal plants in Indonesia which has a high economic value and has many benefits. Ginger contains essential oils With major components zingiberen zingiberol. and gingerol oleoresin With the major components (POM RI 2004) useful for pain relief. anti-inflammatory. and antibacterial (Latif 2002).

Zinc (Zn) is one of micro minerals needed for each cell in the body. Adequacy of these minerals essential in keeping optimal health. Zn deficiency in diabetics can lead to disruption of the immune system (Jacobus 2000). In addition to antioxidant properties of zinc can also reduce levels of cholesterol and triglycerides (Reiterer *et.al* 2005).

In previous study mentioned that the combination of 70% ethanol extract of ginger (*Zingiber officinale* Rosc.) and zinc at a dose of 3 mg / 20g BW + Zn 0.4 mg / 20g BW can lower blood glucose levels. triglycerides. total cholesterol. LDL. and raise levels of HDL (Sunaryo Hadi *et al.* 2013; Sunaryo Hadi *et al.* 2014). If the activity is given the combination of ethanol extract of ginger and zinc have activity as antihypercolesterol antihyperglycemia and this can contribute to the development of drugs by utilizing the results from natural materials. But still uncertain safety of the compound. Therefore. it was conducted one of the pre-clinical trials that subchronic toxicity tests for ensure the safety and the highest dose in the repeated use of a compound. By using the highest therapeutic dose administered repeatedly for 75 days for determine the effect of the combination of ethanol extract of ginger and zinc.

## **MATERIALS AND METHODS**

### **Materials:**

Ginger was obtained from the Institute for Medicinal Plant Research. Bogor. Material after drying in a temperature of about 50°C. powdered extract was then made thicker with the maceration using ethanol 70%.

### Test Animal:

White male mice *Swiss webster* strain. aged 2-3 months weighing about 20-30 grams. As many as 20 mice were divided into one control group and one treatment group.

### Methods:

#### Extract Preparations

As much as 25 kg of dried ginger produces 2.5 kg of powder ginger. Then ginger powder was extracted using maceration method using 70% ethanol as a liquid maserate and produced 18.8 L maserat. Maserate results obtained using a vacuum rotary evaporator and the resulting extract in the oven until viscous.

The test material was administered orally via sonde each day for 75 days. The health condition of the test animals was checked every day to know the symptoms of toxic effects and weighed every day. The control group was given food and drink and the standard treatment group received a dose (ginger 30mg / BW + zinc 0.4mg/20g BW). At the end of 75 days the animals were anesthetized with ketamine then try surgery for blood sampling through the heart to be examined SGOT and SGPT. After the blood was collected through the heart followed by taking the liver and kidneys for making preparations for histopathology as macroscopic observation in a standard way using hematokisilin eosin staining for the presence or absence of histopathologic abnormalities.

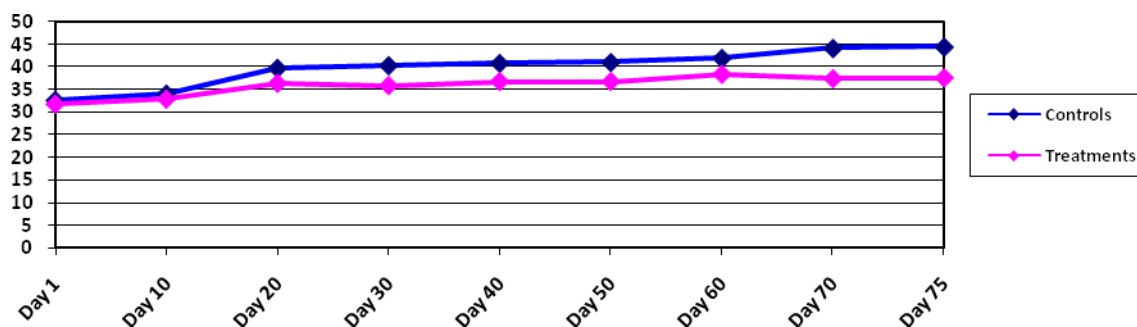
## RESULTS

The experimental results Subchronic toxicity combination ginger extract and zinc are listed in the table.

**Table 1.** The average weight observations mice for 75 days.

Groups	Days								
	1	10	20	30	40	50	60	70	75
Controls	32.7	34.1	39.8	40.4	40.9	41.1	42	44.2	44.5

Treatments	31.9	33	36.5	36	36.9	36.9	38.5	37.5	37.7
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**Figure 1.** The average weight observations mice for 75 days.

**Table 2.** Results of macroscopic examination of the liver and kidneys of mice after 75 days.

Groups	Macroscopic Observation	Liver	Kidney
Control	Color	Normal	Normal
	Form	Normal	Normal
Treatment	Color	Normal	Normal
	Form	Normal	Normal

**Table 3.** The average yield SGOT and SGPT examination mice.

Groups	SGOT	SGPT
Control	13.9794 u/L	8.79 u/L
Treatment	17.6026 u/L	16.5476 u/L

## DISCUSSION

Early stages of this research is the determination. Determination aims to get the true identity of the plants that will be tested its efficacy. so it can be provide the certainty about the truth of these plants. Based on the results of determination of plants



that will be used as the main substance in this study is completely ginger (*Zingiber officinale* Rosc.).

Making the ginger simplicia begins with sorting so ginger wet clean of impurities such as soil attached. then be washed using water flowing. After cleaning ginger sliced with the aim of expanding the material surface to accelerate the drying process. The drying process was done by aerated in order not damage the active substance content of ginger.

The extraction method used in this study is maceration. This method was used because it is easy and simple and did not require heating so it is suitable to active substances which did not resistant heating. The first stage was conducted by soaking the crude drug powder with liquid extract. Extract fluid used was ethanol 70%. because ethanol is more selective against fungi and bacteria so it is difficult to grow. did not toxic and its has well absorb. The water content in 70% ethanol serves to break down the cell walls that contain the active substance resulting in swelling of the cell so that ethanol can enter the cell and the active substance attracted by the solvent. At the time of immersion occasional stirring to flatten the concentration of the solution due to the concentration difference between the solution in the cell and outside the cell solution. Maserat separation using filter paper with no pollen extracts goal that brought into maserat. Maserat obtained was concentrated using a rotary vacuum evaporator so that there was a separation between the active substance and the solvent used is based on differences in boiling point. Concentration using a low temperature process  $\pm 50^{\circ}\text{C}$  in order not to affect the quality of the active substance. Then dried in an oven to remove residual solvent in order to obtain ethanol-free viscous extract.

Viscous extract obtained was conducted phytochemical screening. to determine the compounds contained in the viscous extract. Screening results was obtained a positive result in phytochemical screening are alkaloids. flavonoids and triterpenoids.

In this research sub-chronic toxicity test combination of 70% ethanol extract of ginger with zinc using white mice strains Swiss Webster. Sub-chronic toxicity test was conducted by giving a combination of ethanol extract of ginger with zinc for 75 days and once daily dosing.

The selection of the dose was based on the highest therapeutic dose that did not result in death was obtained in acute toxicity tests. Giving time of ginger extract

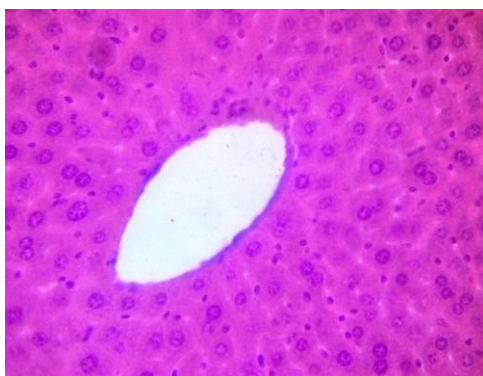
combination With zinc for 75 days. Selection of 75 days to fulfill the requirements of observation subchronic toxicity test which is usually between 4 weeks to 3 months (Donatu 2005). Then did the histology of liver and kidney in the control group and the treatment group and checks the value of SGOT and SGPT. It was conducted to determine the possible target organs affected by a combination of 70% ethanol extract of ginger with zinc and comparing SGOT SGPT of control group with SGOT SGPT of treatment group.

Research using animal testing young adults in a sense still in the process of growth that can be known directly and optimal effect of the test material by using parameters observation of symptoms of toxic effects and weighing during the experiment. It turns out that the results of the experiment shows that the weight of test animals during the 75-day study did not decrease. even increased and the provision of test materials for 75 days did not cause toxic effects on mice.

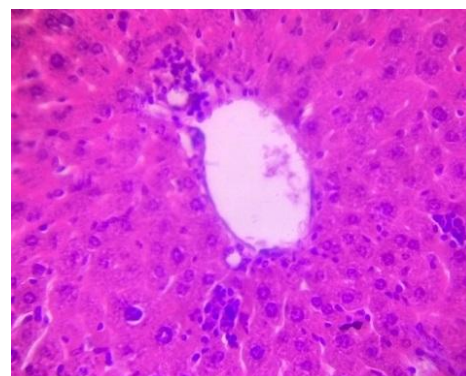
On macroscopic examination of the liver and kidneys of mice was not found specific abnormalities or within normal limits.

Histological examination was conducted on liver and kidney of white mice qualitatively by looking at the composition of the liver structure namely central venous cells and cells of hepatocytes.

While in the kidneys by looking at the structure of the glomerulus. Can be seen in Figure 1. 2. 3 and 4.



**Figure 2. Preparat of histology liver of control group.**



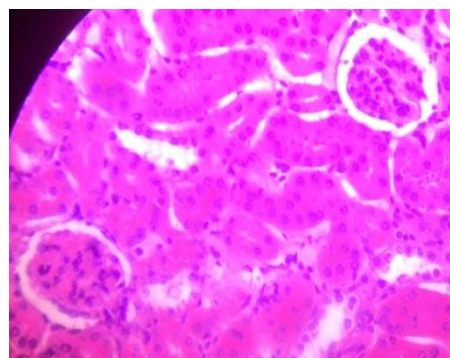
**Figure 3. Preparat of histology liver of treatment group.**

Histological examination of the liver preparations between the control group with treatment group found slightly difference in which in Figure 2 structure central

venous. endothelial cells look normal. while in Figure 3 the structure of endothelium damaged by inflammation so that the structure of central venous becomes damaged.



**Figure 4. Preparat of histology kidney of control group.**



**Figure 5. Preparat of histology kidney of control group.**

Examination of histological preparations renal glomerular structure found slightly difference between the control group to the treatment group. In Figure 4 is a normal glomerular structure. In Figure 5 seen the widening gap between the glomerulus and Bowman's capsule.

Another thing to consider is the examination of SGOT and SGPT. but found no significant difference in the levels of SGOT and SGPT after statistically tested with t-Tests (Table 3).\

## **CONCLUSION**

Giving a combination of extracts of ginger (*Zingiber officinale* Rosc.) with zinc at a dose (ginger extract 30mg / 20g BW mice and Zn 0.4 mg / 20gBW mice) for 75 days. based on the observation of body weight. liver and kidney histopathology. and examination of the value of SGOT and SGPT did not show any toxic effects and damage to vital organs. such as liver and kidney.

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# **THE LEUKOCYTE PROFILE OF INFLAMMATION RABBIT AFTER THE ADMINISTRATION BRANDED KETOPROFEN AND MUCOADHESIVE GRANULE**

**Salman Umar. Suyanda Dwina. Yufri Aldi**

*Faculty Of Pharmacy. Andalas University Padang*

*E-mail address :umar\_salman@yahoo.com*

## **ABSTRACT**

This study was designed to determine the level and AUC profile of total leukocyte and differential leukocytes to inflammatory rabbits after administration of ketoprofen mucoadhesive granules that are formulated and compared with ketoprofen branded. Ketoprofen branded used for comparison is kaltrofen. In the event of inflammation, the immune system will attempt to fight antigens that enter. Leukocytes are included in the non-specific immune system that serves as the first defense when inflammation. Rabbits were used as 6 tails that are divided into 2 groups. The treatment is done with the cross-over method. Induction of inflammatory performed using 1% caragen injected in the knee joints of rabbits. Then performed 1 hour after drug administration. In this study, the mucoadhesive granules of ketoprofen dose is 14 mg / 1.5 kgBW rabbit and kaltrofen as much as 48.02 mg that equivalent to 14 mg / 1.5 kgBW rabbit. The drug is administered orally using capsules. Furthermore, blood was taken after drug administration in hours to 0.5; 1; 1.5; 2.5; 3.5; 5.5; 7.5. It was calculated the total leukocyte and differential leukocyte. The data obtained were analyzed using two ways ANOVA. Anti-inflammatory activity of ketoprofen mucoadhesive granules and branded seen from the leukocyte profile that are indicated from the average value of *Area Under Curve* (AUC). The result showed that time and types of ketoprofen can reduce total leukocyte and some types of leukocytes significantly ( $P < 0.05$ ). When viewed from the AUC values, branded ketoprofen (kaltrofen) have anti-inflammatory activity better than mucoadhesive granules in reducing the total leukocytes, stem neutrophils and segment neutrophil.

Keyword : leukocyte profile, inflammation, ketoprofe

## INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals or microbiological substances. Inflammation is the body's attempt also to inactivate or destroy organisms that attack, eliminating irritants, and set the degree of tissue repair (Mycek.dkk. 2001). Inflammatory phenomena include microvascular damage, increased capillary permeability and migration of leukocytes into tissue (Gunawan. 2009).

Hoarding of white blood cells (WBCs), primarily neutrophils and monocytes at sites of injury, is the most important aspect of an inflammatory reaction. White blood cells (leukocytes) are able to phagocytose foreign material, including bacteria and necrotic cell debris, and lysosomal enzymes contained in it help defend the body in several ways. Some products of white blood cells (leukocytes) are driving an inflammatory reaction, and in certain cases cause significant tissue damage (Robbins & Kumar. 1995).

Anti-inflammatory drugs are a class of drugs that have activity to suppress or reduce inflammation. This activity can be achieved through means which inhibit the formation of inflammatory mediators prostaglandin, inhibit leukocyte migration of cells to the area of inflammation, and inhibit the release of prostaglandins from the cells of a formation. (Arifin. 2009)

Migration is an important feature of leukocytes which is a prerequisite for normal leukocyte function in physiological processes, such as protection against infection or foreign antigens. In pathological conditions, leukocytes enter the local network, produce pro-inflammatory substances and chemokines. This process is known as leukocyte recruitment. Normal ability for active movement is an important prerequisite for the effective inclusion of leukocytes from the circulation microvascular (Friedl. 2008). Blockade of leukocyte migration can cancel the entry of leukocytes and inflammatory processes. Therefore, modulation of leukocyte migration has been proposed as a potential therapeutic approach for the treatment of inflammatory diseases such as psoriasis, eczema, multiple sclerosis and arthritis (Sidiropoulos et al. 2008).

Based on their chemical structure, ketoprofen including non-steroidal anti-inflammatory drug (NSAID) arilasetat acid derivative which is used to relieve pain due to inflammation in various circumstances rheumatic and degenerative disorders of the musculoskeletal system (Siswandono & Sukarjo. 2000). Ketoprofen same efficacy as

drugs NSAIDs such as aspirin in the treatment of rheumatoid arthritis and osteoarthritis. Although the dual effects on prostaglandins and leukotrienes not show other effects that make it superior to other NSAIDs (Katzung, 1998).

The effects of the drugs known as NSAIDs leukocyte migration in vivo is not yet fully known. From the research that has been done by Paskauskas et al in 2011 to get the results that NSAIDs were able to induce a direct inhibitory effect on leukocyte migration both in vitro and in vivo. This effect was dose-dependent and is not limited to certain types of leukocytes. NSAIDs can reduce the migration of leukocytes with strong after topical application. (Paskauskas et al. 2011).

In this study the authors are interested in researching about how much influence on the inhibition of leukocyte migration ketoprofen which causes a decrease in the number of leukocytes in the blood stream of the profile shown in rabbit leukocytes. In this study used granule formulation of ketoprofen ketoprofen trademark (kaltrofen) as a comparison that is administered orally. Ketoprofen mucoadhesive granules are preparations made for the use of modified release mucoadhesive system, where previously ketoprofen formed into a solid dispersion with a polymer. Solid dispersion was made with the aim of increasing the dissolution rate of drugs is one way that can be done to improve the bioavailability problems. One method to increase the dissolution rate of drugs is the formation of poorly soluble drug dispersion in a polymer carrier. One of the polymer carrier that will be used is polyvinyl-pyrrolidone (PVP). With increasing drug dissolution, it is expected that the drug will be quickly dissolved and rapid effect on blood (Sutriyo et al. 2008).

However, in this study the mucoadhesive granules of ketoprofen are used instead of granules are formulated by the author. This research was conducted air-author here only team that examined the influence of this preparation against rabbit leukocytes profile.

Kaltrofen chosen for comparison because these drugs are drugs known as NSAIDs are widely used to treat diseases and disorders remathoid degenerative musculoskeletal system. From this research will compare the effects of two different formulations of ketoprofen in reducing the number of leukocytes in rabbits after inflamed.

Based on the above. the formulation of the problem in this research is how the effect of ketoprofen mucoadhesive granules of the total leukocyte count and differential leukocyte rabbits when compared with branded ketoprofen (kaltrofen) and its AUC profiles in inflammation rabbits.

The purpose of this study was to determine the effect of ketoprofen mucoadhesive granules of the total leukocyte count and differential leukocyte and its AUC profiles when compared with branded ketoprofen (kaltrofen) in inflammation rabbits.

This research is expected to provide information about the author of the immune system response in rabbits given the mucoadhesive granules of ketoprofen and branded ketoprofen (kaltrofen) through picture and profile AUC levels of white blood cells (leukocytes) it.

## **PROCEDURE**

### **Instrument**

Rabbit breeding cages. where food and drinking water. syringes. glass objects. light microscopy. micro tube. a set hemocytometer and labels.

### **Material**

Ketoprofen mucoadhesive granules. kaltrofen 100 mg. rabbit feed and drinking water. 70% alcohol. methanol. cotton. dye staining 10%. distilled water. a solution of Turk. and oil emersi.

### **Treatment of rabbits**

Rabbits were used in this study were 6 tails. Rabbit grouped becoming 2 groups. each group consisting of 3 tails. Group I is the group that will be given the test preparation in the form of granules mucoadhesive ketoprofen at a dose 14 mg / 1.5 kg. while the second is a comparison group given dosage form of branded ketoprofen (kaltrofen) of 48.02 mg / 1.5 kg which is equivalent to a dose of 14 mg / 1.5 kg rabbit. Previous rabbit prepared in individual cages and adapted for one week. Rabbits given feed in the form of vegetables are kale and carrots. On day 0 blood sampling performed on both groups to determine the normal state then do the calculation and observation picture of white blood cells (leukocytes differential). After that on the day of treatment



(day 1) in group I rabbits induced by substance dispersion karagen penginflamasi ie 1% in the rabbit knee joint as much as 0.5 ml. Let stand for 1 hour.

Method of treatment is done in cross over. In period 1. group 1 was given ketoprofen granules are formulated orally at a dose that has been determined. leave 1 hour. after which the observed and calculated the number of leukocytes in the rabbit given time as much as 7 times (0.5. 1. 1.5. 2. 5; 3.5; 5.5; 7.5 hours after drug administration). In group II. Working together with group 1. but using a comparison dosage kaltrofen much as 48.02 mg. after the rabbit rested for 10 days. In period 2. the exchange of the treatment. which in this period one group was given branded ketoprofen (kaltrofen) and group 2 was given ketoprofen mucoadhesive granules.

### **Blood retrieval**

Blood is drawn through the auricular vein at the edge of the rabbit ears. Before the decision. the decision where it is treated first with 70% alcohol and then blood was collected with 1 ml syringe.

*The calculation of the number of leukocytes* Fresh blood that has been given EDTA sucked by a pipette leukocytes to figure 0.5 then sucked turk solution to the 11 subsequent shaken for 3 minutes by means of the pipette 1-2 drops discarded and the haemocytometer counting room dripped one drop. Let the liquid for 2 minutes in order to precipitate leukocytes. The number of white blood cells counted in the four corners of the room count.

*Making preparations for blood pillowcase* Fresh blood dripped on the glass object later with another glass object leveled by placing one end of the object glass both on the first surface of the glass object at an angle 30-45°. Object glass both drawn to touch the drop of blood. blood is left to spread along the edge of the first glass object. Subsequently dried preparations were fixed with methanol for 2-3 minutes and then added to 10% Giemsa stain for 20-25 minutes. After it was washed with running water and then dried.

### **Differential leukocyte**

Mixture commentator who has been stained with Giemsa examined under a microscope with a magnification of 100x using emersi oil. Differential counting of leukocytes based on observations by counting the number of neutrophils. eosinophils.

basophils, lymphocytes and monocytes in 100 grain leukocytes. Leukocyte count results are expressed in percent.

### **Data Analysis**

The data obtained were analyzed using two-way ANOVA test to see the effect of time and type of ketoprofen are used (mucoadhesive granules of ketoprofen and branded ketoprofen ) of the total leukocyte count and various types of (differential) rabbit leukocytes. Furthermore, the value of AUC (Area Under the Curve) of the average number of total leukocytes and various types of (differential) rabbit leukocytes is used as a parameter to see the anti-inflammatory activity between the two preparations.

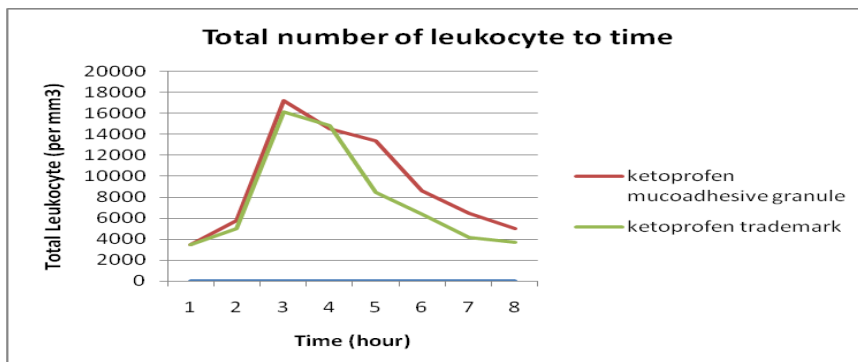
### **RESULT AND DISCUSSION**

Profile leukocytes seen based on the average value of AUC (Area Under the Curve) rabbit leukocyte counts in each treatment. AUC value here is used as one of the parameters to see the anti-inflammatory activity. AUC of white blood cells (leukocytes) describe changes over time in total leukocytes in inflammation. AUC values indicate the amount of anti-inflammatory effects. The larger the AUC value, it can be said that the smaller the effect of a decrease in the number of leukocytes or the smaller the antiinflammatory effect, otherwise the smaller the value of the AUC, the better the effect of a decrease in the number of leukocytes can be said to be better or inhibition of inflammatory.

The influence of time and type of ketoprofen to the total leukocyte count after administration of mucoadhesive granules and branded ketoprofen ( $P < 0.05$ ). According to Campbell (2004), the normal range of the total number of leukocytes rabbits ranging from 6300-10600 microliters / cell and the average number of normal leukocyte cells in rabbits is 3000-12500 / mm<sup>3</sup>.

The average number of total leukocytes in each group are equally increased 2 h after induction. In the group given the mucoadhesive granules ketoprofen, total leukocyte counts returned to normal in the amount of  $8637.5 \pm 3210.49 / \text{mm}^3$  to 3.5 hours after administration, whereas in the group given kaltrofen leukocyte counts returned to normal range is equal to  $8425 \pm 512.59 / \text{mm}^3$  to 2.5 hours after drug administration. Furthermore, when viewed from the average value of AUC (Area Under the Curve) to the total number of leukocytes in the group given the mucoadhesive

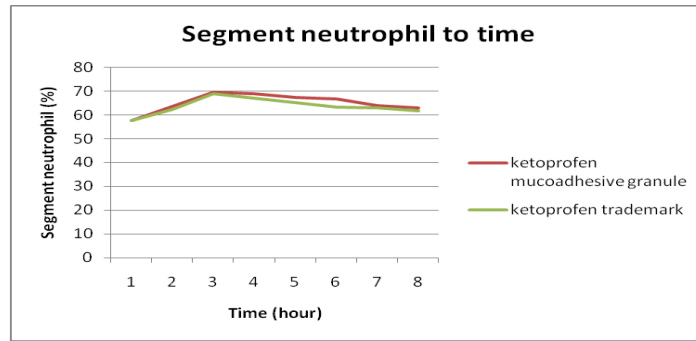
granules of ketoprofen showed AUC values  $68288.96 \pm 9770.96$  and in the group given ketoprofen his trademark AUC value of  $51\ 212.5 \pm 5607.41$ . This means that branded ketoprofen has greater anti-inflammatory activity than the mucoadhesive granules are formulated.



**Figure 1. Graph the total number of leukocytes to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

Pathologically, the increase in the total number of leukocytes in the circulation can be caused by leukocytes active against microorganisms. (Jain, 1993).

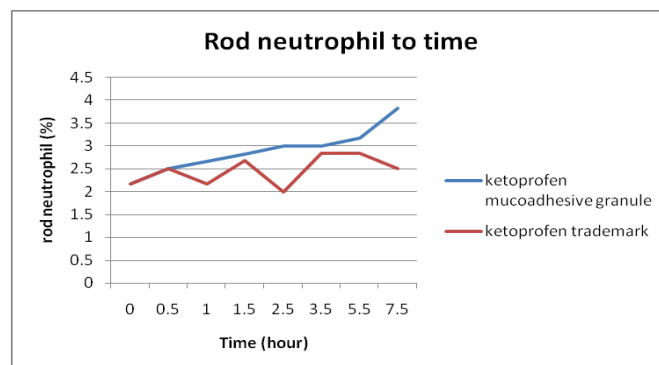
The influence of time and type of ketoprofen are used to segment the neutrophil count ( $P < 0.05$ ). According to Campbell (2004), the normal range rabbit neutrophil number ranging between 1490-3210 cells / microliter and the percentage of the average number of normal neutrophil cells in rabbits is 30-65% of the total white blood cells. Of the two groups seen increased neutrophil segments of normal 2 hours after induction is  $69.5 \pm 3.73$  and  $69 \pm 1.9$ . In the group given the mucoadhesive granules look back neutrophil counts segments are within the normal range of 5.5 hours after drug administration, but in the group given ketoprofen faster decline that is at 3.5 hours after drug administration. When viewed from the type of ketoprofen, an average AUC value of a given group of mucoadhesive granules showed a larger AUC value is  $491 \pm 12.46$  compared to the group given ketoprofen ie  $478.416 \pm 11.98$ . This means ketoprofen better in reducing the number of neutrophils segments compared mucoadhesive granules.



**Figure 2. Graph the amount of segment neutrofil to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

Neutrophils in rabbits often referred to as heterophile. According Tizard (1982). the main function of heterophile is the destruction of foreign materials through phagocytosis which destroy foreign bodies immediately therefore referred to as the first line of defense. Together with macrophages. heterophile attack and destroy bacteria and viruses in circulation.

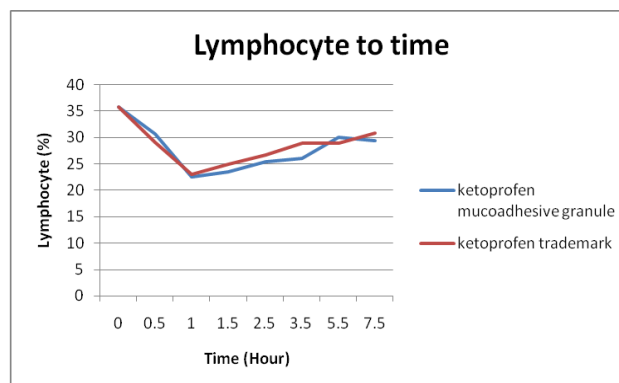
For neutrophils rod. no effect on neutrophil counts stem time after administration of mucoadhesive granules and kaltrofen ( $P > 0.05$ ). but the influence of the type of ketoprofen ( $P < 0.05$ ). Of the two groups were not seen any significant difference stems from neutrophil count every time after induction when compared with controls. When viewed from the type of ketoprofen. AUC values of the group given greater mucoadhesive granules are  $22.917 \pm 4.62$  when compared with the group given kaltrofen is  $19.292 \pm 1.73$ . This means kaltrofen has the effect of lowering the number of neutrophil stem better than the mucoadhesive granules.



**Figure 3. Graph the amount of rod neutrofil to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

This is because in the event of an acute infection, the number of stem increases in circulating neutrophils, but the numbers are not as neutrophil segment. (Dellmann and Brown, 1989). Neutrophil segments showed higher numbers than in circulating neutrophils rod when acute infection.

The influence of time on the number of lymphocytes after administration of mucoadhesive granules and ketoprofen ( $P < 0.05$ ), but not the kind of ketoprofen ( $P > 0.05$ ). According to Campbell (2004), the normal range rabbit lymphocyte counts ranged from 3360-7000 cells / microliter and the average percentage of normal lymphocyte cells in rabbits is 28-85%. The number of lymphocytes was not increased after induction. The number of lymphocytes is diverse but not past the normal limits. When viewed from the value of the AUC, the group given the mucoadhesive granules had AUC values were smaller than the group given ketoprofen ie  $206.833 \pm 6.96$  and  $213.792 \pm 8.77$ .

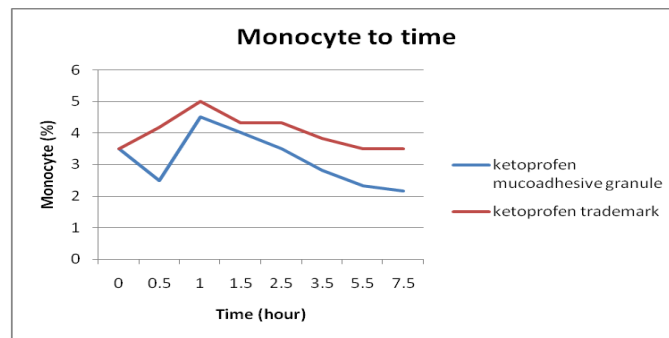


**Figure 4. Graph the amount of lymphocyte to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

The average number of lymphocytes fluctuated but remained within the normal range. Some of the circumstances that led to the high number of lymphocytes (lymphocytosis) is a chronic inflammation, bacterial, fungal, viral, and protozoan Babesia and Theileria particular, injecting ketekolamin, and lymphocytic leukemia (Jain, 1993).

The influence of time and type of ketoprofen are used to the number of monocytes ( $P < 0.05$ ), by Campbell (2004), the normal range of monocytes number of rabbits ranged between 50-540 cells / microliter and the percentage of the average number of normal monocyte cells in rabbits is 2-16% of total leukocytes. The number of

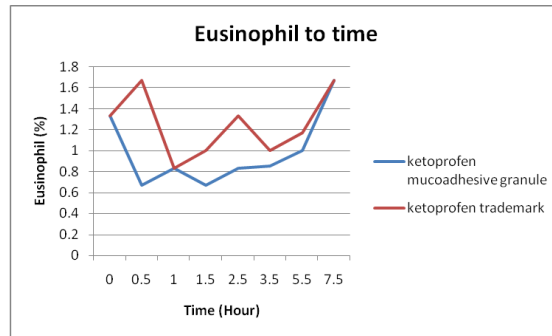
monocytes increased 2 h after induction. Then in the group given the mucoadhesive granules, the number of returns in a normal range of 3.5 hours after drug administration, while in the group given kaltrofen longer that 5.5 hours after drug administration. When viewed from the value of the AUC, the AUC values of the group given the mucoadhesive granules showed lower AUC value is  $21.958 \pm 2.84$  compared with the group given kaltrofen is  $29.292 \pm 2.79$ .



**Figure 5. Graph the amount of monocyte to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

Monocytes are found in the circulation, but in lower amounts than neutrophils (Baratawidjaja, 2004). Monocytes are found many times of chronic inflammation (Mitchell and Cotran, 2003).

The influence of time on the number eusinofil ( $P < 0.05$ ) after administration of mucoadhesive granules and kaltrofen, but no significant effect of the type of ketoprofen ( $P > 0.05$ ). The percentage of the average number of normal cells in rabbits eusinofil is 0.5-5% (Campbell, 2004). Eusiofil number fluctuates every hour, but not increased when compared to the control. If viewed from the AUC, AUC values eusinofil after administration of mucoadhesive granules smaller than the group given kaltrofen is  $7.167 \pm 2.34$  and  $9 \pm 3$ .



**Figure 6. Graph the amount of eosinophil to time after administration of the mucoadhesive granules of ketoprofen and branded ketoprofen**

Total eosinophil after induction is not much different from the blank. This happens because many of these cells in the blood will usually when skin is exposed to parasitic diseases and allergies (Hoffbrand. 2006). The main function is detoxification good eosinophil against foreign proteins that enter the body through the lungs or gastrointestinal tract as well as toxins produced by bacteria and parasites (Frandsen. 1992).

## CONCLUSION

Mucoadhesive granules ketoprofen able to reduce levels of total leukocytes rabbit. but when seen from its profile AUC. AUC value of the average total leukocytes after administration of mucoadhesive granules is greater than branded ketoprofen (kaltrofen). This means antiinflammatory effect was smaller than kaltrofen. Overview concentration and AUC profiles of various types (differential) leukocytes showed that kaltrofen still have a better anti-inflammatory activity primarily to a decrease in the number of total leukocytes. neutrophils and neutrophil stem segments when compared with ketoprofen mucoadhesive granules are formulated.

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# ACUTE TOXICITY STUDY OF ETHANOL EXTRACT OF COATBUTTON (*TRIDAX PROCUMBENS* L.) HERB IN SWISS WEBSTER MICE

Faizal Hermanto\*<sup>1</sup>, Puspa Sari Dewi S<sup>1</sup>, Restu Halistia<sup>1</sup>

Faculty of Pharmacy, University of Jendral Achmad Yani, Cimahi, Indonesia  
[\\*faizalhermanto@gmail.com](mailto:*faizalhermanto@gmail.com)

## ABSTRACT

**Background :** Coatbutton (*Tridax procumbens* L.) is a herb that traditionally has been used to heal many illnesses. Previous studies showed that Coatbutton herb has analgesic, anti-inflammatory, antimalarial, and inhibits the growth of *Trichophyton mentagrophytes* effect.

**Objective:** The research objective was to determine the toxicity of ethanol extract of coatbutton herb in experimental animals.

**Methods:** Ethanol extract of coatbutton herb was prepared using reflux. Acute toxicity test was performed on Swiss Webster mice with a single administration of the ethanol extract at doses of 300, 800, 2000 and 5000 mg/kg bw. Animals were observed for 14 days.

**Outcome measured:** LD<sub>50</sub> of ethanol extract of *T. procumbens* in mice.

**Results:** During 14 days of observation after single oral administration of ethanol extract of *T. Procumbens* up to 5000 mg/kg bw showed no toxicity signs of pharmacological screening, body weight, and indexes of organs.

**Conclusion:** LD<sub>50</sub> of ethanol extract of *T. procumbens* is greater than 5 g/kg bw.

**Keywords:** *Tridax procumbens*, acute toxicity, ethanol extract, LD<sub>50</sub>

## INTRODUCTION

An estimated 400 million inhabitants of the world, that is about 80% of the world's population, are thought to rely chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs (Norman *et al.* 1985). One of the plants that can be used as medicinal herbs is Coatbutton (*Tridax procumbens* L.). In Indonesia, Coatbutton (*Tridax procumbens* L.) is used to treat various diseases such as antidiarrheal, anti-inflammatory, analgesic, antimalarial, hypertension (Depkes, 1989).

Coatbutton (*Tridax procumbens* L.) (Asteraceae) contains saponin, flavonoids, and tannins (Depkes, 1989). Al-Doory (1980) reported Coatbutton has anti-inflammatory and analgesic effects. Patel *et al.* (2011) declared ethanol extract of Coatbutton contains flavonoids which have analgesic activity at a dose of 300 mg/kg bw. Antimalarial activity of ethanol

extract of coatbutton herb has been reported by Hermanto *et al*(2014)with IC<sub>50</sub>was 0.06 µg/mL.

From data above. coatbuton has been shown to have significant effect. but lack of ethno medical verification of safety.The research objective was to determine safety of ethanol extract *Tridax procumbens*Lherb in experimental animal.

## **MATERIALS AND METHODS**

### **Plant collection and determination**

*T. procumbens*herbwere collected from Bogor CentralJava. and determined by School of Life Science and Technology. ITB.

### **Preparation of extract**

Ethanol extract of *T. procumbens*herb was prepared using reflux with ethanol 96% as solvent. Ethanol extract of coatbutton herb used for experimental at doses of 300. 800. 2000 and 5000 mg/kg bw.

### **Animals**

*Swiss Webster* mice (20-30 g) were obtained from Center of Life Sciences. ITB. Bandung. Indonesia.

### **Acute toxicity test**

Acute toxicity test was modified from OECD 420. Acute toxicity test was performed on *Swiss Webster* mice. Animals were fasted for 16 h prior to dosing. Mice received by gavage with a single administration of the water extract at doses of 300. 800. 2000 and 5000 mg/kg bw. Each group consisted of 8 animals.

Four hours after dosing. each animal was observed individually and daily for 14 days. Observations included changes in skin. eyes and mucous membranes. and also respiratory. circulatory. autonomic and central nervous systems. and somatomotor activity and behavior pattern. Individual weights of mice determined for a total of 14 days. At the end of the test. animals were weighed and then humanely killed. Organs. such as lung. heart. kidney. testis. spleen. liver and reproduction organs. were weighed and indexes of organ was calculated based on the ratio between the weight of the organ and the body.

### Statistical analysis

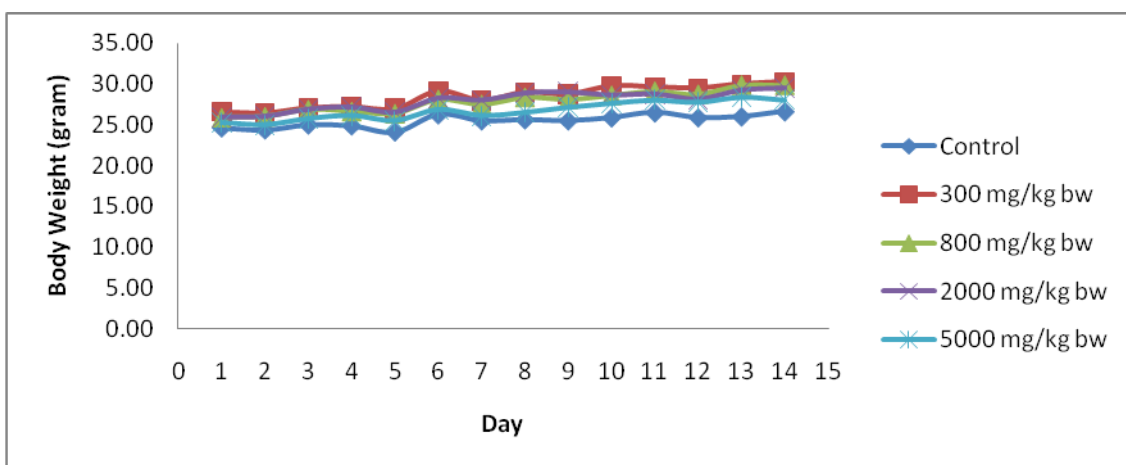
All the values expressed are Mean  $\pm$  S.E.M. Statistical evaluations were performed Student's t-test at the 95% confidence level using an SPSS 19 program for Windows. Values of  $p < 0.05$  were considered significant.

### RESULT AND DISCUSSION

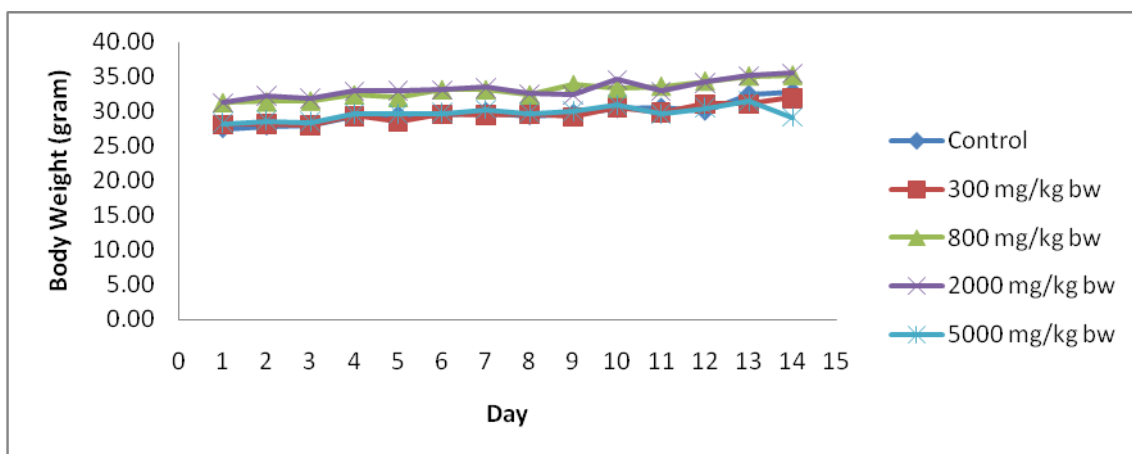
During 14 days of observation after received single oral administration of ethanol extract of *T. procumben* herbup to 5000 mg/kg bw showed no toxicity sign of pharmacological screening. body weight. and indexes of organs.

Observation of pharmacological profile of oral administration of ethanol extract of *T. procumben* herb up to 5000 mg/kg bw in the first 4 hours. and for a total of 14 days showed no signs of toxicity. Observations of Pharmacological screening included changes in respiratory. autonomic and central nervous systems. and somatomotor activity and behaviour pattern. ex. tremors. convulsions. salivation. diarrhoea. lethargy. sleep and coma.

Mean of body weight for male and female mice treated with *T. procumben* herb extract are illustrated in Figure 1 and 2. The body weight of male and female mice has significant difference between doses 2000 mg/kg bw and 5000 mg/kg bw of *T. procumben* herb extract compared to control group.



**Figure 1. Body weight of male mice after 14 days treatment with *T. procumben* herb extract**



**Figure 2.** Body weight of female mice after 14 days treatment with *T. procumbens* herb extract

Abnormalities in organs and tissues were not observed in necropsy after 14 days of acute dosing. Mean of indexes of organ for male and female mice treated with *T. procumbens* herb extract are shown in Table 1 and 2. Observation of indexes of organ of male and female mice showed significant difference for dose 2000 mg/kg bw and 5000 mg/kg bw of *T. procumbens* herb extract compared to control group ( $P > 0.05$ ).

**Table 1.** Indexes of organ of male mice after received single oral administration of *T. procumbens* herb extract

Group	Indexes of organ						
	Heart	Lung	Liver	Kidney	Spleen	Testis	Vesicle seminalis
Control	0.121±0.026	0.195±0.002	1.383±0.418	0.367±0.115	0.238±0.087	0.169±0.025	0.057±0.025
300 mg/kg bw	0.134±0.015	0.208±0.032	1.720±0.267	0.408±0.039	0.256±0.079	0.190±0.019	0.052±0.025
800 mg/kg bw	0.125±0.016	0.216±0.069	1.629±0.371	0.382±0.007	0.240±0.082	0.202±0.016	0.049±0.023
2000 mg/kg bw	0.433±0.030*	0.596±0.056*	5.062±0.917*	1.300±0.138*	0.740±0.167*	0.574±0.072*	0.202±0.087*
5000 mg/kg bw	0.417±0.040*	0.679±0.185*	5.183±0.731*	1.248±0.149*	0.613±0.337*	0.696±0.133*	0.198±0.064*

n=8. \* $P < 0.05$ . compared to the control group using Student's t-test

**Table 2.** Indexes of organ of female mice after received single oral administration of *T. procumbens* herb extract

Group	Indexes of organ						
	Heart	Lung	Liver	Kidney	Spleen	Ovarium	Uterus
Control	0.324±0.0	0.607±0.0	5.090±0.0	0.850±0.0	0.492±0.0	0.023±0.0	0.081±0.0

		061	074	820	140	116	010	048
300	mg/kg	0.327±0.	0.532±0.	4.846±0.	0.840±0.	0.511±0.	0.018±0.	0.085±0.
bw		050	112	886	130	139	009	095
800	mg/kg	0.373±0.	0.619±0.	4.731±0.	0.885±0.	0.628±0.	0.031±0.	0.158±0.
bw		050	127	834	134	266	071	158
2000	mg/kg	0.338±0.	0.556±0.	4.860±0.	0.851±0.	0.572±0.	0.026±0.	0.147±0.
bw		050	080*	665	152	198*	009	149*
5000	mg/kg	0.415±0.	0.735±0.	5.255±0.	1.032±0.	0.838±0.	0.027±0.	0.129±0.
bw		073*	097*	312	127*	236*	016	080*

n=8. \*P<0.05. compared to the control group using Student's t-test

Single administration of *T. procumbens* herb extract at dose 5000mg/kg bw po showed no death effect.. Observation of percent cumulative of death after single oral administration of ethanol extract of *T. procumbens* herb can be seen in Table 3.

**Table 3.** Observation of percent cumulative of death after received single oral administration of *T. procumbens* herb extract

Group	Cumulative death (%) at day -													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
300 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bw														
800 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bw														
2000 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bw														
5000 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bw														

n=8

Based on pharmacological screening. body weight and indexes of organ measurement. and observation of death cumulative. it showed that ethanol extract of *T. procumbens* herb was safe compare to control group and has LD<sub>50</sub> is greater than 5000 mg/kg bw.

### Conclusion

The ethanol extract of *T. procumbens* herb was safe and LD<sub>50</sub> of ethanol extract of *T. procumbens* herb is greater than 5 g/kg bw.

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