ISBN : 978-979-18458-6-1

PROCEEDING

INTERNATIONAL CONFERENCE ON CENTRAL MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION

Grand Cokro Hotel Yogyakarta
May 25th, 2013

THE INTERNATIONAL CONFERENCE ON CENTRAL MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION IN PHARMACY PRACTICE YOGYAKARTA, INDONESIA, 2013

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Published by :
Faculty of Pharmacy, Ahmad Dahlan University
Jln. Prof. Dr. Soepomo, Warungboto, Yogyakarta
Indonesia

ISBN : 978-979-18458-6-1

Welcome Address from Chairman of Organizing Committtee

Honorable Rector of Ahmad Dahlan University,
Dean Faculty of Pharmacy of Ahmad Dahlan University,
Honorable Plenary Speakers
Dear Colleague,
Distinguished Participants,
Ladies and Gentlemen

Assalamu'alaikum warahmatullahi wabarakatuh,

Good morning,

First of all, let us give thanks to Allah, the Almighty God, who has allowed us to attend this conference. Secondly, I would like to welcome everyone to Yogyakarta.

Preparation and reconstitution of drug products are an essential function of hospital pharmacy. The hospital pharmacist should also take notice for central intravenous additive service including cytotoxic reconstitution services. Thus, handling cytostatic in hospital is also crusial in hospital practises. In other words, Cytotoxic Handling should to provide protection to patients, the operator and environment.

Ladies and gentlemen,

To celebrate the 17th aniversary faculty of Pharmacy Ahmad Dahlan University, Yogyakarta, in collaboarting with Bethesda Hospital, Yogyakarta, Indonesia hosted The International Conference on Safety Management of Central Cytotoxic Reconstitution in Pharmacy Practice". The conference is held on 25 May, 2013 at The Grand Tjokro Hotel, Yogyakarta, Indonesia. The conference facilitated some of professional that come from world wide such as academia, researchers, hospital pharmacists, policy maker, and health care professionals. The second agenda is Workshop on Basic Cytotoxic Handling for hospital pharmacists and academia. It will hold on 26-27 May, 2013 in Pharmacy Department, Bethesda Hospital.

I do hope this conference will give a new initiative for practicing sorounding cytotoxic handling and team-work. I thank to conference sponsors and committee members for their support.

Wassalamu'alaikum warahmatullahi wabarakatuh,

Yogyakarta, 25 May 2013

Dr.rer.nat. Endang Darmawan, M.Sc., Apotheker
Chairperson of the organizing committee

ISBN : 978-979-18458-6-1

Welcome Address from Dean of Faculty of Pharmacy, Ahmad Dahlan University

Assalamu'alaikum Wr. Wb.

Dear all participants,

Welcome to the International Conference and Workshop on Safety Management of Central Cytotoxic Reconstitution in Pharmacy Practice. Thank you for participating in this great event. Hopefully, you can enjoy the days of International Conference and Workshop.

The topic of this international seminar is very interesting and important for the development of pharmacists' skills in drug reconstitution, since we know that our new perspective of pharmacy practice is patients' care and patients' safety. Dealing with cytotoxic drug is a kind of collaboration among physician, pharmacist, nurse, psychologist, patient and patient's family. However, as the pharmacists, we have unique skills and knowledge related to the preparation, administration and monitoring of cytotoxic drug administration. Therefore, in a team work, pharmacist has some responsibilities as a leader related to the cytotoxic drug reconstitution.

This international conference and workshop are the collaboration between Faculty of Pharmacy, University of Ahmad Dahlan Yogyakarta as academic institution with the Bethesda Hospital of Yogyakarta as one of the central cytotoxic reconstitution. We also invited Prof A.A. Kaptein, from Leiden University Medical Center, Leiden, The Netherlands and Harbans Kaur Dhillon from University Malaya, Medical Centre, Malaysia as speakers, besides Dra. L. Endang Budiarti, M.Pharm., Apt. as practitioners in Bethesda Hospital of Yogyakarta and Dr. Dyah Aryani Perwitasari, Apt., PhD who wants to share about development of pharmacist's skill in medical reconciliation. After the one day seminar, we invite you to joint with us on the workshop of Central Cytotoxic Reconstitution which will be held in Bethesda Hospital of Yogyakarta. This workshop is useful for us, especially when we want to start the Central Cytotoxic Reconstitution in our hospital.

I hope, The International Conference and Workshop on Safety management of Central Cytotoxic Reconstitution in Pharmacy Practice will inspire us to practice our skills and knowledge in our fields. In the future, we can reach one the goal of pharmacy practice, which is patients' safety.

Have a great conference.

Wassalamu'alaikum wr wb

Dean of Faculty of Pharmacy
University of Ahmad Dahlan, Yogyakarta

Dr. Dyah A Perwitasari, Apt., Ph.D

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COMPLIANCE → ADHERENCE → CONCORDANCE → SELF-MANAGEMENT → QUALITY OF LIFE

Prof. dr. A.A. Kaptein
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Illness elicits an enormous set of behaviours in humans. Perceiving symptoms and interpreting them as necessitating the seeking of medical care is one major step. Enduring diagnostic and therapeutic procedures is another. In chronic illness, long-term use of medication is usually required. Managing the illness, and incorporating the illness and its treatment into daily life, are other major tasks. In short, achieving an acceptable quality of life is a core task in the lives of ill people all over the world.

In this chapter I will discuss three of the topics mentioned above: 'compliance'; self-management; and quality of life ['compliance', adherence, and concordance will be discussed together in one section]. Some theoretical background regarding the three main issues will be provided, with empirical studies presented as illustrations. I will do this from a behavioural medicine perspective as this integrates biomedical, behavioural, pharmacological, and societal views, as well as expertise regarding being ill.

This chapter may serve as a guide for initiating and executing relatively modest research projects for students of medicine, pharmacology, and psychology. Additional sources are relatively easily accessible for this purpose (e.g., French et al., 2010; Ogden, 2012).

ISBN : 978-979-18458-6-1

DEVELOPING PHARMACISTS'SKILLS IN MEDICATION RECONCILLIATION

Dr. Dyah A Perwitasari, Apt., Ph.D
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The role of pharmacists in Indonesia was moved into new perspective, which is patient oriented, since the last decade. Thus the practice knowledge and skills of pharmacy was developed into the aim of improve of patients' care and patients' safety. The American Society of Health System Pharmacist defined that the effective process of medication reconciliation reduces medication errors and support safe medication used by patient. These fact have been supported by many evidences that the role of pharmacists in medical reconcilitiation could reduce the potential adverse drug events, reduce the transtition medication errors and had significant impact in the medical cost. Many experts in The Joint Comission of Accreditation of Healthcare Organization defined that medication reconciliation is *The process of comparing the medications a patient is taking (and should be taking) with newly ordered medications in order to resolve discrepancies or potential problems.* The goal of medication reconciliation are to obtain and maintain accurate and complete medication information for a patient and use the information within and across the continuum of care to ensure safe and effective medication use. By understanding the definition, we know that the main point in the medical reconciliation is the role of pharmacist as a leader in managing of drug administered to the patients. Even though the pharmacist work in a health profesional team, however only pharmacist who has unique knowlede and skills about drug. Thus, the competency of pharmacists as drug experts should be applied in their work fields. To start the medication reconciliation in our fields is not easy, but we can start these activities as the first steps, which are ; reviewing the medication therapy , collecting the personal medical record, applying the medication related action plan and collecting intervention and/or referral documents. In the future, our roles as the pharmacists who have expertise in medication reconciliation, can start to be developed from now, especiallt in the fields of policy and procedure development, implementation and performance improvement, training and competency assurance, information system development and advocacy.

TRAUMATIC EXPERIENCE OF ADOLESCENT FEMALE IN FLOODS OF COLD LAVA AFTER THE ERUPTION OF MOUNT MERAPI IN THE PERSPECTIVE OF GROWTH AND DEVELOPMENT IN MAGELANG REGENCY SHELTER

Retna Tri Astuti[1], Achir Yani S.Hamid[2], Novy Helena C.D[3]
University of Muhammadiyah Magelang

Abstract

Background. *Cold lava flood disaster is catastrophic eruption of Mount Merapi was in Yogyakarta. This disaster cause a traumatic experience for the community including adolescent female.*

Objective. *The Objective of this study was to explore the traumatic experience of adolescent female in floods of cold lava after the eruption of Mount Merapi in the persfective of growth and development in Magelang regency shelter.*

Methods. *Methodology in qualitative research is phenomenological. Subjects in the study were the six partisipans taken purposively with criteria had experienced traumatic.*

Outcome measured. *Impact of Event Scale (IES) for screening traumatic tools and in-depth interviews.*

Results. *Six themes of the picture obtained traumatic for adolescent female, the psychological response, cognitive responses, phychical responses, changes in social relationships, personal growth and rehabilitation of daily living.*

Conclusion. *Research on Adolescent Girls Traumatic Experiences Cold Lava Flood Post Mount Merapi eruption have been identified participants experienced minor trauma is trauma and trauma of the participants were there five participants and there are six major themes, in which the six themes are related to specific objectives.*

Keyword : *Traumatic experience, psychological responses, cognitive responses, physical responses, changes in social relations, achieving personal growth and rehabilitation of daily living*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Problem of prolonged natural disaster in Indonesia has its own impact to the community. One of the biggest natural disasters in 2010 was the eruption of Mount Merapi in Yogyakarta and the subsequent disaster that cold lava flood. This flood caused a variety of damage and trauma itself for the people who experienced it, including for girls. Adolescent girls is one of the most vulnerable groups experiencing traumatic events they experienced. Vulnerability of adolescent girls is not only the physical vulnerability but also emotional or psychological vulnerability and social development. Based on the above phenomenon in this study wanted to explore the traumatic experience of adolescent girls due to cold lava flooding after the eruption of Mount Merapi in the perspective of growth in temporary shelters Magelang regency. The Objective of this study was to explore the traumatic experience of adolescent female in floods of cold lava after the eruption of Mount Merapi in the persfective of growth and development in Magelang regency shelter.

METHODS

This research is a qualitative, descriptive, exploratory using phenomenological methods Election participants in this study used purposive sampling method. Study participants were selected based on the inclusion criteria of this study is that participants were adolescent girls aged 15-18 years, had mild to moderate traumatic disorder, living in temporary shelters ± 1 years, able to communicate well with the use of the Java language as well as Indonesian and able to express thoughts traumatic and adolescent growth and development for our shelter. Participant election results obtained in this study were 6 participants in accordance with the study inclusion criteria above.

Data collection of this research was conducted using face-to-face and in-depth interviews. The time in-depth interviews conducted for approximately 45-50 minutes using the tools of the recorder. Processing of data that researchers are doing by using qualitative analysis. Analysis of the data used in this study based on the analysis phase by Colaizzi (1978, in Polit & Hungler, 2001).

RESULTS

Theme analysis results generated by researchers that there are 6 themes. These themes are (1) psychological response (2) memory of the incident, (3) physical response, (4) changes in social relations, (5) the development of personal growth, (6) rehabilitation of everyday life.

DISCUSSION

Specific Objective 1: Holistic Response to Traumatic Events Teenage Girls Holistic response is a response that includes response to overall physical, psychological, cognitive and social.

Theme 1: Psychological Response

This psychological response is a response that experienced by the participants in which the emotional disturbance that includes the absence of fear, panic and a sense of sadness.

Psychological responses that appear on the participants in this study is one of the symptoms of Post Traumatic Stress Disorder in which the traumatic experience may lead to interference with the integrity of the individual and that individual experiencing fear, helplessness and trauma itself (Townsend, 2009; Varcarolis, 2010). The fear felt by the participants are part of the adaptive response to stress and cognition is a response to this will affect the physical condition of a person who can bring many diseases (Potter and Perry, 2005; Perry, 2003; Wade, 2007; Fuadi, 2011).

Theme 2. Memory for Events

The memory of the events that led to the sub-themes of repetition of traumatic experiences due to cold lava flooding after the eruption of Mount Merapi in the presence of flashbacks and memories shadow of cold lava

flood event. Memory flashbacks experienced by the participants are part of a phenomenon that often occurs in Post Traumatic Stress Disorder (PTSD). Cognitive changes caused by this traumatic event will contribute to the development of an external locus of control (Fontaine, 2009). Negative cognition system that will make people have a negative thought patterns that lead to repeated belief that individuals have a negative effect on the physical condition then individuals and led to many diseases (Fuadi, 2011).

Theme 3: Physical Response

Physical responses experienced by adolescent girls are complaints that they are natural and physical changes. Grievances felt by teenage girls is a sleep disorder and other pain. Changes in body weight and height was also experienced by the participants as expressed by the participants.Traumatic experiences can cause physical changes in the body's process of adaptation to stress. When there is stress, individuals using physiological and psychological energy to respond and adapt. The amount of energy required and the effectiveness of efforts to adapt depends on the intensity, scope and duration of the stressor and the amount of other stressors (Potter & Perry, 2005).

Theme 4: Changes in Social Relations

Changes in social relationships that are formed from two categories: loss of social relationships and togetherness. The existence of strain on relationships with peers will cause interference with the social development. The presence of peers is very meaningful. The presence of peers to be a forum for learning social skills can take on roles in the group (Soetjiningsih, 2007).

Specific Objective 2: Meaning of Traumatic Experiences

Theme 5: Development of Personal Growth This includes the development of personal growth and self-discovery motivation in life. The development of personal growth is a good development consciously or unconsciously within the individual that consists of the development of the mind, physical, emotional, relationship spirit, creativity and interpersonal (Levine, 2006). The advent of personal growth development self motivation self where there is a desire to get up and change the desire for the happiness of the parents and study harder to get better performance.

Specific Objective 3: Teenage Girls Hope Post Traumatic

Theme 6: Rehabilitation of Everyday Life

Themes corresponding to the four specific objectives are the rehabilitation of everyday life. Where the theme is composed of a physical guard, hope in themselves and hope for the government. Physical custody is carried out by participants in meeting the growth and development tasks to undertake efforts to meet the physical needs of participants during their stay in temporary housing. In addition to the physical custody, rehabilitation everyday life by having participants hope given to her include the return to the beginning of life and the desire to grow healthy. It is delivered by five participants.

Participants wanting to grow healthy people eat during their stay at the shelter. The effects of trauma can affect the development of skills such as personality, perception of trauma disorders, cognitive development, psychosocial development, and spiritual development of adolescents (Anderson, 2005). Efforts to improve everyday life expressed by participants in this study in accordance with the theory that girls should have an understanding of self and disaster preparedness and cooperation (Ministry of Social Affairs, 2012).

CONCLUSION

Results of this study identified six themes generated is associated with symptoms of traumatic and change and adaptation mechanisms that exist in the participants. The

resulting six themes is a psychological response, the memory of the events, physical responses, changes in social relationships, personal self development and rehabilitation of everyday life.

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COST ANALYSIS THERAPY OF BREAST CANCER PATIENTS IN Prof. Dr. MARGONO SOEKARJO PURWOKERTO

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Abstract

Background. *Breast cancer is leading cause of death in Indonesia. Treatments for breast cancer is generally in combination therapy i.e chemotherapy, radiation and surgery. Various cancer therapies require a lot of drugs. Various side effects also occurred in cancer therapy and require additional drugs. This leads an increasing in the overall cost of the total cost incurred by cancer patients.*

Objectives. *The purpose of this study was to describe the treatment case, treatment and the average cost of breast cancer patients in Prof Dr. Margono Soekarjo Hospital Purwokerto in 2010.*

Methods. *This research was a descriptive study with retrospective data and used total sampling method to obtain the data from medical records and receipts. The breast cancer patients data from January to December 2010 period was taken.*

Outcomes measured. *Medical and non medical costs are calculated. The average cost was calculated by dividing the total cost of side effects with the total direct costs incurred in cancer patients.*

Results. *The result showed that 39 patients included in the inclusive criteria. The highest number cases was patient in stage IIIC, 23 cases (59.00%). Alkylating and antimetabolit agent combination was the most widely used for chemotherapy, as many as 31 cases (81.57%). Analysis of direct and non direct medical costs : stage IIa third class was Rp. 23,669,472.50. In stage IIb, second class was Rp. 38,062,480.30, and in third class was Rp. 11,063,230.90. In stage IIIA second class was Rp. 22,523,373.00, while for third class was Rp. 20,380,060.73. Stage IIIB for third class was Rp. 13,503,594.00. In stage IIIC second class was Rp. 37,873,859.00 and third class was Rp. 11,256,590.00. Last stage is stage IV, in third class was Rp. 14,345,890.30.*

Conclusion. *The results indicated that hospital need to be more attention for handling breast cancer therapy, particularly, because it has higher therapy cost than anyother disease.*

Key words : *Cost Analysis, Breast Cancer, Prof. Dr. Margono Soekardjo Hospital*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Based on the Hospital Information System (SIRS) data, in 2007, breast cancer was first ranks in hospitalized patients in Indonesia (16.85%) (Anonymous, 2010). In Central Java, according to a report of the District Health Office, in hospitals and health centers in 2006, there were breast cancer cases per 1,000 people (Anonymous, 2006). According to the America Cancer Society (2011), breast cancer is the uncontrolled growth of cells in the breast. Malignant tumor is a group of cancer cells that can be grown and then invade surrounding tissues or spread (metastasis) to distant areas of its origin.

There are four treatment were used in breast cancer, including surgery, radiation, chemotherapy and biological therapy (Balmer et al., 2005). Surgery is usually done by taking some cancerous tissues or whole breast removal, depending on the stage of cancer. Second breast cancer treatment using radiation, with high-tech tools required expertise to operate it. For chemotherapy, the patient using more than one drug (combination drugs). Treatment ussualy in a cycles, depending on the severity or stage of breast cancer patients. Radiation and chemotherapy has side effects such as nausea,vomiting, anemia, etc. thus requiring additional costs to handle this side effect. (Balmer et al., 2005). Therefore, in the treatment of breast cancer required huge cost.

Pharmacoeconomics use to identify the pharmaceutical products and health services cost, which describes the health economic relationship involving drugs, services, and prices used by the public (Jana et al., 2005). In this study, we carried out Pharmacoeconomics study, using Cost Analysis method (Shancez, 2005) to explore the overall cost in the treatment of breast cancer in Prof. Dr. Margono Soekarjo Hospital in January-December 2010.

METHODS

Research design. This research was a descriptive study with retrospective data. We used total sampling method. Data were obtained from medical records, financial receipts and cards in inpatient Margono Soekarjo Hospital, January to December 2010. Research point of view was the perspective of the hospital.

The inclusion criteria used were patients with a primary diagnosis of breast cancer who underwent anticancer treatment in Margono Soekardjo Hospital Soekarjo in 2010 and patients were classified as public health insurance and health insurance category.

Data collection. Data were collected from medical records include number, age, clinical diagnosis, date of entry, date of exit, long of stay, the patient's outcome, and medications used during treatment. Financial receipts include therapeutics costs, hospitalization costs, laboratory costs, medical costs, nutrition costs, side effect costs, and administrative costs. We also obtained data from the drug card in installation of pharmacy hospital included drugs and medical equipment costs.

Data analysis. Research data in the form of patient demographic characteristics (age), characteristics of breast cancer cases (characteristic diagnosis, staging, the last condition of patients, the identification of patterns of breast cancer therapy) and treatment costs were analyzed descriptively. Cost analysis is done by looking at the major components and the cost incurred by patients during treatment.

Calculating total direct costs using the formula:

$$AB = BT+BP+BG+BTn+BPEST+BOA+BRI+BA$$

AB	:	Cost Analysis - Medical and Non-Medical costs
BT	:	Therapeutics Costs
BP	:	Laboratory Costs
BG	:	Nutrition Costs
BTN	:	Medical Costs
BPEST	:	Side Effect Costs
BOA	:	Drugs and Medical Devices Costs
BRI	:	Hospitalization Costs
BA	:	Administrative costs

ISBN : 978-979-18458-6-1

Calculate the average cost using the formula :

$$X = \frac{C}{n}$$

X = The average cost of treatment of breast cancer (IDR) (per therapy/stadium)

C = Total direct costs (IDR) (per therapy/stadium)

n = number of patients (per therapy/stadium)

RESULTS AND DISCUSSION

Data population were 128, five (5) cases with incomplete medical record data, 8 cases with no receipts, and 78 cases were not up to six times chemotherapy treatment cycle so only 39 cases include to inclusion criteria. The distribution of each class were 7 patients class II and 32 patients as class III. The number of breast cancer patients were 39 female patients (100%).

The most patient affected by breast cancer are between the age of 44-52. The American Cancer Society in 2011, stated that cancer was susceptible at above 40 years. Redalli and Radice (2003) in their review article also mentioned that women are usually affected by breast cancer at 40-50 years. This is because of the lack of early detection in every woman in that age, who susceptible to breast. Patient were grouped into two classes, class II and class III.

Treatment class will affect the direct medical costs. In Prof. Dr. Margono Soekarjo hospital there are three classes, class I, II and III. However, in this study, for breast cancer patients was in class II and III. In this study, most patients were treated in the third grade as many as 32 patients (82%) and class II were 7 patients (18%).

The side effects of chemotherapy are effects arising from cytostatika drugs used in chemotherapy in the treatment of breast cancer.

According to Balmer *et al*., (2005) there are several types of side effects caused by chemotherapy, among which bleeding while undergoing chemotherapy, anemia, nausea, vomiting, pain, infections, constipation and fluid retention. The side effects of chemotherapy is the most frequent nausea and vomiting. There were 38 patients (51.35%) with nausea and vomiting caused by chemotherapy. The second highest is anemia by 22 (29.72%) patients.

Treatment for breast cancer patients depends on the. The American Joint Committee For Cancer (AJCC, 2003), grouped the stadium of breast cancer into eight groups. Each stadium had a different treatment. In this study, we found no cases for all stages, only stage IIa, IIb, IIIa, IIIb, IIIc and IV.

The most cases are patients with chemotherapy, 38 cases. In stadium IIIc patient who undergoing chemotherapy are 23 patients. The second type of treatment for breast cancer is surgical with totally 6 cases, 3 radical mastectomy (removal of the affected breast cancer) and 3 incisional biopsy (taking a small portion of solid tumor tissue). According to the National Cancer Institute (2009), the first option of treatment for stage IIa, IIb and IIIa is by radiation or surgery, then chemotherapy may be performed as an adjuvant or neo-adjuvant. Then for stage IIIb and IIIc, first choice treatment is chemotherapy and surgery. But for the surgery, it must be confirmed first, the extent to which cancer cells spread. Then for stage IV or metastatic, the first choice for treatment is use high-dose chemotherapy.

Balmer *et al.* (2005) stated that class of drugs that are still commonly used for breast cancer treatment are antimetabolite, alkilating agents and topoisomerase inhibitors. There are 31 patients in class III, who used antimetabolite, alkilating agent and topoisomerase inhibitors as combination. It is the highest number of patient who use drugs combination compared with other combination. While in class II, there was 4 patients using a combination of a taxane, alkilating agents and topoisomerase inhibitors.

Additional drugs that most commonly used are antiemetics, because the most frequent adverse effects from breast cancer chemotherapy are nausea and vomiting. Drugs used for cytostatica in this study are cyclofosfamid

(?1,500 mg) and dacarbazin that fall into the high risk category (> 90%) cause nausea and vomiting. Doxorubicin and cyclofosfamid <1,500 mg, epirubicin which includes medium category (30-60%) cause nausea and vomiting, 5-Flurourasil, paxus and ebetaxel are in the low category (10-30%) which causes nausea and vomiting. Vincristine is entered in the category of low (<10%) cause nausea and vomiting. This is in accordance with the Hesketh (2008) and Hawkins (2009) research.

Primasari (2009) stated, regarding the evaluation of antiemetic use in Prof. Dr. Margono Soekarjo Hospital Purwokerto, the most widely used antiemetic to overcome the side effects of chemotherapy are dopamine antagonists and histamine antagonists.

We found in our study, the most commonly used antiemetic are dopamine antagonists and histamine antagonists. According to Hesketh (2008), in a review article, the most effective anti-nausea vomiting caused by chemotherapy is serotonin antagonists (5-HT3).

Serotonin antagonist clinically proven to be effective for the treatment of nausea and vomiting caused by chemotherapy in cancer patients. The mechanism that probably involved is when serotonin out of cell enterokromafin then binds to 5-HT3 receptors located on sensory nerve / vagal afferents in the gastrointestinal tract, and delivers stimulation through the nerves to the vomiting center and chemoreceptor trigger zone (CTZ) in the brain postrema. 5-HT3 antagonist (Ondansetron) works by blocked the receptors in the periphery and in the CTZ to prevent vagal stimulus that ultimately prevent nausea and vomiting (Ikawati, 2006). However, in this study, not all patients using 5-HT3 class to overcome the effects of nausea and vomiting due to the price is relatively more expensive when compared with other drugs.

Cost analysis of breast cancer therapy in our study conducted by the hospital perspective focused on direct medical costs and non-medical costs. We explored the data in order to determine what are the components of the costs and how much it costs in breast cancer patients during treatment at the hospital in accordance with patients stadium.

Components of direct medical costs consist of the treatment costs, laboratory costs, physicians costs, side effects treatment cost and medical equipment costs. While the cost of non-medical components are consisting of administrative costs, hospitalization costs and nutrition costs.

Breast cancer treatment costs is a cost incurred for patients undergoing breast cancer therapy (Table I). Included the cost of surgery, radiation and chemotherapy for six cycles every two weeks for 3-5 months.

Table I. Averageof Treatment Cost Based on Stadium in breast Cancer Therapy in Prof.Dr.MargonoSoekarjo Hospital in 2010.

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	15,000.000
IIb	32,329,590	6,957,572
IIIa	14,443,000	14.424.998
IIIb	-	7,611,378
IIIc	30,784,010	5,922,540
IV	-	7,260,000

Expenses are incurred for supporting the breast cancer diagnosis. Includes laboratory tests, examination of the thorax, and the other assesment according to the current stage of patient (Table II.).

Table II. Average Laboratory Cost Based on Stadium of Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010.

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	442,500
IIb	1,563,000	1,718,438
IIIa	2,033,000	2,024,000
IIIb	-	1,563,000
IIIc	1,875,600	1,563,000
IV	-	2,615,000

Expenses in a patient was vary depending on the class and type of therapeutic treatment. Differences in the cost of each class is due to the difference in rates imposed in each class, include physician costs (Table III). The more severe or high stage and type of therapy used, cause many treatment have to take. The longer long of stay the greater costs have to paid.

Table III. Average Physicians Cost Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	210,000
IIb	510,000	423,750
IIIa	660,000	495,000
IIIb	-	735,000
IIIc	462,000	383,055
IV	-	360,000

Side effects costs is a costs incurred as a result of the side effects of treatment (surgery, radiation and chemotherapy) (Table IV). It is include the cost of medicine and blood transfusions.

Table IV. Average of Side Effects Costs Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	140,990.50
IIb	512,132.75	1,013,465.37
IIIa	1,562,935.00	1,175,304.50
IIIb	-	1,388,547.50
IIIc	2,020,057.00	1,247,157.44
IV	-	2,008,952.75

Medical equipment costs are costs incurred to purchase the medical equipment and consumables equipments during the patient hospitalize (Table V.).

Table V. Average of Medical Equipments Costs Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	75,937.50
IIb	376,757.50	280,618.75
IIIa	253,947.50	251,300.00
IIIb	-	255,668.75
IIIc	281,942.56	278,895.83
IV	-	271,937.50

Non-medical costs are costs incurred for administrative interests, the cost of hospitalization and cost of nutrition during patient stay in hospital (Table VI).

Table VI. Average of administrative Costs Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010.

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
I	-	-
IIa	-	10,000
IIb	60,000	63,750
IIIa	70,000	70,000
IIIb	-	60,000
IIIc	60,000	60,000
IV	-	60,000

For patients undergoing chemotherapy, hospitalization costs can be calculated with one cycle during therapy in a span of 3-5 months to six cycles and then calculated the average cost (Table VII.).

Ta ble VII. Average of Hospitalization Costs Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
Ia	-	-
IIa		7,700,000
IIb	2,250,000	1,498,750
IIIa	3,300,000	1,815,000
IIIb	-	1,760,000
IIIc	2,190,000	1,430,000
IV	-	1,320,000

The lowest hospitalization cost is in stage III patients, due on this stage, patients only undergoing chemotherapy for six cycles, each cycle usually takes only two to three days. The other stages are usually needed more than one type of therapy, causing a longer hospitalization, so hospitalization costs become more expensive.

Nutritional costs are costs incurred for the cost of food production and nutrition consultations (Table VIII).

Table VIII. Average of Nutrition Costs Based on Stadium in Breast Cancer Therapy in Prof.Dr.Margono Soekarjo Hospital in 2010.

Stadium	Class	
	II (IDR)	III (IDR)
0	-	-
Ia	-	-
IIa	-	70,000
IIb	204,000	141,250
IIIa	320,500	165,000
IIIb	-	160,000
IIIc	229,700	215,000
IV	-	120,000

Medical and non-medical costs are the direct costs incurred by patients during treatment in hospital (Table IX - Table XIV).

Table IX. Average of Medical and Non Medical Costs in Stadium IIa Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class III	
	IDR	Precentage (%)
Therapy Costs	15,000,000.00	63.42
Hospitalization Costs	7,700,000.00	32.55
Laboratory Costs	442,500.00	1.87
Physician Costs	210,000.00	0.88
Side Effect Costs	140,990.50	0,59
Medical Equipment Costs	75,937.50	0.32
Nutrition Costs	70,000.00	0.29
Administrative Costs	10,000.00	0.04
Total	23,649,428.00	100.00

Table X. Average of Medical and Non Medical Costs in Stadium IIb Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class II		Class III	
	Jumlah (Rp)	Percentage (%)	Jumlah (Rp)	Percentage (%)
Therapy Costs	32,329,590.00	85.51	6,957,572.00	57.51
Hospitalization Costs	2,250,000.00	5.95	1,498,750.00	12.38
Laboratory Costs	1,563,000.00	4.13	1,718,438.00	14.20
Physician Costs	510,000.00	1.34	423,750.00	3.50
Side Effect Costs	512,132.75	1.35	1,013,465.37	8.37
Medical Equipment Costs	376,757.50	0.99	280,618.75	2.31
Nutrition Costs	204,000.00	0.53	141.250,00	1.16
Administrative Costs	60,000.00	0.15	63,750.00	0.52
Total	37,805,480.25	100.00	12,097,594.12	100.00

Table XI. Average of Medical and Non Medical Costs in Stadium IIIa Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class II		Class III	
	IDR	Percentage (%)	IDR	Percentage (%)
Therapy Costs	14,443,000.00	63.78	14,424,998.00	70.63
Hospitalization Costs	3,300,000.00	14.57	1,815,000.00	8.88
Laboratory Costs	2,033,000.00	8.97	2,024,000.00	9.91
Physician Costs	660,000.00	2.91	495,000.00	2.42
Side Effect Costs	1,562,935.00	6.90	1,175,304.50	5.75
Medical Equipment Costs	253,757.50	1.12	251,300.00	1.23
Nutrition Costs	320,500.00	1.41	165,000.00	0.80
Administrative Costs	70,000.00	0.30	70,000.00	0.34
Total	22,643,192.50	100.00	20,420,602.50	100.00

Table XII. Average of Medical and Non Medical Costs in Stadium IIIb Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class III	
	IDR	Percentage (%)
Therapy Costs	7,611,378.00	56.24
Hospitalization Costs	1,760,000.00	13.00
Laboratory Costs	1,563,000.00	11.54
Physician Costs	735,000.00	5.43
Side Effect Costs	1,388,547.50	10.26
Medical Equipment Costs	255,668.75	1.88
Nutrition Costs	160,000.00	1.18
Administrative Costs	60,000.00	0.44
Total	13,533,594.25	100.00

Table XIII. Average of Medical and Non Medical Costs in Stadium IIIc Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class II		Class III	
	IDR	Percentage (%)	IDR	Percentage (%)
Therapy Costs	30,784,010.00	81.21	5,922,540.00	53.35
Hospitalization Costs	2,190,000.00	5.77	1,430,000.00	12.88
Laboratory Costs	1,875,600.00	4.94	1,563,000.00	14.08
Physician Costs	462,000.00	1.21	383,055.55	3.45
Side Effect Costs	2,020,057.00	5.32	1,247,157.44	11.23
Medical Equipment Costs	281,942.56	0.74	278,895.83	2.51
Nutrition Costs	229,700.00	0.60	215,000.00	1.93
Administrative Costs	60.000.00	0.15	60,000.00	0.54
Total	37,903,309.56	100.00	11,099,648.82	100.00

Table XIV. Average of Medical and Non Medical Costs in Stadium IV Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Class III	
	IDR	Percentage (%)
Therapy Costs	7,260,000.00	51.79
Hospitalization Costs	1,320,000.00	9.41
Laboratory Costs	2,615,000.00	18.65
Physician Costs	360,000.00	2.56
Side Effect Costs	2,008,952.75	14.33
Medical Equipment Costs	271,937.50	1.94
Nutrition Costs	120,000.00	0.85
Administrative Costs	60,000.00	0.42
Total	14,015,890.25	100.00

Table XV. Cost Analysis of Medical and Non Medical Costs in Breast Cancer Patients in Prof.Dr.Margono Soekarjo Hospital in 2010

Components	Average of Medical and Non Medical Costs	
	Class II (IDR)	Class II (IDR)
0	-	-
I	-	-
IIa	-	23,649,428.00
IIb	37,805,480.25	12,097,594.12
IIIa	22,643,192.50	20,420,602.50
IIIb	-	13,543,594.25
IIIc	37,903,309.56	11,099,648.82
IV	-	14,015,890.25

In our study, patients with stage IIIb taken surgical therapy and six chemotherapy cycles. Patients usually have to take medication to overcome the side effects caused by chemotherapy.

According Radelli and Radice (2003) the most expensive cost is in the most severe stage, ie stage IV. However, in our study, the greatest of average direct medical costs are among stage IIa (Table 15.). This can be due to the difference of the individual components for treatment in breast cancer patients. This difference can be seen from not every patient get all kinds of tests to support the diagnosis and treatment of breast cancer. Then, the absence of Home Health Care (the control exercised by the hospital for patients undergoing breast cancer therapy). Home health care is the second greatest costs in direct medical cost in Redelli and Radice research.

In our study, the most expensive cost is therapy cost. This result is in good agreement with research by Redelliand Radice (2003). The cost of breast cancer treatment is the cost of chemotherapy, surgery costs, charges and expenses, and radiation in each stage.

CONCLUSION

The results indicated that hospital need to be more attention for handling breast cancer therapy, particularly, because it has higher therapy cost.

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PHARMACIST COUNSELING INTERVENSION BY ORAL CAN INCREASE THE PATIENTS ADHERENCE AND DECREASE SYSTOLIC BLOOD PRESSURE OF AMBULATORY HYPERTENSION PATIENTS AT INTERNAL DISEASE POLYCLINIC PKU BANTUL HOSPITAL, INDONESIA

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Abstract

Background. *The hypertension prevalence in Indonesia in 2004 is 27,5%. High blood pressure can damage arteries and blood vessels. It can also cause coronary artery disease, kidney failure and stroke. It is expected that the appropriate counseling can improve the patients adherence and blood pressure target.*

Objective. *The purpose of this study is to investigate the influence of pharmacist counseling orally on the adherence and systolic blood pressure of ambulatory hypertension patients at internal disease polyclinic PKU Muhammadiyah Bantul Hospital, Indonesia.*

Methods. *This study were conducted with quasi-experimental design. The ambulatory hypertension patients data were collected prospectively during the period of January until April 2013. Sixty patients were divided into 2 groups, 30 (50%) patients were received counseling (intervension group) and 30 (50%) patients were not received counseling (control group). Exclusion criteria were a deaf and pregnant patients. Data collection were conducted by doing interview and completion of Morisky Modification Adherence Scale (MMAS) questionnaire, while the blood pressure data were taken from their medical record.*

Outcome Measured. *Adherence and systolic blood pressure of ambulatory hypertension patients*

Results. *The results showed that oral counseling intervension could increased the patients adherence in the intervension group (66,7%) in comparison to the control group (20%) (p=0,000). Consequently, pharmacist counseling intervension could decreased the systolic blood pressure in the intervention group (17,27 ±14,60 mmHg; p=0,000). There was no decreasing the systolic blood pressure (1.27±19.89; p=0,730) in the control group. Based on the correlation test between MMAS and systolic blood pressure, there were positive correlation in the patients adherence and systolic blood pressure values (p=0,020; r=0,200).*

Conclusion. *Over all it can be concluded that the pharmacist counseling intervension by oral can increase the patients adherence on antihypertension management. Furthermore, it can decrease the systolic blood pressure (p<0,05)*

Key words : *Hypertension, pharmacist counseling, systolic blood pressure, adherence*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Hypertension is as one of the major risk factors for coronary heart disease. Besides causing coronary heart disease, hypertension can cause kidney failure and cerebrovasculer disease. The prevalence of hypertension will increase in line with the life style changes such as smoking, obesity, physical inactivity, and psychosocial stress in many countries. Hypertension has been the public health problem and will be a bigger problem if it is not restrained earlier[1.] The prevalence of hypertension in Indonesia in 2004 is 27,5 %[1]. (Depkes, 2004).

WHO states that non adherence to hypertension therapy is the key factor that inhibit blood pressure control so that it needs intervention to improve the therapeutic adherence. It is estimated that the poor adherence to antihypertensive medication is approximately 30-50 %. The causes of the poor adherence is very complex, including the complexity of medical regiment, cost of medication, age, lowness of social support, and cognitive problem[2]. (Sagate, 2003).

Health people 2010 for hypertension suggest the necessity of a more comprehensive and intensive approach to achieve optimal blood pressure control. The intervention which can be applied by pharmacists to manage hypertension patients is counceling. Counceling can improve the outcome therapy by maximizing the use of appropriate medication[3] (Romtucci, 1997). One of the counseling benefit is improve mediaction adherence, so the mortality rate and detriment (either cost or loss of productivity) can be pressed down[4]. (Palaian *et.al*, 2006).

Self-report scale to evaluate the adherence of antihypertensive therapy was already developed by Morisky *et al* (2008). The study examines the psychometric properties and tests the concurrent and predictive validity of self report medication adherence was test on hypertension patients in 2007 and published in 2008. Self report medication adherence is measured by new 8 item self report Morisky Medication Adherence Scale (MMAS).

Over all, it is necessary to investigate the influence of counceling orally on the adherence and systolic blood pressure of ambulatory hypertension patients at internal disease polyclinic PKU Muhammadiyah Bantul Hospital, Indonesia.

METHODS

It is a prospective study to determine adherence to anti-hypertensive therapy and decreasing the systolic blood pressure in ambulatory patients at internal disease polyclinic PKU Muhammadiyah Bantul Hospital, Indonesia. The study group included 60 patients. They were devided in to two groups as intervension and control group. The intervension group patients received counseling regarding hypertension and hypertension therapy, while the control group not received counseling. The follow up patients were done from baseline to second follow up. The inclusion criteria were patients 18-65 years old with diagnosed to have hypertension and got antihypertensive medication in their prescription. The exclusion criteria were deaf and pregnant patients.

The data were collected from January to April 2013. Data collection was conducted by doing interview and completion of Morisky Medication Adherence Scale (MMAS) questionnaire, while the blood pressure data were taken from their medical record. Validation questionnaire was carried out via conducting pilot study. The pilot study was conducted with 30 patients. The reliability analysis of the questionnaire was performed by calculating cronbach alpha value. The Cronbach alpha value was obtained 0,64 which indicated that the questionnaire was reliable to be used for this study.

The collected data were analyzed and result were expressed as mean ± standard deviation. P value of < 0,05 was considered statistically significant.

RESULTS

Table I. Characteristic of hypertension patients

Characteristic Patients	Intervension Group		Control Group	
	(n=30)	%	(n=30)	%
Sex				
Male	20	66,7	9	30,0
Female	10	33,3	21	70,0
Age (year)				
40-49	4	13,3	5	16,7
50-59	18	60,0	16	53,3
60-65	8	26,7	9	30
Stage of hypertension				
Stage 1	8	26,7	15	50,0
Stage 2	22	73,3	15	50,0
Habit				
Smoking	4	13,3	3	10,0
Not smoking	26	86,7	27	90,0
Education				
<9 year	16	53,3	20	66,7
9-12 year	4	13,3	6	20,0
>12year	10	33,3	4	13,3
Jobs				
Official government	12	40,0	7	23,3
Self employed worker	12	40,0	16	53,3
Labourer	4	13,7	5	16,7
Jobless	2	6,7	2	6,7
Payment				
Self payment	7	23,3	12	40,0
Health Insurance	14	46,7	9	30,0
Jamkesmas	19	30,0	8	26,7
Other Insurance	0	0	1	3,3
Hypertension History				
Yes	11	36,7	9	30,0
No	19	67,3	21	70,0

Sixty patients were included in the pre and post study. At the pre-study, clinical and sociodemographic data of patients were collected. The characteristic data of the subject can be seen on the table I. Based on the characteristic patients, the subject were dominated by male patients (66.7%) for intervension group and female patients (70.0%)

for control group. As for age, both of the intervension and control group were dominated by patients with the ages of 50 to 59 years. As for stages of hypertension, both groups were dominated by patients with hypertension stage two. As for payment, the treatment group was dominated by health insurance (46.7%), where as the control group was dominated by self-payment (40%). In this study also evaluated the characteristic of smoking behaviour, the history of hypertension, education, and jobs. The subject study, either the intervension group or the control one, both were dominated by those who didn't have the history of hypertension, smoking behaviour, self employed workers and education under 9 years.

The result on table II was the adherence of treatment group is higher than the control group (66.7% > 20.0%). The statistical result between MMAS category at the intervension and control group showed difference of the increasing adherence scores 2.67 ± 0.48 and 1.93 ± 0.69 respectively with the significancy 0.000 ($p<0.05$)

The average systolic blood pressure of the intervension and control group pre and post study were 161.60 ± 11.78 mmHg to 144.33 ± 19.42 mmHg and 148.53 ± 20.83 mmHg to 147.27 ± 20.79 mmHg respectively. The average of the decreasing systolic blood pressure of the intervension group were greater than the control group (17.27 ± 14.60 mmHg > 1.27 ± 19.89 mmHg).

The paired samples t test result on systolic blood pressure of the intervension group was highly significant with p value 0,000 ($p<0,050$), meanwhile on the control group was not significant with p value 0.730 ($p>0.05$).

The correlation test result indicates that there was significant correlation between MMAS category and systolic blood pressure decreased of hypertension patients in this study ($p = 0.020$; $r = 0.424$)

DISCUSSION

The medication adherence plays important role in achieving target therapy, especially for chronical disease, such as hypertension. The poor adherence to antihypertensive medication is as one of the cause of poor blood pressure control. One of the ways to evaluate the adherence of hypertension patients in consuming medication is by using Morisky Medication Adherence Scale (MMAS) questionnaire.

The percentage of high adherence on intervension group is greater than the control

Table II. MMAS scores of patients of the intervension group after done counseling and control group in the post study

Group (n=30)	MMAS Scores					
	High adherence		Moderate adherence		Low adherence	
	n	%	n	%	n	%
Control	6	20,0	16	53,3	8	26,7
Intervension	20	66,7	10	33,3	0	0

Table III. The reasons of non adherence patients based on MMAS questionnaire

No	Reason of unadherence	Group		Total (%)
		Intervension	Control	
1	Forgotten	13	10	38,3
2	Intentionally not to take medication	2	9	18,3
3	Feeling inconvenience by the obligation to take medication.	10	12	36,7

group (66.7% > 20.0%) and the significant increase of adherence indicates that counceling by pharmacists can contribute positive impact in increasing the patients adherence on hypertension therapy. Counseling give a good knowledge about hypertension and its medication to patients, so the patient will change their behavioural medication from being not adherence first to being adherence so that desired blood pressure can be achieved. Birader *et al* (2012) states the counceling intervention given by pharmacists can increase the adherence of hypertension patients therapy. Other study performed by Palanisamy and Sumathy (2009)[8] indicates that the rate of adherence from 0% up to 95.4% after being councelled by pharmacists.

The MMAS questionnaire provides information on the behaviour related to the poor adherence which is caused by unintentional factor (ex: careless or forgetfulness to take medication), intentionally (not to take medication as their illness is getting more serious on better), and the lack of knowledge about hypertension and the purpose of its medication. Table III indicates the non adherence of patient to medication because of frequent forgetfulness to take it and their misunderstanding towards hypertension and its medication so they intentionally not to take their medication.

Poor adherence is as challenge for clinicians and pharmacists to determine more effective medication. If pharmacists have ability to identify patients with poor adherence, then accurate and suitable intervention can be done to improve the patients medication adherence. By the existension of counceling by pharmacists hopefully the understanding of patients toward hypertension and its medication will be better.

The systolic blood pressure of the intervension and control group were the same undergo decrease, though, based on the average of systolic blood pressure decrease of the intervension group is greater than the control group (fig. 1). Based on that, it can be concluded that hypertension patients of the intervension group (being councelled by pharmacists) undergo the significant decrease of systolic blood pressure compared with the control group. Ramanath *et al* (2012) indicates that pharmaceutical intervention can improve the blood pressure control of hypertension. In addition the study carried out by Vivian (2002) show that pharmaceutical care can increase the ability to control blood pressure so the normal blood pressure target can be achieved.

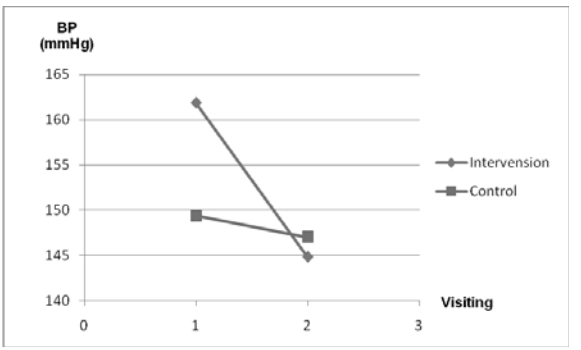

Fig 1. The Average decreased of systolic blood pressure on the intervension and control group

The decrease of blood pressure patients can be influenced by the counceling given by pharmacists to the patients during the study so the patients adherence increase in managing their hypertension, either in the life style modification or accurate use of medication.

The correlation test indicates that their is a significant correlation between the category of MMAS and the decrease of systolic blood pressure of hypertension. The result is in line with the desired outcomes, so there were a significant correlation between the decrease of systolic blood pressure and MMAS category, though correlation coefficient was weak correlation. In this case, it might be reasonable to say that the adherence is not the most dominant to decrease blood pressure. The others factors which influence the decrease of blood pressure, such as the changes of life style and the accuracy of choosing medication. The positive correlation, so it can be conclude that the better

improve of patients adherence will decrease blood pressure better.

CONCLUSION

The pharmacist counseling is effective in improving medication adherence and its associated effect on decreasing systolic blood pressure in patients receiving antihypertensive therapy. Blood pressure control will reduce the risk of coronary artery disease, kidney failure and stroke.

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DECREASING SYSTOLIC BLOOD PRESSURE VIA INCREASE PATIENTS ADHERENCE BY SHORT TEXT MESSAGES (SMS) AND USUAL CARE OF PHARMACIST ON AMBULATORY HYPERTENSION MANAGEMENT AT INTERNAL DISEASE POLYCLINIC, PKU MUHAMMADIYAH BANTUL HOSPITAL, INDONESIA

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Abstract

Background. *Prevalence of hypertension increased in line with changes of life style as smoking, obesity, non physical activity, psycho-social stress in many countries. One of the pharmacist intervention for hypertension management is counseling. This purpose to increase therapeutic outcome and adherence in medicine. World Health Organization (WHO) has suggested that usage text message costly more effective for increasing the adherence to improve the health quality of patients.*

Objective. *This study aim to reveal the impact of pharmacist counseling and reminder motivation via short text messages (SMS) on the adherence and decrease systolic blood pressure of ambulatory hypertension patients in the internal disease polyclinic at PKU Muhammadiyah Bantul Hospital, Indonesia.*

Methods. *Sixty patients were devided into 2 groups, 30 (50%) patients were received pharmacist counseling and reminder motivation via SMS (intervension group) and 30 (50%) were not received pharmacist counseling and reminder motivation via SMS (control group). The ambulatory hypertension patients data were collected prospectively during the period of January until April 2013. Exclusion criteria were patients with pregnant, deaf, illiterate, and have not hand phone. Data were collected by interview and take the quesioner Morisky Medication Adherence Scale (MMAS). Blood pressure data were taken from medical records.*

Outcome Measured. *Adherence and systolic blood pressure of ambulatory hypertension patientns.*

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ISBN : 978-979-18458-6-1

Result. *The results showed that pharmasist counseling and reminder motivation via SMS could improve the patients adherence in the intervension group (83.3%) in comparison to the control group (20%) (p=0.000). Pharmacist counseling and reminder motivation via SMS could also decreased the systolic blood pressure in the intervention group (15.37±23.69 mmHg; p=0.001). There was no decreasing the systolic blood pressure (1.27±18.89 mmHg; p=0.730) in the control group. But, there were no correlation between MMAS and systolic blood pressure (p=0.649; r=0.008).*

Conclusion. *In conclusion, the pharmacist counseling and reminder motivation via SMS can improve adherence on antihypertension management, and decrease systolic blood pressure as well.*

Keywords *hypertension, pharmacist counseling, adherence, systolic blood pressure, short text messages (SMS).*

INTRODUCTION

Hypertension is one of the main factors of coronary heart disease and stroke occurrences. WHO has estimated that about 62% of cerebrovascular disease and 49% of ischemic heart disease all across the world may be caused by blood pressure level. The high blood pressure is estimated to have caused 7.1 million deaths per year (WHO, 2003). Hypertension is also one of the causes of death. Vascular complication caused by hypertension may lead to coronary heart disease, myocardial infarction, stroke, and kidney failure.

The hypertension prevalence increases in line with lifestyle changes such as smoking, obesity, physical inactivity, and psychosocial stress in many countries. Hypertension has become a public health problem and will become even into a bigger one if it is not addressed early on.

Health People 2010 for Hypertension suggests the need for a more comprehensive and intensive approach in order to achieve optimal blood pressure control. Thus, to achieve this objective, active participation of peer pharmacists practicing their profession is needed in every single health care (Ministry of Health, 2007).

One of the possible interventions by pharmacists for the hypertension patients' handling is counseling which is intended to improve therapy result by maximizing the appropriate medications (Rantucci, 1997). One of the benefits of counseling is to improve the patients' adherence of drugs usage. Therefore, mortality rate and losses (both cost and productivity) can be abated (Palaian *et al,* 2006).

The patients' adherence affects on the success of a treatment. The therapy result will not reach the optimal level without the patients' awareness, in fact, it may lead to a treatment failure, and it may also cause complication which is very harmful, then eventually becomes fatal (Hussar, 1995). The safe and effective drugs therapy will happen if the patients are given proper information about the treatment and the usage (Cipolle, Strand and Morley, 2004).

Based on the research result conducted by Palanisamy and Sumathi in 2009, it explains that the intervention by providing education and counseling to the patients is able to improve adherence in the treatment. Pharmacists' intervention improves blood pressure's control in hypertensive patients who have uncontrolled blood pressure (Mehos *et al*, 2000). It is also mentioned in the research of Faekeh *et al.*, 2008 which shows that adherence in the treatment is important to control blood pressure.

World Health Organization (WHO) has also prioritized the new technology usage to help health improvement (WHO; 2010). The potential for benefits is recently being developed in the

mobile phone technology usage to affect health in developing countries (Lester and Karanja 2008). *Mobile text messages* which uses cheap *short message service* (SMS) as well as communications; are able be used to convey health messages to mobile phones' owners (Kaplan, 2006).

Other studies on *text messaging* usage to improve adherence as the primary service has showed that SMS usage has cost effectiveness (*cost effective*) than phone calls usage (Leong, Chen *et al.* 2006). Dunbar, Madigan *et al,* have reported high patients' satisfaction with two lanes SMS, this indicates that SMS application is easier in health improvement.

The research on the effectiveness of information delivery intervention and SMS reminders via *mobile phone* reminders to hypertensive patients in *comparative, controlled, multicenter cluster randomized study* has been conducted by Contreras, Marquez, *et al*, 2004. The research by messages intervention provision (*messages*) and message reminders (*reminders*), which are sent through *mobile phones*, providing adherence level research result of 85.1% (CI 74.9% - 95.3%) in the overall research subjects (N = 67), 85.7% (CI, 70.5% -100.9%) in the control group and 84.4% in the intervention group (CI, 70.7% -95.3%) (Contreras, Marquez, *et al,* 2004).

Self-report scale to assess adherence in antihypertensive drugs usage has been developed by Morisky *et al.*, (2008). The research to examine psychometric and validity prediction of *self-report medication adherence'*s structure was tested to hypertensive patients in 2007 and was published in January 2008. *Self-report* of drugs usage adherence was measured with new *8 item self-report Morisky Medication Adherence Scale (MMAS*).

Based on the above, it is necessary to conduct a research to find out the effect of motivational reminders provision via SMS and pharmacy counseling (*usual care*) on adherence of antihypertensive therapy result in internal medication polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta.

OBJECTIVE

The objective of this study was to determine the effect of motivational reminders provision via SMS and pharmacy counseling (usual care) on adherence of antihypertensive and systolic blood pressure therapy in hypertensive patients in internal medication polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta

METHODS

The research was conducted prospectively, with a quasi experimental design. The research subjects were divided into two groups, namely the group which received pharmacists counseling (usual care) and motivational reminders via SMS (intervention group) and the other one which did not receive counseling and motivational reminders via SMS (control group). The inclusion criteria were adult patients, both men and women; aged between 18 year-old - 65 year-old, who conducted control in internal medication polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta, they were diagnosed by a doctor to have suffered levels I and II hypertension with or without dyslipidemia and diabetic mellitus (DM), they got more antihypertensive drugs more than or equal to 1 antihypertensive drug, they had a mobile communication device in the form of hand phones and they were literate, they were willing to join the research. The exclusion criteria were patients with pregnancy, deaf, illiterate and did not have mobile phones. The data was collected by conducting interviews and filling the adherence questionnaire of Morisky Medication Adherence Scale (MMAS), while blood pressure value was taken from medical records.

Statistical analysis used normality test, if $P > 0:05$ normally distributed, independent t-test and paired t-test, whereas if $p < 0.05$ distribution is not normal; Mann Whitney and Wilcoxon test

is used. $p < 0.05$ significance indicates significant different result between groups.

RESULT AND DISCUSSION

The Patients' Demography

The research was conducted during January to April 2013 in internal medication polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta. The research was conducted prospectively toward hypertension outpatients. Sampling was done by cosecutive sampling method, whereas all subjects came sequentially and met the selection criteria were included in the research until the number of the subjects required fulfilled. The subjects' selection for each group was done randomly, whereas all subjects who met the research criteria were divided into two groups by putting the odd-numbered subjects into the control group, while even-numbered subjects were included into the treatment group.

The research subjects obtained 60 patients who were included in the inclusion criteria. The subjects who joined the research from the beginning to the end of the research were 60 patients, consisting of 30 patients who received pharmacist counseling and motivational reminders via SMS (treatment group) and 30 patients who did not receive pharmacist counseling and motivational reminders via SMS (control group). The socio-demographic and clinical data both on control and treatment groups are on table I. In this research, the relationship of the various subjects' characteristics in the treatment and the control groups obtains a non-significant relationship ($P > 0.05$) among the characteristics of gender, age, education, employment, payment, history of hypertension, smoking habits, and hypertension level (Table I.)

In this research, based on the data characteristics of the patients, it can be seen that the majority of the research subjects; both control and treatment groups are women, each amounted to 21 (70.0%) in the control group and 22 (73.3%) in the treatment group, with the most dominating age is in the range of 50-59 year-old; i.e. 16 (53.3%) in the control group and 13 (43.3%) in the treatment group. The patients' education level dominates on educational age of 0-9 years, 20 (66.7%) in the control group and 14 (46.7%) in the treatment group.

This research also conducted assessment whether there was or there was not an existence of cardiovascular risk factors, namely smoking habits and hypertension history. The majority of the patients did not have smoking habits both in control and treatment groups (90%, 93.3%), while for hypertension history in the treatment group shows higher number; i.e. 66.7% compared to the control group which dominates the patients with no hypertension history previously, which is 30% . Either on the control group or the treatment group shows to have patients with hypertension degree I, whereas 19 (63.3%) in the control group and 17 (56.7%) in the treatment group.

Adherence Assessment

The patients' adherence affects on to the success of a treatment. The therapy result will not reach the optimal level without the awareness of the patients themselves, in fact, it may lead into a treatment failure, and it may also cause highly harmful complication and eventually becomes fatal (Hussar, 1995). Thus, it requires outpatients' non adherence identification to determine the effectiveness of the hypertension control level improvement.

In this research, the patients' adherence parameter was measured using the MMAS both on the control group and the treatment group. MMAS score assessment result is shown in Table II, as following.

Table I. Socio demography

Patients characteristics	Intervention group		control group		P Spearman/ Pearson
	(n=30)	%	(n=30)	%	
sex					
men	8	26.7	9	30.0	0.604
woman	22	73.3	21	70.0	
Age (years old)					
18-29					0.619
30-39	1	3.3			
40-49	9	30.0	5	16.7	
50-59	13	43.3	16	53.3	
60-65	7	23.3	9	30.0	
education level					
0-9 years	14	46.7	20	66.7	0.423
10-12 years	8	26.7	6	20.0	
>12 years	8	26.7	4	13.3	
Job					
Employment civil servants	7	23.3	7	23.3	0.536
enterpreneur	21	70.0	16	53.3	
Labor	2	6.7	5	16.7	
Not work			2	6.7	
payment					
General	10	33.3	12	40.0	0.833
Askes	13	43.3	9	30.0	
Jamkesmas	4	13.3	8	26.7	
Other insurance	3	10.0	1	3.3	
History of hypertension					
Yes	20	66.7	9	30.0	
No	10	33.3	21	70.0	0.097
Smoking habbit					
Smoking	2	6.7	3	10.0	0.053
No smoking	28	93.3	27	90.0	
Degree of hypertension					
Level 1	17	56.7	19	63.3	0.864
Level 2	13	43.3	11	36.7	

Table II. MMAS score assessment

Group	Skor MMAS					
	High Adherence		Moderate adherence		Low adherence	
	∑	%	∑	%	∑	%
Control (n=30)	6	20	16	53.3	8	26.7
Intervention (n=30)	25	83.3	3	10.0	2	6.7
P	0.000*					

The results shows that high MMAS score (MMAS = 8) is bigger in the treatment group than the control group (83.3% > 20.0%). This suggests that pharmacists counseling and motivational reminders provision via SMS have a positive impact in adherence improvement in the treatment group. It is also in accordance with the meta-analysis of the research result on the effect of reminder system on patients' adherence, whereas in the research mentions that the

reminder system may be in the form of via telephone calls, text messages, pagers, video telephone calls, etc. They can significantly improve adherence in treatment group (which received the reminders) compared to the control group (66.61%, 54.71%) (Sarah DF, *et al,* 2012).

Statistical comparison between MMAS value in the control group and the treatment group is done by testing the normality. Kolmogorov Smirnov normality test result shows that the data of control group and the treatment group are not normally distributed; therefore, non-parametric Mann-Whitney test is conducted. Mann-Whitney test result indicates significance of 0.000 ($P < 0.05$), this shows that there is a significant difference between MMAS scores in the control and treatment groups.

Approach to assess treatment adherence may use self-report, pill counts, pharmacy records, drug levels. Measurement uses patient self-report is more concise, quicker and easier to use, but the drawback is more subjective (Cook *et al,* 2005; Garber *et al.*, 2004). Currently, a more effective way in more effective measurement has been developed to evaluate the adherence, namely the new 8-item self-report Morisky Medication Adherence Scale (MMAS) (Morisky, *et al.*, 2008; Garber, *et al*, 2004).

MMAS categorizes the patients' adherence into 3 categories: high (score 8), moderate (grades 6 - <8), and low (score < 6). The research result also shows the patients are included in low adherence category. This becomes a challenge for pharmacists and clinicians in the future to determine effective treatments. The research result suggests many factors that can affect lower adherence in the patients, for example the factors of negligence, depression, the lack of the patients' knowledge about hypertension and its treatment, clinical condition (TD) which is already improved makes the patients decide stopping their treatment, the drugs' side effect, economic factor, as well as excessive prescribing which makes the patients feel tired to take their medication and the patients' life quality themselves.

Blood Pressure Assessment

Hypertension level was assessed at the beginning when the patients entering the research, which was measured by systolic blood pressure and diastolic blood pressure at the first control or disease treatment in internal medication polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta. Hypertension degree identification after systolic and diastolic blood pressure measurement was done on the patients who became the research subjects based on JNC VII classification.

In this research, the majority of the patients were included in the hypertension level 1 and 2. The research result shows that there is no difference in early hypertension level (pre) from the treatment group and the control group based on Mann Whitney analysis (blood pressure systole $P = 0.343$), Table III.

The research result on systolic blood pressure at the beginning of the research (pre) both in the control and treatment groups shows no significant difference in the mean of systolic blood pressure either on the control group

Table III. Adherence and blood pressure in intervention and control group pre counseling and motivational reminders via SMS

Patients characteristics	Intervention group		Control Group		P
	(n=30)	%	(n=30)	%	
Adherence	30	6.45±1.77	30	5.8±1.86	0.172
Blood Pressure	30		30		
Sistole	30	153.53±19.67	30	148.53±20.83	0.343
Diastole	30	89.73±10.34	30	83.76±10.26	0.029*

ISBN : 978-979-18458-6-1

Table IV. the different of blood pressure pre and post in intervention and control group.

Patients characteristics	Intervention group (n=30)		P	Control group (n=30)		P
	Pre	Post		Pre	Post	
	mean±SD	mean±SD		mean±SD	mean±SD	
Tekanan darah						
Sistol	153.53±19.67	138.17±17.64	0.001	148.53±20.83	147.27±20.79	0.730
Diastol	89.73±10.34	83.00±10.22	0.018	83.77±10.26	83.33±9.31	0.786

(148.53 mmHg) and the treatment group (153.53 mmHg) (P = 0.343) Table III.

Systolic blood pressure; both before and after treatment in the control group and the treatment group is shown in Table IV below. Wilcoxon analysis result shows that in the treatment group; significant systolic blood pressure changes happen before and after the pharmacists' counseling (pre) and motivational reminders provision via SMS (P = 0.001), than in the control group (P = 0.730).

This suggests that pharmacists' counseling and motivational reminders via SMS affect on the patients' adherence and they give effect of systolic blood pressure reduction in the treatment group.

Significant blood pressure change in the treatment group is indicated by systolic blood pressure reduction prior to the research / treatment (pre) until after the research / treatment (post) which is of 15.37 points, while the control group experiences non significant reduction of 1:27 points (Table V).

Significant systolic blood pressure reduction in the treatment group shows the influence of the pharmacists' counseling on blood pressure control. This is consistent with the previous research which states that pharmacists' intervention can improve blood pressure in hypertensive patients (Mehos *et.al*, 2000).

CONCLUSION

Based on this research, it can be concluded that counseling provision (usual care) and motivational SMS reminders by pharmacists to hypertensive patients can improve the patients' adherence in undergoing the drugs' therapy, as shown by the reduction of the patients' systolic blood pressure ($p < 0.05$).

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Table V. the different of blood pressure pre and post in intervention and control group

Patients characteristics	Intervention group (n=30) mean±SD	Control group (n=30) mean±SD	P
Blood Pressure			
Sistole	15.37±23.69	1.27±19.89	0.015*
Diastole	6.73±14.72	0.433±8.66	0.048*

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THE USE OF OTC (OVER-THE-COUNTER) DRUGS IN SELF-MEDICATION (SWAMEDIKASI) EFFORT TO THE SOCIETY IN SANTAN SUMBEREJO

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Abstract

Background. *Self-medication (swamedikasi) means treating all self-complaints with medicines bought from the pharmacies or drug store on individual initiative without the advice of a doctor.*

Objective. *Objective of this study is to evaluate the source information that patients gotten.*

Methods : This research is an observational research with cross sectional research design. The numbers of respondents are 60; selected by non-random sampling with predetermined criteria. The technique of data collection is done by using questionnaire and observation.

Outcome Measured. *Source drug information for self medication.*

Result. *The result of the research shows that 83.3% of people in Santan Sumberejo District do the swamedikasi or self-medication and 75% of people buy the medicine in the shop. 50% of people in Santan Sumberejo buy OTC drugs because they are cheap. In selecting the medicines, they know from their friends or relatives that is 41.7%, and matters which are noticed by the people in Santan Sumberejo is the efficacy of the medicines (55%). 96.7 % of people in Santan Sumberejo have been appropriate in medicines use. They know the rules of medicines use by reading the medicines package (70%). 93.4% of people in Santan Sumberejo never made mistakes in treatment. OTC drugs which are often bought in Santan Sumberejo are headache medicines by 46.1% respondents.*

Conclusion. *If they have not recovered, 71.7% of people in Santan Sumberejo choose to go to the doctor as a solution. Level of knowledge of people in Santan Sumberejo is included in good category in which 76.25% of respondents answer the questions correctly.*

Keywords : *drugs use, knowledge level of society, OTC drugs*

ISBN : 978-979-18458-6-1

INTRODUCTION

The Republic of Indonesia government, in this case the Ministry of Health, trying to improve the quality of Health Care of the People. Swamedikasi can be interpreted simply as a person attempts to self-medicate. Swamedikasi be an alternative that many people selected to relieve or cure minor health complaints or to increase the affordability of access to treatment. Communities should be given the opportunity to swamedikasi (Tan et al, 1993). Based on the results of the National Socio-Economic Survey (NSES) in 2009, BPS noted that there are 66% of people ill in Indonesia doing swamedikasi. This figure is relatively higher than the percentage of people who went to the doctor (44%). Swamedikasi who do people often do on free drug classes and drug-free is limited. The efficacy and safety of drug-free would be good only if used properly according to the instructions and warnings for use of the drug and are not necessarily free to use without rules. Improper use of OTC drugs can still be harmful to users, at least not effective for the treatment (Widodo, 2006).

Objectives

1. This research was conducted using a quantitative approach to the type of descriptive and survey methods. Survey conducted by the method of cross-sectional approach.
2. Technique using non-probability sampling (non-random sampling)
3. Sample of this research is in the public environment Sumberejo Santan village with age limit 18-60 years. This study used 60 samples.
4. The timing of the research conducted during March and April 2012

METHODS

Instruments used in data collection for the study was a questionnaire. Data analysis was conducted in research using Microsof Excel program.

RESULTS

Dissemination of questionnaires given to the respondent response obtained as follows:

Table I. Community Action If you experience pain

Answer	Frequency	Percentage
Going to the doctor	8	13%
Go to the healer / alternative medicine	2	4 %
Treat yourself to buy medicine	50	83%

Table II. Reason for using OTC

Answer	Frequency	Percentage
more practical	13	22%
cheap	30	50%
more effective	0	0 %
Easily obtained	17	28%

Table III. Information drug selection

Answer	Frequency	Percentage
Ever received a prescription	13	21%
Knowledge from the mass media	25	42 %
Buying drugs themselves because they know of friends	22	37%
Attractive drug packaging	0	0%

Table IV. Knowledge level survey data

No	question	Frequency	percentage
1.	The level of public knowledge about the definition of drug-free	56	92%
2.	Level of public knowledge about the use of drug-free	37.6	63%
3.	The level of public knowledge about drug safety	37.5	63%
4.	The level of public knowledge about drug-free information	53	88%
		53	88%

Discussion

Can be seen as much as 76.25%. The level of knowledge is included in the category of "good" because according to (Arikunto, 2002), the level of knowledge is quite good if the respondents who answered correctly between 76% to 100%.

Factors influencing knowledge among education, occupation, age, interests, experiences, cultural surroundings, information, ease of obtaining information. Most of the respondents' answer is to do a self-medication to cope with illness (83%). This is in accordance with national socio-economic data (NSES) in 2009 BPS note that in Indonesia who do their own treatment reached 66%. This happens for several reasons, namely (Anonymous, 2012): Socio-economic factors, lifestyle, Ease of obtaining medicinal products, environmental health factors And the availability of new products.

CONCLUSION

Based on the results of research in the Santan Environment can be concluded that:

1. Society in Santan Environment Sumberejo who do swamedikasi or treat yourself as much as 83% with most drugs where to buy in the shop as much as 75%.
2. The reason most people in the neighborhood Santan Sumberejo use OTC drugs as low as 50%
3. In the selection of drugs, people in the neighborhood know Santan Sumberejo much of the mass media with a percentage of 42% and it is often considered in the use of drugs in the community is neighborhood Santan Sumberejo drug efficacy (55%).
4. A total of 97% of the people in the neighborhood have been appropriate in the Santan Sumberejo drug. They know the rules of the use of drugs by reading the packaging of drugs (70%).
5. A total of 93% of the people in the neighborhood Santan Sumberejo never made

a mistake in treatment. Drugs are often purchased at the Santan Sumberejo Environment is a headache medicine by as much as 46% of respondents. If it has not healed as much as 71.7% of the people in the neighborhood Santan Sumberejo choose to go to the doctor as a solution.

6. The level of knowledge society in Santan Environment Sumberejo included in either category with 76.25% of respondents answered the question correctly.

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IMPACT OF PHARMACIST COUNSELING AND BOOKLET INTERVENTION ON PATIENT'S ADHERENCE AND SYSTOLIC BLOOD PRESSURE OF AMBULATORY HYPERTENSION PATIENTS IN INTERNAL DISEASE POLYCLINIC PKU MUHAMMADIYAH BANTUL, HOSPITAL, INDONESIA

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Abstract

Background. *Prevalence of hypertension increased in line with changes of life style as smoking, obesity, non physical activity, psycho-social stress in many countries. Comprehensive and intensive antihypertension intervention could be more control of the blood pressure of patient. Counseling and provision of appropriate information by pharmacists is expected to improve adherence of patient on drug therapy in decreasing the blood pressure.*

Objective. *The aim of this study is to investigate the influence of pharmacist counseling and booklet on the adherence and decrease systolic blood pressure of ambulatory hypertension patients at internal disease polyclinic PKU Muhammadiyah Bantul Hospital, Indonesia.*

Methods. *This study was conducted with quasi-experimental design. The ambulatory hypertension patients data were collected prospectivelly during the period of January until April 2013. Sixty patients were divided into 2 groups, 30 (50%) patients receive a pharmacist counseling and booklet (intervention group) and 30 (50%) without a pharmacist counseling and booklet (control group). Exclusion criteria was pregnancy, deaf and illiterate. Data collection were conducted by interview and completion Morisky Medication Adherence Scale (MMAS) adherence questionnaire, while values of blood pressure were taken from medical records.*

Outcome Measured. *Adherence and systolic blood pressure of ambulatory hypertension patients.*

Results. *The results showed that pharmacist counseling and booklet could increased the patients adherence in the intervention group (66.7%) in comparison to the control group (20%) ($p=0.001$). Accordingly, pharmacist counseling and booklet intervention could decreased the systolic blood pressure in the intervention group of (15.2 mmHg, $p=0.000$). There was no decreasing the systolic blood pressure (1.27 mmHg, $p=0.730$) in the control group. There were positive correlation in patients adherence and systolic blood pressure value ($p=0.024$, $r=0.410$).*

Conclusion. *In Summary, the pharmacist counseling and booklet intervension can increase the patients adherence on antyihypertension management. Moreover, it can decrease systolic blood pressure patients ($p<0.05$).*

Keywords : *hypertension, pharmacist counseling, adherence, systolic blood pressure, booklet.*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Hypertension is one of the main factors triggering *coronary heart disease* and stroke. WHO has estimated about 62% of *cerebrovascular disease* and 49% of *ischemic heart disease* in the world can be caused by blood pressure levels. High blood pressure is estimated to cause 7.1 million deaths per year (WHO and IPF, 2006).

In many countries, the prevalence of hypertension increases with lifestyle changes such as smoking, obesity, physical inactivity, and psychosocial stress. In Indonesia, there are no complete national data concerning the prevalence of hypertension. The National Household Health Survey (*Survei kesehatan Rumah Tangga* (SKRT)) in 1995 found that the prevalence in Indonesia was 83%. The prevalence of hypertension in the ages of more than 50 years ranged between 15% - 20% (The Department of Health, 2007).

Hypertension attacks human body very slowly. Patients with hypertension may have no symptoms for years. This latency period covers the development of the disease until significant organ damage occurs. If any symptoms are indicated, those signs are usually non-specific ones such as headaches or dizziness. If hypertension remains unknown and untreated, this results in death due to heart failure, myocardial infarction, stroke, or kidney failure. However, early detection and effective intervention of hypertension can reduce the amount of morbidity and mortality. Thus, regular blood pressure checks have significance in the intervention of hypertension (Brown, 2006).

Healthy People 2010 for Hypertension suggest a more comprehensive approach and intensive in order to achieve optimal blood pressure control. So to achieve that goal, it needs the active participation of pharmacist in implementing the practice of his profession at every place of health care. Pharmacist can work with doctors in providing education to patients about hypertension, monitoring the response of patients, adherence to drug therapy and non-drug, detect and identity side effect early reaction, and prevent and or solve problems associated with providing drugs (The Department of Health, 2007).

One of the possible interventions by pharmacists for the hypertension patients' handling is counselling. Counseling intended to improve therapy result by maximizing the appropriate medications (Rantucci, 1997). One of the benefits of counseling is to improve the patients' adherence of drugs usage. Therefore, mortality rate and losses (both cost and productivity) can be abated (Palaian *et al*, 2006). Not only held verbally, counseling can also accompanied by written matter that serves to increase knowledge and strengthen what is delivered by farmasis when counseling (The Department of Health, 2007).

Based on the findings of the research by (Palanisamy and Sumathi in 2009), it is explained that the intervention by providing education and counseling to patients can improve adherence to intervention. The interventions of pharmacists improve blood pressure control of the patients with hypertension who have uncontrolled blood pressure (Mehos *et al,* 2000).

The findings of the research carried out by (Mansoor *et al.* 2008) suggested that written materials accompanied by verbal consultation can have a positive impact in improving adherence. The research by Mulyasih, (2011) showed that the provision of verbal counseling and leaflets can affect adherence and lower systolic and diastolic blood pressure until it reaches the target. Based on the research by Rostikarina, (2011), the provision of *Home Pharmacy Care (Booklet)* contributed significantly to increase knowledge about the use of oral antihypertensives in patients with hypertension.

Self-report scale to assess adherence with the use of antihypertensive drugs have been developed by Morisky *et al*, (2008). Reseach to test the psychometric and predictive validity of the structure of self-report medication adherence

in hypertensive patients tested in 2007 and published in January 2008. Adherence with the use of drugs was measured with the new 8-item self-report Morisky Medication Adherence Scale (MMAS).

Based on the information above, it is necessary to study to see the effect of the provision of pharmacist counseling and booklets on the adherence and the therapy result (systolic blood pressure) of hypertension intervention in internal disease polyclinic PKU Muhammadiyah Bantul Hospital, Yogyakarta.

OBJECTIVE

1. To investigate the influence of pharmacist's counseling and *booklet* on the adherence and therapy result (systolic blood pressure) in hypertensive patients in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta
2. To investigate the relation of adherence with the terapy result in hypertensive patients in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta.

METHODOLOGY

The research belongs to a quasi-experimental research. Subjects were all hypertension patients in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta period January to April 2013. They were divided in to two groups as intervention group and control group. The intervention group is the group of patients who received a pharmacist counseling and *booklet* and control group patients is the group of patients who did not recieve a pharmacist counseling and *booklet*. Data were collected prospectively and research result was presented descriptively. All patients received information regarding the objectives of the study and gave their written informed consent.

Data collection were conducted by interview and completion *Morisky Medication Adherence Scale* (MMAS) adherence questionnaire, while values of blood pressure were taken from medical records. The inclusion criteria were adults patients, both men and women with age 18-65 years old, who conducted control in PKU Muhammadiyah Bantul Hospital, Yogyakarta, they were diagnosed by a doctor to have suffered levels I and II hypertension with or without dyslipidemia and diabetic mellitus (DM), they got more antihypertensive drugs more than or equal to 1 antihypertensive drug, not illiterate and gave their written informed consent. The exclusion criteria were patients with pregnancy, deaf, and illiterate. After all required data is obtained, which is from medical record data, health assessment, MMAS questionnaire, blood pressure, do data processing with statistical methods using SPSS version 16. Statistical analysis used normality test, if $P>0.05$ normally distributed, independent t-test and paired t-test, whereas if $p <0.05$ distribution is not normal, Mann Whitney and Wilcoxon test is used. $p <0.05$ significance indicates significant different result between groups.

RESULT AND DISCUSSION

The research was conducted during January to April 2013 in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Yogyakarta. The research was conducted prospectively toward hypertension outpatients. Sampling was done by cosecutive sampling method, whereas all subjects came sequentially and met the selection criteria were included in the research until the number of the subjects required fulfilled. The subjects selection for each group was done randomly, whereas all subjects who met the research criteria were divided into two groups by putting the odd-numbered subjects into the control group, while even-numbered subjects were included into the intervention group.

The subjects who joined the research from the beginning to the end of the research were 60 patients, consisting of 30 patients who received a pharmacist counseling and *booklet* from the

researcher (intervention) and 30 patients who did not receive a pharmacist counseling and *booklet* (control).

1. The Patients' Demography

The distribution of the variety subjects characters of the intervention group to the control group was shown on table I.

In this research, the relationship of the various subjects characteristics in the intervention and the control groups obtains a non-significant relationship (P>0.05) among the characteristics of gender, age, education, employment, payment, history of hypertension, smoking habits, and degree of hypertension..

In this research, based on the data characteristics of the patients, it can be seen that the majority of the research subjects; both control and intervention groups are women, each amounted to 21 (70.0%) in the control group and 19 (63.3%) in the intervention group, with the

Table I. Distribution of subjects characters in intervention group to control group

Subjects characters	Intervention Group		Control Group	
	(N=30)	%	(N=30)	%
Gender				
Man	11	36.7	9	30
Women	19	63.3	21	70
Age (years old)				
18-29	1	3.3	0	0
30-39	3	10	0	0
40-49	8	26.7	5	16.7
50-59	10	33.3	16	53.3
60-65	8	26.7	9	30
Education				
0-9 years	18	60	20	66.7
10-12 years	7	23.3	6	20
>12 years	5	16.7	4	13.3
Employment				
Civil Servants	3	10	7	23.3
Entrepreneur	18	60	16	53.3
Labor	8	26.7	5	16.7
Not Work	1	3.3	2	6.7
Payment				
General	7	23.3	12	40
Askes	8	26.7	9	30
Jamkesmas	13	43.3	8	26.7
Other Insurance	2	6.7	1	3.3
History of Hypertension				
Yes	17	56.7	9	30
No	13	43.3	21	70
Smoking Habit				
Smoking	5	16.7	3	10
No smoking	25	83.3	27	90
Degree of Hypertension				
Level I	11	36.7	19	63.3
Level II	19	63.3	11	36.7

most dominating age is in the range of 50-59 year-old; i.e. 16 (53.3%) in the control group and 10 (33.3%) in the intervention group. The patients' education level dominates on educational age of 0-9 years, 20 (66.7%) in the control group and 18 (60%) in the intervention group.

This research also conducted assessment whether there was or there was not an existence of cardiovascular risk factors, namely smoking habits and hypertension history. The majority of the patients did not have smoking habits both in control and intervention groups (90%, 83.3%), while for hypertension history in the intervention group shows higher number; i.e. 56.7% compared to the control group which dominates the patients with no hypertension history previously, which is 30%. In the control group shows to have patients with hypertension degree I 19 (63.3%) and patients with hypertension degree II 19 (63.3%) in the intervention group.

2. Adherence Assessment

The patients' adherence affects on to the success of a intervention. The therapy result will not reach the optimal level without the awareness of the patients themselves, in fact, it may lead into a intervention failure, and it may also cause highly harmful complication and eventually becomes fatal (Hussar, 1995). Thus, it requires outpatients' non adherence identification to determine the effectiveness of the hypertension control level improvement.

In this research, the patients' adherence parameter was measured using the MMAS both on the control group and the intervention group. MMAS score assessment result is shown in Table II.

The MMAS questionnaire had a total score of 8, with the category of high adherence (MMAS value=8), moderate adherence (MMAS score=6 <8) and low adherence (MMAS value=<6). The assessment of adherence using the MMAS was done until the second visit both to the control group or the intervention group. The results presented in Table II explain that the high adherence category (MMAS score=8) is greater in the intervention group that the one in the control group (66.7% or 20 patients>20% or 6 patients). This suggests that counseling from pharmacists and booklets contribute positively to the adherence of the patients in the intervention group.

Statistical comparison between MMAS value ??in the control group and the intervention group is done by testing the normality. The results of *Kolmogorov Smirnov* normality test showed that the data of both the control group and the intervention group were not normally distributed therefore a non-parametric test using the Mann-Whitney test was conducted. Based on the test results, the significance value of 0.001 ($p<0.05$) was obtained. Therefore, it can be

Table II. MMASScores on HypertensionPatients in Intervention and Control Group after Get Counseling in the Final Research.

Group	MMAS Scores					
	High Adherence		Moderate Adherence		Low Adherence	
	∑	%	∑	%	∑	%
Control (n=30)	6	20	16	53.3	8	26.7
Intervention (n=30)	20	66.7	10	33.3	0	0
P value	0.001*					

Description :

MMAS : Morisky Medication Adherence Scale

ISBN : 978-979-18458-6-1

Table III. The Reasons for non-adherence of patients based on a questionnaire MMAS.

No	Reasons for non-adherence	Group		Total (%)
		Intervention	Control	
1	Forgot	0	8	13.3
2	Deliberately not taking medication	1	4	8.3
3	Being distracted by having to take medication	3	8	18.3

concluded that there are significant differences between the MMAS scores of the control group and the MMAS scores of the intervention group.

Approach to assess intervention adherence may use *self-report*, *pill counts*, pharmacy records, drug levels. Measurement uses patient *self-report* is more concise, quicker and easier to use, but the drawback is more subjective (Cook *et al,* 2005; Garber *et al.*, 2004). Currently, a more effective way in more effective measurement has been developed to evaluate the adherence, namely the new 8-item self-report Morisky Medication Adherence Scale (MMAS) (Morisky, *et al.*, 2008; Garber, *et al*, 2004).

Many factors have been reported can affect lower adherence in the patients, for example the factors of negligence, depression, the lack of the patients knowledge about hypertension and its intervention, clinical condition (TD) which is already improved makes the patients decide stopping their intervention, the drugs' side effect, economic factor, as well as excessive prescribing which makes the patients feel tired to take their medication and the patients' life quality themselves.

MMAS provides information on the habits associated with low adherence that may be caused by the coincidence (e.g. negligence), accidentally (not taking medication when feel it getting worse or improved). Table III shows some patients not adherent to therapy caused patients often forget to take the medication and understanding patients are wrong about their disease so that they deliberately not to take their medicine. Patients who do not comply assume that after a patient taking the drug antihipertensi and there has been a decrease in blood pressure, patients feel the ailment had healed and do not need to take drugs again. Then patients will take drugs again when the symptoms of elevated blood pressure, for example pain in the back of the head or feel dizzy. Non-adherence in taking the drug also caused due to a lack of understanding about the risks that will happen if blood pressure patients aren't reach the target set.

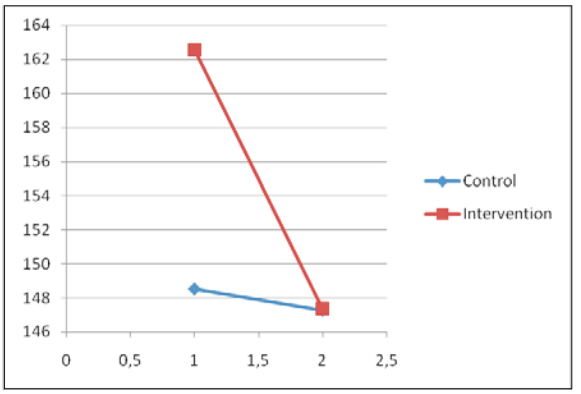
The low adherence becomes a challenge for pharmacists and clinicians in the future to determine effective interventions. If pharmacists s is to identify patients who have adherence low, so pharmacists can intervene in an imprecise manner and appropriate. With a counseling pharmacists, the patient is given the understanding that the disease hypertension incurable and when his blood pressure not reach the target applied will damage occurs on vital organs other bodies.

3. Blood Pressure Assessment

Hypertension level was assessed at the beginning when the patients entering the research, which was measured by systolic blood pressure at the first control or disease intervention in PKU Muhammadiyah Bantul Hospital, Yogyakarta.

The results of the normality test using the *Kolmogorov Smirnov* concerning systolic blood pressure showed that both the control group and the intervention group at the beginning and in the end of the study were normally distributed ($p>0.05$). Thus, to examine the differences between the initial condition and the final condition of the study in both the intervention group and the control group, a parametric

ISBN : 978-979-18458-6-1



Piture 1. The mean of Systolic Blood Pressure at the Beginning and in the End of the Research of both the Control Group and the Intervention Group

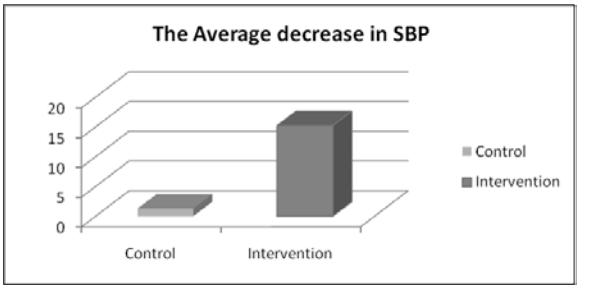
Group	The Beginning	The End	P Value
Control	148.53	147.27	0.730
Intervention	162.57	147.37	0.000*

Description :

* : *Using Paired Samples t-Test*

statistical test with *Paired Samples t-Test* was administered. In accordance with the results of the test conducted in the intervention group, the p value of 0.000 ($p<0.05$) was obtained, meaning that there are significant differences in systolic blood pressure measurements at the beginning and in the end of the study. Whereas in the control group, the p value of 0.730 ($p>0.05$) was obtained, meaning that there is no significant difference in systolic blood pressure measurements during and in the end of the study.

The normality test on the mean of the SPB changes using the *Kolmogorov Smirnov* for systolic blood pressure showed that the data of both the control group and the intervention group were normally distributed ($p>0.05$), so as to know the difference between the control group and the intervention group, the parametric statistical test of *the Independent Samples t-Test* was conducted. The test results generated the p value of 0.008 ($p<0.05$). Therefore, it can be concluded that the patients with hypertension in



Picture 2. The average decrease in (Ä) Systolic Blood Pressure of the Patients with Hypertension of both the Control Group and the Intervention Group at the Beginning and in the End of the Research

Group	The average decrease in (Δ) SBP (mmHg)	P Value
Control	1.27	0.008*
Intervention	15.2	

SBP : Systolic Blood Pressure

* Using *Independent Sampels t-Test*

the intervention group (that were facilitated with pharmacist counseling and booklets) encountered more significant systolic blood pressure decreases than the control group.

4. Assessment Correlation the result of terapy with Adherence

The correlation test in to analyze the correlation between the result of the terapy with adherences. The result of therapy which is correlated with adherence is reduction of blood pressure systolic with MMAS category. Statistical correlation test was Spearman test. This test was chosen because the variables are tested in the form of rasio with ordinal variables and were not normally distributed.

The results of this test showed significant correlation between decreasing systolic blood pressure with MMAS score of pharmacist patient with hypertension who become research in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Indonesia. This is an accordance with the condition expected, namely a significant relationship

Tabel IV. The Corelation of changes of SBP with a MMAS category of patient hypertension in PKU Muhammadiyah Bantul Hospital.

Blood pressure	MMAS score		Conclussion
	Value P	Value P	
Changes of Systolic blood pressure	0.024	0.410	There is strong significant correlation, the power of correlation weak, the direction of the positive correlation

between reduction results therapy with MMAS score, although the correlation coefficient showed a weak relationship. This matter could probably cause counseling pharmacist is not dominant as a factor of decreasing systolic blood pressure. The direction of correlation is positive, which means the greather the difference in a person's blood pressure will be higher his MMAS score.

CONCLUSION

1. Pharmacist counseling and booklets had a positive and significant effect (p=0.001) on adherence of ambulatory hypertension patients in internal disease polyclinic in PKU Muhammadiyah Bantul Hospital, Indonesia
2. Compliance has a positive and significant (p=0.024, r=0.410) for systolic blood pressure reduction so that the higher the level of patient compliance, the greater the decrease in blood pressure.

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DISORDER OF PURINE AND PYRIMIDINE NUCLEOTIDE METABOLISM AND ITS THERAPY

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Abstract

Background. *Metabolism disorder means indicates a change in a normal metabolism either in qualitative or in quantitative manner. This disorder can be identified through the changes of metabolism productions, possibility of the increase or the decrease of the relevant enzymes activities, or even the absence of an enzyme.*

Objective. *To study the disorder of purine and pyrimidine nucleotide metabolism, the arising disease and its therapy*

Method. *The review is used the 4 books and 2 articles related to the disorder of purine and pyrimidin nucleotide metabolism, the arising disease and its therapy.*

Outcome Measured. *Nucleotide degradation and enzyme activity.*

Results. *This article presents metabolism/catabolism/anabolism and the management of purine and pyrimidine nucleotide metabolism are followed by the metabolism disorder and its therapy. Following this, the nucleotide degradation and the disorder of purine and pyrimidine metabolism in the form of the increasing activity of enzymes or the absence of certain enzymes along with the mechanism of its therapy are presented.*

Conclusion. *The nucleotide degradation and the disorder of purine and pyrimidine metabolism in the form of the increasing activity of enzymes or the absence of certain enzymes along with the mechanism of its therapy are presented*

Keywords : *metabolism disorder, purine nucleotide, pyrimidine, and nucleotide degradation*

ISBN : 978-979-18458-6-1

INTRODUCTION

Metabolism disorder refers to the change in a normal metabolism and occurs in a qualitative or in a quantitative manner. The PRPP synthetase enzyme experiences an increasing activity due to a quantitative change. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) enzyme that plays a role in non-Inosinate-guanilate salvage pathway biosynthesis shows a qualitative change.

This study is aimed to discuss the purine and pyrimidine metabolism disorder. Nucleotide consists of base N binding sugar (ribose) in C1 and phosphate in C5'. Purine Base N includes adenine and guanine. Meanwhile, pyrimidine is included in cytosine, uracil, and thymine.

Purine and pyrimidine nucleotide has a number of important roles and functions. It is including (a) as a universal energy such as *adenosin trifosfat* (ATP); (b) as monomer from nucleic acid both as ribonucleic acid (RNA) and as deoxyribonucleic acid (DNA); (c) active intermediate (zantara) in many biosynthesis processes such as diacylglycerol -diphosphatase (CDP-diacylglycerol) intermediate in phosphor glycerol biosynthesis; (d) component of coenzyme such as oxidized Nicotinamide adenine dinucleotide (NAD^+), flavin adenine dinucleotide (FAD) and (e) metabolic regulator such as cyclic adenosine mono phosphate (cAMP).

Purine nucleotide biosynthesis (Cory, 2006; Elliot and Elliot, 1997; Berg, Tymoczko and Stryer, 2002; Smith and Clark, 2011).

de novo pathway, It is initiated from Phosphoribosyl pyrophosphate (PRPP) to be Inosinate acid (IMP) and then to be Adenosine monophosphate (also known as 5'-adenylic acid), and guanosine monophosphate (GMP):

Purine nucleotide biosynthesis (Cory, 2006; Elliot and Elliot, 1997; Berg, Tymoczko and Stryer, 2002; Smith and Clark, 2011).

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PRPP a→ (+Glutamine – Glutamate/Amino phosphoribosyltransferase) 5-phosphoribosyl-1-amine b→ (+Glycine +ATP-ADP-Pi) Glycinamide Ribonucleotide c→ (+N^{10}-Formil THF-THF) Ribonucleotide formiglicynamide d→ (+Gln+ATP-Glu-ADP-Pi) Ribonucleo tida formilglisinamidin e→ (-H_2O) Ribonu cleotide 5-aminoimidasol f→ (+CO_2) Ribonucleotide 5-aminoimidazole 4-carboxylate g→ (+Asp+ATP-ADP-Pi) Ribonucleotide 5- aminoimidazole l-4-N-succino carboxamide h→ i→ (-Fumarate) Ribonucleotide 5-aminoimidazole -4- carboxamides j→ (N^{10}-Formil THF –THF) Inosinate/IMP.

IMP k→ (+Asp+GTP –GDP-Pi) Adenylosuccinate l→ (-Fumarate) Adenylate /AMP.

IMP m→ (+NAD+ -NADH) xantilate n→ (+Gln +ATP –Glu-AMP-PPi) Guanilate/ GMP.

The involved enzymes: (a). glutamine PRPP amidotransferase, (b). GAR synthetase, (c). GAR transformylase, (d). FGAM synthetase, (e). AIR synthetase, (f). AIR carbocslase, (g). SAICAR synthetase, (h). adenylosuccinate liase, (i). AICAR transformylase, (j). IMP cyclohydrolase, (k). adenylosuccinate synthetase, (l). Adenylosuccinate, (m). IMP dehydrogenate, and (n). GMP synthetase.

Other pathway: Salvage Pathway

1. Adenine + PRPP a→ Adenylate + PPi
2. Hypoxanthine + PRPP b→ Inosinate + PPi
3. Guanine + PRPP b→ guanylate + PPi
   a. Adenine Phosphoribosyl transferase
   b. Hypoxanthine-guanine phosphoribosyltransferase (HGPRTase)

ISBN : 978-979-18458-6-1

Arrangement of Purine Nucleotide Biosynthesis (Cory, 2006; Berg, Tymoczko and Stryer, 2002)

- Ribose 5-phosphate → PRPP → Phosphoribosylamine → 1 IMP → Adenylosuccinate → AMP
- 2. IMP → Xanthylate → GMP
- R 5-F → PRPP is inhibited by IMP, AMP, and GMP
- PRPP → Phosphoribosylamine is inhibited by IMP, AMP, and GMP
- IMP → Adenylosuccinate is inhibited by AMP
- 1 MP → Xanthylate is inhibited by GMP

Pyrimidine Nucleotide Synthesis (Cory, 2006; Elliot and Elliot, 1997; Berg, Tymoczko and Stryer, 2002; Kegg[b], 2013)

Here, it is started from Carbamoyl phosphate to be orotat and afterward to be Uridylic acid (UMP).

Carbamoyl phosphate + aspartate a→ (-Pi) N- Carbamoyl aspartate b→ ($+H^+$ $-H_2O$) Dihydroorotate c→ ($+NAD^+$ $-NADH_2$) Orotat d→ (+PRPP –PPi) orotidylate e→ ($+H^+$ $-CO_2$) uridylate /UMP.

- The involved enzymes:

  a. aspartate transcarbamoylase, b. Dihydroorotase, c. Dihydroorotate dehydro genase, d. Orotate phosphoribosyltransferase, e. orotate Decarboxylase

Interconversion:

UMP + ATP → UDP + ADP (UMP kinase)

AMP + ATP → 2 ADP (Adenylate kinase)

XDP + YTP → XTP + YDP

UDP + UTP → UTP + ADP

CTP Synthesis with amination of UTP:

UTP → (+Glutamine +ATP + H_2O –Glutamate –ADP –Pi $-2H^+$) CTP

Arrangement of Purine Nucleotide Biosynthesis is done by inhibiting the feedback: CTP inhibits aspartate transcarbamoylase (ATCase); UMP inhibits aspartate transcarbamoylase.

Degradation of purine and pyrimidine nucleotide (Cory, 2006; Berg, Tymczko and Stryer, 2002, Kegg[a], 2013, Kegg[b], 2013).

Nucleic acid a→ Nucleotide Guanine b→ Guanosine c→ Guanine d→ Xanthine e→ Uric Acid.

- The involved enzymes:

  a. nuclease, b. nucleotidase, c. purine nucleoside phosphorylase, d. guanase (-NH4+), e. Xanthine oxidase.

Nucleic acid a→ Adenine nucleotide b→ Adenosine c→ Inosine d→ Hypoxanthine e→ Xanthine f→ Uric acid.

Adenine nucleotide g→ IMP h→ Inosine

- The involved enzyme:

  a. nuclease, b. nucleotidase, c. Adenosine deaminase, d. purine nucleoside phosphorylase, e. Xanthine oxidase, f. Xanthine oxidase, g. AMP deaminase, h. nucleotidase.

Metabolism Disorder (Cory, 2006)

Disorder indicates the existence of a change either in quantitative or in qualitative manner. In this part, a number of qualitative disorders and quantitative disorders will be discussed.

1. **The increasing activity of synthetase PRPP** can cause the increase of the concentration of intracellular PRPP.
2. **The activities of HGPRTase are influenced in the *pathway for de novo synthesis of purine nucleotides.*** A. The decrease of salvage pathway for hypoxanthine and guanine, B. The decrease of salvage pathway for hypoxanthine, IMP, and GMP

3. **Deficiency of glucose 6-phosphate** can cause the increase of PRPP concentration (related to the PRPP amidotransferase).

   With the three disorders above, Gout disease can be emerged. It is remarked through the high concentrate of uric acid (either in blood or in urine). The increasing rate of purine nucleotide synthesis (*de novo* synthesis) causes the increase of synthesis followed by its degradation into uric acid. The medical treatment through some medicines includes colchicines, antihyperuricemic agents, and allopurinol. Allopurinol and the result of its metabolism, alloxanthine is an effective xanthine oxidase inhibitor causing the decrease of uric acid level. Meanwhile, the medical treatment with Allopurinol can cause the decrease of the level of uric acid and the decrease of purine nucleotide synthesis.

4. **The absence of HGPRTase protein means the absence of HGPRTase activities.**

   This disorder can bring an effect of the emergence of *Lesch-Nyhan* syndromes that include

   - Remarked by hyperuricemia
   - Causing the neurological problems, one of which is mental disorder
   - The role of HGPRTase has an effect on the reaction of nucleotide synthesis from hypoxanthine and guanine.
   - The absence of HGPRTase of Hypoxanthine and guanine has resulted in the absence of any reaction.
   - Salvage causes the increase of PRPP concentrate and the decrease of IMP or GMP
   - Both cause the increase of *de novo* synthesis of purine nucleotide.
   - The activity of IMP dehydrogenate in brain is very low and the absence of HGPRTase can cause the decrease of the number of intracellular GTP that in turn cause the decrease of salvage pathway of guanine. GTP refers to the candidate of Tetrahydrobiopterin cofactor essential in both neurotransmitter synthesis and protein synthesis.
   - The medical treatment with Allopurinol will decrease the number of uric acid formation, and releases the problem that can cause the formation of uric acid sodium.
   - Patient with Lesch-Nyhan is marked with the decrease of HGPRTase activity, the absence of salvage pathway of hypoxanthine and guanine, the dysfunction of PRPP making the *de novo* synthesis of purine nucleotide unstoppable.
   - Until recently, no any solution has been found for solving the neurological problem. Patients are dead for kidney damage due to the deposit of uric sodium.

5. **The increasing activity of *cytosolic 5'-nucleotidase***

   - The substrates for the enzymes are 5'AMP or 5'UMP in which the activity can increase 6 – 10 higher
   - This disorder can marked with the slower improvement, *ataxia, seizures, severe language deficit*, *hyperactivity, short attention span,* and less social interaction
   - The increase of 5'nucleotidase activity can cause the deficiency of nucleotide → The medical treatment is done by giving uridine orally.

6. **The disorder of purine nucleoside degradation**

   - The disorder can cause the immunodeficiency:
     a. Adenosine deaminase deficiency, also called ADA deficiency
     b. Purine nucleoside phosphorylase deficiency (PNP-deficiency)
   - Substrate for ADA : adenosine and deoxyadenosine

- Substrate for PNP: Inosine, Guanosine, Deoxyinosine, Deoxyguanosine.
- Deficiency of ADA is related to the immunodeficiency including the function of cell T and cell B.
- Deficiency of PNP is related to the immunodeficiency including the function of cell T.
- Immunodeficiency in concentration of intracellular dATP and S-adenosylhomocysteine is very increasing/large
- Hypothesis:
  a. Deficiency ADA → high dATP concentration inhibits Ribonucleotide reductase with a consequence of inhibiting the DNA synthesis.
  b. Deoxyadenosine inactivates S-adenosylhomocysteine hydrolase making the decrease of S-adenosylhomocysteine used in the methylation from base N in RNA and DNA.
  c. The increase of adenosine concentration leads to the increase of cAMP.
- Enabling the dysfunction of immune system
- Medical treatment on children with deficiency of ADA is by blood transfusion, bone marrow transplant, ADA-PEG, gene therapy.
- Each of them has weakness

7. **The increase of nucleotide degradation**
   - Nucleotide is from degradation of nucleate acid originating from the death of cell.
   - The medical treatment for cancer patients with radiation therapy or with chemotherapy will cause the concentration of uric acid in blood increase.
   - The medical treatment to decrease the concentration of uric acid with Allopurinol
   - The increase of uric acid is due to the degradation of purine nucleotide resulting in the increase of uric acid level due to the degradation of purine nucleotide that results in xanthine and is catalyzed by xanthine oxydase resulting in uric acid.
   - Allopurinol refers to a compound inhibiting xanthine oxydase.
8. **The increase of purine nucleotide synthesis on child**
   - Increasing four times higher than the normal one
   - The increasing uric acid concentration in urine
   - Autism
   - The condition of mental introversion is reflected to egoism and unawareness
9. **The disorder of *de novo* synthesis of pyrimidine nucleotide**
   - Characteristic: slow growth, increasing concentration of orotat acid in urine
   - Disorder of Orotat phosphoribosyltransferase or Orotidine decarboxylase,
   - Combined with synthetase UMP
   - Therapy with uridine per oral
   - Uridine → UMP → UDP furthermore → UTP inhibits Carbamoyl phosphate synthetase →orotat synthesis.

CONCLUSION

By studying the disorder of metabolism/anabolism/catabolism, it is important to explain the mechanism of the disease in order to overcome or conduct a therapy and its therapy mechanism.

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WATER FRACTION OF SAMBILOTO (ANDROGRAPHIS PANICULATA NEES) ETHANOL EXTRACT EFFICACY IN INDUCING THE NUMBER OF MACROPHAGE, NEUTROPHIL, AND THE LEVEL OF TNF-α ON WISTAR RATS

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Abstract

Background. *The study of the effect of water fraction of Sambiloto (Andrographis paniculata Nees) ethanol extract on the number of macrophage, neutrophil, and level of TNF-α in the body fluid of male Wistar rats after Staphylococcus aureus induction.*

Methods. *Animals were divided into 3 groups, namely: negative control (NC), treatment (T), and positive control (PC) group. The NC, T and PC groups were administered by 0.9% NaCl, water fraction of Sambiloto ethanol extract of 40mg/200g BW dose, and ibuprofen of 7.2 mg/200g BW dose, respectively. The treatment was performed for 7 consecutive days. The number of macrophages, neutrophils, was identified using light microscope whereas the TNF-α level was identified using ELISA.*

Outcome Measured. *The number of macrophage, neutrophil, and level of TNF-α in the body fluid of male Wistar rats after Staphylococcus aureus induction.*

Results. *The number of macrophages in the KC, the T, and the PC groups were 2.33 ± 1.58; 3.80 ± 4.06; and 2.67 ± 1.55 cells, respectively. The number of neutrophils were 0.83 ± 0.33; 0.58 ± 0.32; and 1.33 ± 0.38 cells respective to the KN, T, and PC groups. The level of TNF-α were 17.78 ± 11.67; 23.48 ± 15.95; and 27.90 ± 30.54 pg/ml respective to the KN, the T, and PC groups.*

Conclusion. *This study indicated that water fraction of Sambiloto ethanol extract able to increase the number of macrophage and TNF-α level yet decrease the number of neutrophil in the body fluids of male Wistar rats after Staphylococcus aureus induction.*

Keywords : *macrophages, neutrophils, TNF-α, Herba Sambiloto, ibuprofen*

ISBN : 978-979-18458-6-1

INTRODUCTION

Inflammation is a local response to infection or tissue injury. It may occur as a local, systemic, acute, and chronic disorder that lead to the pathologic condition. Inflammatory response precedence by the activation of cell mediated immune system. The cells of immune system respond to the foreign substances that invade the body by several mechanisms, such as chemotaxis, in which the immune cells move to the site of infection, followed by an increase of vascular permeability, and subsequently changes of blood osmotic pressure proceed. Those mechanisms facilitate leucocytes migration to the site of infection (Abbas *et al* 2007).

Development of the inflammatory response plays an important role in initiating pathological condition. Therapeutic approaches to reduce the inflammatory response caused (one of them) by the *Staphylococcus aureus* need to be conducted (Baratawidjaja and Rengganis 2012). Medication choice of inflammation may utilize synthetic drugs and/or natural ingredients. The long term uses of synthetic drugs may initiate unwanted effects on the human body. Therefore, natural ingredients are now becoming chosen alternatives as a healing therapy of inflammatory condition. Some examples are the leaves of Salam (*Syzygium polyanthum*), herbs of Sambiloto (*Andrographis paniculata* Nees.), leaves of Cassava (*Manihot utilissima* Pohl), leaves of Red Betel (*Piper crocatum* Ruiz & Pav), leaves of breadfruit (*Artocarpus altilis*), and others.

Sambiloto (*Andrographis paniculata Nees*) is a natural ingredient which is widely studied because of its efficacy as medicine.Sambiloto (AP) herbs have several active compounds. One of the compounds is known as Andrographolide which posses anti-inflammatory activity (Tewari et al 2010). Previously, it was identified that Andrographolide in ethanol extract of AP herbs inhibited inflammation in Wistar rats (Evacuansiany and Soebiantoro, no date). Other studies mentioned that AP herbs active as anti-inflammation by inhibiting the production of radical oxygen of the neutrophil. Moreover, AP inhibited macrophage migration, and production of TNF-α and IL-12 (Lin and Chao 2010) in the animal serum that treated with AP herbs water fraction containing 10% Andrographolide (Raharjo *et al* 2009).

Among the previous studies of AP anti-inflammatory activity, study of AP especially on phagocytic cells including: monocyte, macrophage, neutrophil, and eosinophil have not yet been conducted. We, therefore, identified the AP anti-inflammatory activity in male Wistar rats.

The objective of this study is to conduct a local inflammation by infecting rats with *Staphylococcus aureus* and identify the response of immune system cells that react on the infected bacteria after an hour. The number of phagocytic cells, namely macrophages and neutrophils were measured as parameter. Since the cells will release cytokines following their activation, therefore, levels of cytokines (TNF-α) also be counted. TNF-α is the main cytokine that is released during acute inflammation against bacteria and other microbes by phagocytic cells (Baratawidjaja and Rengganis 2012). The level of TNF-α measured by ELISA (Enzyme Linked Immunosorbent Assay) method. This study identified the effect of AP herb water fraction of ethanol extract compared to ibuprofen on alleviating local acute inflammation after *Staphylococcus aureus* infection.

METHODS

The apparatuses: distillation equipment, percolator, rotary evaporator (Buchi Rotavapor R-124); separating funnel, chamber, ose, tube, centrifuge apparatus (Hettich Zentrifugen), Eppendorf, syringe, pipette volume, light microscope (Olympus); microplate reader (Thermo Scientific Multiskan-Go, USA).

The materials: Andrographidis Herbs that were collected and determined in the Materia Medica, Batu, East Java; ibuprofen; distilled water; ethanol 96%; ammonia; $CHCl_3$;

HCl 10% v / v; klorhidrik alcohol; $FeCl_3$; gelatin solution; Steasny reagent; Na acetate; Na hydroxide; ether; acetic acid glacial; Mayer reagent; Dragendorf solution, n-hexane, ethyl acetate; methanol; butanol, acetic acid; kuercetin; ; *Staphylococcus aureus* (ATCC 25923); MSA medium; BaCl2 1%; concentrated H2SO4; 0.9% NaCl; Giemsa dye; TNF-α ELISA kit (Abcam, USA).

Animal handling

Animal used were 18 male Wistar rats 2 - 3 months of age and approximately 150-200 g of weight with healthy as well as normal activities. Prior to use, rats were adapted for a week to the new environment. The inclusive criteria of animals were: no symptoms of illness and uniform body weight. Prior to the experiment, rats were fasted for 18 hours (Winter in Hadisoewignyo 2010). Rats were divided into 3 groups of 6 animals. Group I was the negative control (NC), group II was the AP water fraction treated (AP), and group III was treated by ibuprofen (PC).

RESEARCH METHODS

Preparation and Fractionation of AP Crude Extracts

The study was preceded by maceration of crude AP powder by 96% ethanol for 24 hours at room temperature. The obtained extract was filtered and evaporated with a rotary evaporator. The condensed extract was dissolved in 50 ml of methanol:water (7:3). Subsequently, the solution was separated by adding 100 ml of n-hexane and shook in a separator flask for 60 minutes. The formed second layer was separated. The n-hexane addition was repeated for three times. The methanol layer was further separated by ethyl acetate. Ethyl acetate was added to the water layers and shook in a separator flask for 60 minutes. Then, the formed second layer was separated. This process was repeated for three times.

Anti-inflammatory Activity

In order to conduct anti-inflammatory activity, the rats were injected with *Staphyloccocus aureus* (SA). The SA preparation preceded with the rejuvenation of SA in MSA medium and incubation at 37°C for 24 hours. The rejuvenated SA, was mixed to 0.9% NaCl. Subsequently, the turbidity of SA-NaCl suspension was compared to Mc Farland I, in which contained 3 × 108 cfu/ ml of bacteria. Before injected to the rats, the suspension was incubated for 2 hour at 37° C (Wasito et al 2008).

Rats were allowed to stand for an hour after SA injection before conducting dissection. Subsequently, the rats were allowed to stand for 5 minutes after 0.9% NaCl injection. Rats that were ready for dissection were anesthetized with ether. Peritoneal fluid was taken from the abdominal cavity. The peritoneal fluid was subsequently smeared and fixed by absolute methanol for 5 minutes. Finally, the fixed peritoneal fluid was stained with Giemsa for 20 minutes, rinsed and dried. The stained peritoneal fluid were identified under a light microscope (400x magnification) and counted for the number of macrophages (Kusmardi et al 2006).

To study the number of the neutrophiles, the blood was taken from the heart, then disposed in a tubes contained EDTA. Prior to centrifugation, part of the blood was taken and subjected to be smeared, and stained as the peritoneal fluid. Subsequently, the stained blood was identified under light microscope (magnification of 400x) to count the number of neutrophil cells. The remaining of the blood was centrifugated at 2,000 rpm for 20 minutes. After centrifugation, serum was taken to be stored at -20° C for measuring the level of of TNF-α by ELISA method (Abcam, 2012).

ISBN : 978-979-18458-6-1

Table I Standardisation of AP Herbs

No.	Parameter	Qualifications (IHD 1979)	Test results
1	Ashes	< 12%	11.13 ± 0.35
2	Water soluble extract	≥ 18 %	17.03 ± 0.38
3	Ethanol soluble extract	≥ 9.7%	13.53 ± 0.10

IHD : Indonesian Health Departemen

Table II. Rf of AP Herbs Extract and Water Fraction under UV (254 and 366 nm) and UV Visible

Extract	UV λ 254 nm		UV λ 366 nm			UV visible		
	Rf	Compounds	Rf	Color	Compounds	Rf	Color	Compounds
AP Exstract	0.77	Flavon	0.60	Blue	Flavanon	0.56	Yellow	Flavanon
	0.87	Isoflavon	0.79	Blue	Flavanon	0.69	Yellow	Flavanon
			0.87	Red	Isoflavon	0.87	Brown	Isoflavon
AP-water Fraction	0.62	Flavanonol	0.6	Grey	Flavanonol	0.56	Brown	Flavanonol
	0.75	Flavon	0.81	Blue	5-deoksiisoflavon	0.69	Brown	Flavanonol
	0.87	Isoflavon						
Andrografolide	0.81	Andrographolide	0.25	Blue	Andrographolide			

Table III. The Number of Macrophage, Neutrophil, and TNF-α Leve

Groups	Macropage	Neutrophil	TNF-α
Negative control	2.33 ± 1.58	0.83 ± 0.33	17.7 ± 11.67
AP Herbs	3.80 ± 4.06	0.58 ± 0.32	23.48 ± 15.96
Positive control	2.67 ± 1.54	1.34 ± 0.38	27.90 ± 30.54

AP Herbs: AP Water fraction

ISBN : 978-979-18458-6-1

Table IV. ***One Way Anova Analysis*** **(α=0,05) of Macrophage, Neutrophil, and TNF-α level Between Groups**

Note	F	F table	Significancy
Macrophage	0.42	3.59	0.67
Neutrophil	4.85	4.07	0.04
TNF-α	0.07	3.34	0.94

Table V. LSD Analysis of the Number of Neutrophil

Groups	Compared to	Sig.	Note
Negative control	AP Herbs	0.34	NS
	Positive control	0.07	NS
AP Herbs	Negative control	0.34	NS
	Positive control	0.01	S
Positive control	Negative control	0.07	NS
	AP Herbs	0.02	S

NS: not significant S: significant AP Herbs: AP Water fraction

Results

Before used, several parameter of AP water fraction (AP WF) was standardized including level of ashes, level of water soluble extract, and level of ethanol soluble extracts (Table I). The Indonesian Health Department (1979) qualified that level of AP WF ashes, water soluble extract, and etanol soluble extract were less than 12%, more than 18%, and more than 9.7%, respectively. The levels of crude AP herbs water-soluble extract (17.03 ± 0.38) were not reach the qualification, therefore, the study was continuously undergone subsequent extraction steps used ethanol as the solvent.

This study used 1 kg of dried AP herbs that macerated by ethanol. The macerated extract obtained was 245 g (24.5%). The extract was subsequently undergone organoleptic test for the color, taste, and smell parameter. The ethanol extract of dried AP herbs was dark green in color, bitter in taste and specific in odor which were qualified according to the previous mentioned data (Indonesian Health Departement 1979).

Following the organoleptic test, the ethanol extract was fractionated extracts by water. The fractionated extract was then subjected to Thin Layer Chromatography (TLC) with butanol: acetic acid: water (3: 1: 1) as mobile phase. The TLC plate was dotted and eluated until reacted with ammonium and so produced a yellowish brown color which was a specific indication of the presence of flavonoids (Harborne 1987) (Table II).

Table II showed the results of Rf level of AP extract and water fraction compared to Andrographolide. When observed at λ 254 and 366 nm, the Rf level of AP extracts and water fractions were different to Andrographolide. It was predicted due to the flavonoids remained in both of AP extracts and water fraction, thus the Andrographolide was not clearly stained. The Rf following observation under the UV visible showed no Rf of andrographolide. It was due to the ammonium vapor was reacted solely to the flavonoids while Andrographolide is belongs to the diterpenoid classes (Lin and Chao 2010).

Table III showed the number of macrophages which was higher than the negative and positive control group. It was probably caused by the injected *Staphylococcus aureus* (SA) were produced an exfoliative which was able to activated macrophages, therefore macrophages were stimulated to release TNF-α, IL-6 and pyrogenic toxin superantigens that subsequently induce the activation of T cells and other macrophages to move to the site of infection (Hidayani no date). The result indicated that AP WF treatment may reduce nitric oxide production and increase macrophage activity (Guan, *et al.* 2012). Moreover, the increased number of macrophages was not different statistically compared to the negative control group that may be caused by the AP WF bacteriostatic and bactericidal activity (Anonymous 2005) which may encounter SA following injection, thus prevent the movement of macrophages to the site of infection. The number of macrophages in the positive control group, that contained ibuprofen, was lower than the AP WF group. This was an indication of less anti-inflammatory activity conducted by ibuprofen since approximately 90% of ibuprofen was bound to protein serum. Therefore, it would be more difficult for ibuprofen to penetrate to the cell membrane to produce anti-inflammatory effects (Wilmana and Gan, 2007).

The number of neutrophil of the AP WF group was lower compared to the negative and positive control group which can be explained due to the antioxidant activity of AP WF that may suppress the synthesis of nitric oxide (NO) and thus less NO will be produced and neutrophil adhesion will be inhibited, consequently (Ezeamuzie, *et al*. 2009). In the positive control group, the number of neutrophil was higher than the negative control and the AP WF group. This was explained due to the action of ibuprofen that affect the biosynthesis of several inflammatory mediators such as prostaglandins and leukotrienes (Wilmana and Gan 2007) that stimulate the increased number of neutrophils (Baratawidjaja and Rengganis 2012).

The levels of TNF-α of the AP WF and the positive control groups showed higher level than the negative control group. This is may be caused by macrophages that activated and released TNF-α into the blood circulation (Abbas, et al. 2007) since TNF-α is known as the major cytokine in the acute inflammatory response to bacteria and microbes. In acute inflammation condition, the TNF-α and endothelial leukocytes are working in coordination. Thus, the increased of macrophages is accompanied by elevated level of TNF-α (Baratawidjaja and Rengganis 2012).

CONCLUSION

Conclusively, this study indicate that AP WF may increase the number of macrophages in the peritoneal fluid that had been infected by *Staphylococcus aureus* and consequently increase the level of TNF-α in the serum of the Wistar rats. However, decrease the number of netrophils in the seum of the Wistar rats.

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ACUTE TOXICITY TEST OF RAMBUTAN LEAF (*Nephelium lappaceum* L) EXTRACT IN MICE

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Abstract

Background. *Rambutan (**Nephelium lappaceum** L.) has been widely and easy to obtain, and also recognized by people of Indonesia. As empirically, rambutan leaves used to treat diarrhea, fever, and black hair. The preclinical studies have shown that rambutan leaf extract has activity as lowering blood glucose levels (Kusuma, 2008). However, the scientific studies have not found rambutan leaf safety. To be eligible in the formal health care system, it must meet the requirements of traditional medicine quality, safety, and efficacy.*

Objectives. *This study aimed to test the acute toxicity of rambutan leaf extract in mice. The study followed unidirectional random pattern.*

Methods. *The study use 25 Swiss Albino male of mice, which is divided into 5 dose groups. The dose used in the acute toxicity test of rambutan leaf extract are 1, 2, 4; 8 g/kgBBmice and a negative control group (0.5% CMC-Na) as orally. The inspection do the 24-hour by intensive inspection on the first 3 hours.*

Outcome Measured. *The observations made as clinical observations that toxic symptoms occur, the number of dead mice and biochemical examination of ALT levels, AST, and serum creatinine. The LD_{50} calculations performed using the Thompson-Weil.*

Results. *The results showed toxic symptoms were observed in mice during acute toxicity test include uncontrollable way, restlessness, passivity motion, no reactivity to stimuli, and sleepy. The test compound caused a significant increase in ALT and AST levels ($p < 0.05$) at a dose of 8 g/kgBB, but the test compound did not cause death in test animals.*

Conclusion. *The test compound can be categorized as "Practice Not Toxic".*

Keywords : *toxicity, extract, rambutan, Nephelium*

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ISBN : 978-979-18458-6-1

INTRODUCTION

In recent years, due to side effects of synthetic products, herbal medicine are gaining popularity in the world, particularly plant drugs for their primary healthcare. That is documented that 80% of the words population has faith in herbal medicine (Dubey *et al.*, 2004). Rambutan leaf (*Nephelium lappaceum* L) has been widely recognized by Indonesia people to treat various of diseases. As empirically, rambutan leaf has been used to treat diarrhea, fever and black hair (Dalimartha, 2004). The preclinical studies have shown that rambutan leaf extract have antimicrobial activity, antioxidant, and antiatheroschlerosis (Mohamed *et al,* 1994; Istikharah, 2007; Singhatong et al., 2010). Another studies have been conducted by Kusuma (2008) and Manaharan (2012) suggests that rambutan leaf has activity as lowering blood glucose in mice. Rambutan leaf has been suggested as source of potential useful antidiabetic drugs. However the toxicity of rambutan leaf has not been extensively studied. The numerous warnings regarding the potential toxicity of these therapies is needed for practitioners to keep abreast of the reported incidence of hepatotoxicity, nephrotoxic and cardiotoxic caused by the ingestion of herbal medicine (Saad *et al.*, 2006). This effort is very important that could be used rambutan leaf as standardized herbs that has been tested the efficacy and the safety.

Objectives

The general objective of this study was to determine the toxicity level of rambutan leaf (*Nephelium lappceum* L) extract in mice. Spesific objective is to determine the acute toxicity value (LD50) of rambutan leaf (*Nephelium lappaceum* L) extract in mice.

METHODS

Plant Material

Rambutan leaf were collected at Ngluwar Muntilan, Indonesia and were authenticated at Pharmacy Biological Laboratory, Islamic University of Indonesia based on Flora of Java book (Backer and Van der Brink, 1965). Powdered leaf of Rambutan (145 g) was macerated of ethanolic 70% at room temperature for 1 day and continued was percolated for 3 days. The resulting extract was concentrated to dryness to obtain a mass of ethanol extract (29.3 g) with the extraction yield of (20.21%).

Animals

The experimental animals used in this study were 25 Swiss Albino mice of male sexes each weighing 20-30 g and aged 8-10 weeks (Tedong, *et al.*, 2007). All animals were available at Integrated Research and Testing Laboratories, Gadjah Mada University. The animals were randomly distributed into five groups. All animals (mice) were maintained at constant temperature and humidity.

Acute Toxicity

In order to study any possible toxic effect or changes in normal behaviour, four groups of 5 mice were used in this experiment. The acute toxicity of the plant was studied by preparing four different concentrations of the extract (1.0, 2.0, 4.0, 8.0 g/Kg body weight) respectively was administered orally, to the animals as a single dose. The control group was given diluted solution in water. The symptoms of toxicity such as motor activity were cheked on the first 3 hours (Tedong *et al.*, 2007). The animals were observed for 24 hours and the number of dead mice was recorded and used in the calculation of the acute toxicity value (LD50).

Statistical Analysis

Statistical analysis was carried out using One-Way Analysis of Variance (ANOVA) continued Kruskal-Willis test. P values of less than 5% ($P < 0.05$) was considered statistically significant differences between the groups (Tedong *et al.,* 2007).

RESULTS

In this study, there were no mortality (expressed as LD50) after oral administration of single doses of rambutan leaf up to 8 g/kg (Table 1). However, the biochemical parameter (ALT, AST) increased progressively with increasing dose (Table 2). The reduction of motor activity (uncontrollable way, restlessness, passivity motion, no reactivity to stimuli, and sleepy) were seen since at dose of 1 g/kg up to a dose of 8 g/kg.

DISCUSSION

Acute treatment of the mice with rambutan leaf extract at doses of 1.0, 2.0, 4.0 g/kg for 24 hours did not affect biochemical parameters. On the other hand, administration of rambutan leaf extract at dose of 8 g/kg for 24 hours resulted in significant changes in the levels of transaminases (ALT, AST), and creatinine. There were good indicators of liver and kidney functions (Tedong *et al.*, 2007). It is reasonable to deduce that the rambutan leaf extract induce damage to liver and kidneys. The changes in the biochemical parameter has been studied by Tedong *et al.* (2007) and Mohammed *et al.* (2012) by histopathological examination of selected organs showed liver infiltration and congestion, kidneys' mesangial expansion and nucleus pycnosis. Histopatological indicated mild vascular degenerative changes and necroses to liver and kidney when compared to that of control group. The change biochemical parameters of rambutan leaf extract may be related to its tannin content. Importanly, so many species which contain tannin have been shown to display a wide spectrum of toxicological activities. Diets tannin had deleterious effects on liver of the rabbits and rats (Obidah *et al*., 2010; Fayemi *et al*., 2011).

The ethanolic rambutan leaf extract (1, 2, 4, 8 g/kg) produced a reduction in spontaneous motor activity, motor coordination, sleepy and depressant. Preliminary qualitative chemical studies of some plants indicated tannin in the extract suggested that contains some active

Table I. Acute Toxicity of Rambutan leaf (*Nephelium lappaceum* L) extract in mice

Number of mice	Dose of extract g/kg	Number of mice dead	Percentage of mice dead
5	0.0	0	0
5	1.0	0	0
5	2.0	0	0
5	4.0	0	0
5	8.0	0	0

Table II. Biochemical Animal Test After 24 hours administration

Dose of extract g/kg	*SGPT (u/l)*	*SGOT (u/l)*	Kreatinin (mg/dl)
0.0	*56.0*	*111*	0.3
1.0	*49.5*	*93.5*	0.2
2.0	*49.0*	*81*	0.4
4.0	55.0	105.5	0.3
8.0	122.0	227.0	0.2

principles which possess potential CNS-depressant action, decreased loco-motor activity, produced muscle relaxation and showed antianxiety activity (Bhosale et al., 2011; Habib et al., 2011; Raju *et al.*, 2011).

In this study, there was no lethality observed for all the tested doses throughout the 24 hours period as well as tannins at *Cinnomomum iners* leaves was no lethality (Mustaffa *et al*., 2010).

CONCLUSION

On the basis of present study, it can be concluded that rambutan leaf extract appears to be free from acute toxicity and relatively safe within the normal doses. However it is recommended for the next studies:

a) To determine histologically the acute toxicity effects of rambutan leaf the internal organs of mice

b) Acute toxicity test used more various dose and more obeserved day .

c) Sub-acute and chronic toxicity tests is planned in order to determine the long-term effects of the extract.

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EVALUATION OF ANTIHYPERTENSIVE DRUGS UTILIZATION IN HOSPITALIZED HYPERTENSION PATIENS (ICD I.15-2) AT X HOSPITAL BANTUL YOGVYAKARTA IN 2010 AND 2011 BY ATC/DDD METHOD

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Abstract

Background. *Hypertension is the most prevalent cardiovascular disease in the world. Hypertension is now often compelled with Diabetes Mellitus because of changing in life style.*

Objectives. *This study was aimed to know the description of the use of drugs that includes the classification of drugs, the most widely used antihypertensive, compliance with the National Essential Medicines List (DOEN) in 2008, JNC-7 and Hospital Formulary at X Hospital in Bantul Yogyakarta during 2010 and 2011 using ATC / DDD.*

Methods. *The study was conducted by descriptive design using retrospective method. Information about antihypertensive data was obtained from the Installation Inpatient Medical Records. All the data was then processed to determine the quantity of use of antihypertensives in units of stay and profiles DDD/100 Drug Utilization (DU 90%), compliance with Hospital Formulary, type of appropriate to the National Essential Drugs List (DOEN) and JNC 7 Guideline.*

Outcome. *The purpose of the ATC/DDD system is to serve as a tool for drug utilization research in order to improve quality of drug use*

Results. *The results showed that the widely used antihypertensive drugs per 100 bed days in 2010 and 2011 was amlodipin, which were 91,45 and 60,61, respectively. The utilization of antihypertensive drugs was changed in 2010 and 2011, based on number of antihypertensive drugs within DU 90% segment. Types of antihypertensive appropriate to the DOEN were captopril, lisinopril, nifedipine and diuretic were furosemide and spironolacton in 2010, however in 2011 for the diuretic were furosemide, spironolactone and hydrochlorothiazide. The utility of antihypertensive drugs which were not appropriate with JNC-7 were valsartane, irbesartane, candesartane (Antagonist Agiotensine II), amlodipin (CCB) and spironolactone (Antagonist Aldosterone). Some antihypertensive not appropriate to the Hospital Formulary.*

Conclusion.*The widely used antihypertensive drug was amlodipin. The utilization of antihypertensive drugs was changed in 2010 and 2011. Types of antihypertensive appropriate to the DOEN, however not appropriate to JNC 7 Guideline and the Hospital Formulary.*

Key words : *antihypertensive drugs, methods of ATC / DDD, DU 90%*

ISBN : 978-979-18458-6-1

INTRODUCTION

Hypertension is a disease that suffered by many people in Indonesia and all over the world. The increasing quality of life brings changes in individual life pattern. Hypertension with diabetic will increase cardiovascular disease, it needs choosing the right medicine. Drug use evaluation by ATC/DDD methods, which will be done in X Hospital in Bantul Yogyakarta.The overall goal of treating hypertension is to reduce hypertension associated morbidity and mortality.

This study was aimed to know the description of the use of drugs that includes the classification of drugs, the most widely used antihypertensive, compliance with the National Essential Medicines List (DOEN) in 2008, JNC-7 and Hospital Formulary at X Hospital in Bantul Yogyakarta during 2010 and 2011 using ATC / DDD.

METHODS

The study was conducted by descriptive design using retrospective method. Information about antihypertensive data was obtained from the Installation Inpatient Medical Records. All the data was then processed to determine the quantity of use of antihypertensives in units of stay and profiles DDD/100, *Drug Utilization* (DU 90%), type of appropriate to the National Essential Drugs List (DOEN), compliance with JNC 7 Guideline and Hospital Formulary.

RESULTS AND DISCUSSION

The results of this study showed in these tables below:

1. Appropriate to the National Essential Drugs List (DOEN)

From Table I, there were 5 drugs included in the DU 90%, the rank order decreased of the utilization antihypertensive drug was Amlodipin, Furosemide, Irbesartan, Captopril and Valksartan, percent utility of 26,90, 21,61, 20,32, 14,15 and 5,99, respectively.

From Table II, there were 4 drugs included in the DU 90%, the rank order decreased of the utilization antihypertensive drug was Amlodipin, Irbesartan, Captopril and Furosemide, percent utility of 30.24, 23.16, 19.06, and 15.96, respectively.

Amlodipin was the highest percentage to use because it is effecacious in improving left ventricular function in hypertension and ischemic heart (Alam *et al.*, 2009; Pimenta,

Table I. The value of DDD/100days and DU90% in 2010

Generic name	DDD/100days	% of used	Cumulative percentage	Segment
Amlodipine	91,45	26.90	26.90	DU 90%
Furosemide	73.45	21.61	48.51	
Irbesartan	69.09	20.32	68.83	
Captopril	48.09	14.15	82.98	
Valsartan	20.36	5.99	88.97	
Ramipril	15.27	4.49	93.46	10%
Nifedipin	6.18	1.76	95.22	
Candesartan	6	1.76	96.98	
Bisoprolol	3.64	1.07	98.05	
Diltiazem	3.51	1.03	99.08	
Lisinopril	1.27	0.38	99.49	
Spironolactone	1.21	0.36	99.87	
Nicardipin	0.44	0,13	100	

Table II. The value of DDD/100days and DU 90% in 2011

Generic name	DDD/100days	% of use	Cumulative percentage	Segment
Amlodipin	60.61	30.24	30.24	DU 90%
Irbesartan	46.42	23.16	53.40	
Captopril	38.20	19.06	72.46	
Furosemide	31.97	15.96	88.42	
Diltiazem	7.87	3.93	92.35	10%
Imidapril	4.24	2.11	94.46	
Valsartan	2.88	1.44	95.90	
HCT	2.12	1.06	96.96	
Lisinopril	1.82	0.91	97.87	
Nifedipin	1.82	0.91	98.78	
Bisoprolol	1	0.50	99.28	
Spironolactone	0.76	0.38	99.66	
Ramipril	0.61	0.30	99.96	
Nicardipin	0.13	0.06	100	

2009). In JNC 7, for compelling indication diabetes, the first choice is ACEI (Angiotensin Coverting Enzyme Inhibitor) or ARB (Angiotensin Receptor Blocker), because both tend to renoprotective effect, then used diuretic thiazide and the last BB (Beta Blocker) or CCB (Calcium Channel Blocker) (JNC 7, 2003). Based on this study, it can be concluded that the utilization of antihypertensive drugs was changed in 2010 and 2011, based on number of antihypertensive drugs within DU 90% segment.

2. The use of antihypertensive compliance

a. Antihypertensive drugs utilization

Antihypertensive drugs utilization at X Hospital Bantul Yogyakarta were appropriate to the DOEN; were, captopril, lisinopril, nifedipin, furosemide, spironolactone (Depkes, 2008) and captopril, lisinopril, nifedipin, furosemide, hydrochlorothiazide and spironolacton (Depkes, 2011).

b. JNC 7

From Table III, it appeared that there were two classes of antihypertensive drugs were not appropriate with JNC 7, namely Ca antagonist and Antagonist aldosteron. Both dihydropyridine and non-dihydropyridine calcium channel blockers (CCBs) effective in lowering blood pressure, but only non-dihydropyridine CCBs (diltiazem and verapamil) which may reduce over proteinuria and improve glomerular size selectivity in patient with nephropathy due to type 2 diabetes (Giuseppe *et al*., 2002.; Burney and Bakris, 2010).

The precence of diabetic nephropathy should influence the choice of an ACE inhibitors versus an ARB. Amlodipin an Calcium Channel Blocker (CCB) (dihydropyridine) are second line to ACEI or ARB. Data are emerging for combined therapy (Dipiro, 2008).

In cardiac disease cases, for blood pressure management: a given lower goal than for essential hypertension, often requires more antihypertensive medications. Hypertension regimen should include an ACE inhibitor or ARB (Dipiro, 2008). Thiazide diuretics, ARB, and ACE inhibitors may be the best first-line although other agents are usually necessary and goals may not be achieved even with three or four agents. Aggressive blood pressure control may be the most important factor in preventing

ISBN : 978-979-18458-6-1

Table III. Antihipertensive compare to JNC 7 in 2010 and 2011

Types	ATC code	Name/ dosage form	Appropriate to JNC 7	
			2010	2011
ACEI	C09AA01	Captopril/ tablet	√	√
		Farmoten/ tablet		√
	C09AA03	Interpril/ tablet	√	√
		Noperten/ tablet	√	
	CA09AA05	Hyperil /tablet	√	√
		Cardace/ tablet	√	
	C09AA16	Tanapress/ tablet		√
Loop diuretic	C03CA01	Furosemide/ injeksi	√	√
		Furosemide tablet	√	√
		Farsix/ injection	√	
		Farsix/ tablet	√	√
		Lasix/ injection	√	√
		Lasix / tablet		√
Thiazide Diuretic	CA03AA03	HCT/ tablet		√
ß blocker	C07AB07	Bisoprolol/ tablet	√	√
Angiotensin Antagonist II	C08CA01	Valsartan/ tablet	√	
	C09CA03	Valsartan tablet		√
	C09CA04	Irtan/ tablet	√	√
		Irvask/ tablet	√	√
		Iritensa/ tablet	√	√
	C09CA06	Blopress/ tablet	√	-
Ca Antagonist	C08CA01	Intervask/ tablet	-	-
		Amlodipine/ tab	-	-
		Cardisan/ tablet	-	-
		Divask/ tablet	-	-
Aldosteron Antagonist	C03DA01	Spironolakctone/ tablet	-	-
CCB	C08CA04	Perdipine/ tablet	√	√
	C08CA05	Nifedipine/ tablet	√	√
	C08DB01	Diltiazem/ tablet	√	√
		Herbesser/ tablet	√	√
		Herbesser CD/ tablet		√

adverse outcomes in patients with type 2 diabetes (Vijan and Hayward, 2003).

It was recommended to publish standard of therapy as a guideline for the physicians in prescribing.

ISBN : 978-979-18458-6-1

Table IV. Antihipertensive compared to Hospital Formulary in 2010 and 2011

Types	ATC code	Name/ dosage form	Apropiate to hospital formulary	
			2010	2011
ACEI	C09AA01	Captopril /tablet	√	√
		Farmoten /tablet		√
	C09AA03	Interpril /tablet	√	√
		Noperten/ tablet	-	
	C09AA05	Hyperil /tablet	√	√
		Cardace /tablet	-	
	C09AA16	Tanapress/tablet		-
Loop diuretic	C03CA01	Furosemide /injection	√	√
		Furosemide/ tablet	√	√
		Farsix /injection	√	
		Farsix/ tablet	√	√
		Lasix/ injction	√	√
		Lasix/ tablet		√
Thiazide diuretic	CA03AA03	HCT/tablet		√
ß bloker	C07AB07	Bisoprolol/ tablet	√	√
Angiotensin II antagonist	C08CA01	Valsartan/ tablet	√	
	C09CA03	Valsartan/tablet		√
	C09CA04	Irtan /tablet	-	-
		Irvask/ tablet	√	√
		Iritensa/ tablet	√	√
	C09CA06	Blopress/ tablet	√	
Ca Antagonist	C08CA01	Intervask/ tablet	-	-
		Amlodipine/ tablet	√	√
		Cardisan/ tablet	√	√
		Divask/ tablet	√	√
Aldosterone antagonist	C03DA01	Spironolactone/ tablet	√	√
CCB	C08CA04	Perdipin/ tablet	√	√
	C08CA05	Nifedipin/ tablet	√	√
	C08DB01	Diltiazem/ tablet	√	√
		Herbesser/ tablet	√	√
		Herbesser CD/ tablet		√

c. Hospital Formulary

From Table IV, showed that antihypertensive drugs compared to Hospital Formulary were not appropriate namely Cardace, Noperten, Irtan, Intervask in 2010, not appropriate was 16%. Also showed that antihypertensive drugs compared to Hospital Formulary were not appropriate namely Tanapress, Irtan, Intervask in 2010, not appropriate was 10%.

In accordance with results it was suggested to review Hospital Formulary in order

to make drug inventory more effective and efficient.

CONCLUSION

The general results lead to conclusion that the widely used antihypertensive drug was amlodipin. The utilization of antihypertensive drugs was changed in 2010 and 2011. Types of antihypertensive appropriate to the DOEN, however were not appropriate to the Hospital Formulary and JNC 7 Guideline.

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UTILIZATION ANALYSIS OF ANTIBIOTICS FOR TYPHOID FEVER IN HOSPITALIZED PATIENT IN 2010 AND 2011 AT X HOSPITAL IN BANTUL WITH ATC/DDDMETHOD

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Abstract

Backgroud. *Typhoid fever caused by Salmonella typhi is still endemic disease in Indonesia. Antibioticis a group of drugs often used today to treat typhoid fever.*

Objectives. *The purpose of this study was to determine the pattern of antibiotic use for treatment of typhoid fever in hospitalized patients, the suitability of the use of antibiotics were comparedto the hospital formulary and DOEN.Futher more the change of antibiotic usage patterns of typhoid fever in hospitalized patients in 2010 and 2011 atX hospital in Bantulwas seen from the DU90%.*

Method. *The study design was descriptive.We used ATC/DDD as means to increase the quality use of medicines with an average maintenance dose per day was estimated for the adult indication. The study subjects were all data of antibiotics used for treatment of typhoid fever in adult patients hospitalized at X hospiltal in Bantul in 2010 and 2011were obtained in medical record.*

Outcame :*This study of ultization medicine was to increase quality use of antibiotic for thypoid fever treatment especialy for hospitalized patient.*

Results. *The antibiotic use for treatment of typhoid fever in hospitalized patientsin 2010 were ceftriaxone (45,83 DDD/100days), cefrozil (19,87 DDD/100days), ciprofloxacin (11,28 DDD/100days), cefixime (6,73 DDD/100days), levofloxacin (3,21 DDD/100days), ceftazidime (1,60 DDD/100days), cefadroxil (0,64 DDD/100days). In 2011 antibioticsuse for treatment of typhoid fever in hospitalized patients were ceftriaxone (96,79 DDD/100days), ciprofloxacin (15,51 DDD/100days), levofloxacin (12,82 DDD/100days), cefotaxime (11,54 DDD/100days), azithromycin (3,21 DDD/100days), ceftazidime (2,56 DDD/100days), metronidazol (2,56 DDD/100days), cefadroxil (1,28 DDD/100days), ofloxacin (1,28 DDD/100days).*

Conclusions. *Most antibiotic used in 2010 and 2011 was ceftriaxone. In 2010 the appropriate antibiotic usedaccording to hospital formulary were 89% while in 2011,reached into 100%. The use of antibiotics that also existed in DOEN were cefriaxone and ciprofloxacin. There were two different antibiotic used in 2010 and 2011, which were levofloxacin and cefotaxim (2010) and cefprozil and cefixime (2011). However ceftriaxone and ciprofloxacin still used in 2010 and 2011.*

Keywords : *Typhoid Fever, Antibiotics, ATC/DDD, DU90%.*

ISBN : 978-979-18458-6-1

INTRODUCTION

Typhoid fever still remains a health problem in developing countries, mostly in the tropics such as in Indonesia, where the case number increase in the season and at the beginning of the rainy season. Incidence number of typhoid fever in Indonesia on average 900,000 cases / year with a mortality rate more than 20,000 which 91% of cases occur in age of 3 -19 years.

In the last four decades, typhoid fever has become a global health problem. The incidence number of this disease estimated reach 13 – 17 million cases worldwide with a mortality rate of up to 600,000 people per year. Endemic typhoid fever spread across various continents, from Asia, Africa, South America, the Caribbean, to Oceania. The majority of cases (80%) are found in developing countries, such as Bangladesh, Laos, Nepal, Pakistan, India, Vietnam, and including Indonesia.

Antibioticis a group of drugs often used today to treat typhoid fever. Selection of appropriate antibiotics in patients with typhoid fever is very important, because it can prevent complications and reduce mortality.

OBJECTIVES

The purpose of this study was to determine the pattern of antibiotic use for treatment of typhoid fever in hospitalized patients, the suitability of the use of antibiotics were compared to the hospital formulary and DOEN.Futher more the change of antibiotic usage patterns of typhoid fever in hospitalized patients in 2010 and 2011 atX hospital in Bantulwas seen from the DU90%.

METHODS

The study design was descriptive.We used ATC/DDD as means to increase the quality use of medicines with an average maintenance dose per day was estimated for the adult indication. The study subjects were all data of antibiotics used for treatment of typhoid fever in adult patients hospitalized at X hospiltal in Bantul in 2010 and 2011 were obtained in medical record.

Outcame

We hope this study of utilization drug can increase knowledge about application methods ATC / DDD in the study of drug use.Provide an overview of the suitability of antibiotic therapy in the treatment of typhoid in the hospital formulary and its concept.Provide an overview of information regarding the use of antibiotics in the treatment of typhoid patients was particularly useful for pharmacy in pharmaceutical management.This study was expected to be the input for other researchers on the analysis of ATC / DDD.Also the important was to increase the quality use of antibiotic for thypoid fever treatment especialy for hospitalized patient.

RESULTS

Types and quantity of antibiotics for thypiod fever treatment of adult patients hospitalized were obtained from medical record card X hospital in Bantul. Rute of administration of antibioticmustobtain concernbecause in the ATC / DDD there are some medications with different values DDD between oral and parenteral administration.To determine the quantity use of antibiotic in thypiod fever patients using DDD based WHO Collaborating Center for Stasistic Methodology in 2010. Number of inpatient days in this study were obtained from the number of days of hospitalization entire adult typhoid fever patients for one year. Adult typhoid fever patients hospitalized at X hospital in Bantul in 2010 was 468 days whereas in 2011 was 468 days. Data on the number of stay is needed to calculate the use of antibiotics in the inpatient unit of DDD/100.

Antibiotic utilization data shown on the table below.

ISBN : 978-979-18458-6-1

Table I. Antibiotic use for typhoid fever in 2010

Generic name	DDD/100 bed days	% use	Cumulative	Segment
Ceftriaxone	45.83	51.40	51.40	DU 90%
Cefrozil	19.87	22.29	73.69	
Ciprofloxacin	11.28	12.65	86.34	
Cefixime	6.73	7.55	93.99	
Levofloxacin	3.21	3.60	97.59	10%
Ceftazidime	1.60	1.79	99.37	
Cefadroxil	0.64	0.73	100	

Table II. Antibiotic use for typhoid fever in 2011

Generic name	DDD/100 bed days	% use	C umulative	Segment
Ceftriaxone	96,79	65,60	65,60	DU 90%
Ciprofloxacin	15,51	10,51	76,11	
Levofloxacin	12,82	8,69	84,80	
Cefotaxime	11,54	7,82	92,62	
Azithromycin	3,21	2,18	94,80	10 %
Ceftazidime	2,56	1,73	96,53	
Metronidazole	2,56	1,73	98,26	
Cefadroxil	1,28	0,87	99,13	
Ofloxacin	1,28	0,87	100	

From the table I and the table II it can be seen that thehighest antibiotic use for patients with typhoid fever are 3rdcephalosporins, namely ceftriaxone, this may be due to sefalosforin have high stability against gram-negative and gram-positive thus more effective in killing the bacteria that causes typhoid fever (Tan and Rahardja, 2007).

In small-scale study (Sutardi 2010), ceftriaxone was more effective when given only 2-3 days although recurrence rates with short-term treatment can not always be measured.Some studies show the superiority of ceftriaxone as antibiotic selected for typhoid fever.

In 2010 antibiotics were included in segment DU90% is ceftriaxone, cefrozil, cefixime and ciprofloxacin. Whereas in 2011 the antibiotics included in the segment DU90% is ceftriaxone, cefotaxime, ciprofloxacin and levofloxacin. There was a difference when compared segment DU90% use of antibiotics in the treatment of typhoid fever between 2010 and 2011. The use of levofloxacin increased in 2011, this shows a shift in the use of antibiotic class quinolones is more widely used for the treatment of typhoid fever than the epidemic in 2010.

Table III. Antibiotic use in 2010 compare to hospital formulary and DOEN

Types	Patent name	Generic name	Rute	Hospital formulary	DOEN
1st cephalosporins	-	Cefadroxil	Oral	√	
2nd cephalosporins	Lizor	Cefrozil	Oral	√	
3rd cephalosporins	-	Ceftriaxone	Parenteral	√	√
	Zidifec	Ceftriaxone	Parenteral	√	√
	Elpicef	Ceftriaxone	Parenteral	√	
	-	Cefixime	Oral	√	
	Fixiphar	Cefixime	Oral	-	
	Maxpro	Cefixime	Oral	√	
Quinolon	-	Ciprofloxacin	Oral dan parenteral	√	√ (oral)
	Cravox	Levofloxacin	Oral	√	

√ = appropiate , - = not appropiate

From the table III, use of antibiotics in 2010, there was 1 (11%) types of antibiotics that are not included in the hospital formulary, which Fixiphar that is the trademark of cefixime. While in 2011 (table IV) all of antibiotics (100%) which is used to treat typhoid fever was accordance with the hospital formulary. It has a good indication, means the doctor has complied with the hospital formulary and ensure patients obtain prescription drugs.

Table IV. Antibiotic use in 2011 compare to hospital formulary and DOEN

Types	Patent name	Generic name	Rute	Hospital formulary	DOEN
1stcephalosporins	-	Cefadroxil	Oral	√	
3rdcephalosporins	-	Cefotaxime	Parenteral	√	
	-	Ceftazidime	Parenteral	√	
	-	Ceftriaxone	Parenteral	√	√
	Elpicef	Ceftriaxone	Parenteral	√	√
	Strarxon	Ceftriaxone	Parenteral	√	√
Quinolon	-	Ofloxacin	Oral	√	
	-	Ciprofloxacin	Oral dan parenteral	√	√ (oral)
	Cetafloxo	Cetafloxo	Parenteral	√	
	-	Levofloxacin	Oral	√	
	Cravox	Levofloxacin	Parenteral	√	
	Cravit	Levofloxacin	Oral	√	
Makrolide	-	Azithromycin	Oral	√	
Imidazole	-	Metronidazole	Parenteral	√	√

√= appropiate , - = not appropiate

DOEN was an essential drug list which was very important for supply drug in Indonesia. DOEN were structured to ensure the availability of drugs that seem to be more equal and accessible by the public. Antibiotics used for typhoid fever in hospitalized adult patients at X hospital in Bantul in 2010 and 2011 were ceftriaxone and ciprofloxacin contained in the list.

CONCLUSIONS

Most antibiotic used in 2010 and 2011 was ceftriaxone. In 2010 the appropriate antibiotic used according to hospital formulary were 89% while in 2011, reached into 100%. The use of antibiotics that also existed in DOEN were cefriaxone and ciprofloxacin. There were two different antibiotic used in 2010 and 2011, which were levofloxacin and cefotaxim (2010) and cefprozil and cefixime (2011). However ceftriaxone and ciprofloxacin still used in 2010 and 2011.

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ANACTINOMYCETES (ISOLATE T34) AS ANTIBIOTIC PRODUCER AGAINTS *Staphylococcus aureus* AND BIOAUTOGRAPHY ANALYSIS

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Abstract

Background. *Actinomycetes is a group of microorganisms producing many kinds of active compounds. One of them is antibiotic.*

Objective. *The purpose of this study is to determine the potency of Actinomycetes (isolate T34)as antibioticproducer against Staphylococcus aureus and to know the spot of thin layer chromatography showing activity as antibiotic based on bioautography test.*

Methods. *The Actinomycetes (isolate T34) was taken from rhizosphere of Tin (Ficuscarica L.) plant which is grown on Starch Nitrate Agarmedium.The broth culture was prepared using 25 ml of Starch Nitrate Brothmedium having been givena quarter plate of the Actinomycetes isolate and then shaken for 5days.After that, the25 µl of actinomycetesbroth culture was put on the well of plate that had been planted with S. aureus and incubated at 37 °C for 24 hours to measure the inhibition zone diameter.The active broth culture was extracted using ethyl acetate solvent. Components in the extract were analyzed by thin layer chromatography and followed by bioautography process on Mueller Hinton medium having been cultivated with S. aureus for 30 min and incubated for 24 hours.*

Outcome measured. *Inhibition zone diameter against S.aureusgrowth.*

Results. *The results shows that the Actinomycetes (isolate T 34) can inhibit S. aureus with 24,2 mm of inhibition zone diameter. The result of the thin layer chromatography using silica gel GF254 as stationary phase and n-hexane : ethyl acetate (2:1) as mobile phase shows that one spot appears by UV 254 detection and two spots appear by UV 366. One of spots with Rf of 0.56 shows activity as antibiotic.*

Conclusion. *Actinomycetes (isolate T 34)produces antibiotic against S. aureus.*

Keyword : *Actinomycetes, bioautography, Staphylococcus aureus*

ISBN : 978-979-18458-6-1

INTRODUCTION

Infectious disease is one of the problems in the health sector that continues to grow. Infection can be transmitted from one person to another, from animals to humans. Some microorganisms that cause infections such as bacterial, viral, rickettsial, fungal, and protozoal (Gibson, 1996). One of them is *Staphylococcus aureus. S. aureus* can cause infections in humans both intissues and organs and cause typical signs such asinflammation, necrosis and abscess formation. Infection can bemild to the skinfurunclestobe afatalpiemia (Nelnick, 1994). This bacterium was resistant to most antibiotics. MRSA (methicillin resistant *S. aureus*) were obtained from the hospital environment and occurs due to the exposure of a semisynthetic penicillin and methicillin (Enright, 2003). Sulistyani *et al.* (2009) have isolated bacteria from hospital sewage and found a lot of resistance occurs in *S. aureus* primarily on Betalaktam and erythromycin derivatives.

With increasing misuses of antibiotics, the serious problems of antibiotic resistance are developing at an alarming rate. Hence, intensive search for new antibiotics has become imperative worldwide (Haque*et al.,* 1995; Oskay*et al.,* 2004; Parungao*et al.,* 2007) especially from actinomyceteswhich is known as the greatest source of antibiotics (Ogumnwonyi, 2010).Actinomycetes is best known for their ability to produce antibiotics and Gram-positive bacteria consists of a group of unicellular microorganisms branched. They produce mycelium branches consisting of two types: the substrate mycelium and aerial mycelium (Sivakumar, 2010).

The purpose of this study was to find out whether actinomycetes (isolate T34) were isolated from the rhizosphere of plants Tin (*Ficuscarica* L.) has potential as an antibiotic against *S. aureus*. In addition, to determine which patches of thin-layer chromatography shows potential as an antibiotic against *S. aureus*.

MATERIALS AND METHOD

A. Materials

Materials used in this study are sterile distilled water, the bacteria *S. aureus*, Nitrate Starch Agar (SNA) medium, Starch Nitrate Broth (SNB), Mueller Hinton agar (MH), Brain Heart Infusion, Standard McFarland, nystatin 100 mg / mL, glycerol 20% v / v, n-hexane, ethyl acetate, silica gel GF254.

B. Procedure

1. Turification of the Actinomycetes (isolate T34).

   Actinomycetes isolates cultured from the rhizosphere samples of tin plant (*F.carica* L.) was done by streaking on SNA plate (Rante, 2010), then incubated at 28 ° C for ± 10 days. Observation of morphological characteristics of Actinomycetes (isolate T34) was carried out toward the pigment, aerial mycelium and vegetative mycelium.

2. Preparation of *S.aureus* culture

   Several colonies of bacteria growing on agar taken 24 hours,were suspended in 1 mL of BHI broth and incubated 4-8 hours at 37 ° C. The suspension was added with sterile distilled water up to a certain turbidity in accordance with the standard concentration of 10^8 CFU/mLof bacteria.

3. The activity test of antibiotic-producing Actinomycetes with the wells diffusion method a quarter plate of isolates included in 25 mL of Starch Nitrate Broth (SNB) medium, incubated at room temperature for 5 days with the shaking. To obtain the cell-free supernatant, the culture broth was centrifuged at 8000 rpm for 10 min. The culture of *S.aureus* with concentration of 10^8 CFU/mL was spread using sterile cotton on Mueller Hinton Agar (MH), and then made three wells with diameter of 5 mm and each well wasloaded with 25 µL of the clear

supernatant. The dishes were preincubated at 4.0 °C for 2 hours to allow uniform diffusion into the agar, then followed by incubation for 24 hours at 37 º C, then measured the diameter of inhibition zone (Oskay, 2009).

4. Extraction of secondary metabolites the broth cultures were centrifuged, then the supernatant was extracted with ethyl acetate using a ratio of 1:1 (Sulistiyani, 2006). The extraction was conducted twice (Rante, 2010), strongly shaken and then left in place to form ethyl acetate phase and liquid phase. Phases were separated and the ethyl acetate phase was evaporated in a hood.

5. Thin-layer chromatography and bioautography

   Bioautography test was conducted to determine spotting potentially active compounds as antibiotics by using thin layer chromatography. Bioautography was done with spotting of extract on silica gel GF254 plate, then developed with the appropriate mobile phase for the separation of compounds contained in the fraction. The mobile used phase in this study is n-hexane: ethyl acetate (2:1). Chromatogram plate were placed on a agar surface that has been spread with *S. aureus* suspension, chromatograms were left clinging on agar for 30 minutes so that the active compound diffuses into the agar medium, then carefully removed and the dish was incubated for 24 hours. Furthermore, it can be seen patches that provide clear zone (zone of inhibition) that suggest the potential as antibiotics.

RESULTS AND DISCUSSION

1. Isolation and Purification Actinomycetes

The actinomycetes (isolate T34) was isolated from the rhizosphere of Tin plant (*Ficuscarica* L.) were obtained from Gergunung (North Klaten). The rhizosphere taken was located 5-10 cm below the soil surface. In the process of isolation, the rhizosphere samples were dried at room temperature until completely dry or no water content again, evidenced by the absence of water absorbed by the paper after tested with put on paper. Then the dried soil samples was carried propagules sediment extraction by making serial dilutions 10^{-1}-10^{-5}, after that, each dilution was inoculated on media SNA to do selective isolation and purification Actinomycetes.

Actinomycetes are soil bacteria that have a slower growth rate when compared with other soil bacteria that isolation requires a technique that allows maximum bacteria isolated Actinomycetes. Some methods used are: pre-treatment by heating the sample at a temperature of 50 º C for 10 minutes to prevent the growth of other bacteria, the addition of nystatin 100 mg/mL in order to prevent the growth of fungus and selective use of media that can eliminate the growth of other bacteria. Selective media were used to grow Actinomycetes in this research that Starch Nitrate Agar (SNA), this medium can be used by microorganisms including Actinomycetes as a source of nitrogen is a nutrient for growing Actinomycetes. Thus, the purification of Actinomycetes is done by planting the alleged Actinomycetes isolates on solid medium SNA.

In the process of isolation of Actinomycetes from tin plant rhizosphere (*F. carica* L.), Actinomycetes colonies grow slowly, showing the consistency of powdered, firmly attached to the surface of the agar and have a different appearance from each other (Rao, 1994), including a variety of different colors both the vegetative mycelium and aerial mycelium (Holt *et al*., 1994).

2. The morphology of colonies of Actinomycetes (isolate T34)

Gram staining is one way for microscopic identification of Actinomycetes. Actinomycetesisa group of Gram-positive bacteria. Gram staining was conducted to determine whether the classification of

ISBN : 978-979-18458-6-1

Gram-positive microorganisms (results are purple) or Gram negative (red result). The Gram staining result showed that the Actinomycetes (isolate T34) has the characteristics of class members Actinomycetes that have branched mycelium and purple. Picture of the Gram staining result of Actinomycetes (isolate T34) is presented in Figure 1.

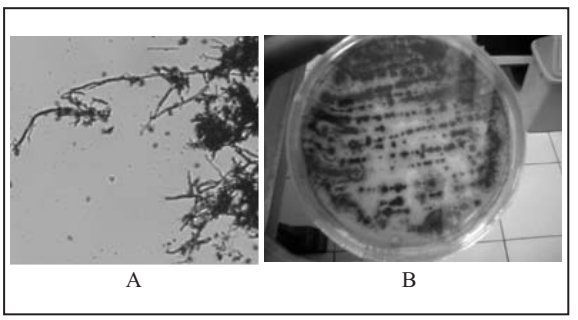

Figure 1. The morphology of Actinomycetes (Isolate T34), microscopic (A) and the colony (B)

Figure 1 shows there sult of painting is colored purple orare Gram positive, so that it can be concluded that these isolates have characteristics of Actinomycetes asgram-positive bacteria. The observation of the morphological characteristics of Actinomycetes (isolate T34) suggests that it has the characteristics of class members of Actinomycetes that their colonies are initially relativelys mooth surface but then form a kind of wovenaerial my celium which can manifest granularnya like powder, velvet, produces pigments that cause colors inaerial my celium and vegetative my celium. This Actinomycetes isolate has moss green color aerial my celium and brownish green color vegetative my celium.

3. The Potency of Actinomycetes (Isolate T34) as Producer Antibiotics against *S. aureus*

In this study, the activity test of Actinomycetes as antibiotics producers use *S. aureus* as test bacteria. The method used to test the activity of antibiotic-producing Actinomycetes is wells diffusion method. The wells diffusion method is done by making a hole in the solid medium that had been inoculated with bacteria. The number and location of holes were adapted to the purpose of the study, then the hole is filled with the sample to be tested. After the incubation, bacterial growth was observed to determine the presence or absence of inhibition areas around wells. The advantage of this method is much easier to measure the diameter of inhibition zone formed by the activity of the compounds in the test sample that is not only on the surface of the nutrient agar but also get to the bottom (Kusmayati and Agustini, 2007). The figure of potential test results of Actinomycetes (isolate T34) as producers of antibiotics against *S. aureus* is presented in Figure 2.

Figure 2.The Activityof Actinomycetes (Isolate T34) against *S. aureus*

The strength of antibiotics to inhibit the growth of bacteria is classified by Davis Stout as very strong (inhibitory area 20 mm or more), strong (inhibitory region 10-20 mm), medium (5-10 mm inhibitory region) and weak (local inhibitory 5 mm or less) (Sulistiyani, 2006). At the test potential of Actinomycetes (isolate T34) activity as a producer of antibiotics, it is done three planting replication in a liquid culture plate. The first major inhibitory diameter was 23.7 mm, the diameter of the second inhibitory diameter is 24.5 and the third inhibitory diameter was 24.5 mm. Based on this measurement, it is known that the Actinomycetes (isolate T34) has the potential to produce antibiotic categorized very strong inhibitory activity in inhibiting the growth of *S.aureus* (average diameter of 24.2 mm barrier region (not including the 5 mm

diameter wells)) with SD values of 0.46 and 1.9% CV values.

4. Thin Layer Chromatography (TLC) and Bioautography

Before doing Bioautography-TLC, it is previously carried out the extraction of metabolite from the culture broth of Actinomycetes (isolate T34) using ethyl acetate.The metabolite extractyield results are 0.102% w/v. Antibacterial activity of this extract then is tested by bioautography, the method previously performed TLC to separate the good patches for gettinge specially active spot as an antibacterial. Bioautography-TLC testinthis studyaims to determine the spots of TLC which shows activity as antibiotics against *S.aureus*.The TLC mobile phase used in this study are n-hexane: ethyl acetate in the ratio of 2:1 and the stationary phase is silica gel GF254. The TLC results shows the presence of one spot that appears on the detection using a 254 nm UV light and two spot on observations with the 366 nm UV light. The bioautography results indicate a potential spot as the antibiotic on the spot with Rf of 0.56. The figure of bioautography chromatogram and test results are presented in Figure 3.

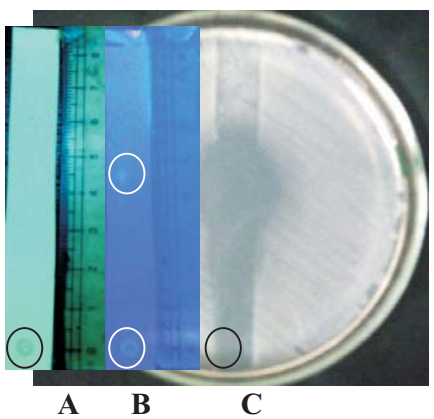

Figure 3. The chromatogram of ethyl acetate extractof Actinomycetes (isolate T34) culture brothon detection with 254 nm UV light (A), UV 366 nm (B) and the results of the TLC bioautography against *S. aureus* (C)

Bioautography method is done bychromatography plate is placed on the surface of the media, so that the compounds that have been separated into spots on the chromatogram will be able to diffuse into the agar medium. Chromatography plate affixed for about 30minutes on solid medium previously spread *S.aureus*.During attachment, the content of the compounds contained in the chromatogram spots diffuses into the agar medium. If the sepatches have potential as antibiotics,it will form a clear zone, which is an inhibitory zone of the chromatogram spot of metabolites extract of Actinomycetes(isolate T34) culture broth against *S.aureus*.

CONCLUSION

Based on theresults ofthe studyit can be concludedthat:

1. The Actinomycetes (isolate T34) produce antibiotics that inhibitsthe growth of *Staphylococcu saureus.*
2. The TLC with mobile phase of n-hexane: ethyl acetate in the ratio of 2:1 and the stationary phase of silicagel GF254 shows that the TLC patch of ethyl acetate extract of Actinomycetes (isolate T34) culture broth containing antibiotics against *S.aureus* has Rf of 0.56.

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TLC SCREENING FOR ANTIOXIDANT ACTIVITY OF HENNA (*Lawsonia inermis* L.) LEAF EXTRACT

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Abstract

Background. *Antioxidants have an important role to health, for example is the implications of the oxidation reaction in the body that can lead to cardiovascular disease, cancer, and aging. Natural antioxidants in plants can come from one or more components such as in Henna leaf, its compound can inhibit the oxidation reaction.*

Objective. *The aims of this study is to determine the chemical compound groups in Henna leaf extract that have antioxidant activity.*

Methods. *Powder of Henna leaf were macerated and frequently shaken with various of solvent such as water, methanol and chloroform for an hour. The filtrate of each extract was made up to concentration 10% w/v. Five microliter of each extract were spotted on silica gel F254 plates and eluated with mixture of chloroform: methanol 17:3 v/v as mobile phase. Spots that appear on the chromatogram were identified for flavonoids, naphthoquinone, polyphenols and also were sprayed with DPPH reagent to determine the spot that active as antioxidants.*

Outcome measured. *The active antioxidant Henna leaf constituents were detected as yellowish white spots produced by bleaching of DPPH by resolved bands on the TLC plates. All spot that have active antioxidant were identified by Rf value, characteristics spot under UV254, UV365, and spot colour after sprayed with specific spray reagent.*

Result. *The results of chromatogram showed that water extract, methanol extract and chloroform extract have antioxidant activity and were identified as flavonoids, naphthoquinone and polyphenols group. Potential antioxidant from the lowest respectively were chloroform extracts, water extract and methanol extract. Methanol extract have 4 spots, water extract have 3 spots and chloroform extract only have 1 spot were detected have antioxidant activity.*

Conclusion. Methanolic extract of Henna leaf had the greatest antioxidant activity than water or chloroform extract. The active compounds as antioxidant were flavonoids, naphthoquinones and polyphenols group.

Keyword : *Lawsonia inermis L, antioxidant, DPPH, TLC.*

ISBN : 978-979-18458-6-1

INTRODUCTION

Antioxidants have an important role to health, for example the implications of the oxidation reaction in the body that can lead to cardiovascular disease, cancer, and aging (Nelson *et al.*, 2003). Some synthetic antioxidant such as BHA and BHT are carcinogent and potentially toxic. Previous studies indicated that a high intake of antioxidants was positively associated with the reduced risk of coronary heart diseases and ageing related diseases (Flora, 2007; Valko *et al*., 2007). Indonesia has a fairly extensive tropical forests and biodiversity, both flora and fauna. One of the plants with the potential to be developed as antioxidant is henna (*Lawsonia inermis* L.). Natural antioxidants, such as in henna leaves, can inhibit the oxidation reaction.

L. Inermis L. have many biological activities besides used as a dye. Water and methanol extracts of the henna leaves have a high potential as an antioxidant and can simultaneously inhibit oxidative cell toxicity of MDA-MB-435S and pBR322 DNA induced Cr (VI) (Guha *et al*, 2009). The antioxidant activity of the methanol extract is higher than the water extract. This compares with total phenolic compounds extracted with methanol greater than the water extract of 2.56 mg / g and 1.45 mg / g calculated as tannins with Folin-Ciocalteu method (Hosein & Zinab, 2007). Henna plant have broad antimicrobial activity including as antibacterial, antiviral, antimycotic and antiparasitic (Babu and Subhasree, 2009). Chloroform extract can inhibit the growth of Malassezia in concentrations of 3 and 4 (v / v%), methanol extract at 0.25 and 3 (v / v%), while the water extract at 0.25 and 0.5 (v / v%) (Berenji, *et.al.*, 2010). Based on the description above, so in this experiment were used various of solvent such as water, ethanol and chloroform for henna leaves powders extraction to produce extract that have antioxidant activity. So the results of this study can be used as a reference for the preparation of henna extracts with high antioxidant activity.

MATERIALS AND METHODS

1. General

Silica gel 60 F254 TLC plates (Merck, Germany) were used for TLC bioautography analysis. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH_) purchased from Sigma-Aldrich (Steinheim, Germany). All solvents used for chromatography were pro analytical grade and extraction solvent used pharmaceutical grade.

2. Plant Materials

Leaves of *L. inermis* L. was collected from Yogyakarta province, Indonesia, in April 2012, and authenticated in Biology Phaculty of Ahmad Dahlan University, Yogyakarta, Indonesia.

3. Preparation of Henna Leaves Powder

Fresh free disease henna leaves was collected from Celeban Baru, UmbulHarjo, Yogyakarta then washed with running water and drained. The leaves material was then dried in oven at temperature 50^{o}C for 2 day until completely dry or easily crushed by hands. Dried leaves were powder by mechanicaly.

4. Preparation of water, methanol, and chloroform extract from henna leaves.

Five hundred milligrams of henna leaf powder was macerated using 5 ml of a suitable solvent while shaken for 1 h. The filtrate obtained by filtration using filter paper then volume was made to 5.0 ml by adding appropriate solvent.

5. Preparation of standard solution

Standard solution was made 0.1% w/v concentration in methanol.

6. Thin Layer Chromatography of Bioautographic Antioxidant Assays

A set of four plates of silica gel 60 F254 were used one plate for detection of bioautographic antioxidant assay and three another plates for detection of flavonoids,

polyphenols and naphthoquinones groups in each extract. Five µl 20% w/v of water, MeOH, and chloroform extract, were applied on TLC plate 1 and also 5.0 µl of naphthoquinone (lawsone) standard solution. On TLC plate 2 and 3 was used 5.0 µl of quercetine as standard solution. On TLC plate 4 was used lawsone and quercetine as standard solution. The plates were then developed with chloroform-MeOH (17:3, v/v) (Zainab, 2012). The dried TLC plates 1-4 were inspected under UV light (254 nm and 366 nm). TLC plate 1 was visualization with 5% KOH methanolic for naphthoquinone identification. TLC plate 2 was visualization with 1% $AlCl_3$ for flavonoids identification. TLC plate 3 was visualization with 1% $FeCl_3$ for polyphenol identification. TLC plate 4 was sprayed with 0.05% DPPH solution in methanol and allowed to stand for 30 minutes at room temperature. Spots which active compound as an antioxidant will be detected as a yellowish white spot with a purple background. All spots that have antioxidant activity were recorded the Rf values, characteristic of the spots under UV and given a score. Quercetin and lawsone were used as a positive control, and blank TLC plate was taken as negative control (Kannan *et al,* 2010).

RESULTS AND DISCUSSION

1. Determination plant

Determination was performed at Faculty of Biology, University of Ahmad Dahlan, Yogyakarta. The Results of determination is: 1b - 2b - 3b - 4b - 12b - 13b - 14b - 17b - 18b - 19b - 20b - 21b - 22b - 23b - 30b - 31a - 32a - 33a - 34a - 35b - 37b - 38b - 39b - 41b - 42b - 44b - 45b - 46a - 50a - 51b - 53b - 54b - 56a - 57b - 58b - 59b - 72B - 73b - 74a - 75b - 76a - 77a - 78b - 103A - 104b - 106b - 107b - 186b - 287b - 288b - 289a - 290b - 291a - 292b - 293a - Lythraceae - 1a - 2a - Lawsonia inermis L. (Backer, 1965).

Based on the result of determination, it can be seen that the plant will be determined and used in this study is correct species of *Lawsonia inermis* L.

2. Preparation of henna leaves powder

The Henna leaf was taken on April 2012 in Celeban Baru, Umbulharjo, Yogyakarta. The main material is taken from a certain place designed to avoid variations in the chemical constituents of plants. If the plants are taken from different places, then the influence of climatic and environmental conditions can lead to variations of active compounds in plants.

The dried leaves was powdered with blender and then sieved with sieve flour to get uniform powder size. The powder size was influence the effectivity of solvent contact with the powder, more small powder more effective so the active substance more extracted to the solvent. From 316.0 g of henna fresh leaves obtained 98.6 g of dried henna leaves, so the obtained yield was 31.20%. Dried henna leaf powder then was measured by graphimetric method and obtained 3.45% value of lost on drying.

3. Phytochemical Screening with Thin Layer Chromatography

3.1. The results of TLC screening for naphthoquinone identification

Identification of naphthoquinone groups compounds in water, methanol and chloroform extract was done by TLC with silica gel F254 as stationary phase and mixture of chloroform: methanol (17:3) v/v as mobile phase. The results of TLC test can be seen in Table I. All the spots of naphthoquinone showed quenching in UV-254 nm. After sprayed with 10% methanolic KOH reagent, naphthoquinone showed red fluorescence in UV-366 nm and red to red-brown in visible light (Wagner and Bladt, 1996).

ISBN : 978-979-18458-6-1

Table I. Result of thin layer chromatography of naphthoquinone identification

No	Rf value			Detector			Naphtho-quinone
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + KOH	
1	-	0,05	-	Q	orange	red orange	+
2	0,09	0,09	-	Q	orange	red orange	+
3	-	0,14	-	Q	yellow	yellow	-
4	0,19	0,19	0,19	Q	orange	red orange	+
5	0,23	0,23	-	Q	blue	yellow	-
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	red orange	+
8	-	0,47	0,47	Q	blue	yellow	-
9	-	-	0,66	Q	blue	yellow	-
Rf standard of lawsone : 0,19				Q	orange	red orange	+

Note:
Q = Quenching

The chromatogram results of naphthoquinone identification showed that water, methanol and chloroform extract contained of naphthoquinone groups. The water extract showed 2 spots at Rf 0.09 and 0.19, methanol extract showed 4 spots at Rf 0.05, 0.09; 0.19, 0.39 and chloroform extracts showed 2 spots at Rf 0.19; 0.39 which is detected as naphthoquinone groups.

3.2. The result of TLC screening for flavonoids identification

Identification of flavonoids groups compounds in water, methanol and chloroform extract was done by TLC with silica gel F254 as stationary phase and mixture of chloroform: methanol (17:3) v/v as mobile phase. The results of TLC test can be seen in Table II. All the spots of flavonoids on the chromatogram shows

Table II. Result of thin layer chromatography of flavonoids identification

No	Rf value			Detector			Flavonoids
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + $AlCl_3$	
1	-	0,05	-	Q	orange	-	-
2	0,09	0,09	-	Q	orange	-	-
3	-	0,14	-	Q	yellow	yellow	+
4	0,19	0,19	0,19	Q	orange	-	-
5	0,23	0,23	-	Q	blue	yellow	+
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	-	-
8	-	0,47	0,47	Q	blue	yellow	+
9	-	-	0,66	Q	blue	yellow	+
Rf Quercetin Standard: 0,25				Q	yellow	yellow	+

Note:
Q : Quenching

ISBN : 978-979-18458-6-1

Table III. Result of thin layer chromatography of polyphenol identification

No	Rf value			Detector			Polyphenol
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + $AlCl_3$	
1	-	0,05	-	Q	orange	grey	+
2	0,09	0,09	-	Q	orange	grey	+
3	-	0,14	-	Q	yellow	grey	+
4	0,19	0,19	0,19	Q	orange	grey	+
5	0,23	0,23	-	Q	blue	grey	+
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	grey	+
8	-	0,47	0,47	Q	blue	green	+
9	-	-	0,66	Q	blue	green	+
Rf standar Quercetin : 0,25				Q	yellow	grey	+

Note
Q : Quenching

queching under UV-254 nm light. Depending on the type of flavonoids structure, flavonoid spots will show yellow, green-yellow, green, blue, dark violet, orange or red fluorescence under UV-366 nm and turn yellow after sprayed with citroborat or $AlCl_3$ reagent in visible light (Wagner and Bladt, 1996; Markham, 1988).

The results of chromatogram showed that water, methanol and chloroform extract contained of flavonoid groups. Water extract had one spot Rf 0.23, methanol extract had three spots at Rf 0.14; 0.23; 0.47 and chloroform extracts had two spots at Rf 0.47; 0.66 were detected as flavonoids groups.

3.3. The result of TLC screening for polyphenol identification

Identification of class of polyphenolic compounds content in water extract, methanol and chloroform was done by TLC with silica gel F254 stationary phase and mobile phase mixture of chloroform: methanol (17:3) v / v. TLC test results can be seen in Table III.

All the spots on the chromatogram shows the outage polyphenols under UV-254 nm. Depending on the type of structure of polyphenols, spots of polyphenols can fluorescence or not under UV-366 nm and blue-black or green-black after being sprayed with $FeCl_3$ reagent under visible light (Wagner and Bladt, 1996; Markham, 1988).

The results of polyphenolic identification on water, methanol and chloroform extract are shown in Table III. All of extract contained polyphenols group. The water extract had three spots Rf 0.09; 0.19 and 0.23 that detected as polyphenol. Methanolic extracts had seven spots that detected as polyphenols at Rf 0.05, 0.09; 0.14; 0.19, 0.23; 0.39 and 0.47. Chloroform extracts had four spots were detected as polyphenols at Rf 0.19, 0.39; 0.47 and 0.66.

3.4. The Results of Bioautografi Antioxidant Henna Leaves

Thin-layer chromatography bioautography antioxidant was used to rapidly detect chemical components in the extract that have antioxidant activity. This method components that have antioxidant activity with the ability to capture free radicals DPPH marked as yellowish white spots with purple background (Gu, *et al.*, 2009). Capture reactions of free radicals by antioxidant compounds can be seen in Figure 1. Results of thin-layer chromatography detection

of antioxidant compounds can be seen in Table IV.

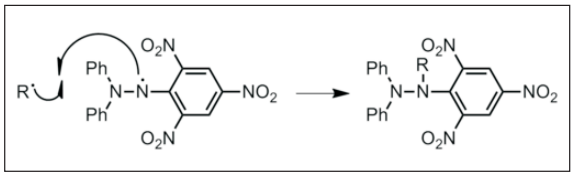

Figure 1. DPPH free radical capture reaction by an antioxidant compounds
(Gu, *et al.*, 2009)

Table IV showed that the water, methanol and chloroform extract contained an active component as an antioxidant. In the water extracts was detected 3 spots that active as an antioxidant. There were 2 spots that identified as naphthoquinone with Rf 0.09 and 0.19 and 1 spots detected as flavonoids with Rf 0.23. In the methanol extracts was detected 4 spots that active antioxidant. There were 2 spots with Rf 0.05, 0.09 detected as naphthoquinone and 2 spots were detected as flavonoids with Rf 0.19 and 0.23. In the chloroform extract was detected only 1 spot that has antioxidant activity at Rf 0.19 and was detected as naphthoquinone. Compared among of the three extracts the components of methanol extract have greates of antioxidant activity with the highest intensity of the yellow color. Results of other studies also showed that the water extract and methanol extract of henna leaves has great potential as an antioxidant and can simultaneously inhibit oxidative cell toxicity of MDA-MB-435S and pBR322 DNA induced Cr (VI). This compares with total phenolic compounds extracted with methanol greater than the water extract of 2.56 mg / g and 1.45 mg / g of tannins equivalen with Folin-Ciocalteu method (Guha *et al,* 2009; Hosein & Zinab, 2007). So that the methanol extract was more potent as an antioxidant than the water and chloroform extract.

CONCLUSION

In general water, methanol and chloroform extract of henna leaves had antioxidant activity and were identified as flavonoids, naphthoquinone and polyphenols groups. The potency of antioxidant from the lowest respectively were chloroform extracts, water extract and methanol extract. Methanol extract have 4 spots, water extract have 3 spots and chloroform extract only have 1 spot were detected have antioxidant activity. Our finding suggested that methanolic extract of henna

Table IV. Result of thin layer chromatography of antioxidant identification

No	Rf value			Vis + DPPH
	Water Extract	MeOH Extract	$CHCl_3$ Extract	
1	-	0,05	-	yellow
2	0,09	0,09	-	yellow
3	-	0,14	-	-
4	0,19	0,19	0,19	yellow
5	0,23	0,23	-	yellow
6	-	0,26	-	-
7	-	0,39	0,39	-
8	-	0,47	0,47	-
9	-	-	0,66	-
Spots active antioxidant	3 spots	4 spots	1 spot	
Score	+++	++++	+	

leaves more suitable to used as further research for antioxidant activity.

ACKNOWLEDGEMENT

The author wishes to thank Faculty Pharmacy of Ahmad Dahlan University and Research and Development Departement of Ahmad Dahlan University for the support in this research.

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LIPOSOM FORMULATION AS A THYMOQUINON NANO-CARRIER TO INCREASED THE ANTICANCER ACTIVITY

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Abstract

Background. *The use of liposom in drug delivery system have been developed rapidly in order to increase terapeutics effect of anticancer.*

Objective. *This research was aimed to find an optimal condition of thymoquinon liposom formulation which have a good characteristic of liposom i.e. liposom size, polidispersibility index (PI), and potential zeta, which can increase a terapeutics effect.*

Methods. *The formulation method of thymoquinon liposom was Mozafari methode which had been modified. In this research the concentration of phospholipid and thymoquinon had been optimized. Characterization of liposom used the Particle Sizer Analysis (PSA) for determination the size of liposom and polidispersibility index, Zeta Sizer Analysis for determination the potential zeta of liposom, and Transmition Electron Microscopy (TEM) for describe the morphology of liposom.*

Outcome measured. *liposom size, polidispersibility index (PI), and potential zeta*

Results. *The result showed that the formulation of thymoquinon liposom had 80,8 nm in liposom size (a good liposom have 10-100 nm in size), 0,348 in polidispersibility index (PI) (a good PI is under 0,5), and -15,34 in potential zeta (its related to the liposom stability). From the TEM analysis could be seen the morphology of thymoquinon liposom.*

Conclusion. *From this result could be conclude that thymoquinon liposom has a good characteristic so it can be developed as a potent anticancer agent.*

Keywords *thymoquinon, liposom, nano-carrier, anticancer*

ISBN : 978-979-18458-6-1

INTRODUCTION

Liposomes have long been recognized as drug delivery vehicle for cancer therapy (Khan, 2010; Khan et al., 2008; Wang et al., 2008; Allen and Cullis, 2004). They can accommodate both hydrophilic and hydrophobic drugs by storing them either in their internal aqueous core or their phospholipid bilayer, respectively. Liposomes are generated from phospholipids, that makes them an ideal candidates for drug delivery systems because they are nontoxic, biocompatible, biodegradable and nonimunogenic (Wang et al., 2009; Washington et al., 2001). Liposomal treatment has been shown capability to reduce some of the traditional side effects associated with chemotherapy when compared to unencapsulated drugs. An important physical aspect associated with the clinical successes of liposomese-based drugs is the size of the nanocarrier (Ian MacLachlan, 2007).

Thymoquinon is the main bioactive component of the volatile oil of the black seed (*Nigella sativa*, Linn). Previous studies reported that thymoquinone exhibited inhibitory effects on cell proliferation of many types of cancer cell lines and can induce cells apoptosis (Gali-Muhtasib et al., 2006; Yi et al., 2008; Ivankovic, 2006; El-Mahdy, 2005).

Cancer cell can effluxs the drug or another substances which entered to it, so the maximum therapy cannot be achieved, but nanoparticle can entered to the cancer cells easily (Yuan et al., 2010). To solve this problem, preparation of nanocarrier as drug delivery system have been developed. In this research thymoquinon as an active ingredient had been formulated to nanoliposomes which have good characteristic i.e. liposomes size, polidispersibility index (PI), and potential zeta, which can increase a terapeutics effect as anticancer. The formulation method of thymoquinon liposomes was *Mozafari methode* which had been modified. In this research the concentration of phospholipid and thymoquinon had been optimized.

METHODS

1. Thymoquinon Nanoliposomes Formulation (Mozafari et al., 2008)

Formula optimization had been done with two factor were optimized, phosphatidilcholine concentration (9,5 mg/mL and 19,5 mg/mL) and thymoquinon concentration (5 mg/mL dan 10 mg/mL). Formula 1 (F1) with 9,5 mg/mL fosfatidilkolin and 5 mg/mL thymoquinon, and Formula 3 (F3) with 19,5 mg/mL fosfatidilkolin and 10 mg/mL thymoquinon. The characteristic of liposomes thymoquinon had been measured, that were the size of liposomes, polidispersibility index, potensial zeta, and morphological of thymoquinon liposomes.

Lipid phase had been made with mixture the soya phosphatidilcholine with cholesterol 1:1, on the *hotplate stirrer* (e.g. RET basic IKAMAG 1 Safety Control, IKA), the speed was 1000 rpm, and the temperature was 40^{o}C during 45-60 minutes, under the nitrogen circulation. Then glyserol was be added (the final concentration of glycerol was 3% v/v). Thymoquinon was be added after the temperature was decrease, and the condition was under the nitrogen circulation until 1 hour to get a stabil nanoliposomes.

2. Characterization of Thymoquinon Nanoliposomes (Wang *et al.*, 2009)

a. Measurement of liposomes size and liposomes size distribution (*polidispersibility index*) used the *Particle* Size Analyzer (PSA) and for measured the potential zeta was used *Nano Series ZS Zetasizer* (Malvern Instruments Ltd, Worcestershire, UK)

b. Determination the liposomes morphology used the *negative staining* Transmission Electron Microscopy (TEM).

ISBN : 978-979-18458-6-1

RESULT AND DISCUSION

The result showed that the 1 Formula (F1) had 175 nm in liposomes size, with 0,356 in polidispersibility index, and the potential zeta was -12,58. The liposomes size distribution can be seen in figure 1.

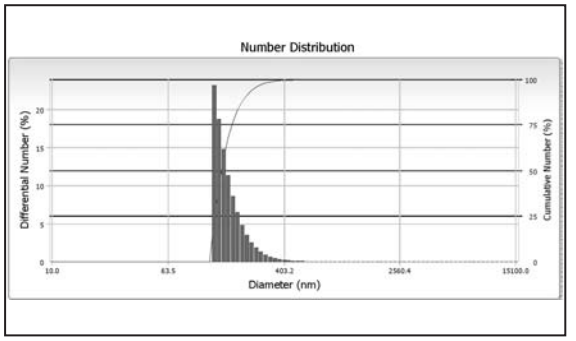

Figure 1. Liposomes size distribution for F1

Formula 3 (F3) showed that the liposomes size was 80,8 nm, polidispersibility index was 0,348, and potential zeta was -15,34. The liposomes size distribution can be seen in figure 2.

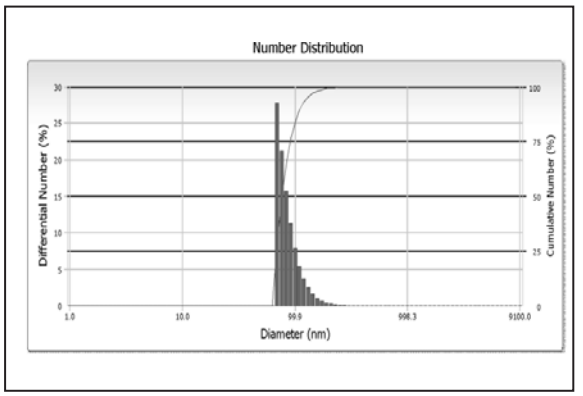

Figure 2. Liposomes size distribution for F3

The comparation between F1 and F3 showed that liposomes size of F3 was smaller than F1, that is 80,8 nm (< 100 nm), so F1 was better to be developed as anticancer than F3. Liposomes with 10-100 nm in size have better to be accumulated in cancer cell. In fact, previous studies have shown that liposomes which have size over from 100 nm are removed fast from circulation. These systems remain in circulation long enough such that they can be accumulated within tumor tissue at levels great enough to have the intended cytotoxic effect. An important physical aspect associated with the clinical successes of liposomes-based drugs is the overall size of the nanocarrier (Wang et al.,2009 ; Zauner et al.,2001).

Polydispersibility index (PI) describe the size distribution of liposomes. PI < 0,5 is called good, liposomes size distribution is homogeneous. Besides that the potential zeta is an important factor because its related with liposomes stability. The higher of potential zeta (negative or positive) showed the better stability of liposom (Couvreur et al., 2002; Parida, 2011).

From the TEM determination we can see the result in figure 3 and 4.

From the figure above, we can see the morphologic of the thymoquinon liposomes, its

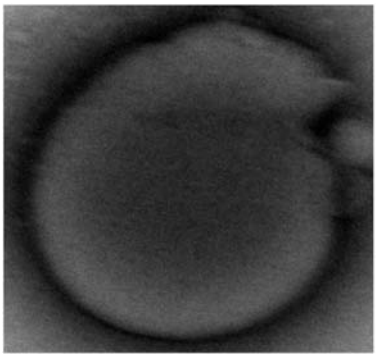

Figure 3. Result from F1 TEM determination

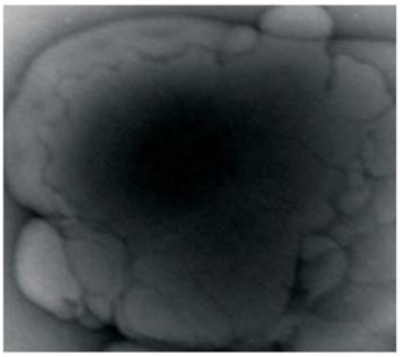

Figure 4. Result from F3 TEM determination

described the lamellarities of liposomes, and the drug can be entrapped into the liposomes.

Conclusion

From the formula optimization between F1 and F3, it could be concluded that F3 is the better formula than F1, because F3 has smaller size of liposomes (80,8 nm) than F1 (175 nm), and the potential zeta of F3 (-15,34) is more negative than F1 (-12,58), so thymoquinon liposomes of F3 can be developed as a good anticancer which has capability to increase the anticancer activity.

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FORMULATION OF PROPANOLOL CREAM WITH VCO (VIRGIN COCONUT OIL) CONTAINED BASE

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Abstract

Background. *Propranolol is a β-blocker agent with low oral bioavailability (15-23%) so that transdermal route can be used as alternative drug delivery.*

Objective. *This study was purposed to know the influence of VCO (Virgin coconut Oil) to the propranolol release rate in cream base preparation.*

Methods. *Cold cream base in various conentrasion of VCO, i.e 0%(F0), 14%(F1), 28%(F2), 42% (F3) contained 7% propranolol HCl were tested their dissolution using dissolution tester (paddle shaped stirrer). Acetat buffer solution 0,01 M pH 5 was used as medium, the temperature was set up at 36°C, stiring rate was controlled at 100 rpm. The parameter of this study was DE (dissolution efficiency) value.*

Outcome measured. Dissolution Efficiency value (DE_{30})

Results. *The result showed that DE_{30} value (in %) of F0, F1, F2, and F3 were $4,73x10^{-3}$±0,53 $x10^{-3}$; 6,41 $x10^{-3}$±0,63 $x10^{-3}$; 9,18 $x10^{-3}$±1,78 $x10^{-3}$; 12,65 $x10^{-3}$±2,08 $x10_{-3}$ respectively.*

Conclusion. *It can be concluded that VCO increase propranolol dissolution significantly ($p<0,05$) and the optimum VCO concentration as cold cream base was 42% with DE30 propranolol value 267% compare with control formula.*

Keywords : *Propanolol , VCO (Virgin Coconut Oil), Dissolution, Cream, Cold Cream.*

ISBN : 978-979-18458-6-1

INTRODUCTION

Propranolol is an unselective ?-antagonist with low oral bioavailability (15-23%) (Katzung, 2001), so that it is needed to develope an alternative delivery. Transdermal delivery can be choiced, because of its adventages, i.e. (1) it is convenient because of non invasive, (2) it can be used by patient as self delivery, (3) it can be design as one daily dose or less frequent, and (4) the plasma level can be maintened at constant value because of its zero order kinetic delivery (intra venous infusion like kinetics) (Nugroho, 2005).

There are many kinds of dosage form used transdermally. Cold cream is suitable dosage form to deliver propranolol transdermally because it is lipophyl, while propranolol is hidrophyl. This contradictive propertiescan enforce propranolol release from its base and difuse through the skin.

The dissolution is the first step of absorbtion, and it makes available the drug to be absorbed. This process is releasing the drug from the cream base on the interphase of the cream and thin film water on the skin. Since the drug on this interphase was releasing, the drug concentration on this interphase was below than in the bulk phase, and the diffusion from bulk phase to this interphase will be happened simultanously (Martin et al, 1993).

In the cold cream dosage form, oil is needed as disperse medium. In this research, VCO (Virgin Coconut Oil) was used. VCO contains 92% saturated fatty acid, composed of 48% - 53% lauric acid, 1.5% - 2.5% oleic acid, and anothers, i.e. caprilic acid and capric acid (Syah, 2005). Oleic acid and lauric acid enhance the rate of permeation of estradiol, progesteron, acyclovir, 5-fluorouracyl, salicilic acid (Niazy, 1991), and piroxicam (Santoyo and Pygartua, 2000) through the skin. It is hoped that propranolol cold cream has a good dissolution and permeation properties. In this research, dissolution properties was observed.

MATERIAL AND METHOD

Material

Pharmaceutical grade of propranolol HCl was purchased from PT. Indofarma, Jakarta.VCO *(Virgin Coconut Oil)*is Vinuto® produced by Wira Husada Yogyakarta was purcahsed from Apotek Nurani, Godean, Yogyakarta. As base materials were used pharmaceutical grade of cera alba, cetacium, olive oil, and aquadest.The acetic buffer pH 5, 0,01 M (made from E Merk analytical grade glacial acetic acid and acetic sodium) was used as dissolution medium. The dissolution observation was performed using using paddle stirrer DissolutionTester(Erweka DT600) as shown in figure 1. The cream was filled in the diffusion cell and covered by stretched cellophane membrane. The propranolol concentration was measured using Spectrophotometer UV-Vis (Shimadzu 1700).

Method

Propranolol Coldcream Formulation

The propranolol coldcream composition was listed in table I. Cera alba and cetacium were melted in water steamer, than olive oil and VCO were added and mixed in mortir porcelen. Propranolol HCl was dissolved in warm water

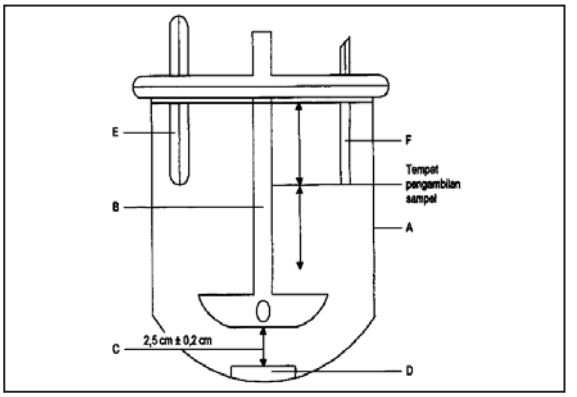

Figure 1. Erweka Dissolution Tester,A: chamber dissolution medium, B: *Paddle*, C: space betwen paddle and dissolution cell,D:Dissolution cell, E: Thermometer,F: sampling line.

ISBN : 978-979-18458-6-1

and added to the oil phase. The strong blending will homogenize this coldcream.

Table I. The propranolol coldcream formulation

Material	Concentration in coldcream (%)			
	F0	F1	F2	F3
Propranolol HCl	7	7	7	7
VCO	-	14	28	42
Olive oil	56	42	28	14
Cera Alba	12	12	12	12
Cetaceum	12,5	12,5	12,5	12,5
Aquadest	14	14	14	14

Propranolol Coldcream Physical Characteristic Observation

Before dissolution observation, the physical characteristics of propranolol coldcream were determine, including spreadability, adhesiveness, water protection, and its pH.

Propranolol Coldcream Dissolution Observation

The propranolol coldcream was filled in cilindric diffusion cell (2 cm in diameter and 0,4 cm height). The cellophane was swollen by soaking in aquadest for 30 minutes, than put on the diffusion cell to cover the coldcream. This cell was put in Erweka Dissolution Tester, the paddle was moved down, and stirred 100 rpm. The themperature was maintened in 37°C ± 0,5°C. The dissolution was began by filling the dissolutin chamber with acetic buffer pH 5, 0,01 M. The aliquot 5,0 ml of dissolution samples were wthdrawn in various minute: 0, 5, 10, 15, 20, 25, 30, 60, 90, and 120. The propranolol concentration in these samples were determined using spektrofotometer UV-Vis (Anggraeni *et al*., 2012). The propranolol HCl solution (40 µg/ml) in 0,01 M acetic buffer pH 5 was scanned its absorbance at 200 – 300 nm to identify its maximum wavelength. The same solution, in various concentration of propranolol HCl (1 – 100 ìg/ml), was determined its absorbance at maximum wavelength to construct calibration curve.

RESULT AND DISCUSION

Propranolol Spectrum

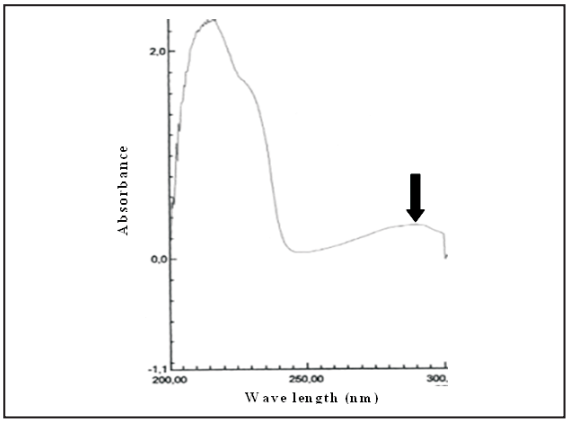

Figure 2. The spectrum of propranolol HCl 40 µg/ml in 0,01 M acetic buffer pH 5. It is shown (↓) that this solution has maximum wavelength at 288 nm.

Propranolol Calibration Curve

The calibration curve was needed to calculate the concentration of propranolol. The absorbance of propranolol HCl solution in various concentration was recorded in 288 nm based on the maximum wavelength shown in figure 2. The linear curve and its regresion-correlation between absorbance and concentration was shown in figure 3. This callibration curve has a high corellation since

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$r_{calculated}$ (0,993) is higher than r_{table} for 12 degree of freedom (0,532) and can be used to calculated the propranolol concentration.

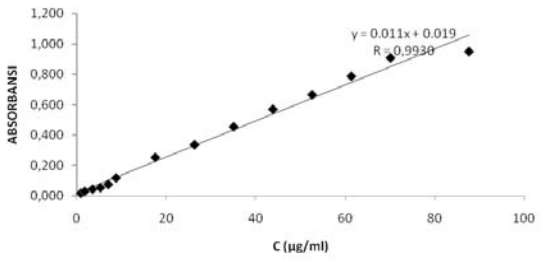

Figure 3. The calibration curve of propranolol has a linear regression y = 0,011x + 0,019.

Physical Characteristics of Propranolol Coldcream

Physical characteristic of cream support patien convenient and delivery of the drug to and through the skin. This coldcream characteristics were listed in table II. The VCO has less consistency than olive oil, so that the higher the VCO concentration the higher the spreadability, and the cream is more easy to use. The adhesiveness of these coldcream, insures the enought contact time betwen cream and skin to deliver propranolol. This coldcream is w/o emulsion base, so that it can resiste the water difusion. It is the adventage of coldcream, because of its capability to increase the skin hidration. The skin hidration enforce the skin fluidity and enhance the drug permeability.

Propranolol Coldcream Dissolution Observation

Propranolol coldcream dissolution make available the propranolol on moleculer size in water layer of skin. Based on Fick's Law, concentration is the driving force of simple diffusion process. Enhancing the rate of dissolution make the higher concentration of propranolol available to be absorbed, so that the rate of absorbtion will be increase. In this research, the dissolution of coldcream tested using Paddle Dissolution Tester, and the datas were shown in figure 4.

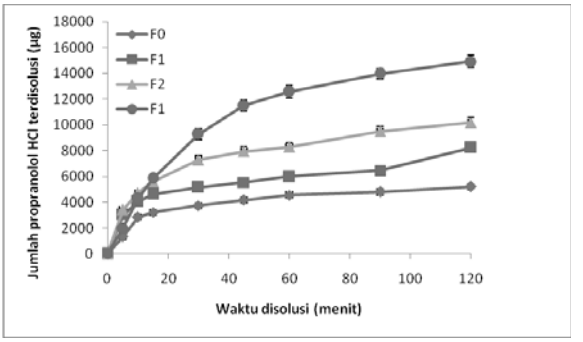

Figure 4. Dissolution profile of propranolol coldcream in average from 5 replication. The standard deviation (SD) was shown by É symbol.

It is shown in figure 3 that the dissolution profile is affected by VCO concentration in the coldcream formula. The higher the concentration of VCO, the better the dissolution profile, and from the value of standard deviation, it can be predicted that their differences are significant. In formulas 1, 2, and 3, some olive

Table II. Physical characteristics of propranolol coldcream

Formula	Spreadability (cm^2)	Adhesiveness (minutes)	Protection (for 5 minutes)	pH
F0	3,1	>15	able to protect	about 5
F1	5,9	>15	able to protect	about 5
F2	6,8	>15	able to protect	about 5
F3	10,2	>15	able to protect	about 5

ISBN : 978-979-18458-6-1

oil was repleaced by some VCO increase respectively. VCO has saturated fatty acid (92%) (Syah, 2005) more than olive oil. Since the saturated fatty acid is more lipophilic than unsaturated fatty acid, the oil with higher

45 minutes dissolution test in a cm^2 area of dosage form-medium contact. The value of DE_{30} and $t_{45\ minute}$ of propranolol were shown in table III.

Table III. Dissolution expression of propranolol coldcream

No	Formula	Average DE_{30} (%) + SD (n=5)	Average t_{45} minute (mg/cm^2)+ SD (n=5)
1	F0	$4{,}73x10^{-3} \pm 0{,}53x10^{-3}$	1,326433 ± 0,17803
2	F1	$6{,}41x10^{-3} \pm 0{,}63x10^{-3}$	1,764129± 0,279653
3	F2	$9{,}18x10^{-3} \pm 1{,}78x10^{-3}$	2,529994± 0,59868
4	F3	$12{,}65x10^{-3} \pm 2{,}08x10^{-3}$	3,660567± 0,729533

containing of fatty acid will be more lipophilic, and the hidrofilic drug, including propranolol HCl, will be released more rapidly. The other reason is the spreadability of coldcream. It is shown in table 2 that formulas with VCO have the higher value of spreadability. This phisical characteristic is linear with coldcream fluidity, and the fluidity is linear with diffusion coeffisien (D). From the Fick's equation (Martin et al, 1993), it can be evaluated that the diffusion will be easier in an unviscous matterial.

The disolution parameter can be calculated from its dissolution profile. There are many kind of dissolution parameters, including DE (dissolution efficiency), $t_{x\%}$ (time necessary to release x % of drug), and t_{xmin} (percent drug released in x minute) (Costa and Lobo, 2001). The DEx parameter is a ratio between area under curve of dissolution profile for x minute and area of rectangule shaped by times of x minute dissolution time and amount of drug in tested pharmaceitical dosage form (Khan, 1975). The parameter t_{xmin} is the simplest expression and frequently used by pharmacopies (i.e. t_{45}=80%). But this expression is powerless if aplicate in semisolid dosage forms dissolution test using cell diffusion which are the mass of dosage forms introduced in medium can be varieted, because only some of the dosage form contact with dissolution medium in a constant area. In this research we introduced new definition of $t_{45\ minute}$ in semisolid dosage form as the drug disolved in

Statistical analysis (Anova test followed by Tukey HSD) showed that there are no significant differences in DE_{30} and $t_{45\ minute}$ between formula 1 and control (formula 0), but the formula 2 and 3 showed the significant higher value in DE_{30} and $t_{45\ minute}$ compare with control (F1).

CONCLUTION

The VCO (Virgin Coconut Oil) can be used as base material for propranolol coldcream instead of olive oil. VCO containing propranolol coldcream has the better dissolution characteristics and spreadability compare with olive oil alone, but they have the same characteristic in adhesiveness, water protection, and pH.

AKNOWLEDMENT

The author would like to thank to Muji Lestari, Iis Wahyuningsih, and Tedjo Yuwono who has found time to discus about this research.

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ANTIOXIDANT ACTIVITY ASSAY OF ETHANOLIC EXTRACT OF SIRSAK (*Annona muricta* L) LEAVES

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Abstract

Background. *Free radicals cause cell`s damage in the body which manifestate as disease. The increase prevalence of degeneratife diseases caused by free radicals in Indonesia has motivated scientists to explore natural antioxidant compounds. Sirsak (Annona muricata L) is one of plant used as anticonvulsant, antioxidant, and anticancer.*

Objective. *This study was purposed to investigate antioxidant activity of ethanolic extract of Annona muricata L*

Methods. *This research comprised ethanolic extract of Sirsak leaves using maceration method and antioxidant in vitro examintation used 2,5; 5; 10; 20; and 40 ug/mL of DPPH. The method used for antioxidant assessment was true ability of antioxidant to capture DPPH.*

Outcome measured. *ES_{50} of DPPH technique*

Results. *The ES_{50} result of ethanolic extract on Sirsak leaves was 22.23 ± 0,64 µg/mL*

Key words : *Annona muricata, DPPH, antioxidant*

ISBN : 978-979-18458-6-1

INTRODUCTION

The diseases caused by ffree radical comprise the degeneratife illness, early aging and the general inflammation. Free radicals able to interfere the healthy cell`s nucleus then initatite mutation wich transform the cell into tumour cell or cancer (Thompson, 2004). Apart from that, free radical damages blood vessel wall because it creates inflammation which finally resulted in the coronary heart disease. It also causes the hypertension illness, stroke and diabetes mellitus (Adeyemi *et al.*, 2008).

Sirsak (*Annona muricata* L) is one of the plants that is used for detoksication, antioksidan and anti-cancer. The scientific data about the in vitro and in vivo anti-cancer activity of Sirsak is still very limited (Fang *et al*., 2009). *Annona muricata* is used as antioxidant, medicine anticonvulsant, the stamina enhancer, anti-cancer, and cytotoxic. The compounds which are contained in *Sirsak* (*Annona muricata*) are flavonoid and acetogenin .(Adewole and Ojewole, 2009).

The *Sirsak* (*A. muricata* L) has the anticonvulsant activity, anti-cancer, and antiinflammasi. The leaves extract has the anti-cancer activity HEP2 (Human epidermoid cancer cells) that shows the strong cytotoxic activity. The cytotoxic activity or anti-cancer is also due to the antioxidant effect. This is because cancer is also caused by oxidants or the free radicals (Baskar *et al*., 2007)..

The DPPH method is based on the capacity of antioxidant to hinder the free radical to donorthe hydrogen atom. The change in the purple DPPH colour became purple reddish/yellow indicate the activity of the compound antioxidant. This method uses the positive control as the standard to asertain theantioxidant activity of the sample. The antioxidant activity assay DPPH method used *1,1-difenil-2-pikrilhidra-zil* (DPPH) as the free radical. The principle is that the scavengers of hydrogen by DPPH from the antioxidant compound will change it into *1,1-difenil-2-pikrilhidrazin* (Sharma and Bhat, 2008). The scavenging activity of antioxidant toward free radicals is calculated into Electron scavenging 50 (ES_{50}) (Locatelli *et al*., 2009).

Based on the background that has been presented, the research question is outlined which is how many ES_{50} of ethanol extract of sirsak leaves in capturing DPPH. It is hoped that in the future, the phytopharmacy agency of Sirsak can be patented.

METHODS

Materials and Methods

Instrument

The instruments which were used for the antioxidant assay of ethanol extract of sirsak leaves were glass equipment, balance, electricity stove, blender, electric stirer, separation funnel, Buchner funnel, uv vis spectrofotometer.

Materials

The material that were used for the leaves ethanol extraction of sirsak leaves was quality materials technic, that was: aquadestilata, ethanol. The material for the antioxidant assay were aquadest, DPPH, and ethanol p.a.

Research Procedures

1. Plant Determination

This stage aims to validate the main material which was Sirsak (A murricata). This activity was conducted in the Farmakognosi Laboratory, Ahmad Dahlan University. Sirsak determination was conducted using the guidance of the Flora book of Java (Van steenis, 2000)

2. Extraction

Two hundred fifty gram of Sirsak was macerated using 2 litres of ethanol 70%, the maceration process was maximised using electric stirer for 3 hours, then was kept in room temperature for 24 hours. The filtrate was received used a Buchner funnel and was called as

first maseration. Extract residue resulted from the first maceration process then was macerated again using ethanol 70% totalling 1 litre, so that second and third maceration were obtained. Ekstrak liquid ethanol evaporated to obtain thick extract. This process is illustrated in figure 1.

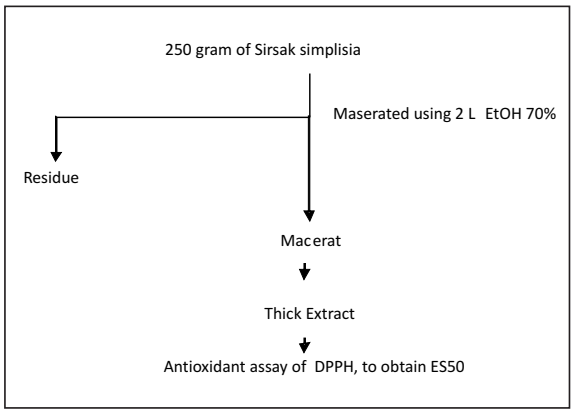

Figure 1. Ethanol Extract and antioxidant Activity Assay

3. Antioxidant assay

a. Operating time determination

Each of 1,0 ml the sample solution was stired with 1,0 ml of the DPPH solution 0.15 mM, afterwards it was observed absorbacy him for 60 minutes in long the wave 517 nm.

b. The determination of Maximum Absorbancy Wave Length.

The determination of wave length (λ) of the maximum absorption of the DPPH was carried out as follows: l,0 ml the DPPH solution 0.15 mM was combined with 1,0 ml of absolute ethanol,and was mixed homogeneous then was measured by the absorption of 400-600 nm wave lenght.

c. The determination of free radicals scavenger absorbation using DPPH method

Each of 1,0 ml the sample solutions and the solution to the standard with various concentration was mixed with 1.0 ml the DPPH solution 0.15 of mM. This mixture was kept in the dark place during operating time. Afterwards the absorbansi was measured to ascertain the maximal DPPH absorption with spectrofotometer UV-Vis. Blanko solution which was used is abolut ethanol

d. Data Analysis

The data obtained using the previous procedure was % ES_{50} and the concentration of the tested compound afterwards was analyzed using linear regression to obtain the concentration of the the radical scavenger 50 % (ES_{50}).

$$\% \text{ free radical scavenger} = \left(\frac{\text{negatif control absorbance - sampel absorbasnce}}{\text{negatif absorbance}} \right) \text{x } 100\%$$

The value of free radical percentage was regress toward the log of concentration . The value of ES_{50} which was obtained from the antilog x where Y=5.

RESULT AND DISCUSSION

The mechanism of the occurrence of the illness was often caused by the existence of the oxidant that was abundant in the body. These illnesses including hypertension, cancer, diabetes melitus, and the decline illness in the degenerative function of the other body. Up til now investigation antioxidant has been carried out by looking for the synthesis compound and from the nature material (Richards *et al.,* 2009). The need was received antioxidant especially from the nature material was caused by the source that often was available in Indonesia.

The mechanism of the occurrence of the illness was often caused by the existence of the oxidant that was abundant in the body. These illnesses including hypertension, cancer, diabetes melitus, and the degenerative function of the body. Up til now investigation antioxidant has been carried out by looking for the synthesis

compound and from the nature material (Schiffrin, 2010).

The main content from the leaves sirsak that played a role as antioksidan was flavonoid. This flavonoid had many double bonds and the hydroxil cluster that could play a role as free radical scavenger (Hidalgo *et al.*, 2010). After scavenge the free radical it change itself into radicals however it could stabilise itself via autoresonance. The extract was tested by the activity antioxidant by testing towards the ability of DPPH free radical scavenging. The assay was carried out by 3 times so as to be received by the ES_{50} value.

Based on the data the ES_{50} can be obtain by making the curve of log relations the level of

Table I. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant first replication.

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,773	4,6072	3,36
5,000	0,718	11,3945	3,77
10,000	0,625	22,8712	4,26
20,000	0,484	40,2715	4,75
40,000	0,198	75,5656	5,71

Table II. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant second replication.

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,770	4,9774	3,36
5,000	0,735	9,2966	3,66
10,000	0,637	21,3904	4,19
20,000	0,482	40,5183	4,77

Table III. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant third replication

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,780	3,7433	3,25
5,000	0,729	10,0370	3,72
10,000	0,641	20,8968	4,19
20,000	0,479	40,8885	4,77
40,000	0,206	74,5784	5,67

ISBN : 978-979-18458-6-1

Table IV. The result of ES_{50} 1, 2, dan 3 replication of ethanol extract toward DPPH

Replication	ES_{50} (µg/mL)	Average	SD	%CV
1	21.57	22,23	0,64	2,88
2	22.85			
3	22.26			

vs probit. The ES_{50} value is ilustrated in the Table IV.

Based the result of ES_{50} which was 22.23 ± 0,64 ug/mL it can be concluded that the antioxidant capacity of ethanol extract of Sirsak is relatively low as the standard of antioxidant which usually has ES_{50} less than 2 µg/mL. Further research to lower the ES_{50} value by carrying out purification of the extract is recommended.

CONCLUSION

The extract of leaves ethanol sirsak has the ES50 of 22.23 ± 0,64 µg/mL towards the scavenger of the free radical DPPH.

ACKNOWLEDGEMENT

Thank you to the UAD Research and Development Agency which gave the research funding during 2012/2013 budget year to make this research possible.

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ANTI ANGIONESIS ACTIVITY OF ETHANOL EXTRACT OF GREEN ALGAE (*Spyrogyra* sp) *Purified* WITH *Chorio Allantoic Membrane* (CAM) METHOD

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Abstract

Background. *Angiogenesis plays an important role in tumor progression. Modern treatment of cancer currently has an alternative way to inhibit cancer through antiangiogenesis process. It is expected to inhibit the formation of new blood vessels around the tumor, the supply of nutrients and oxygen by the blood to the tumor can be inhibited.*

Objective. *This study aims to determine the antiangiogenesis activity of ethanol extract of green algae (Spyrogyra Sp) Purified by using the method of chorio allantoic membrane (CAM).*

Methods. *In this study used 8 groups: Group I was a control that content only paper discs, paper discs contain a group II + PBS, group III contain a paper disc + PBS + bFGF, group IV, V, VI, VII, VIII as a treating were given 10, 20, 40, 160, and 320 ug / ml of ethanol extract of green algae respectively. CAM is Obtained from embryonated chicken eggs aged 8 days. Incubated for 3 days afterward. The macroscopic observation is used to see the inhibitory activity of blood vessels. Statictical analysis used Kruskal-Wallis test to compare among each other Significantly, continuated by Mann Whitney test (P <0.05).*

Outcome measured. *Inhibitory activity (IC_{50})*

Results. *The results showed that the ethanol extract of green algae purified with concentration 10 ug / ml (p = 0:03) can inhibit angiogenesis of embryonated chicken eggs Compared to bFGF control.*

Conclusion. *In conclusion, the ethanol extract of green algae has antiangiogenesis activity in chorio allantoic membrane.*

Keywords : *antiangiogenesis, CAM, green algae, Spyrogyra Sp*

ISBN : 978-979-18458-6-1

INTRODUCTION

Cancer is a disease characterized by the uncontrolled proliferation of cells and the cells can invade and damage other part of bodies. The disease is still one of the leading causes of death in developed countries and the second leading cause of death in developing countries (Ahmedin, *et al*., 2011).

Melatonin is an alkaloid compounds and potent as inhibitors of cancer activity (Veronique *et al*., 2004). Melatonin works with three action in inhibiting cancer activities, which working with specific cytotoxic, slow cleavage (*proliferation*) of Cancer cells, and negate the toxic effects of heavy metals (epigenetic carcinogens). Mechanism of the latter action is because melatonin can stimulate cancer cells to synthesize *metallothioneins* (MTs) (Imbesi *et al*., 2008). These compounds are intracellular proteins that can protect cells by removing toxic effects of metals (*metal*) And other epigenetic agents (Korkmaz *et al*., 2008). Melatonin produced by the pineal gland in the human body and vertebrate animals. However, melatonin production in the body is very low (less than 100 mg) (Lerner *et al*., 1960). Based on this, it is necessary to melatonin intake from outside the body to inhibit the activity of the cancer and to eliminate the effects of toxic substances epigenetic.

Green algae (*Spirogyra sp*.) is a plant with a *phytomelatonin* content (Kolar and Machackova, 2001). *Phytomelatonin* is compound *melatonin* contained in the plant (*phyto*). Levels of compounds *phytomelatonin* in green algae at 240 ug / kg wet weight (Kolar and Machackova, 2001). *Phytomelatonin* in green algae can be isolated by using thin layer chromatography (TLC) (Lerner *et al*., 1960). Usefulness of these compounds can be seen as a barrier to cancer activity (*cancer inhibitor activity*) To test the cytotoxicity and antiproliferative test. Cytotoxicity test using the IC_{50} parameters or the concentration of the active substance that kills cells by 50% from the baseline, whereas the antiproliferative test using the parameter *doubling time* or length of time required by the cell in order to splitting into two times of the original amount (Katzung, 1995).

This study aims to investigate anti-angiogenesis effect of ethanol extract of purified green algae. Purification is done to obtain the fractions that containing of more melatonin and separated from the other components. By knowing how to purify green algae and determine the activity of anti angiogenesis then be developed the ethanol extract of green algae with melatonin standardized. This standardized extract may be developed as an anti angiogenesis in cancer therapy.

METHODS

Tool :The tools used in this study include the chamber chromatography, silica gel GF 254, the Soxhlet set, watch glass, rotary evaporator, glass objects, incubator, mini drill, LAF, camera, injection syringes, Eppendorf, micropipette, scissors and tools glassware.

Ingredients: Materials used in this study is the ethanol extract of green algae (*Spirogyra sp*.) Were extracted by the sohxlet method in Chemistry Laboratory of Pharmacy Faculty, Ahmad Dahlan University, bFGF, chorio allantoic membrane (CAM) of chicken eggs with 8 days ages, *paper disc*. Other chemicals were 96% ethanol, antiseptic solution, PBS, ethyl acetate, petroleum ether, and sterile aqua .

Green Algae Extract preparation: Green algae plants cleaned and put in a container filled with water, allowed to stand for 24 hours: 12 hours of irradiation phase and 12 hours of phase embezzlement. Algae taken from the container after 5-6 hours of embezzlement phase and then dried under the sun with a black cloth covered. Algae pollinated, pollen extracted by Soxhlet device using 96% ethanol. Pollen was evaporated to obtain thick extract.

Ethanol extract purified preparation: A total of 1000 mg of ethanol extracts obtained added with 15%acetic acid as much as 15 ml.

The solution then filtered by using filter paper and a Buchner funnel. The filtrate was extracted with 15 ml of petroleum ether 3 times. Acidic water fraction added NH4OH until become pH 10, then extracted again with ether. Sari ether evaporated until obtained the thick extract. Test solutions were made in PBS solution.

Identification of Melatonin on green algae: Fraction of the ether which is obtained from thick extract spotted on silica gel KLT plate using capillary tube. The first spotted is the standard phytomelatonin, second spotted is purified of ethanol extract and third spotted is ethanol extract. Further silica gel GF 254 plates petted with a mobile phase *n*-Butanol-acetic acid-water (12:3:5). The results identified under UV light (254 nm).

Preparation of bFGF as an inductor of angiogenesis: Created stock levels of 50 ng /mL using a solution of 10 mM Tris-HCl pH 7.5 and then diluted to obtain a level of 0.5 ng / mL. BFGF levels are given for each treatment induced egg is 10 ng (Sun *et al*., 2004).

The inhibition of angiogenesis test: SPF chicken eggs that have been purchased, age 0 day immediately incubated in a laboratory incubator at a temperature of 390c. After eggs is 8-9 days old, the embryos location known through *candling* the egg. Egg shell at the pole containing the air space and egg shell above the embryo disinfected with iodine solution. Later in the two areas was made drill a small hole using a mini. The air from air chamber Aspirated with a rubber ball to move from the shell poles to the top of the egg. This treatment is done with egg horizontal position, in the dark, and through *candling*, and then the artificial air space formed in the embryo can be seen. Egg shell above the embryo was cut with a saw (mini drill) to make a rectangular window with an area of ??1x1 cm. Through this window, bFGF and isolates test was implanted into the *chorio allantoic membrane* (CAM). Eggs subjects test were divided into 8 groups (each treatment consisted of 4 eggs), as follows:

1) Group 1 is the egg with the implantation of paper disc.
2) Group II group with implantation of paper disc + Solvent (PBS) 10 mL.
3) Group III bFGF control group + solvent is the group of eggs with the implantation of the *paper disc* which is to content 10 ng bFGF + solvent (PBS) 10 mL.
4) Group IV, V, VI, VII and VIII are eggs used to see the inhibitory effect of the test solution with levels of variation is equal to 10 ug / ml, 20 ug / ml, and 40 ug / ml, 160 ug / ml and 320 ug / ml extracts.

Eggs in this treatment group were given implantation *paper disc* and added 10 ng bFGF and test solutions of polar fractions of green algae with concentration various by 10 mL.

Once treated, the eggs were incubated at a temperature of 37-39 ° C with a relative humidity of 60% for 3 days or 72 hours (Ribbati *et al*., 1997), then the egg is opened (age 12 days) and the contents of eggs laid. After that *chorio allantoic membrane* (CAM) which is attached to the shells was observed macroscopically and counted the number of new blood vessels grow on the surface and the edge of the paper disc. Macroscopic observation can be done with the aid of a magnifying glass (Jennie *et al*., 2006).

Data Analysis: Parameters observed in the study is the number of new blood vessels at the edges and surfaces of *paper disc*. To reduce the subjectivity of observations, then the observations made by three people, so the result is an average of the three observations. Observation of new blood vessels that form on the edges and surfaces of *paper disc*, must be distinguished from the main vein / origin of CAM. Where the main vein in CAM has a larger size, while the new blood vessels are arteries finer / smaller (Ribatti et al., 1999). After that the data obtained were analyzed statistically. Results of quantify the amount of new blood vessels can be calculated % inhibition by using the formula:

ISBN : 978-979-18458-6-1

$$\% \text{ Inhibition} = \frac{\sum \text{control blood vessel of bFGF} - \sum \text{treatment blood vessel}}{\sum \text{New blood control vessel}} \times 100\%$$

Results quantify the amount of new blood vessels then statistically analyzed using the Mann Whitney and Kruskal Wallis method (p = 0,05).

RESULTS AND DISCUSSION

Identification of melatonin Compound on ethanol extract purified

Test compound is a ethanol extract compound purified because it was partitioned by using several solvents in order to obtain extracts whose composition is almost pure. The stage of purification performed with ethanol extract and added 15% acetic acid to its alkaloid content tends to acidic. Subsequently partitioned with Petroleum Ether. Acetic acid extract was taken and added NH4OH to pH 10. Then the solution was extracted with ether and the solvent was evaporated to obtain a purified extract.

Identification of purified ethanol extract was carried out by thin layer chromatography method. On the identification of stationary phase used was silica gel GF254 while mobile phase used was a mixture of n-butanol ratio: 15% acetic acid: water (12:3:5). Identification results can be seen in Figure 1. Based on the identification can be seen that the ethanol extract of purified yield results spotted amount less than the ethanol extracts alone and active content of compounds melatonin in the ethanol extract is purified still look dominant.

Parameters on thin-layer chromatography is *retardation factor* (Rf), a comparison of the distance of analyte from the origin to the distance from the point of origin of the mobile phase. In the result it shows one spot on the standard and some spots on the samples, but one sample patches have the same Rf price with the Rf price of standard patches 0.68. This suggests that in the test solutions there are melatonin compounds.

Description:

a = melatonin standard

b = ethanol extract purified

c = ethanol extract

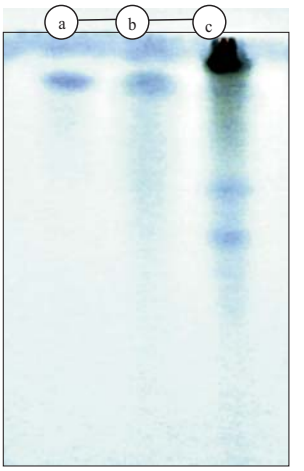

Figure 1. Qualitative identification of the ethanol extract, ethanol extract purified by comparison melatonin. = Silica gel stationary phase and mobile phase = 254 n-butanol: 15% acetic acid: water (12:3:5)

Rf values 0,68 on samples compounds are different due to different and vary levels of polar compounds. Polar compounds with polar stationary phase will be retained on the stationary phase. It can also be caused due to the non-polar compounds with non-polar mobile phase will elute higher than compounds that are more polar. In the picture looks not just one spot melatonin had with the test compounds but also there are some spots that appear on the elution results, it can be caused due to a lack of purification so that not only melatonin fraksinated. Such compounds may be able to support or reduce the activity of melatonin as an anticancer so we need more research to determine the contained of compounds in the purified ethanol extract.

Antiangiogenesis test

Angiogenesis is the formation of new blood vessels from existing blood vessels. The formation of blood vessels occurs either due to the effects of physiological and pathological

effects, one of which occurs in cancer cells. Cancer cells secrete bFGF to form new blood vessels, so the research is conditioned as in cancer cells. bFGF is one of the pro-angiogenic factor that plays a role in the process of new blood vessel formation. bFGF is used as an inductor of angiogenesis in the test object. BFGF induction for each egg is equal to 10 ng. This dose has given the differences significant growth of blood vessels when compared with controls (Sun *et al*., 2004). The results of the calculation the average number of blood vessels in each control group and the group fed ethanol extract of green algae purified can be seen in Table I.

Table I. The results of the calculation of average number of blood vessels in each treatment group.

Treatment group	The average number of blood vessels (X ± SD)
Paper disc	0
PBS	0
bFGF	20:00 ± 0
10 ug / ml	4:33 ± 0:57
20 ug / ml	3.67 ± 12:57
40 ug / ml	2.67 ± 12:57
160 ug / ml	1:33 ± 0:57
320 ug / ml	1:00 ± 0:57

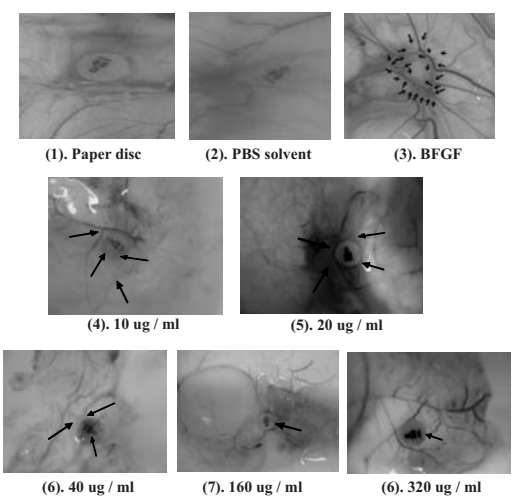

Figure 2. Observations macroscopically CAM

Macroscopically observations indicate that the test compound is purified ethanol extract could inhibit the growth of new blood vessels or angiogenesis in the CAM induced by bFGF. Test results angiogenesis purified ethanol extract showed the higher levels, the higher the inhibition of angiogenesis that occurs in bFGF-induced CAM is characterized by a decrease in the density of new blood vessels score. On eggs with test compound concentrations of 10 ug / ml seen the growth of new blood vessels in the area around the paper discs were decreased when compared with controls bFGF. The growth of new blood vessels is experiencing a reduction in the concentration of 20 ug / ml, 40 ug / ml, 160 ug / ml and 320 mg / ml.

Price% inhibition can also be obtained by finding the difference between the number of blood vessels in the bFGF and control the amount of blood vessels in each test solution concentration, divided by the number of blood vessels in the control group bFGF and multiplied by 100%. Price% inhibition of purified ethanol extract is shown in Table II.

Table II. % (percent) inhibition of purified ethanol extract

Concentration of Test Solution	% Inhibition of Blood Vessels (X ± SD)
10 ug / ml	78.33 ± 2.89
20 ug / ml	81.67 ± 2.89
40 ug / ml	86.67 ± 2.89
160 ug / ml	93.33 ± 2:22
320 ug / ml	95.00 ± 00:00

Purified ethanol extract have antiangiogenesis activity with the highest percent inhibition at a concentration of 320 ug / ml. Percent inhibition proportional to the concentration of the test solution, the higher the concentration of the test solution % inhibition of the higher percent.

Test Result Statistics

In this study, the data obtained were normally distributed, but the data are not homogeneous, so that the analysis followed by Kruskal Wallis and Mann Whitney. This analysis was conducted to determine differences in treatment between groups. Mann Whitney test results showed a significant difference between groups. Here is a summary of the results of the Mann Whitney test for each treatment group.

Comparisons between the control group *paper disc* with bFGF control group showed significantly different results. This suggests that bFGF used in the study actually influence the process of angiogenesis. In contrast to the results of the analysis between the control group *paper disc* with PBS control. The analysis showed that there was no significant difference between the two treatment groups, so it can be concluded that the PBS solvent used in the study does not give effect to the formation of new blood vessels.

In the ethanol extract purified with various concentration 10 ug / ml, 20 ug / ml, 40 ug / ml, 160 ug / ml and 320 ug / ml showed significantly different results with the significance of bFGF control which means it can provide as an antiangiogenesis effect, this directly proportional to the inhibition of the growth of new blood vessels at every level variations in the concentration of ethanol extract purified.

In this study, purified ethanol extract shown to have activity as an inhibitor of angiogenesis inhibition mechanism itself, although it is not certain. Mechanism of inhibition of new blood vessel formation by purified ethanol extract test solution can not be determined related to the method used, the limitations of the method *chorio allantoic membrane* (CAM). This method is only aware of the antiangiogenesis effects based on the number of existing blood vessels after treatment, while the other responses that influence the angiogenesis inhibitor compounds can not be known. However, the mechanism of inhibition of a compound that may occur to the formation of new blood vessels associated with angiogenesis mechanism itself, among other mechanisms of inhibition of angiogenesis is the inhibition of invasion, motility, and cell adhesion, inhibition of endothelial cell activation, interfere with angiogenic growth factors or their receptors,

Table III. Summary of statistical analysis of antiangiogenesis test group and the control group treated with a test compound using *Mann-Whitney* test.

Variation	Control *paper disc*	BFGF control	PBS control	10 ug / ml	20 ug / ml	40 ug / ml	160 ug / ml	320 ug / ml
BFGF control	S							
PBS control	TS	S						
10 ug / ml	S	S	S					
20 ug / ml	S	S	S	TS				
40 ug / ml	S	S	S	S	S			
80 ug / ml	S	S	S	S	S	S		
160 ug / ml	S	S	S	S	S	TS		
320 ug / ml	S	S	S	S	S	S	TS	

Description: S = Significant contrast / contrast Meaningful (p = 0.05),
Unlike TS = Not Significant / Contrary Not Meaningful (P> 0.05)

inhibition the enzymes involved in the process of angiogenesis, and *vascular targeting* (Liekens *et al*., 2001).

In this study, which gives the effect of antiangiogenesis compounds in purified ethanol extract allegedly not only indicated by the presence of melatonin in the compound tested solutions but can also be supported by the presence of other compounds contained in the test solution. This can be seen from the results shown on the identification of melatonin has been done but more research needs to be done to determine the types of compounds contained in the test solution.

CONCLUSION

Ethanol extract of purified compounds containing melatonin have antiangiogenesis effect on *chorio allantoic membrane* (CAM) of chicken egg-induced *basic fibroblast growth factor* (BFGF) levels begin 10 ug / ml. The higher concentration of ethanol extract of purified, the higher the inhibition angiogenesis.

ACKNOWLEDGEMENT

This study was supported by HIBAH UNGGULAN PT 2011 from Directorate General of Higher Education Of Republik Indonesia

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PREVALENCE AND SUPPORTIVE FACTORS OF GERIATRIC SELF MEDICATION IN PHARMACIES GUNUNGKIDUL REGENCY AT MAY-JULY 2012

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Abstract

Background. *The increased of geriatric population is increased along with life expectation. Geriatric population is identical with the decline of physiological functions that can cause various diseases.*

Objective. *This study aim to investigate self medication in geriatrics requires special care from the pharmacist.*

Methods. *This study was observational study carried out by survey. Subjects of this study were geriatric patients aged 60 years or more who did self medication in pharmacies which involved in this study. The pharmacies used were representing the northern, eastern, southern, western, and central area in Gunungkidul regency. Data was collected in May-July 2012 using questionnaire. The percentage of geriatric self medication prevalence was formulated by comparing between the number of geriatrics who did self medication with the overall population who did self medication. Supportive factors of geriatric self medication were analyzed with chi square test with 95% confidence level, followed by binary logistic test to determine the most supportive factors.*

Outcome measured. *Supportive factors : monthly income, distance of residence from the nearest health services, and the types of illness*

Results. *There were 154 respondents of this study. The prevalence of geriatric patient who did self medication was 10,11%. The chi square test's result that monthly income, distance of residence from the nearest health services, and the types of illness were supportive factors of geriatric patient self medication in pharmacies Gunungkidul regency ($p < 0.05$) and binary logistic test's result that distance of residence from the nearest health services was the most supportive factors ($p<0,05$) with OR value was 6,727 (confident interval 95%: 1,163-38,911).*

Conclusion. *The geriatric who did self medication were affected by supportive factors*

Keywords : *geriatric, self medication prevalence, supportive factors*

ISBN : 978-979-18458-6-1

INTRODUCTION

WHO declares that elderly in indonesia will reach 11,34 % in 2020 (Anonim, 2009). Increased life expectancy will affect the growing of geriatric population. It is not directly and epidemiology contributes to degenerative diseases, chronic diseases and non-communicable diseases, including can not be cured and need more time recovery diseases. This condition Influences on the increase of health service needs (Megawati, 2004).

Geriatric population is a population that has experienced a decline of physiological functions. With the decline in physiological function, the process of absorption, distribution, metabolism and excretion of drugs will also change (Prest, 2003). Kuntjoro states (2002), there are four characteristics that can be categorized as a Geriatric patients and Psikogeriatri:

a. Body functional limitation associated with the increasing of age.

b. Accumulation of degeneratif deseases.

c. Elderly are psichosocially in crisis if : there is dependence on others (need others' services), isolate themselves from social activities.

d. The things that can cause impaired balance (homeostasis) so it brings geriatric towards damage / decrease (deterioration) which are mainly progressive especially sudden psychological aspects, such as confusion, panic and depression.

Previous research in Jakarta states that the public interest do self medication in pharmacy is increasing, the majority of drug selection served by assistant pharmacists (95%) and by pharmacists (5%) (Purwati et al., 2004). Self medication according to the WHO definition is the selection and use of modern medicine, herbal or traditional medicine by an individual to cope with the disease or symptoms of diseases (WHO, 1998). Self medication is considered by the World Health Organization (WHO) to be an international health policy, because self medication not only reduce the burden on health care costs but also to increase patient compliance and improve treatment outcome (You *et al*, 2011).

According to the newest data of Department of Social Province DIY 2010, the total of elderly in Province DIY is 29.724 people, while 11.565 people (38,88%) are in Gunungkidul regency (Anonim, 2011). The aim of this reseach is to know prevalency and supporting factors of geriatric patient self medication in pharmacies Gunungkidul regency.

RESEARCH METHODOLOGY

Research design : research design used is observational with survey method. The data are taken as prospective in 3 months, started on May 2012. Evaluation is conducted based on questionnaire on May to July 2012.

Research population: the population of this research is 60 years old and above of geriatric patients in Gunungkidul regency who come to the pharmacies Gunungkidul regency to buy medicine without doctor prescription on May to July 2012.

Research Subject: the sample of this research is geriatric patient in Gunungkidul regency who come to pharmacies Gunungkidul regency to buy medicine without doctor prescription on May to July 2012 with criteria inclusion and exclusion. Inclusion criteria is that geriatrics who are able to fill the questionnaire correctly while exclusion criteria is geriatrics who are not able to fill the questionnaire of this research.

In the research:

The research is conducted at pharmacies Gunungkidul regency and uses sampling area technique. The data of Health Department year 2011 shows that there are 22 in pharmacies Gunungkidul regency. The total sample minimum pharmacy is taken by using formula + $\sqrt{n}$ + 1 ($\sqrt{22}$ + 1= 6 pharmacies). Choosing research subject as respondent in this research is decided by sampling purposive with the geriatric

ISBN : 978-979-18458-6-1

Table I. Prevalence of self medication geriatric patients in pharmacies Gunungkidul regency

Region	Patients with self medication		Prevalence (%)
	Geriatric	All patients	
Northern	9	365	2,47
Eastern	4	720	0,60
Southern	6	1379	0,44
Western	15	776	1,93
Central	120	2566	4,67
	Total		10,11

criteria above 60 years old people who come to the pharmacies to conduct self medication and they agree become the subject of this research.

The data taken are data from the complete questionnaire filled by the research subjects at the pharmacies. Questionnaires are distributed continually on May to July 2012 to all of pharmacies in this research. Self medication prevalence of geriatric patient is shown in percentage. Statistic test uses SPSS 16.0 program for Windows. Supportive factors of geriatric patients self medication in Gunungkidul regency were analyzed with chi square test with 95% confidence level.

Research Obstacle

The obstacles this study are not all pharmacies located in Gunungkidul are willing to participate in this research and not all of geriatric patients who come to self medication are willing to be the subjects in this study so that the sample may not have been able to represent the actual population size.

RESULT AND DISCUSSION

The result of this research shows the prevalence of self medication geriatric patients in pharmacies Gunungkidul regency is 10.11% (presented in Table 1). The data were obtained from nine pharmacies that meet the sampling area; the northern, eastern, southern, western, and central to the inclusion and exclusion criteria. Compared with other regions, the central regency has the highest percentage, it is 4.67%. The high of self medication prevalence of geriatric patients in the central regency is due to the number of samples used pharmacies in the central regency more than other regency. Middle regency which is the capital of Gunungkidul is urban areas, where the practicality and the speed become everyday lifestyle including the efforts of curing the disease.

The total of geriatric patients who participated in this study is 154 respondents. Geriatric patients who do self medication in pharmacies Gunungkidul regency was 44.59% (presented in Table II). The results are similar to studies conducted Vacas,. Et al (2009) in primary health care (PHC) center Spain, it is known that 31.2% of patients on independent treatment, where pharmacies are the most common sources for getting medications (49.3%).

This is understandable because the drug is one of human rights, the provision of essential medicines is an obligation for the government and health care institutions, both public and private (Anonim, 2008). Provision of essential drugs in health services such as pharmacies will provide easy access to their own medical needs or self medication.

In this study as much as 96.75% of the respondents expressed the drug information by officers while performing their own treatment. In addition as many as 96.75% respondents expressed the needs for information on the independent medication treatment. The

ISBN : 978-979-18458-6-1

Table II. Self medication in pharmacies Gunungkidul regency

Self medication	number	percentage (%)
Option for getting medications if sick		
Self medication	70	44,59
Other health services	87	55,41
Self medication frequency per month		
Not sure / if sick	17	10,76
1x/ month	59	37,34
2x/ month	47	29,75
1-2x/ month	4	2,53
3x/ month	9	5,70
2-3x/ month	2	1,27
>3x/ month	9	5,70
Availability of drug information when self medication		
Yes	149	96,75
No	2	1,30
Need of drug information on self medication		
Yes	149	96,75
No	3	1,95
Satisfaction with the self medication		
Satisfied	149	96,20
Not satisfied	2	1,27

existence of drug information by pharmacists is expected to increase patients' knowledge about drugs so that patients are able to use drugs rationally to maximize therapy and minimize side effects. Research conducted by Satibi and Oetari (2001) shows that the medicine information affects the selection of and the use of caught medicine in the independent treatment in the Godean regency . Amoako EP et al (2003) in his study concludes that geriatric may not be aware of the risks associated with poor concurrent use of pain medications, alcohol, high blood pressure medications, and the regular use of caffeine.

As 96.20% of the patients in this study express satisfaction with independent medical care. This is understandable because it will facilitate the acquisition of OTC drug (over the counter-drug) availability and drug cost savings.

Results of analysis using the chi square test shows that there are 3 factors that support self medication significantly of geriatric patients ($p < 0.05$), such as the amount of income each month, distance of residence from the nearest health services, and types of illness (presented in Table III).

The Public Health Action Support Team (PHAST) in 2011 proposed the health aspects that can be considered in four things: 1). Effectiveness, related to the health benefits measured through improved health, 2). Efficiency, associated with the cost and the health benefits, 3). Acceptability, related to social acceptance, psychological and ethical about the way people are treated in health, 4). Equity, related to the fair distribution of health among individuals or groups.

Table III. Results of analysis using the chi square test of supporting factorsself medication geriatric patients in pharmacies Gunungkidul regency

supporting factors self medication geriatric patients		Number of respondents		p-value
		self medication	Other health services	
Sex	Male	43	56	0,809
	Female	25	30	
Job	Entrepreneur	48	49	0,099
	Does not work	18	33	
Amount of monthly income	<1.000.000	7	4	0,035*
	>= 1.000.000	8	21	
Distance of residence from the nearest health services	<= 1Km	26	52	0,003*
	>1 Km	33	23	
Insurance ownership	ASKES/Jamkes	14	21	0,296
	No	52	58	
Types of illness	Chronic	39	70	0,001*
	Not Chronic	29	16	
Amount of illness	1 illness	62	75	0,285
	>1 illness	5	11	
Originally financing medication if sick	self/family	62	73	0,403
	company /ASKES	6	12	
Completeness facilities of pharmacies	complete	65	83	0,209
	not complete	2	0	
Provision of information by the pharmacist	Yes	66	83	0,333
	No	0	2	
Need of drug information on self medication	Important	66	83	0,918
	Not Important	1	2	
Satisfaction with the service of self medication at the pharmacy	Satisfied	66	83	0,918
	Not satisfied	1	1	

Self medication is one of treatment option that can reduce the cost burden on health services as disclosed by You et al, (2011), this is in accordance with the PHAST which include efficiency as assessed health aspects. Independent health cost can not be separated from the income of an individual, in this case the geriatric patients. It shows that income is one factor supporting self medication. Through this research it can be concluded that the amount of monthly income can significantly affects s self medication of geriatric in pharmacies Gunungkidul regency. Another study conducted by Atmoko and Indria (2009) states that the cost of expensive treatment gives positive effects to the consumer decision to do self medication.

Geriatric patients who have more than a kilometer to the nearest health facilities are more often do self medication in pharmacy because of the pharmacy is considered closer to making it easier and faster to get the medicine. Decreased physiological conditions that ultimately decrease the geriatric patient's physical condition will cause their limited space, so the distance of pharmacy where they do self medication

relatively close to their house will be very supportive of the actions of undertaken self medication of geriatric patients if they are sick.

Types of illness factors significantly influence geriatric self medication in pharmacies Gunungkidul regency. Types of illnesses suffered by elderly patients who do self medication are degenerative diseases such as hypertension, gout, rheumatism, and so on. The degenerative diseases requiring long-term medication and under a doctor's supervision as prone to complications and require monitoring of drug side effects that may occur as infrequently do not need a lot of medication at the same time.

The use of drugs in degenerative diseases can not be separated from the drugs included in the list of mandatory drug pharmacy, which can be submitted without a prescription at pharmacies with medication administration records only on the basis of the doctor's treatment replicates (Kemenkes, 1999). Christiane, et al., (2008) states that there are 30% of advers drug reactions that cause elderly patients hospitalized due to NSAID.

The role of the pharmacist at the time self medication is needed in terms of drug selection and dosage appropriate circumstances to the geriatric patients. Provision of clear information regarding the efficacy, rules of use, security, drug side effects, drug interactions, and storage. The purpose of giving information is to make sure patients are able to use the drugs properly so the goal of treatment can be achieved.

CONCLUSION

It can be concluded that:

1. Self medication prevalence of geriatrics in pharmacies Gunungkidul regency is on May to July 2012 is 10, 11%.
2. Supporting factors that support geriatric Patient to do Self medication in in pharmacies Gunungkidul regency is monthly income, distance of residence from the nearest health services, and the types of illness ($p < 0,05$).

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THE ANALYSIS OF QUALITY OF LIFE IN DIABETIC PATIENTS CONSUMING ORAL DIABETIC AGENTS

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Abstract

Background. *Diabetes mellitus is one of chronic metabolic disorder characterized by hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism because of insulin insufficiency. Diabetes mellitus is a chronic disorder which have consequencies in patients' quality of life.*

Objective. *The research aimed to find out the description about quality of life in diabetic patients and the influence of delivering drug information to the quality of life in diabetic patients who got oral diabetic agents in Puskesmas Umbulharjo 1 Yogyakarta.*

Methods. *The research was a cohort study at Puskesmas Umbulharjo I. The inclusion criteria were all diabetic patients who got oral diabetic agents and had been receiving the medication for at least one month in Puskesmas Umbulharjo I, patients who are willing to be the respondents, dan the living place of the patients can be reached by the researcher. The exclusion criteria was a patient who had complicated disorder. There were 18 patients in the research who had inclusion criteria. The data being analyzed was a primary data to identify quality of life that was measured using SF-36 questionnaire Indonesian version.*

Outcome measured. *Quality of life domain in diabetic patients*

Results. *The result of the research indicated that 8 domains were a (1) general health perceptions (2) physical health problem (3) physical activities (4) emotional problems, (5) social activities (6) bodily pain (7) vitality and (8) general mental health. The result from eight domains showed that there was no corellation between the provision of drug information and quality of life in diabetic patients ($p > 0.05$). In the other results showed that there was a significant corellation between patient's adherence and quality of life in diabetic patients in three domains such as general health perceptions, vitality, and general mental health ($p < 0.05$).*

Conclusion. *In conclusion, the provision of drug information did not affect the quality of life of patients with diabetes mellitus ($P>0.05$), whereas the effect of compliance to quality of life resulted that compliance of patients significantly influenced the quality of life of patients with diabetes mellitus in 3 domains, namely physical activities, vitality, and general mental health ($p <0.05$).*

Keywords : *Diabetes mellitus, quality of life, delivering drug information , SF-36 questionnare*

ISBN : 978-979-18458-6-1

INTRODUCTION

Diabetes mellitus is defined as a disease or a chronic metabolic disorder with multiple etiology characterised by high blood sugar levels accompanied with disturbance of carbohydrate metabolism, lipid and protein as a result of insulin function insufficiency. Insufficiency of insulin function may be caused by impaired or deficient production of insulin by the beta cells of Langerhans pancreas gland, or due to the lack of responsiveness of body cells to insulin (Anonim, 2005).

The development of the concept of HRQOL (Health-related Quality of Life) or the relationship of health and quality of life improved in line with world health clinical research in healthcare. HRQOL instruments are divided into general and disease specific instrument. Instruments Study Short Form 36 (SF-36) is one common instrument that has been widely used to measure the HRQOL disease chronic pain and other common illness (Magnus et Al, 2007).

Research on quality of life is expected to obtain an overview of the level of quality of life of patients with diabetes mellitus who consumed oral hypoglycemic drugs, so it will obtain the suitable model for the provision of drug information on patients with diabetes mellitus.

METHODS

Subjects

The study subjects were all the patients of diabetes mellitus and get oral hypoglycemic drugs in *Puskesmas Umbulharjo I* in April-May 2012 who meet the criteria for inclusion and exclusion criteria. Inclusion criteria for the study were all patients of diabetes mellitus that receive oral hypoglycemic drugs and were undergoing treatment at least 1 month in *Puskesmas Umbulharjo I*, patients who were willing as a respondent, and the respondent could be reached by researchers. Exclusion criteria for the study were patients who experienced complications of other diseases.

Research Data Collection

Data collected in the form of primary data obtained from research subjects by using an instrument that has been tested, the Indonesian version of the.SF-36. The patient's quality of life data collection done at home while visiting a patient's home, to see the remaining amount of oral hypoglycemic drugs that have been drunk. Before conducting interviews and questionnaires, it should be ensured that the selected respondent is in compliance with the criteria of inclusion.

Analysis of results

In this stage, the processing and analysis of the results of the study are to analyze the influence of drug information and adherence to the quality of life of patients with diabetes mellitus who consumed oral hypoglycemic drugs. Quality of life of patients assessed by the SF-36 questionnaire, which contained 8-will do assessment domains, namely the domain of general health perceptions, physical health problem, physical activities, emotional problem, social activities, bodily pain, vitality, and general mental health. From the domains, then do the scoring with a scale of 0 to 100 with 100 being the highest level.

RESULTS AND DISCUSSION

This study was conducted on 18 patients who fit the inclusion and exclusion criteria. Measurement of quality of life is intended to compare and differentiate between two different therapy with moral values and high morbidity (Spilker, 1996). To measure the quality of life of the patients, the study used the SF-36 instrument. The SF-36 instrument is a general instrument that can be used to measure the quality of life of patients in various clinical disorders whether the type is chronic illness or specific diseases.

Diabetes mellitus is a chronic disease type, so it can analyze the patient's quality of life using SF-36 instrument. The SF-36 has 8-scale profile of functional health; the limitation of physical activity due to existing health problems,

social activity limitations due to physical and emotional problems, whole body pain, general mental health, restriction of daily activities due to emotional problems, vitality of life, and general health outlook (Wata, 2007). Seven domains are analyzed by the Independent T-test with a level of 95% while the emotional state of domain analyzed by 2-related samples.

In Table I are data on the effect of drug information to the quality of life. Provision of drug information was limited to how to take the medicine and the rules of use, due to time constraints. The results showed that administration of drug information does not differ significantly (P> 0.05) on the quality of life of patients in either eight domains, namely general health, physical functioning, physical condition, emotional state, physical function, bodily pain, vitality, and mental state.So it can be concluded that the provision of drug information to patients does not affect the patient's quality of life. This can occur because the information presented is only how to take the medicine and the rules of use, so there are some other things that can affect the patient's quality of life such as compliance of the patient.

Influence patient compliance to the quality of life can be seen in Table II. Compliance is defined as the extent to which a person's behavior in terms of using medication (is defined by how far a person's behaviour in terms of drugs usage), following a diet, or

Table I. The Effect of Drug Information on Patient's Quality of Life

	Function Domain	Average ± SD	P	Significance
With Drug Information	KU	56.93±17.19	0.817	Not Significant
Without Drug Information		58.79±16.33		
With Drug Information	FF	67.22±33.64	0.242	Not Significant
Without Drug Information		82.22±15.43		
With Drug Information	KF	38.88±39.74	0.675	Not Significant
Without Drug Information		30.55±42.89		
With Drug Information	KE	363.495±125.896	0.181	Not Significant
Without Drug Information		363.495±125.896		
With Drug Information	FS	72.22±27.79	0.696	Not Significant
Without Drug Information		76.38±14.58		
With Drug Information	NT	58.61±22.77	0.657	Not Significant
Without Drug Information		63.05±18.65		
With Drug Information	V	53.33±19.36	0.704	Not Significant
Without Drug Information		56.66±17.13		
With Drug Information	KM	69.77±19.09	0.854	Not Significant
Without Drug Information		68.00±21.07		

changing inlife style in accordance with medical advice or health workers advice. As increasing patient compliance does not arise, it is expected that drug resistance can be detrimental (can harm/can be harmful) to the patient itself, the environment, recurrence and death (Melanie, 2009).

Oral hypoglycemic drug compliance seen from the remaining number of oral hypoglycemic drugs provided by the pharmacists in *Puskesmas* Umbulharjo I that were drunk by patients, whether in accordance with the amount that should be taken or not, and be seen as doing the interview at the patient's home.

From the results gained, showing that compliance was not effected significantly ($P > 0.05$) in five domains, namely the domain of general health, physical functioning, emotional health, social functioning, and bodily pain. But compliance significantly effects ($P < 0.05$), on the 3 domains, namely the domain of physical health, vitality, and mental health. It can be concluded that compliance significantly influence the quality of life of patients.

Overall, based on the test results of the Independent T-test, it showed that the provision of drug information and patient compliance give no significant effects on the public health domain. Itcan be seen in the table the value of

Table II. Compliance Influenceon Patient'sQuality Of Life

	FunctionDomain	Average ±SD	P	Significance
Obedient	KU	59.58±18.11	0.632	Not Significant
Disobedient		55.71±14.61		
Obedient	FF	85.00±10.27	0.064	Not Significant
Disobedient		61.87±35.14		
Obedient	KF	52.50±44.79	0.032	Not Significant
Disobedient		12.50±18.89		
Obedient	KE	362.995±126.224	0.181	Not Significant
Disobedient		362.995±126.224		
Obedient	FS	78.75±15.64	0.345	Not Significant
Disobedient		68.75±27.54		
Obedient	NT	61.50±17.00	0.082	Not Significant
Disobedient		60.00±25.10		
Obedient	V	62.50±16.20	0.042	Significant
Disobedient		45.62±15.90		
Obedient	KM	78.80±11.63	0.011	Significant
Disobedient		56.50±20.88		

significant effect of drug information on the quality of life of patients is 0.817 (> P 0.05) and the value of the significant influence of compliance to the quality of life of patients, namely 0.632 (> P 0.05).

In the domain of physical function, it can be assessed how the ability of a patient's daily activities such as walking, bending, and lifting heavy loads,whether the health conditions of physical function may result in limitations of the patient through physical activity. From the research, the provision of drug information does not affect the physical function domain. It can be seen the value of significance is 0.242 (P > 0.05), and compliance did not affect the physical function domain, can be seen the significant value is 0.064 (P > 0:05). It can be concluded that the provision of drug information and patient compliance has no effect on physical function domain, whereas Wiyanti (2012) stated that the physical function domain effect on age.

In the domain of physical conditions can be assessed how much physical state interfere in the work and activities of daily living. From the research data that the provision of drug information does not affect the physical state visible domain of significant value in the table is 0.675 (P > 0.05), whereas the effect on patient compliance domain visible physical state of significant value in the table is 0.032 (P > 0.05), so it can be concluded that the given drug information or not given drug information does not affect the quality of life of patients but blindly obedient or not patients have a significant effect on the quality of life of patients is the physical state that disrupt their work and daily activities.

In the emotional state domain, it used statistical analysis with 2 related samples, it can be seen some aspects of the patient-related mood in doing the work and activities of daily living. Provision of drug information and patient compliance has no effect on the emotional state of the domain, can be seen from the data table of significance values are 0.181 and 0.181 (P> 0.05), so it can be concluded that with the drug information was given or not, and how the patient obedient or not, can not affect the patient's emotional state. From patient interviews, most of them stated that emotional disorders such as anxiety do not interfere the patient in performing daily activities or their work.

In the domain of social function, it can be evaluated that the patient's health or emotional disturbance can disrupt activities or social activities of the patient's family, friends, neighbors, or groups. Provision of drug information and patient compliance has no effect on physical function domain can be seen in the table of significance values 0.696 and 0.345.It can be concluded that given or not given the drug information and how the patientobedient or not, has no effect on social functioning of patients. And from the interviews, it is found that many patients say health or emotional disorder that is often felt they did not interfere with patient's activity or social activities to family, friends, neighbors, or groups.

In the bodily pain domain, it can evaluate pain intensity and impact of pain on daily activities both at work and activities inside and outside the home. Provision of drug information and patient compliance has no effect on bodily pain domain views of significant value in the table, namely 0.657 and 0.882. It can be concluded that given or not given the drug information and how the patientobedient or not had no significant effect on the patient's pain.

In the domain of vitality, it evaluate the level of fatigue, fatigue, and lethargy are often perceived by the patient. Provision of drug information does not affect the vitality domains, but the effect on patient adherence vitality domain can be seen from the significant value of 0.657 (P> 0.05), and 0.042 (P <0.05). How obedient or not the patient will have an impact on patient quality of life, especially its vitality because one of the complaints experienced by patients with diabetes mellitus is weak body, then the non-adherent patients taking the medication more frequently complain of weak entities.

In the mental health domain, it evaluate general mental health including depression, anxiety, and behavior control emotions by the patient. Provision of drug information does not affect the mental health domain, but the effect on patient compliance mental health domain, it can be seen from the significant value of 0.854 (P> 0.05), and 0.011 (P <0.05). How obedient or not the patient will have an impact on the quality of life of patients, especially in mental health patients, as diabetes mellitus is a disease that is difficult and long to be cured, it can make mental health patients reduced the impact on patient adherence. In the study of Wiyanti (2012), quality of life of diabetes mellitus patients, there are 2-type influential domains, one of them is mental health, that is influenced by the duration of the disease.

CONCLUSION

Based on the results of a study of 18 patients with diabetes mellitus and the use of oral hypoglycemic drugs, showed that of the 8 domains tested, provision of drug information does not affect the quality of life of patients with diabetes mellitus (P> 0.05), whereas the effect of adherence to quality measurement life result that compliance of patients significantly influence the quality of life of patients with diabetes mellitus in 3 domains, namely physical health, vitality, and mental health (p <0.05).

RECOMMENDATION

It is still needed to conduct research on patients' compliance and quality of life with diabetes mellitus for evaluating treatment with time to improve the provision of information and frequency of medications, as well as increasing the number of research samples.

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EVALUATION ON THE IMPLEMENTATION OF DRUG INFORMATION SERVICE AT PHARMACY IN YOGYAKARTA

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Background. *The drug information service is a part of pharmaceutical care to increase the quality of life of patient. To prevent unappropiated drug use and to improve patients knowledge and understanding on drug use leads to the treatment compliance and the success of therapeutical management. Therefore, it is extremly important to give drug information service for the patients and their family.*

Objective. *This study was aimed to identify the realization of drug information service implementation at pharmacy in Yogyakarta.*

Methods. *This study was non-experimental study with descriptive observational design and quantitative approach*

Outcome measured.: *Drug information aspects : the name of the drug and aim of treatment, the rule of drug use, the schedule of drug use, and the period of using the drug.*

Result. *The result showed that the implementation of drug information service at pharmacy in Yogyakarta had been conducted well. There were four information aspects was gived to the patients. Based on the observation, 70 % patients experienced drug information service on the name of the drug and aim of treatment, the rule of drug use, the schedule of drug use, and the period of using the drug. However, information on the side effect of the drug and the drug storage were ungived. There were less than 70 % patient giving the information. Moreover, the number of patients giving information on drug toxicity and things to do related to drug toxicity reached merely 33,33%, whereas patient who come to pharmacy is out-patient who is responsible to the use of the drug.*

Conclusion. *Implementation of drug information service at pharmacy in Yogyakarta had been well conducted. Nevertheless, some aspects of drug information were not implemented optimally.*

Keywords : *implementation, drug information, pharmacy*

ISBN : 978-979-18458-6-1

INTRODUCTION

Strategy implementation is the total activities and choices which were required for the execution of a strategic plan. It is the process which objectives, strategies, and policies a process are put into action trough the development of programs, budgets, and procedures. Although an implementation is usually considered after strategy has been formulated, an implementation is a key part of strategic management. Strategy implementation involves establishing programs to create a series of organizational activities. The purpose of a program is to make a strategy action oriented. The evaluation of strategy implementation is a process that monitors activities and actual performances result which can be compared with the standard (Hunger and Wheelen, 2006).

Pharmaceutical care was including the drug production, quality control of pharmaceutical preparations, safety, procurement, storage and drug distribution, drug management, drug prescription, drug information services, drug development, and traditional medicine. Pharmaceutical care is a direct and responsible service to patients, relating to pharmaceutical preparations in order to achieve results that are sure to improve the quality of life of patients (Anonymous, 2009)

The prices of drug and added costs of health care more expensive, causing people tried to self medication with drugs that are sold over the counter in pharmacy or the market (Sulistyarini, 2010). Before tried to self medication some one must be recognize that happened complaint, then can choose what is appropriate remedy to overcome the such complaints, and knowledge when to the drug used.

The drug information service is an activity to give accurate drug information and objective in relation to patient care, drug information services is essential in order to support the management culture and rational drug used. (Julianti and Widayanti, 1996). Pharmacist must give the drug information is a true, clear and easy to understand, accurate, unbiased, ethical, thoughtful and up to date. The drug information service to patients is high quality of information includes a description of drugs that includes basic information about a drug component.

Pharmaceutical care has shifted from the drug oriented to the patient oriented. The consequence of changes in oriented, pharmacists are required to improve the knowledge, skills and behaviors in order to carry out the direct interaction with the patient. The other forms of interaction between the implemention drugs information, monitoring the drug used to determine the final destination as expected and well documented (Anonymous, 2008).

The standard of pharmaceutical care in pharmacy, the pharmaceutical care has developed standards that have been set out in the decree of the Ministry of Health of the Republic of Indonesia No 1027/MENKES/SK/IX/2004 is a parameter used to assess the quality of pharmaceutical care in the pharmacy (Anonymous, 2004). The drug information services and aspects of drug information give by pharmacists is part of pharmaceutical care at the pharmacy. Pharmacists practice in pharmaceutical care is integrated activity with the aimed of identifying, preventing and resolving related problems to health, especially in terms of drug used.

The role of pharmacist in the pharmacy, drug information services is extremly important to give for the patients and their family patients, especially for self medication patients. The self medication patients get the drug information from the experience of others, advertising in the mass media are often less complete, less precise, even plunged (Sulistyarini, 2010). We was known for many drugs in circulation and reach thousands of types of drugs, so as to confuse the public in choosing the right drug and safety drug for him. For that pharmacists should give accurate drug information and objective so that patients can use the drug correctly.

The study was aimed to identify the realization of the drug information service

implementation at pharmacy in Yogyakarta. From the result, it is expected for pharmacy can select the right step. So that the drug information services as the strategic plan to improve the pharmacy service quality, that has been planned can reach the target that has been established. So it can support the pharmaceutical care in pharmacy that has high quality to increase the quality of life of patient.

RESEARCH METHODOLOGY

This type of research is non experimental study with observational descriptive design with quantitative approach. The data was obtained from the questionnaires distributed to respondents, patients with a doctor's prescription and self medication patient at pharmacy in Yogyakarta. The observation of the drug information services by pharmacist and the required patient. While primary data was taken form answereds the questionnaire respondents.

The tools of this study used the questionnaires, the questionnaires used to determine the implementation of drug information services give and needed patients at pharmacy. The questions contain questionnaires used to regarding the factual drug information services give and required patient.

The study used non experimental design. The quantitative data was obtained from filled patients questionnaires. The sampling was conducted using purposive sampling or aimed sampling. The samples were patients and their family was comed to the pharmacy. The inclusion criteria of adult patients aged over 17 years, the prescription patients or self medication patients who is willing to be respondents and exclusion criteria are not willing to be a respondent. The amount of data based on Roscoe who said the amount of data should be more than 30 and less than 500 (Sekaran, 2003).

The study was conducted at pharmacy in Yogyakarta, the pharmacy criteria is a pharmacy where located represents the area of the city in Yogyakarta. Each of the two pharmacies, located in the center, north, east, south and west of the city in Yogyakarta. Where the pharmacy has been providing counseling space, at least two pharmacists practice.

Data as analyzed descriptive statistics methode to identify the drug information services implementation. In the analisys, the data of drug information services implementation that is was obtained from the questionnaire respondents. Data factual description of drug

Table I. Factual description of Respondents

Factual description of Respondents		Total	Percentage (%)
Visit to the pharmacy before	Often visit	90	90.91
	Rarely visit	9	9.09
Drugs purchased in pharmacy	Prescription drug	36	36.36
	Self medication	63	63.64
Status of the respondents	Their self	67	67.68
	Other self	32	32.32
Need for drug information services	Need	99	100.00
	Needn't	0	0.00
Ever get the full drug information services	Ever	58	58.59
	Never	41	41.41

Table II. Aspects of Drug Information Service

Aspects of Drug Information	Percentage (%)	Parameter
The information of drug name and aimed of treatment	89.90	Pharmacists give the information of drug name, efficacy and aimed of treatment
The information of frequency or schedule of drug use	93.94	Pharmacists give the information of frequency or schedule of drug use and drug dose
The rule of drug use information	93.94	Pharmacists give the rule of drug use, especially for special drug use
The period of drug used	75.76	Pharmacists give the period of drug used information
Adverse effect of the drug	67.68	Pharmacists give the information at general adverse effect of the drug in most of the patients drug used
The drug toxicity and things to do related to drug toxicity	33.33	Pharmacists give the drug toxicity information and things to do related to drug toxicity
The drug storage	60.61	Pharmacists give the drug storage information

information services give and needed patients are presented in table.

RESULT AND DISCUSSION

The result showed that the implementaion of drug information service at pharmacy in Yogyakarta had been conducted well. The respondents involved in this study were 99 respondents, with a factual overview of the respondents are presented in table I

The factual of respondents in Table I showed that 90.91% of respondents were costumers pharmacy where research, their often visit the pharmacy. Respondents drugs purchased at pharmacies without a prescription or self medication as much as 63.64%, is greater than the prescription drugs that reached 36.36%. The majority of respondents status were patients on drugs purchased by 67.68%, which is only 58.59% of respondents who had give the full drug information services. From the statement of 100% of respondents require drug information services.

The implementation of the drug information service at the pharmacy, was description in Table II. The drug information services was observed form aspects of drug information.

The implementation of the drug information service at the pharmacy in Table II, showed that the aspects of drug information had been conducted well. There were four information aspects was give to the patients. Based on the observation, 70% patients experienced drug information service on the name of the drug and aimed of treatment, the rule of drug used, the schedule of drug used, and the period of drug used. However, the information on the adverse effect of the drug and the drug storage were not implemented optimally. There were less than 70% patient giving the adverse effect of the drug and drug storage information. Moreover, the number of patients giving information on drug toxicity and things to do related to drug toxicity reached merely 33,33%, whereas patient who come to phramacy is out patient who is responsible to the drug used.

ACKNOWLEDGEMENTS

Limitations of this study, the data as analysed using descriptive statistic analysis. The

next study can used another methods to analysed, and completely analysis of the data.

CONCLUSION

Implementation of drug information service at pharmacy in Yogyakarta had been well conducted. Nevertheless, some aspects of drug information were not implemented optimally

1. Patients at pharmacy in Yogyakarta overall needs drugs information services
2. There were four information aspects was give to the patients
3. Implementation drug toxicity and things to do related to drug toxicity information reached merely 33.33%

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EFFECT OF TURMERIC (Curcuma Domestica Val.) RHIZOME ETHANOLIC EXTRACT TO PLASMA LIPID PEROXIDE LEVEL ON WISTAR RAT INDUCED BY TRIMETHYLTIN

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Abstract

Background. *Oxidative stress is one cause of death of neurons in the brain. The reduced number of neurons from oxidative stress disrupt memory functions of the brain that causes dementia. Turmeric (**Curcuma domestica** Val.) contains curcumin, which has antioxidant activity.*

Objective. *This study aims to determine the effect of ethanol extract of turmeric (**Curcuma domestica** Val.) to plasma lipid peroxide level on Wistar rat induced by trimethyltin chloride.*

Methods. *This study performed 45 male Wistar rats, divided into 6 groups randomly. Group I was a healthy control group, which the rat given CMC-Na solvent. Group I as a negative control was given trimethyltin (TMT) 12.5 mg/kg intraperitonially on 9th day. Group III as a positive control, was given by Piracetam dose 500 mg/kgBW. The groups IV, V and VI were given turmeric ethanol extract dose 120 mg/kgBW, 240 mg/kgBW and 480 mg/kgBW orally. All treatments carried out for 8 days. On 9^{th} day, groups II, III, IV, V and VI induced by 12.5 mg/kg TMT intraperitoneally. After 24 h of TMT injection, the blood were taken to measure malondialdehyde (MDA) level which is a biomarker of lipid peroxidation using $TBARSC_{18}$ (Thiobarbituric Acid Reactive Substance C_{18}) method. MDA levels of data were statistically analyzed using the test for normality and homogeneity test followed by ANOVA and Post Hoc Least Significant Difference (LSD).*

Outcome measured. *MDA levels using TBARSC18 (Thiobarbituric Acid Reactive Substance C18) method*

Results. *The results showed that administration of ethanol extract of turmeric for 8 days can reduce plasma MDA level on rat induced by TMT. Piracetam dose 500 mg/kg body can lower MDA level 85.53%. While turmeric ethanol extract dose 120, 240 and 480 mg/kgBW lowered MDA levels respectively 46.80%, 61.36% and 81.17%.*

Conclusion. *Ethanolic extract of turmeric for 8 days can reduce plasma MDA level on rat induced by TMT*

Keywords : *lipid peroxides, antioxidants, dementia, trimethyltin, ethanol extract of turmeric*

ISBN : 978-979-18458-6-1

INTRODUCTION

Oxidative stress is an important pathogenesis of dementia. Brain cells are particularly vulnerable to oxidative damage because of their high utilization of oxygen and the substantial polyunsaturated fatty acid content, and this organ has limited ability to combat oxidative stress (Halliwell and Gutteridge, 1985; Halliwell, 2001). Oxidative damage to lipid (lipid peroxidation) and protein (protein carbonyl formation) can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and finally cell death. Oxidative damage to lipid can lead to formation of breakdown products such as malondialdehyde (MDA), 4-hydroxy-2, 3-nonenal (HNE), acrolein, etc. Antioxidant agents from diet have a significant therapeutic influence on various neurodegenerative disorders associated with oxidative stress (Ahmad *et al.*, 2005; Ishrat *et al.*, 2006).

Turmeric contains curcumin which has been known to have antioxidant activity (Rochmaulana, 2009). Curcumin can reduce oxidative stress and amyloid pathology associated with Alzheimer's dementia (Menon and Sudheer, 2007). This study aim is to determine the antioxidant effects of turmeric (*Curcuma domestica* Val.) ethanol extract on MDA plasma levels of rat dementia model induced by trimethyltin.

METHODS

1. Material

Turmeric were obtained from Samigaluh, Kulonprogo, Trimethyltine (TMT) were purchased from Sigma Chem. Other chemicals such as curcumin, H_3PO_4, Tiobarituric Acid, Tetraetoxiprophylene were obtained from Integrated Research and Testing Laboratory, Gadjah Mada University.

2. Animal Treatment

Male adult Wistar rats of 100–20 g BW were used for all experiments. They were housed in separate cages under 12 h light and 12 h dark periods. Rats had free access to standard food and water ad libitum. 45 male Wistar rats, divided into 6 groups randomly. Group I was a healthy control group, which the rat given CMC-Na solvent. Group I as a negative control was given trimethyltin (TMT) 12.5 mg/kg intraperitonially on 9^{th} day. Group III as a positive control, was given by Piracetam dose 500 mg/kgBW. The groups IV, V and VI were given turmeric ethanol extract dose 120 mg/kgBW, 240 mg/kgBW and 480 mg/kgBW orally. All treatments carried out for 8 days. On 9^{th} day, groups II, III, IV, V and VI induced by 12.5 mg/kg TMT intraperitoneally. After 24 h of TMT injection, the blood were taken to measure malondialdehyde (MDA) level which is a biomarker of lipid peroxidation using $TBARSC_{18}$ (*Thiobarbituric Acid Reactive Substance* C_{18}) method

3. Extraction of turmeric

A total of 250 grams of turmeric powder were weighed and then put into a stirred maserator electric, added 1.0 liters of 96% ethanol, stirred for 3 hours, allowed to stand for 24 hours. The filtrate was evaporated, weighed, and then counted its yield.

4. TLC densitometry-test

TLC test aims to ensure the presence of curcumin in turmeric extract and determine levels of curcumin in the extract. Previously made standard stock solution of curcumin concentration of 1 mg / ml. The standard stock solution was diluted to 0.125; 0.25, 0.5, 1, 2, 4 mg / ml. Sample solution was prepared by dissolving 100 mg of the extract into 10 ml of 96% ethanol. Standard solution and the sample spotted on the fixed phase 60 F_{254} silica gel and eluted with mobile phase chloroform: methanol with a ratio of 9: 1. Paches were read by TLC-densitometer at a wavelength of 426 nm.

ISBN : 978-979-18458-6-1

5. Determination of MDA Level

Levels of MDA was determined using the or Reactive Substance C_{18} ($TBARSC_{18}$) method (Wuryastuti, 2000). Lipid peroxide precipitation is done by mixing 0.75 m H_3PO_4, TBA 0.25 mL, 0.05 mL of sample / blank / standard TEP and 0.45 mL H_2O with a vortex for 2 minutes, then heated in a waterbath for 60 min at 100 ° C. The mixture then was cooled for 1-2 hours until the temperature reaches 30°C. The mixture was eluted on C_{18} column, then placed in a 1 cm cuvette for absorbance read with a spectrophotometer at a wavelength of 532 nm. MDA levels is in units (mmol / L)

RESULT AND DISCUSSION

The yield of 96% ethanol extract of the rhizome of turmeric (Curcuma domestica Val.) suggests that as many as 1000 g powder turmeric extract obtained weighing 297.7 g extract. While based on the TLC test showed spots of compounds curcumin extract is at Rf 0.65 and

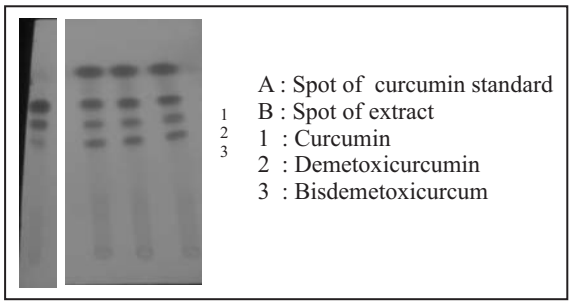

Figure 1. The Profil of Thin Layer Chromatography of Turmeric Extract

turmeric extract containing curcumin compound, with curcumin levels were obtained at 15.78% w / w.

The level of MDA was determined by $TBARSC_{18}$ method using a spectrophotometer with a maximum wavelength of 532 nm. Principle of the assay by reading the absorbance of the complex MDA-TBA2 (pink). The complex formation reaction MDA-TBA2 was shown in Figure 2.

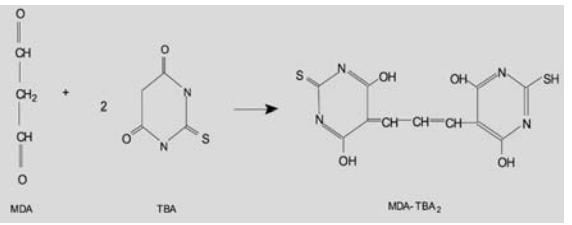

Figure 2. Reaction between MDA and MDA-TBA into TBA2 (Grotto *et al.*, 2009)

The reaction between one molecule of malondialdehyde (MDA) and two molecules tiobarbiturat acid (TBA) as a nucleophilic attack involving all five of TBA carbon and carbon to one of MDA followed by dehydration and the same reaction occurs in the molecule into two TBA-MDA complex formed TBA2 pink (Grotto *et al.*, 2009).

Based on statistical tests showed that trimethyltin administration can significantly increase plasma levels of MDA. TMT selectively

Table I. The average of MDA level in all groups

Group	Treatment	Average of MDA levels (mmol/L) ± SD
I	Control	0,0808 ± 0,0392
II	TMT group	2,0742 ± 0,0996
III	Pirasetam group	0,3000 ± 0,1095*
IV	Extract 120 mg/kgBB	1,1035 ± 0,0860*
V	Extract 240 mg/kgBB	0,8014 ± 0,0752*
VI	Extract 480 mg/kgBB	0,3905 ± 0,0643*

*differnce significant with TMT group ($p<0,05$)

standard curcumin is at Rf 0.65. It is proved that can reduce the population of neurons in the brain,

especially the hippocampus formation that plays a role in the memory process. Trimethyltin lead to activation of NMDA receptors and cainic acid toxin that produces a number of free radicals or reactive oxygen species ROS group (Shin *et al*., 2005). NMDA receptor activation causes the increasing of ROS. ROS are free radicals of oxygen reactive group that includes the triplet ($3O_2$), single (singlet/$1O_2$), superoxide anion (O_2. -), hydroxyl radical (-OH), nitric oxide (NO), peroxynitrite (ONOO-), hipoklorus acid (HOCl), hydrogen peroxide (H_2O_2), alkoxyl radical (LO-), and peroxyl radicals (LO-2) (Hadyathma, 2010). Free radicals can attack polyunsaturated fatty acids (PUFA) in the nerve cell membrane and induce lipid peroxidation resulting in increased lipid peroxidation product such as malondialdehyde. The increasing of malondialdehyde has been reported to occur in patients with Alzheimer's dementia (Devore *et al.*, 2010).

The groups administered by turmeric extract dose 120 mg / kg, 240 mg / kg, 480 mg / kg can decrease MDA levels as many 46.80%, 61.36%, 81.17% respectively compared with TMT group.

Turmeric extract can reduce levels of MDA plasma. The largest decrease was shown in the group given 480 mg kg / BW of turmeric extract. Turmeric containing curcumin known as an antioxidant. Curcumin is a lipophilic compound that can enter the blood-brain barrier. The antioxidant activity of curcumin the methoxy group and phenols that can capture free radicals, such as superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals (Chattopadhyay *et al.*, 2004). Binding of free radicals by curcumin produce more stable products such as vanillin, ferulic acid and curcumin dimer (Fujisawa *et al*., 2004). Decrease the amount of free radicals by curcumin prevents oxidative stress resulting in lower risk of lipid peroxidation and increased lipid peroxidation products such as malondialdehyde. Therefore, turmeric is expected to be used for the treatment of dementia.

In the group given pirasetam indicate that piracetam can also reduce levels of MDA. Pirasetam (2-oxo-pyrrolidone) is nootropik drug which is structurally related to GABA. Pirasetam have various effects on glutamate neurotransmission at micromolar levels while also piracetam potentiate potassium-induced release of glutamate from hippocampal nerve. Pirasetam have an effect on the subunits of glutamate NMDA receptors are involved in learning and memory. Pirasetam can give comprehensive effect on brain neurotransmission via modulation of ion channels (ie, Na +, K +). Additionally, pirasetam protects neurons against oxidative stress by normalizing activity associated cell membrane (Alkuraishy *et al.*, 2012).

CONCLUSION

The results showed that administration of e turmeric ethanol extract for 8 days can reduce plasma MDA level on rat induced by TMT. Pirasetam dose 500 mg/kg body can lower MDA level 85.53%. While turmeric ethanol extract dose 120, 240 and 480 mg/kgBW lowered MDA levels respectively 46.80%, 61.36% and 81.17% compared with TMT group.

Table ll. The percentage of the decreasing of MDA levels

Group	Treatment	The percentage of decreasing MDA levels
III	Pirasetam group	85,5%
IV	Extract 120 mg/kgBB	45,80%
V	Extract 240 mg/kgBB	61,36%
VI	Extract 480 mg/kgBB	81,1%

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THE STUDY OF EFFECTS ETHANOL EXTRACT *Mimosa pudica* L. and *Manihot utilissima* Pohl. AS AN ANTIHYPERURICEMIC IN MALE POULTRY WITH INDUCED CHICKEN LIVER JUICE

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Abstract

Background. *Hyperuricemia and gout appear to be rapidly increasing worldwide and frequently cause symptom metabolic syndrome. Mimosa pudica L and Manihot utilissima Pohl have flavonoid can decreasing hyperuricemia.*

Objective. *Aim of study was investigate the effects ethanol extract Mimosa pudica L and Manihot utilissima Pohl for antihyperuricemic in poultry.*

Methods. *Extraction compounds from* ***Mimosa pudica*** *L and* ***Manihot utilissima*** *Pohl by maceration method. Identification flavonoid using spectrophotometer UV with the scan lambda max. The study were divided 20 poultry in five groups. Group I is a health control was given food and drink, group II is hyperuricemia control was given food, drink and chicken liver juice with concentration 100% orally for 2 weeks, group III is positive control was given food, drink, poultry liver juice with concentration 100% orally for 2 weeks and allopurinol 10 mg/kg BW, group IV ethanol extract Mimosa pudica L control was given food, drink, poultry liver juice with concentration 100% orally for 2 weeks and ethanol extract Mimosa pudica L 93 mg/kg BW and group V ethanol extract Manihot utilissima Pohl control was given food, drink, poultry liver juice with concentration 100% orally for 2 weeks and ethanol extract Manihot utilissima Pohl 93 mg/kg BW. Analyze data using Mann-Whitney Test and ANOVA with P 95%.*

Outcome measured. *On day 15 checked uric acid on period I 30 minutes after treatment and period II 60 minutes after treatment in using Easy Touch Glucose Cholesterol Uric Acid kit.*

Results. *The data decreasing uric acid level from ethanol extract Mimosa pudica L 1,70 mg/dl (22%) and ethanol extract Manihot utilissima Pohl 1,43 mg/dl (21%).*

Conclusion. *Oral administration of ethanol extract Mimosa pudica L and ethanol extract Manihot utilissima Pohl was be able to reduce uric acid levels in hyperuricemic poultry with no significant effects compare with allopurinol.*

Keywords : *Hyperuricemia, Mimosa pudica L, Manihot utilissima Pohl.*

ISBN : 978-979-18458-6-1

INTRODUCTION

The development of modern life increasingly demanding technological advances human participation to more quickly in the activities. This creates a fully practical lifestyle including in controlling the intake of unhealthy food. Lifestyle such as this increase the occurrence of various diseases, one of which is marked by an increase of gout uric acid in the blood.

Uric acid is the substance of the final product or the nucleic acid purine metabolism in the body. Uric acid is produced from the primary metabolism of purine nucleoside purine bases hipoxantin late, xantin and guanine. When there is deviation in the process will increase levels of uric acid, called hiperurisemia conditions (Murray *et al.*, 2009).

Hiperurisemia an early sign of gout or pirai. Gout is closely linked to disorders of purine metabolism which raises the level of serum uric acid that is> 7.0 mg / dl (Wells *et al*., 2000). Hiperurisemia can give rise to kidney stones result in kidney failure. Uric acid is a risk factor for coronary heart disease. Unexpectedly uric acid crystals will damage the endothelial or vascular coroner. Based on clinic patient data Cipto Mangunkusumo Hospital (RSCM), Jakarta, patients with uric acid appears to seven percent of the patients suffering from rheumatic diseases. Hiperurisemia prevalence of approximately 2.6 to 47.2%, which varies in different populations. While the prevalence of gout varies between 1 to 15.3%. In a study obtained 4.9% incidence of gout in the blood uric acid> 9 mg / dl, 0.5% at a rate of 7 to 8.9% and 0.1% at a rate of <7 mg / dl. The cumulative incidence of gout achieve up to 22% after 5 years, at the rate of uric acid> 9 mg / dl (Hidayat,2009).Increase the incidence of dementia (Herlina, 2010).

Treatment chemically expensive and take a long time and side effects of medicines gout (eg intoxification acute kolkisin, gastritis on NSAID's (Non Steroid Antiinflamation Drugs), dermatitis allergic to probenecid) (Katzung, 2002) make the community look for drug alternative. Indonesian nation ancestors leverage available in the natural plant as medicine. Plants used traditionally to reduce the levels of uric acid is the daughter of shame and yam leaves. Mimosin contains compounds shy daughter, pipekolinat acid, tannins, alkaloids, saponins, triterpenoids, sterols, polyphenols, and flavonoids. Meanwhile yam leaves contain flavonoids (Anonymous, 2000a).

According to the Coss *et al*. (1998), several flavonoid and alkaloid compounds can inhibit oxidase xantin work so as to inhibit the formation of uric acid in the body. Susanti study (2006) showed that plants contain flavonoids inhibited oxidase activity xantin work can decrease levels of uric acid in the blood. Research Anderson *et al*. (2001) etanolik fractions containing flavonoids chysin shy daughter, apigenin, luteolin, kaempferol, galangin, quercetin, rhamnetin, myricetin, gossypetin.

Based on the theoretical background and above, it can be concluded that the hypothesis of ethanol extracts of herbs giving daughter embarrassed and ethanol extracts of leaves of sweet potatoes can lower uric acid induced chicken liver juice. Provide alternative information for gout particularly shy daughter herba and cassava leaves. Enriched scientific data on the benefits of traditional medicinal herbs daughter particularly shy and cassava leaves on gout treatment in order to develop herbal medicine.

METHODS

Material

Reagent needed include: Powder shy daughter herbs and leaves yams, ethanol extracts of herbs and shy daughter yams leaf ethanol extract, ethanol, diethyl ether, anhydrous Na_2S_{O4}, male chickens with body weight 1.5 kg, chicken liver juice , 100 mg allopurinol with potential.

Tool

Research tools needed include: analytical scales to weigh the powder material simplicia (OHAUS), glass ware, magnetic strirrer, rotary evaporator, reflux, filter paper, container form squares, Buchner funnel, porcelain mug, animal weighing test, oral spuit 10 ml, uric acid plumb Easy Touch GCU (Glucose Cholesterol Uric Acid), lancette needle, alcohol as a cleaner, gout strip, UV spectrophotometer.

Extract Proces

Extracts made by dipping respective shy daughter of herbal powder and yam leaves with ethanol extractor squares as possible using a container with magnetic stirring strirrer. Extraction is done until all flavonoids extracted by using FeCl3 checked. Filtrate obtained filtered using filter paper with a Buchner funnel and vacuum relief. Dioven then dried, dry powder is calculated as the yield. Once the powder is dried and direfluks dikerik for 1 hour and dievaporasi use rotar evaporator to make it a thick liquid, then evaporated on a waterbath to extract thick

Pharmacological Test

Experimental animals used in this study were male chickens weighing 1.5 kg. experimental animals first adapted to the environment for 7 days. Chicken uric acid increased (made hiperucemia) by way of chicken liver juice for 14 days 2 times a day for peroral and on the 15th day is done checking uric acid increased and given medication. The checking is done of uric acid in the II period. Period I made a check at 30 minutes after treatment and period II performed at 60 minutes after treatment. Experimental design was used with the group treated with the negative control group and comparing the levels of uric acid was treated before and after being given treatment.

Induction Juice Chicken Liver

Used chicken liver juice concentrate 100% 5 ml / kg body weight morning and evening for 2 weeks.

Data Analysis

Difference form of the data obtained decrease levels of uric acid. statistical test data with the Mann-Whitney test and ANOVA. Results and discussion determination of group.

Identification is done to prove that the powder used in this study is shy daughter herbal powder and leaves yams. Microscopic identification is done in Laboratory Faculty of Biology University of Ahmad Dahlan. Based on the results obtained that the identification of the powder used in this study are herbal shy daughter (Mimosa pudica L.) and cassava leaves (Manihot utilissima Pohl.).

Extract Yield

Manufacture of ethanol extracts of herbs and leaves shy daughter performed in laboratory yams Phytochemistry Faculty of Pharmacy University of Ahmad Dahlan. Obtained extract is made using maceration method ie with stirring and soaking liquid extractor rhizome powder that is 96% ethanol. Ethanol to semipolar compounds have properties that can be extracted semipolar nature. A part from that mold and bacteria grow in the privacy of 20% ethanol, ethanol can be mixed with water in a variety of comparisons, as well as a good solvent for hydrophilic and lipophilic compounds. Extraction method used is maceration. This method is done by soaking the plant material that has been refined / milled in the selected solvent, and then stored in a certain period in a dark place. Gain maceration method is simple, and cheap. Maceration method that is also a lack of a long time (Kiranmai *et al.*, 2011). Extracts tested qualitatively using UV spectrophotometer to perform a scan on the lambda max produced from the extract.

ISBN : 978-979-18458-6-1

Measurement of uric acid

After administration of ethanol extracts of herbs and leaves shy daughter yams, do check levels of uric acid. Effect of ethanol extracts of herbs and leaves shy daughter yams seen from a decrease levels of uric acid. I average yield reduction of uric acid period after administration of ethanol extracts of herbs and leaves of yam shy daughter on oral test animals can be seen in Table I and Figure 1.

Analysis of the data with the Kolmogorov-Smirnov test showed that the distribution of long data retention mice in this study is not normal. This is demonstrated by the significant value of p = 0.000 (p <0.05) for the six groups. Test of homogeneity (Levene test) also showed the value of p = 0.000 (p <0.05) which means that the distribution of the data to the six groups are not homogeneous. From the results of the tests of normality and homogeneity, then for subsequent data analysis using non-parametric analysis Kruskal Wallis and Mann-Whitney test. Kruskal Wallis test results show that there is no significant difference in the decrease in uric acid group mice between 3 and 4, 3 and 5, 4 and 5. This is indicated by the value of significance (p>, 05). Then performed Mann-Whitney test to compare differences between groups long retention results can be seen in Table II.

Based on the results of statistical tests in healthy controls compared with the control group of drugs (allopurinol), control herbaceous shy daughter and gained control of cassava leaf significance <0.05, which means there is a significant difference in lowering uric acid to the group. Whereas in the control group with the control group herbal medicines shy daughter gained significance> 0.05, which means there is no significant difference in lowering uric acid in both groups. Control the drug can lower uric acid by 4.93 mg / dl (63%), ethanol extract shy daughter herb can lower uric acid by 1.53 mg / dl (21%).

On drug control group with the control group gained significance cassava leaves> 0.05, which means there is no significant difference in lowering uric acid in both groups. Control medication (allopurinol) can lower uric acid by

Table I. Uric acid reduction of the period I

Group	Dose (mg/kg body weight)	Average Uric Acid mg/dl ± SD		
		Uric Acid Level	Reduction Of Uric Acid	Margin decline
Normal	-	3,16±0,51	2,90±0,45	0,26±0,05
Sick	-	8,73±3,25	8,40±3,30	0,30±0,05
Allopurinol	10	7,73±0,66	2,80±0,17	4,93±0,83
Shy daughter herba	93	7,43±2,11	5,90±2,16	1,53±0,71
Cassava leaves	93	6,56±2,10	5,00±1,68	1,43±0,61

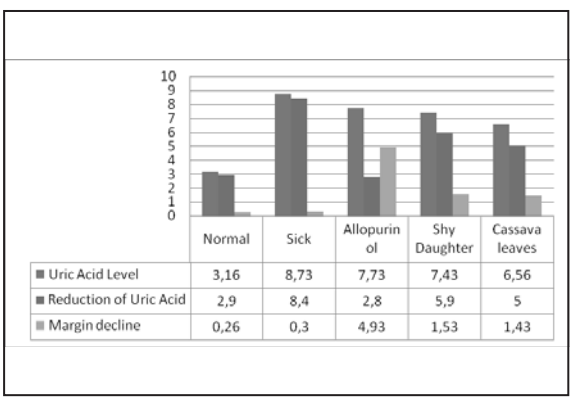

Figure 1. Histogram purata decrease uric acid period I

Table II. Mann-Whitney test results decreased uric acid period I

Compared Group	Significancy	Results	Mean
1 vs 2	0,197	No significant	1 = 2
1 vs 3	0,046	Significant	1 < 3
1 vs 4	0,046	Significant	1 < 4
1 vs 5	0,046	Significant	1 < 5
2 vs 3	0,046	Significant	2 < 3
2 vs 4	0,046	Significant	2 < 4
2 vs 5	0,046	Significant	2 < 5
3 vs 4	0,050	No Significant	3 = 4
3 vs 5	0,050	No Significant	3 = 5

4.93 mg / dl (63%), ethanol extract of cassava leaves can lower uric acid of 1.43 mg / dl (21%).

Based on the results of statistical tests in healthy controls compared with the control group of drugs (allopurinol), control herbaceous shy daughter and gained control of cassava leaf significance <0.05, which means there is a significant difference in lowering uric acid to the group. Whereas in the control group with the control group herbal medicines shy daughter gained significance> 0.05, which means there is no significant difference in lowering uric acid in both groups. Control the drug can lower uric acid by 4.93 mg / dl (63%), ethanol extract shy daughter herb can lower uric acid by 1.53 mg / dl (21%). On drug control group with the control group gained significance cassava leaves> 0.05, which means there is no significant difference in lowering uric acid in both groups. Control medication (allopurinol) can lower uric acid by 4.93 mg / dl (63%), ethanol extract of cassava leaves can lower uric acid of 1.43 mg / dl (21%).

Hyperuricemia control group with the control group cassava leaves gained significance> 0.05, which means there is a significant difference in lowering uric acid in both groups. Control of herbal extracts shy daughter can lower uric acid by 1.53 mg / dl (21%), ethanol extract of cassava leaves can lower uric acid of 1.43 mg / dl (21%). Qualitative test results generated from scans lambda max in ethanol herb extract shy daughter and cassava leaves indicate the presence of flavonoid compounds. Ability to reduce uric acid produced

Table III. decreasement uric acid period II

Group	Dose (mg/kgBW)	Average Uric Acid mg/dl ± SD		
		Uric Acid Level	Reduction Of Uric Acid	Margin decline
Normal	-	3,43±0,15	2,86±0,34	0,56±0,05
Sick	-	8,53±3,05	7,90±3,03	0,60±0,05
Allopurinol	10	7,73±0,66	2,36±0,32	5,36±0,40
Shy Daughter Herbs	93	7,43±2,11	5,73±2,24	1,70±0,60
Cassava Leaves	93	6,80±2,51	5,36±2,46	1,43±0,30

ISBN : 978-979-18458-6-1

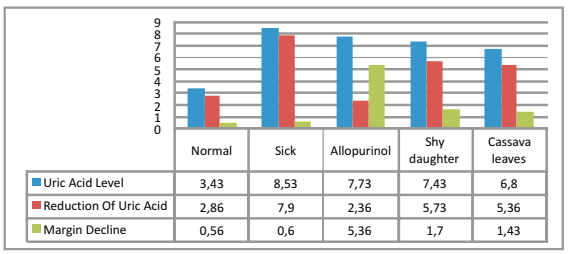

Figure 3. Histogram purata period II decreased uric acid

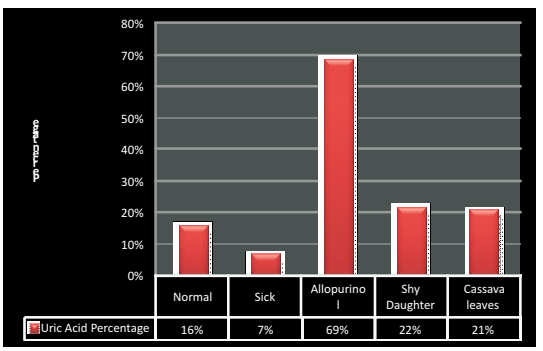

Figure 4. Histogram of the percentage difference in uric acid decreased during the period of II

by the ethanol extract of herbs and shy daughter cassava leaves made possible due to the activity of the flavonoid compounds.

Results Uric Acid Measurement Period II

Uric acid measurement period II performed at 60 minutes after treatment meant to know are there any decrease in uric acid levels after the first period at the time of 60 minutes.

Kolmogorov-Smirnov test results data is the difference in reduction of uric acid in this study are normally distributed with (p> 0.05), whereas the acquired Levene test (p> 0,05). Subsequent data analysis using parametric analysis one way ANOVA post hoc test followed SLD. In the ANOVA significance value of 0.000 (p <0.05), which means that there are significant differences in the numbers purata difference between groups decreased uric acid. LSD test is then performed to determine differences in error rate of each rat kelompok.Hasil LSD test are shown in Table IV.

Based on the results of statistical tests in healthy controls compared with the control group of drugs (allopurinol), control herbaceous shy daughter and gained control of cassava leaf significance <0.05, which means there is a significant difference in lowering uric acid to the group. Whereas in the control group with the control group herbal medicines shy daughter gained significance <0.05, which means there is a significant difference in lowering uric acid in both groups. Control the drug can lower uric acid by 5.36 mg / dl (69%), ethanol extract shy daughter herb can lower uric acid by 1.70 mg / dl (22%).

On drug control group with the control group gained significance cassava leaves <0.05, which means there is a significant difference in lowering uric acid in both groups. Control the drug can lower uric acid by 5.36 mg / dl (69%), ethanol extract of cassava leaves can lower uric acid of 1.43 mg / dl (21%).

Hyperuricemia control group with the control group cassava leaves gained significance> 0.05, which means that each contained a significant difference in lowering uric acid in both groups. Control of herbal extracts shy daughter can lower uric acid by 1.70 mg / dl (22%), ethanol extract of cassava leaves can lower uric acid of 1.43 mg / dl (21%).

Qualitative test results generated from scans lambda max in ethanol herb extract shy daughter and cassava leaves indicate the presence of flavonoid compounds.

Ability to reduce uric acid produced by the ethanol extract of herbs and shy daughter cassava

Table IV. LSD test results difference decreased uric acid period II

Compared Group	Significancy	Results	Mean
1 vs 2	0,822	No significant	1 = 2
1 vs 3	0,000	Significant	1 < 3
1 vs 4	0,003	Significant	1 < 4
1 vs 5	0,013	Significant	1 < 5
2 vs 3	0,000	Significant	2 < 3
2 vs 4	0,004	Significant	2 < 4
2 vs 5	0,020	Significant	2 < 5
3 vs 4	0,000	Significant	3 > 4
3 vs 5	0,000	Significant	3 > 5
4 vs 5	0,377	No Significant	4 = 5

leaves made possible due to the activity of the flavonoid compounds.

CONCLUSION

Based on the results of this study concluded that:

1. Ethanol extract herbal shy daughter dose 93 mg / kg and ethanol extract of leaves of cassava dose 93 mg / kg body weight can lower uric acid induced in chicken liver chicken juice.
2. Purata decrease uric acid levels obtained by the ethanol extract herbal shy daughter dose 93 mg / kg by 1.70 mg / dl (22%), cassava leaves ethanol extract dose 93 mg / kg BW of 1.43 mg / dl (21%)

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THE EFFECT OF INFUSA OF *Zingiber officinale* ROXB TO THE IBUPROFEN TABLET BIOAVAILABILITY IN MALE RABBITS

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Background. *The tendency to use of herb drug simultaneously with synthetic drug may result in an interaction. Red ginger contains gingerol and shagaol proven to increase gastric emptying time and have antiplatelet activity, which causes the blood becomes more dilute, the heart of the work is lighter, so the smooth blood flow, which in turn may affect the bioavailability of furosemide.*

Objective . *This study aims to determine the difference between the effect of 2 hours before, 4 hours after coadministration with infusa and red ginger (Zingiber officinale Roxb.) on the bioavailability of the tablet furosemida in male rabbits.*

Method. *The cross over design with the same subject was used in this study. The samples consisted of 80 mg furosemide with 0%b/v, 5%b/v, 7,5%b/v and 10%b/v red ginger infusa, 2 hours before administration furosemida tablet, 4 hours after administration of tablets furosemida. Blood sampling is done throught marginalis vein and performed at the 0,5; 0,75; 1; 1,5; 2; 3; 4; 6; 8; and 24. Furosemida levels in blood plasma using the Kelly's method modified by Hakim. Bioavailability parameters are determined directly from the graph the relationship Cp vs t while the AUC determined using the trapezium method.*

Outcome measurement. *The results then were used to evaluated the bioavailability parameters: $t_{maks,}$ $Cp_{maks,}$ and $AUC^{o-\infty}$ and were analyzed using ANOVA test in the same way as well as 95% trust standart.*

Result. *Value $Cp_{max,}$ from treatment respectively for 43.2 ug / ml, 58.3 μg / ml, 182 μg / ml, and 119 μg / ml. Tmax value of treatments A, B, C, and D, respectively for 5.4 hours, 5.2 hours, 5 hours, and 3.6 hours. Value $AUC^{o-\infty}$ of treatments A, B, C, and D, respectively for 668 μg.jam / ml, 867 μg.jam / ml, 1808 μg.jam / ml, and 1459 μg.jam / ml.*

Conclusion : The results showed that infusa red ginger (Zingiber officinale Roxb.) with a variation of the timing of 2 hours before, 4 hours after, and together with the provision may affect the bioavailability of the tablet furosemida, where prices Cp_{maks} and AUC on all three treatments is greater than the control and in each treatment there were significant differences ($P < 0,05$), while the price tmaks did not differ significantly with control or equal to controls ($P > 0,05$).

Key words : *Furosemida, red ginger (**Zingiber officinale** Roxb.) Bioavailability.*

ISBN : 978-979-18458-6-1

INTRODUCTION

Millions of people use herbal medicine in conjunction with synthetic drugs without the doctor's recommendation (Gohil and Patel, 2007). The general public thinks, herbal medicines can reduce the side effects of drugs taken and can improve the effectiveness of treatment (Inamdar *et al.,* 2008). Although considered natural, many medicinal herbs that can interact with other medications causing harmful side effects and or reduce the benefits of the drug (Gohil and Patel, 2007).

The number of pharmacologically active compounds in herbal medicine, the interaction is likely to increase. Theoretically herbal drug interactions with synthetic drugs is higher than synthetic drugs because the interaction of two synthetic drugs usually contain only a single chemical constituents (Izzo, 2004). The use of herbal medicine in conjunction with synthetic drugs is generally not supervised by the physician or practitioner of herbal medicine, it can result in harm to the patient, if they use herbal remedies and potential drug interactions sintetiknya have. This interaction is generally not known until the patient is experiencing pain or serious incident occurs that threatens the patient's life (Gohil and Patel, 2007).

Herbs can interact with medications through pharmacokinetic interactions and synthetic or pharmacodynamic (Rodda et al., 2010). Pharmacokinetic interactions lead to changes in absorption, distribution, metabolism or excretion of synthetic drugs or herbal medicines that can affect drug action quantitatively. Pharmacodynamic interactions qualitatively affect drug action, either through enhancing effect (synergistic or additive action) or antagonist effects (Gohil and Patel, 2007). Interactions between herbal medicines and antibiotics, such as ginger and metronidazole, may increase the toxicity of metronidazole, due to ginger lowers kliren and elimination of metronidazole (Okonta *et al,* 2008). On the other hand, according to Young *et al,* (2006) Ginger has a synergistic effect given together with nifedipine in inhibiting platelet aggregation in hypertensive patients.

Furosemide chosen as a model drug in this study because it is one powerful diuretic drug that is the first line treatment of hypertension according to JNC VII level I (Chobanian *et al,* 2003). Furosemide categorized as BCS class II drug because of poor solubility. Furosemide has a dose-response curve is steep and high protein binding, ie by 95% so that furosemide as a potential drug interactions with objects on drugs, drugs with food (Mc Evoy, 2002). Red ginger contains gingerol and shogaol proven to increase gastric emptying time and have antipletet activity, which causes the blood becomes more dilute, the heart of the work is lighter, so the smooth blood flow, which in turn can affect the bioavailability of furosemide tablets.

MATERIALS AND METHODS

The main material used is a 9 month old red ginger obtained from Bantul area. The chemicals used were pa furosemide powder, furosemide tablets (Indofarma), ethyl acetate pa (E Merck), HCl pa (E Merck), phosphate buffer pH 8 (NaOH and KH2PO4), NaOH pa, heparin (Invicolt), distilled water (Asia Lab.), local strain male rabbits aged 3-4 months weighing 1.5-2 kg (CV <5%). This study used 5 male rabbits of local strain (n = 5) weighing between 1.5-2 kg (CV <5%), were studied using a cross-over design (Table I) with 6 kinds of treatment.

Before the treated rabbits were fasted for 1 day. For each treatment was given 7 days washout (t1 / 2 preliminary experiments (control 1) is 8.15 hours). After drug administration, blood was collected at 0.5 hours, 0.75, 1, 1.5, 2, 3; 3.5; 4; 8 and 24, via Marginal ear vein of rabbits. Blood collected in tubes that had been given heparin ependrof. The blood levels of furosemide in accordance with the method set out Kelly et al. modified by the Judge (1996). Blood plasma (250 mL) plus 0.1 N HCl (50 mL) and then mixed disari with ethyl acetate (3.0 mL) using a vortex for 2 minutes. The organic layer

Table I. The design of Cross Over in determining the bioavailability of the furosemide tablet infusion on coadministration of red ginger

Rabits	Week					
	I	II	III	IV	V	VI
1	F0	F1	F2	F3	F4	F5
2	F1	F2	F3	F4	F5	F0
3	F3	F4	F5	F0	F1	F2
4	F4	F5	F0	F1	F2	F3
5	F5	F0	F1	F2	F3	F4

F0: furosemide 80 mg tablet (control).

F1: Giving with 5 mL of red ginger infusion 5% with furosemide tablets.

F2: Giving with red ginger infusion 5 mL of 7.5% with furosemide tablets

F3: Giving red ginger infusion 5 mL 10% 2 hours before furosemide tablets

F4: Giving with red ginger infusion 5 mL 10% with furosemide tablets.

F5: Giving red ginger infusion 5 mL 10% 4 hours after furosemide tablets Note: delivery volume of 5 mL infusion administered orally.

(2.0 mL) were taken, added to it a solution of 0.1 M phosphate buffer pH 8 (2.50 mL), and then in-vortex for 2 min and in-centrifuged (2500 rpm, 10 min). Buffer layer is taken 2.0 mL acidified with 0.5 N HCl (1.0 mL). Subsequently the solution was measured at a wavelength of excitation intensity and maximum emission using spectrofluorometer (Hitachi F 2500).

Determination of bioavailability parameters of furosemide

Bioavailability parameters include tmax, Cpmaks, and $AUC^{0-\infty}$ obtained directly from the curve relationship between drug concentration and time, and $AUC^{0-\infty}$ obtained by the trapezoidal method.

RESULTS AND DISCUSSION

Determination of furosemide levels in the blood carried by spectrofluorometer that had been previously validated. The result of the excitation and emission wavelengths of furosemide in blood plasma obtained respectively 273 nm and 408 nm. Linear regression equation to calculate the levels obtained: Y = 1.7448 X + 14.5463, with a correlation coefficient of 0.9970 is greater than r table 0.754 (n = 9 and 95% confidence level). Recovery value of 104.84 ± 1.3 to 118.53 ± 2.42%. Value of plasma levels of furosemide in blood at any given time are presented in Table II.

With fluctuating results in Figure 5 has area under the curve (AUC) was determined using the trapezoidal method, the average of the levels obtained, while t_{max} and Cp_{maks} directly from the graph of the results of the assay furosemide in blood plasma for each treatment. More results can be seen in Table III.

From previous experiments on the effect of food on the bioavailability of furosemide, said that the food does not significantly affect the bioavailability of furosemide (Kelly et al, 1974). Unlike the other experiments (McCrindle et al, 1996) that furosemide 40 mg were given and analyzed using HPLC, the food may decrease the

ISBN : 978-979-18458-6-1

Table II. Value of furosemide levels in blood plasma (purata ± SE)

Time (hours)	Consentration (µg/mL) ±SE					
	F0	F1	F2	F3	F4	F5
0	0	0	0	0	0	0
0.5	10.08±3.99	9,71±3.59	16.55±3.10	8.12 ± 0.41	20.13±4.57	38.69 ± 4.72
0.75	12.64±10.87	12,92±3.74	19.33±2.95	8.47 ± 0.64	28.10±5.02	49.76 ± 4.53
1	14.73±4.11	17.77±5.02	22.14±3.47	11.92 ± 1.55	40.54±9.90	53.67 ± 6.82
1.5	25.03±9.23	25.53±10.24	40.89±12.88	14.48 ± 2.37	55.87±15.71	64.84 ± 7.59
2	25.67±9.24	31.12±13.17	49.32±14.30	22.97 ± 1.80	76.32±20.93	77.77 ± 6.30
3	38.84±15.04	47.53±16.76	64.74±14.02	37.25 ± 3.77	100.78±22.27	104.79 ± 11.17
3.5	45.07±14.91	41.27±11.02	72.77±17.36	46.84 ± 2.03	119±19.34	105.65 ± 5.28
4	50.97±14.53	47.99±12.32	82.35±20.28	56.22 ± 5.53	121.85±17.67	71.52 ±4.54
8	58.97±11.27	61.61±9.41	59.57±16.69	44.16 ± 5.29	128.05±29.23	55.52 ± 6.22
24	13.52±2.09	11.13±3.75	9.43±3.34	10.2 ± 1.73	13.26±4.34	18.57 ± 0.79

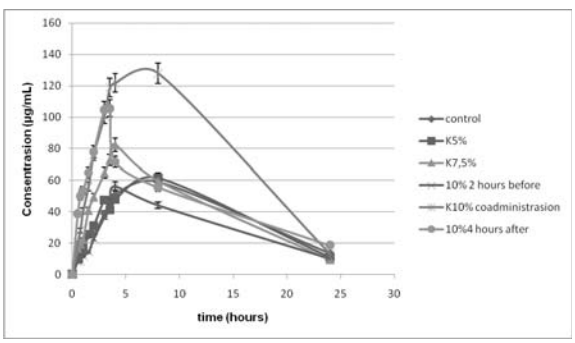

Figure 1. Furosemide concentration curve (purata ± SE) in plasma as a function of time, with each male rabbits treated orally at a dose of 80 mg.

bioavailability of furosemide by 30% and other studies also suggested breakfast can decrease the bioavailability of furosemide by 30%, but is not affected by the long Gastric emptying time (Beerman & Midskov, 1986).

From the results of research conducted, to see the effect of ginger rhizome infusion concentration given that the concentration of 5%, 7.5%, and 10% w / v with furosemide, the price obtained is obtained from the smallest tmax furosemide treatment concentration of 7.5%, which is 4 , 4 hours. This indicated that the coadministration treatment of red ginger infusion concentration of 7.5%, furosemide is absorbed more quickly, made possible because the content of oleoresin of red ginger (gingerol and shogaol) (Yamahara *et al,* 1990) increases the speed of gastric emptying (Hu *et al,* 2011). Speed affects the rate of gastric emptying unstable drug degradation in the stomach (Devissagoet, 1982).

Area Under the Curve ($AUC^{0-\infty}$) largest obtained from coadministration treatment of red ginger infusion concentration of 10%, ie 1996.46 ug / mL.jam, this is due to the possibility of active substance (gingerol and shogaol) highest, which is known to serves as an antiplatelet (Young *et al,* 2006). Next will cause the blood thinner, lighter work of the heart and blood flow to be smooth, so that the displacement of furosemide from the intestine into the systemic circulation has increased.

The maximum concentration (Cp_{maks}) mainly from coadministration treatment of red

ISBN : 978-979-18458-6-1

Table III. Purata furosemide bioavailability parameter values ??in male rabbits

Bioavailability Parameters	Treatment					
	F0	F1	F2	F3	F4	F5
Cpmaks (µg/mL)	61,36± 12,77	70,07±11,48#	87,45±18,05#	58,3 ± 3,9#	154,28±23,49*	119 ± 7,3*
tmaks (Jam)	7,2± 0,80	6,2± 1,11#	4,4± 0,98#	5,2 ± 0,4#	5,5± 1,02#	3,6 ± 0,6*
AUC $0^{-\infty}$ (µg/mL.jam)	1186,26± 150,45	1071,75± 155,22#	1096,58± 194,28#	867 ± 61#	1996,46± 290,08*	1459 ± 64#

Remark s: * No significant difference (p <0.05) with the control treatment
No significant difference (p> 0.05) with the control treatment

ginger infusion concentration of 10%. Cpmaks will increase with an increase in $AUC^{0-\infty}$, because the concentration of the drug will show the amount of drug that will be absorbed by the body.

From the results of the study to see the effect of the timing of the price obtained furosemide smallest tmax obtained from 4 hours after treatment administration is 3.6 hours. Area Under the Curve ($AUC^{0-\infty}$) largest obtained from coadministration treatment of red ginger infusion with furosemide tablet, which is 1996.46 ± 290.08 mg / ml.jam. The maximum concentration (Cp_{maks}) mainly from coadministration treatment of red ginger infusion concentration of 10% concurrently with furosemide tablet, which is 154.28 ± 23.49 mg / ml.jam.

Decrease or increase of Cp_{maks}, t_{max}, and AUC0-∞ possible because of several factors such as the surface area of the intestinal wall that allows the drug to the colon greater contact, the speed in which delay gastric emptying slows gastric emptying and drug absorption furosemide at acidic pH height / base more in the form of ions while the drug is absorbed in the intestine molecular form (Banker and Rhodes, 1996). The movement of the digestive tract and into the blood stream absorption and decreased movement of the drug from the gut can also affect the amount of drug absorbed, and the presence of the drug with mineral complex will also affect drug dissolution rate and amount of drug absorbed becomes smaller, so that everything is not directly affect the bioavailability of the drug. Not only influenced by it alone, the stability of the drug and the disease causes a decrease in organ function in test animals are not known to also affect drug absorption (Devissaguet, 1982).

These results indicate that infusion of red ginger affect the bioavailability parameters Cpmaks and AUC0-∞, but does not significantly affect the parameters tmax, this can happen due to many factors that affect the bioavailability of a drug, among others, note that furosemide in Biofarmasetika Classification System (BCS) including two classes. Ie drugs that have a high permeability while the low solubility or dissolution. The active substance of red ginger rhizome causing increased permeability. This is due to the active substances in addition to increase gastric emptying time, as well as antiplatelet that is a blood thinner and blood flow more smoothly. If the blood flow smoothly shift from gastrointestinal to systemic furosemide increased.

Since the results indicate that furosemide when administered concomitantly with red ginger infusion significantly cause changes in bioavailability parameters Cp_{maks} and AUC0-∞, then the use of furosemide should be careful when consumed with red ginger infusion.

CONCLUSION

At the conclusion of research conducted as follows:

1. There is no significant difference in the effect of red ginger infusion by 5 ml at a concentration of 5% w / v and 7.5% w / v in conjunction with the bioavailability of furosemide tablets furosemide, but the effect on the concentration of 10% Cp_{maks}, and $AUC^{0-\infty}$.

2. There is no significant difference in the timing of the effect of red ginger infusion 5 ml of 10% w / v two hours before, simultaneously, and 4 hours after, the bioavailability of furosemide tablets.

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SUB CHRONIC EFFECT OF ETHANOL EXTRACT OF NUTMEG (*myristica fragrans* Houtt) SEED IN RAT KIDNEY

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Abstract

Background. *Nutmeg (Myristica fragrans) is original plant from Indonesia has sedative, hepatoprotective, and anti-seizure activities.*

Objective. *The aim of this study was to determine the toxic effects of ethanol extract of nutmeg seed on the rat kidney.*

Methods. *Male rats grouped into 4 groups and each group consists of 7 animals. Group I as the control group 0.5% CMC-Na. Group II, III, and IV were given ethanol extract of nutmeg dose of 50 mg / kg, 100 mg / kg, and 200 mg / KgBW respectively. Samples were administered for 31 days, on the 32th day was taking blood from the orbital sinus eye for BUN, creatinine assay and histopathology examination.*

Outcome measured. *BUN, creatinine, and histopathological profile*

Results. *The results showed all doses of ethanol extract of nutmeg seed causes an increase BUN levels. While on the creatinine levels between the control group and the treatment group showed no significant difference. Histopathological examination showed no change in renal morphology was given ethanol extract of nutmeg seeds.*

Conclusion. *The conclusions this research that the ethanol extracts of nutmeg (Myristica fragrans Houtt) seed did not cause toxic effects on the kidney.*

Keyword : *Biji Pala (**Myristica fragrans** Houtt.), BUN, Creatinine, Histopathological morphology*

ISBN : 978-979-18458-6-1

INTRODUCTION

Nutmeg (Myristica fragrans) is original plant from Indonesia, which originated from the archipel Malaise Banda and Maluku islands then spread and grow into islands such as Sumatera, North Sulawesi, and Papua. Nutmegs including Family Myristicaceae grow to five genuses of 250 species (Drazat, 2008). Olaleye *et al.* (2006) reported nutmeg seed has antioxidant activity, anti diarrhea, carminative and stimulant. Also has activity as sedative (Sonavane *et al.*, 2001), anti microba (O'Mahony *et al*., 2005), anti depressant (Dhingra and Sharma, 2006), anti diabetic (Han *et al*., 2008), aphrodisiaca (Tajuddin et al., 2005), *cytotoxicity* (Lee *et al*., 2005), hepatoprotective (Morita et al., 2003). Nutmeg seed induces apoptosis in leukemia cells (Chirataworn *et al*., 2007), anti inflammatory (Lee and Park, 2011), anti hyperlipidemia (Kareem *et al.,* 2009).Sonavane *et al*. (2002) proved nutmeg seed has anticonvulsant activity. Ethanol extract of nutmeg has anti-seizure activity in mice-induced pentylenet etrazoles (Saiful bachri,M.and yuliani,S., 2011). Nutmeg has pharmacological activity, also has toxic effect. Aromatic compounds myristicine, and safrole elimycine are found 2-18% in seeds and are inducing hallucinations. Maximum consumption of 5 grams of powder or nutmeg oil lead poisoning is characterized by vomiting, headache and dry mouth (Nurdjannah, 2007). Myristicine and elimycine have toxic effect (Jukic *et al.*, 2006).

Materials or medicinal compounds of plant or animal must go through a series of pre-clinical tests such as pharmacology tests, toxicology testing and clinical trials for use in formal treatment. One of the toxicology tests are subchronic toxicity tests to get information of potential toxicity when used in long term. Among much toxicity evaluation parameters are biochemical parameters such as creatinine and blood urea nitrogen are useful to evaluate the condition of the kidney (Henry JB. 2001,).

It is necessary to study sub-chronic administration of ethanol extract of nutmeg and investigated the kidney histopathology.

MATERIALS AND METHODS

Animals

Male wistar (SD) (200±10 g) rats were purchased from Gadjah Mada university. All animals were maintained in the institutional animal facility. Animals were acclimatized for a week before starting the experiments with condition, light/dark cycle: 12 hr, in university animal room and with free access to rodent food and water ad libitum throughout the experimental period.

Preparation of the extract

Nutmeg seed powder was purchased from a Beringharjo market in Yogyakarta Indonesia. Samples were processed into extract in Biology Pharmacy Department of faculty of Pharmacy, Ahmad Dahlan University Yogyakarta, Indonesia. The 3 kg of powder dried samples was dissolved three times in 6 liters of ethanol for 3 days, filtered, and evaporated to obtain the crude ethanol extract (25 g). The crude ethanol extracts freeze dryer for 3 days to get 15 g dried powder.

Animal Groups and experimental treatment

Animals were divided into four groups with seven animals in each group; Group I, Control rats treated with the vehicle only (CMC-Na 0.5%); Group II, rats treated with EtOH extract of Nutmeg Seed (50 mg/kg); Group III, rats treated with EtOH extract of Nutmeg seed (100 mg/kg); Group IV, rats treated with EtOH extract of Nutmeg seed (200 mg/kg). On day 32th, take the blood from orbital sinus for analysis of creatinine and BUN activities with spectrophotometer. Then the rats sacrificed, take the kidney for histopatological examination. Histopathologic examination was performed in Pathology Laboratory Animal of Veterinary medicine faculty, Gadjah Mada University to

determine the possibility of damage to the kidney.

RESULT AND DISCUSSION

BUN dan Creatinine Analysis

Table I showed the average BUN levels between the control group and the treatment group there is a difference or increasing. Increased BUN levels can be caused by loss of extracellular fluid and plasma volume (such as bleeding, shock, excessive vomiting, lack of water, salt), which decreased glomerular filtration rate but no kidney disease. BUN levels can also be increased with an increase in protein catabolism as occurs in the gastrointestinal tract bleeding or tissue (Aslam 2003). Another factor that led to increased BUN levels, the lack of nutrients and hepatotoxicity are common effects of some toxicant (Lu, 2010).

Increased levels of BUN in nutmeg ethanol extract at a dose of 100 mg / kg and 200 mg / kg body weight may be due to damage to the kidneys according to existing studies showed elevated levels of BUN at a dose of 400 mg / kg - 1000 mg / kg (Eweka, *et al.*, 2010; Olaleye *et al*., 2006). Whereas at a dose of 50 mg / kg had no increasing effect when compared with controls.

Urea is the final product of amino acid catabolism. In the process of solving the amino acids will form ammonia compounds that are toxic to the human body. Furthermore ammonia will be converted into non-toxic compounds, namely in the form of urea through the urea cycle formation. Urea in the blood will be reabsorbed into the renal medulla and immediately excreted through the urine (Poedjiadi *and* Supriyanti 2006). The presence of urea in the blood (calculated as Blood Urea Nitrogen, BUN) and urea in the urine can be used to determine the effectiveness of renal function (Lu, 2010). On the condition of impaired renal function, plasma urea concentration increases because of the decrease in glomerular filtration process (Anonymous, 2006b).

Table I show that the average creatinine levels between the control group and the treatment group showed no significant difference. Normal levels of creatinine in mice are about 0.2-0.8 mg / dl (Malole and Pramod, 1989). The result on creatinine, the average levels of creatinine showed all the groups are still in the normal range. Creatinine is a metabolite of creatine and excreted entirely in the urine via glomerular filtration. Thus, increased levels of creatinine in the blood are an indication of damage to kidney function (Lu, 2010). Serum creatinine is considered to be more sensitive and specific indicator of kidney disease compared with BUN test. The increase in creatinine levels themselves are not affected by the intake of food or drink (Kee, 2008).

The results of this research of BUN and creatinine levels can be seen that the ethanol extract of nutmeg seed cause elevated levels of BUN in the dose group 100 mg / kg and a dose of 200 mg / kg compared with the control group (CMC-Na), whereas serum creatinine levels did not increase or decrease that considered normal. Increased levels of BUN with normal creatinine levels usually become clues to the cause of non-renal uremia. Increased levels of BUN values could be due to loss of extracellular fluid and plasma volume as lack of water or salt and can also occur due to increased protein catabolism that occurs in the gastrointestinal tract or body tissues (Aslam, 2003).

Synthesis of creatinine is relatively constant, therefore the blood creatinine levels can describe of renal creatinine clearance. Creatinine excretion was excreted through glomerular filtration process of kidney. If serum creatinine levels rise mean creatinine clearance decreased (Wildmann, 1995).

The research shows that the value of normal creatinine levels, not an increase, so that the ethanol extract of nutmeg is subchronic for 31 days did not affect renal function, seen from the blood chemistry parameters levels of urea and creatinine values, because the normal creatinine levels indicate that the kidneys are also normal. creatinine clearance suggests that

ISBN : 978-979-18458-6-1

Table I. Mean ± SD of BUN dan Creatinin Level caused nutmeg seed administration

Group	Dose (mg/KgBW)	BUN (mg/dl)	creatinine (mg/dl)
Kontrol	CMC-Na	39.47 ± 4.24	0.8 ± 0.15
EtoH extr.	50	42.95 ± 3.92	0.8 ± 0.16
	100	45.54 ± 2.12 *	0.7 ± 0.12
	200	44.88 ± 3.84 *	0.8 ± 0.14

* different compare with control group p<0.5

the glomerular filtration rate. The Circumstances where the normal creatinine levels while increasing BUN levels can be caused by lack of fluids in the body or dehydration (hypovolemia) (Kee, 2008).

Histopathological examination showed no change in renal morphology was given ethanol extract of nutmeg seeds (picture not shown).

The Conclusions this research that the sub chronic administration of ethanol extracts of nutmeg (Myristica fragrans Houtt) seed did not cause toxic effects on the kidney.

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ANTICONVULSANT EFFECT OF ETHYL ACETATE FRACTION AND UNSOLVED ETHYL ACETATE FRACTION FROM SIRSAK LEAF (*Annona muricata*, L.) ON PENTYLENTETRAZOL INDUCED IN MICE

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Background. *Ethanol extract from sirsak leaf (**annona muricata** L.) reported have anticonvulsant activities. For studying it deeply ethanol extract must do fractination by using ethyl acetate, thus resulting ethyl acetat fraction and unsolved ethyl acetat fraction.*

Objective. *This research aimed to know about anticonvulsant activities from each fraction.*

Methods. *It research conducted by using mices which divided is to 8 groups, each groups consist 7 mices which pentylentetrazol induced 90 mg/kg bw. Doses ethyl acetate fraction Groups (FE) are 100, 200, 400 mg/kg bw, and unsolved ethyl acetate fraction groups are 100, 200, 400 mg/kg bw. Control group just added Na CMC 0.5% and comparation group added by Phenobarbital dose 50 mg/Kg Bw by using peroral technique. Both ethyl acetat and unsolved ethyl acetat fractions were given quantificacy test of total flavonoid content by Chang method, calculated as quercetin. All data should be analyzed by Kruskal Wallis test which continued with Man-Whitney by using reliable level 95%.*

Outcome measured. *The parameters anticonvulsant activities are clonic and tonic onset, clonic and tonic incidence , clonic frequency, time and incidence of mortality.*

Results. *All fractions delayed the onset of tonic and reduced tonic and mortality incidence. The most decrease of mortality incidence in FE400, while longest delayed the onset of tonic in FT400. The latency of mortality possessed in FE400, FT100, FT200, FT400 with the higest at FT400. Total flavonoid of FE is 1,31% and FT is 2,6%.*

Conclusion. *In conclusion, that all fractions are appeared have anticonvulsive activities mean while not yet equal Phenobarbital in dose material treatment.*

Keywords : *Annona muricata, Ethyl acetat fraction, unsolved ethyl acetat fraction, Anticonvulsant, pentylentetrazol*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Epilepsy is one of the most common serious neurological disorders. Treatment with anti-epileptic drugs (AEDs) is generally chronic even life long. The drugs are synthetic molecules that exert serious adverse effects, such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity, and megaloblastic anemia (Namara, 2003). Plant extracts can be an important source for the development of better and safer drugs for the treatment of epilepsy. The plant material can be selected, such as sirsak (*Annona muricata*). The plant has been used medicinally in many tropical African countries for an array of human ailments, especially for parasitic infections and cancer. It has also been used in some African herbal medicine systems for its sedative, antispasmodic and convulsive seizure properties (Taylor, 2002). N'Gouemo *at al*. (1997) have reported that ethanol extract from sirsak leaf (*annona muricata* L.) has anticonvulsant activities. For studying it deeply ethanol extract must do fractination by using ethyl acetate, thus resulting ethyl acetat fraction and unsolved ethyl acetate fraction. This research aimed to know about anticonvulsant activities from each fraction.

METHODS

Material

Fresh sirsak leaves are dark green obtained from Kuningan in September 2011. Sirsak leaf were identified in the laboratory of Plant morphology, Department of Biology, Ahmad Dahlan University Yogyakarta. Comparative material, used phenobarbital tablets (KF) obtained from the Apotek Mitra Bahagia Cirebon, Na CMC obtained from the Laboratory of Pharmaceutical UAD. Pentylentetrazol (PTZ) from Sigma Co.

Animal

Healthy, male Balb C mice (*Mus domesticus*) weighing 20-30 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity and light (12-hour day/12-hour night cycle); and were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were broadly divided into 8 groups, each groups consist 7 mices which pentylentetrazol induced 90 mg/kg bw. Doses ethyl acetate fraction Groups (FE) are 100, 200, 400 mg/kg bw, and unsolved ethyl acetate fraction groups are 100, 200, 400 mg/kg bw. Control group just added Na CMC 0.5% and comparation group added by Phenobarbital dose 50 mg/Kg Bw by using peroral technique.

Phytochemical screening

The methods of Culvenor-Fitzgerald (1963) and Simes *et al*., (1995) were used to screen the Annona muricata leaf used in this study for its chemical constituents. The findings of the phytochemical screening are shown in Table 1.

Preparation of extract and fractionation

One kilogram (1 kg) of sirsak leaves was air-dried at room temperature. The plant was milled into fine powder in a blender and then macerated in ethanol 96% and extracted twice, on each occasion with 5 litre at room temperature for 3 x24 h with occasional shaking. The extract was concentrated under reduced pressure in a rotary evaporator at 50^{o}C. The resulting ethanol extract 146 g (14,6 %). The extract was separated into ethyl acetat soluble and insoluble parts. thus resulting ethyl acetat fraction and unsolved ethyl acetat fraction. Furthermore, each faction is made in 3 doses, a dose of 100, 200, and 400 mg / kg.

Thin-layer chromatography

TLC was conducted on sirsak leaf ethanol extract, ethyl acetate fraction, unsolved ethyl acetate fraction. Stationary phase such as silica gel 254 nm, mobile phase BAW upper layer (butanol, acetate, water = 4: 1: 5). Stains used are $FeCl_3$, ammonia vapor, UV light 254 by comparison quercetin.

Standardization of active substances

Standardization of the active flavonoid substances conducted by Chang method with a spectrophotometer Shimadzu UV-1800. Total flavonoid content of ethyl acetate fraction and unsolved ethyl acetate fraction of sirsak leaves is calculated as quercetin.

Evaluation of anticonvulsant property

The anticonvulsant testing method of Amabeoku *et al.*, (1998) and Visweswari *et al.*, (2010) with slight modifications. Each dose administered orally in animal experiments in accordance with the group for 7 days and 0.5% NaCMC as well as controls. Phenobarbital 50 mg / kg given orally on seventh day to 30 minutes before the administration of PTZ. PTZ 90 mg / kg dissolved in physiological saline was given ip. in all groups 30 min after administration of each fraction on the seventh day. Experimental animals were observed for 30 minutes after administration of PTZ. Parameters observed in the form of onset and incidence of clonic, onset, duration and incidence of tonic, clonic frequency, time of death and mortality.

Clonic PTZ seizures were defined as an episode of muscle spasms involving fore limbs with or without the loss of the righting reflex. Tonic PTZ seizures were characterized by an initial ventroflexion followed by full fore limb and hind limb extension (N'Gouemo, *et al.*, 1997).

Statistical analysis

Data were analyzed with the Kruskal Wallis test followed by Mann Whitney test at 95% confidence level.

RESULTS AND DISCUSSION

Results of determination to indicates that the true leaves of the sirsak (*Annona muricata*). Furthermore, phytochemical screening exhibit phenolic compounds are indicated by the formation of a greenish black color by reaction with FeCl3. Flavonoid compounds identified by reaction with Mg and concentrated HCl which gives red color. With shaking formed foam that indicate the presence of saponin compounds. Alkaloids and steroids were not identified, although some studies reported isokuinolin

Table I. hRf value and color spot from four samples

samples	hRf	Color spot without stain	Color spot with UV 254	Color spot with $FeCl_3$	Color spot With amonia
Ethanol	85	Yellow green	Slight green		Yellow brown
extract	76	Yellow green	Slight green		
	65		Slight green		
	23	Yelloe green	purple		yellow
FE	85	Yellow green	green	Black blue	Yellow brown
	76	Yellow green	Slight purple		Slight brown
	65		Slight purple		Slight brown
FT	41	yellow	Purple	Black blue	Yellow
	23	yellow	purple	Black blue	Yellow
kuersetin	76	yellow	green	Black blue	yellow

FE = ethyl acetat fraction

FT = unsolved ethyl acetat fraction

alkaloids and phytosterols (Watt and Breyer-Brandwijk, 1962, cit Adewole and Ojewole, 2009).

TLC

TLC results in the form of the value of the hRf and color spot of the four samples that ethanol extract, ethyl acetate fraction (FE), unsolved ethyl acetate fraction (FT), and quercetin can be seen in Table I.

Table I shows that the FE and FT contain polyphenolic compounds indicated by bluish black spots after being sprayed with FeCl3 (Harborne, 1987). Quercetin as a comparison also shows the same color although with different color intensity. Provision of ammonia vapor produces color yellow spots, yellow brown, and brown weak. This means there are flavonoids at all the sample. According to Harborne (1987), Flavonoids are phenolic compounds, therefore it is the color change when added alkaline or ammonia. The resulting color depends flavonoids. According Leabouf *et al*., (1982), Watt and Breyer-Brandwijk, (1962) cit Adewole and Ojewole, (2009), sirsak leaves contain flavonoid.

Quercetin as a comparison was not detected its presence in all samples, although the value of the HRF there is similarity in the three samples is 76 but of different color spots.

Results of active substances standardization

Measurement results of total flavonoid content of unsolved ethyl acetate fraction is 2.62% and ethyl acetate fraction is 1.31%. Unsolved ethyl acetate fraction is more polar than ethyl acetate fraction. Thus, flavonoids which are water-soluble compounds (Harborne, 1987) more in the unsolved ethyl acetate fraction.

Results of testing the anticonvulsant effect

The test results showed that the anticonvulsant effect of different factions and different doses up to 400 mg / kg BW can not delay the onset of clonic and do not cause a decrease in the incidence of clonic seizures that occur in mice. Based on the results of the Mann Whitney test for the onset of clonic seizures at various doses of the fraction of ethyl acetate and ethyl acetate insoluble fraction showed no significant differences due to the significant value of more than 0.05 ($p > 0.05$) compared to the control group.

Tonic incidence decreased with increasing dose, although the ethyl acetate insoluble fraction doses of 100 and 200 mg / kg have the same percentage. To the onset tonic, giving ethyl acetate fraction and insoluble fraction of ethyl acetate at different doses may prolong the onset of tonic or may delay the occurrence of tonic with a significance value of less than 0.05 ($p <0,05$). Thus, all the fractions showed anticonvulsant activity. According to Adeyemi, *et al.,* (2007) and Ojewole, (2008) the ability of plant extracts to prevent seizures or prolong the onset of tonic seizures indicate anticonvulsant activity. Observation of the duration of the tonic, ethyl acetate fraction dose of 400 mg / kg body weight can significantly shorten the duration of tonic, while the dose of 100 and 200 mg / kg have not been able to reduce the duration of tonic. For the insoluble fraction of ethyl acetate, the shortest duration of tonic occurred at a dose of 200 mg / kg, which is 20.20 seconds, but statistically the results do not differ significantly in comparison with controls. According Kasture *et al*. (2000), the anticonvulsant activity can be demonstrated by a decrease in the duration of tonic in experimental animals. Thus the only anticonvulsant activity shown by the ethyl acetate fraction dose of 400 mg / kg.

The test results showed the number of seizures that occurred an average frequency of seizures that occur higher than the control group than the treatment dose FE 100. However, this value is statistically significantly different from no more significance than 0.05 ($p> 0,05$). So, there was no effect of administration of ethyl acetate fraction and ethyl acetate insoluble fraction soursop leaves the number of seizures that occurred. Thus, the parameter number of

seizures can not provide information regarding the anticonvulsant activity of both fractions.

However, this value is not significant.

Observation of the time of death showed that the ethyl acetate fraction may extend the time of death at a dose of 400 mg / kg bw, while the insoluble fraction of ethyl acetate all treatment doses of 100, 200, and 400 mg / kg bw able to extend the time of death compared to the control. To the number of deaths, compared with the control group, all treatments can reduce the number of deaths caused by mice with PTZ-induced seizures decrease in the number of deaths was highest in ethyl acetate fraction dose of 400 mg / kg. Data of all test parameters are presented in Table II.

Of all the parameters that are used to look anticonvulsant activity, most of the parameters are the parameters of onset, incidence, and duration of tonic, time of death and the number of deaths to support the conclusion that the fraction of ethyl acetate and ethyl acetate insoluble fraction showed anticonvulsant activity.

Anticonvulsant activity of the ethyl acetate fraction and ethyl acetate insoluble fraction thought to be related to the presence of flavonoids in the both fraction. Flavonoids are a large group of plant polyphenols. Flavonoids have a variety of activities, such as antioxidant (Winarsi, 2005). The antioxidative properties due to the position of hydroxyl groups capable of scavenging free radicals. Flavonoid initially oxidized by radicals, then changed to a more stable and less reactive radicals. Thus flavonoids can stabilize reactive oxygen compounds (Korkina & Afanas'ev, 1997 cit Winarsi, 2005). Adewole and Ojewole (2009), reported a decrease in reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS) in rats administered a aqueous extract of leaves of the sirsak, it means there are antioxidant

Table II. Recapitulation of Research Data on multiple anticonvulsants Parameter Testing Activity Induced in Mice PTZ

Groups	clonic onset (sec)	Clonic Inci-dence	Tonic onset (sec)	Tonic Inci-dence	Tonic Duration (sec)	Time of death (sec)	Morta-lity	Frequensi Of seizure
control	92,43 ± 23,28	7/7 (100 %)	266,50 ± 47,91	6/7 (86%)	25,20 ± 3,11	306,17 ± 60,24	6/7 (86%)	2,43 ± 0,79
Fenobarbital	1800*	0%*	1800*	0%	0*	1800*	0%*	0
FE 100	106,14 ± 12,17	7/7 (100 %)	399,00 ± 43,71*	4/7 (57%)	24,25 ± 1,71	424,67 ± 178,00	3/7 (43%)	2,00 ± 0,89
FE 200	86,40 ± 11,46	6/6 (100 %)	564,00 ± 98,99*	3/6 (50%)	19,00 ± 4,36	561,00 ± 470,71	3/6 (50%)	3,75 ± 2,22
FE 400	131,00 ± 39,84	7/7 (100 %)	722,00 ± 83,44*	3/7 (43%)	18,67 ± 3,21*	553,00 ± 185,26*	2/7 (29%)*	3,00 ± 1,22
FE 100	72,00 ± 27,12	7/7 (100 %)	443,67 ± 28,43*	5/7 (71%)	21,00 ± 2,24	908,75 ± 201,78*	5/7 (71%)	3,43 ± 1,13
FE 200	90,57 ± 36,71	7/7 (100 %)	532,00 ± 244,99*	5/7 (71%)	20,20 ± 5,07	831,50 ± 302,97*	5/7 (71%)	4,20 ± 0,45
FE 400	98,67 ± 42,33	7/7 (100%)	976,50 ± 132,23*	3/7 (43%)	21,67 ± 2,08	1134,50 ±37,48*	3/7 (43%)	2,67 ± 1,37

*p < 0,05 to control (PTZ 90 mg/kg bw i.p + CMC 0,5%)

activity of aqueous extracts of sirsak leaves. According Winarsi, (2005) based on its mechanism of antioxidants can be divided into three, namely: primary antioxidants (endogenous antioxidants / antioxidant enzymatic); antioxidant secondary (exogenous antioxidants / antioxidant nonenzimatis) and tertiary antioxidants (antioxidants that act repair biomolecules damaged by free radicals) .

Oxidative stress is an etiological factor in the occurrence of epileptic seizures (Shin *et al.*, 2011) including PTZ-induced seizures. Several studies have demonstrate this. Bashkatova et al., (2003) reported the increase in the formation of nitric oxide (NO) fivefold, and TBARS more than twofold in the brain cortex of rats after administeration of PTZ 120 mg / kg subcutaneously. NO is a free radical with a short half-life. NO is considered a disorder of molecules that play a role in the pathophysiology of Alzheimer's, Parkinson's, stroke, trauma, and seizures. Other studies have shown that administration of PTZ resulted in an increase in malondialdehyde (MDA), and decreased glutathione (GSH) (Mehla, *et al.*, 2010; Ilhan, *et al.,* 2005; Gupta, YK *et al.*, 2003) and increased catalase (Sharma *et al*., 2010). PTZ also known as a selective blocker of the chloride ion complex for GABA-A receptor and cause a decrease in function GABAergik (Ilhan, *et al.*, 2005).

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) such as O_2-*,*OH, *NO cellular level with a higher cellular antioxidant defenses. ROS generation ubiquitous nature as a result of aerobic metabolism such as oxidation in mitochondria. In order to scavenge ROS, different defense systems in the brain in the form of enzymatic, nonenzymatic, and antioxidants in the form of food. If ROS are not effectively eliminated, it can cause oxidative injury is peroxidation of cell membrane phospholipids, proteins (receptors,enzymes) and DNA. Brain tissue is very vulnerable to ROS because, (1) The brain produces very high ROS as a result of aerobic metabolism is high, whereas relatively few enzymatic antioxidant defenses, (2) with a lipid-rich brain is vulnerable to oxidative damage, and (3) DNA nerve damaged in adults ineffective repaired because there is no DNA replication in the brain.

In this study the ethyl acetate fraction showed a stronger anticonvulsant activity mainly on the parameter number of animal mortality. Ethyl acetate fraction dose of 400 mg / kg body weight can reduce the number of mortality dropped 86% in controls to 29%. On the other hand, levels of flavonoids from the ethyl acetate fraction lower than the insoluble fraction of ethyl acetate. Therefore, the estimated potential of antioxidant fraction of ethyl acetate and ethyl acetate insoluble fraction is not the only cause of the anticonvulsant effects of the two fractions.

CONCLUSION

All fractions are appeared have anticonvulsive activities mean while not yet equal phenobarbital in dose material treatment.

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ANTI CONVULSANT EFFECT OF *Centella asiatica* FRACTIONS AND HISTOPATOLOGY STUDY OF LIVER AND KIDNEY

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Abstract

Background. *Seizure is an neurologik issue that relatively much be found. Treatment with conventional chemical remedies are more expensive beside their limited distribution. conventional remedies have a lot of side effects. Therefore, needed to find a new alternative treatment that more safe, effective and selective to suppress seizure as anti convulsant. Pennywort (Centella asiatica (b) urb) has effect as anti convulsant. Ethanol extract of Pennywort herb also known could have sedative effects on mice because it contains with brahminoshide and brahmoshide glycosides using their cholinergic mechanism.*

Objective. *The aim of this research is to know whether ethyl acetat fraction and unsoluble ethyl acetate fraction of pennywort herb can be used as anti convulsant.*

Methods. *This research conducted using mice which divided in eight groups which consisted of 7 mices per group. The classification of the group consists of negative control (suspension of CMC 5 %), ethyl acetate fraction group with dose 100mg/KgBW, 200mg/KgBW, 400mg/KgBW, unsoluble ethyl acetate fraction group with dose 100mg/KgBW, 200mg/KgBW and 400mg/KgBW, and positive control group (fenobarbital 100mg/KgBW). To make convulsion condition, mice induced using PTZ dose 80Kg/BW. This test lasted for 7 days. The result was analyzed by using post hoc test and Mann Whitney method were also used to compare each sample. The significant value was accepted if $P<0.05$.*

Outcome measured. *The parameters of anti convulsant including time of duration. The histopatology tested on liver and kidney.*

Results. *Unsoluble ethyl acetate fraction dose 400 mg/KgBB has the ability to reduce duration time. Histopathology test showed that ethyl acetat fraction dose 100mg/KgBW and unsoluble ethyl acetat fraction dose 400mg/KgBW significantly increase repairment of kidney damage induced by PTZ.*

Conclusion. *Conclusion that not all fractions have the ability as anti convulsant.*

Keywords : *Anti convulsant, Centella asiatica, ethyl acetate fraction, unsoluble ethyl acetate fraction, Phentylenetetrazole (PTZ).*

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ISBN : 978-979-18458-6-1

INTRODUCTION

A seizure is a neurologic issue relatively much be found. Almost 5 % child under 16 years old at least experienced once seizure during his life (Schweich and Zempsky, 1999). Seizure treatment with anticonvulsant drug sometimes affect on cognitive (Aldenkamp *et al.,* 1993). Although it is known that the result of seizures conventional treatment is so beneficial clinically but there has cognitive side effect of the drug at dose of therapy. Because their side effects and the treatment cost, the new treatment options that are safe, effective and selective to suppress seizures is crucial to trying to accomplish.

One of medicine plant which alleged has an effect as anticonvulsant is pennywort (centella asiatica (b.) Urb.). Some studies on the pharmacology effects of pennywort, it is known that those plant indicates the activity as anticonvulsant with the additional benefit of preventing cognitive decline (Gupta *et al.,* 2003).

The ethyl acetat and unsolube ethyl acetat fractions of pennywort against onset time, duration time and the number of seizures, mortality, and also liver and kidney histophatologi on male mice induced PTZ was studied in this reserach. So it can be used as medicine alternative which effective as anticonvulsant drug and can be useful in the development of traditional medicine in Indonesia.

METHODS

Materials and Instruments

Materials: Pennywort herb (*Centella asiatica* (L) Urb), male *Swiss* mice (5-6 weeks) with body weights approximately 25-35 g, ethanol 70%, ethyl acetat, penthilenetetrazole (PTZ) (Sigma Co), phenobarbital (Bratachem) dan CMC Na (Bratachem), NaCl 0,9%, formaldehyde 10%, hematoxilin-eosin stain.

Instruments: milled machine, sieve which mesh number 40, oral injection, vacum rotary evaporator, analytical scale, spectrophotometer UV-1800 Shimadzu.

Sample Preparation

The pennywort powder sifted by using mesh 40 then will be extracted. The process of maceration is carried out by using ethanol 70% for 24 hours until the solvent is clear sight. Fractination of ethanol extract using ethyl acetate solvent, where there will be two parts those are the soluble part of ethyl acetate fraction and insoluble ethyl acetate fraction.

The sample of ethyl acetat and insoluble ethyl acetat fractions tested for flavonoid total using spectrophotometer UV-1800 Shimadzu, where determination of the flavonoid total according to the Chang *et al* method's (2002).

Adaptation of the Mice

Adult male *Swiss* mice with body weights approximately 25-35g were kept in room temperature and standard (natural) photoperiod of approximately 12h of light alterating with approximately 12h of darkness. The mice were maintained on standard mice feed and potable water which were made available *ad libitum*.

The test of this research was conducted to 8 groups of mices which consisted of 7 mices per group. Each group consisted of positive control (phenobarbital 100mg/KgWB), negative control (CMC 0,5%), ethyl acetat fraction group with doses 100mg/KgBW; 200mg/KgBW; 400mg/KgBW, insoluble ethyl acetat fraction group with doses 100mg/KgBW; 200mg/KgBW; 400mg/KgBW. Factions was given for seven days while positive control group phenobarbital given at the 7th. On the 7th day one hour after PTZ induction, observation was done on onset, duration, number of seizures, mortality and histopathology observation of liver and kidney.

Statistical analysis

Statistical analysis was done by using Mann-Whitney Results are expressed as the

mean ± SD. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSIONS

From the maceration process, rendemen of extract was obtained 18,54%. Fractination was done on pennywort extract using ethyl acetat which having rendemen value about 4,12%. Insoluble ethyl acetat fraction has rendemen 13,4%. Rendemen value of insoluble ethyl acetate more higher than rendemen of ethyl acetate fraction. This is because ethanol 70% extract more polar than ethyl acetate fractions. Was known that the largest component in ethanol extract compound is triterpen compounds that are polar.

Total Flavonoid

The levels of total flavonoids was measured on ethyl acetat and insoluble ethyl acetat fractions using UV-Vis spectrophotometer. The purpose of determination of flavonoid total levels is to fullfil standardization extract or faction. Differences of growing places and environmental conditions affect the levels of active substances from plants even on the same plant species. The largest of total flavonoid level contained in ethanol extracts there is 3,419%. Total flavonoids of insoluble ethyl acetate fraction (1,465%) have higher levels than ethyl acetate fraction (0,323%). The polarity of ethyl acetate is lower than ethanol 70%, so not all of the extract could dissolved in ethyl acetate solvent which called insoluble ethyl acetat fraction.

Have been researched previously by zainol, et al (2009) that some compounds of flavonoids contained in pennywort are naringin, routine, quercetin, catechin, luteolin, and apigenin, kaemferol. This flavonoid compounds have the effect as an antioxidant. Flora and gupta's (2007) research concluded that flavonoid of a fraction pennywort to give the effect protection against toxicity cells of the neurons in mice by antioxidant mechanism.

So can be concluded that high flavonoid can increase the activity of the neuron cell protection, in the end can serve as agents of an anti seizure.

Ethyl Acetat and Insoluble Ethyl Acetat Fractions of Pennywort Herb As Anticonvulsant

Has been researched before by Ganachari *et al* (2004) that ethanol extract 100mg/KgBW showed potential to extend sleeping time on experiment animals were given sodium pentobarbiton and has the activity as anticonvulsant. Hopefully when ethanol extract was fractionated with ethyl acetat, it will get specific compound that will enhance the activity of pennywort herb as an anticonvulsant.

None of the groups that have different values significantly to negative control, this indicates that all the factions have not been able to reduce onset time of seizure activity. Insoluble ethyl acetate fraction group dose 400mg/KgBB showed decreasing the time of duration better than the other groups. It characterized by significant differences of duration time compared to a negative group.

Table I .Onset and Duration Average Time

Group	Onset (second)	Duration(second)
NEGATIVE CONTROL	172,14±133,74*	333,33±86,22*
POSITIVE CONTROL	0,00±0,00#	0,00±0,00#
EAF 100mg/KgBB	120,83±48,21*	530,00±29,44*#
EAF 200mg/KgBB	130,00±20,98*	507,50±137,75*
EAF 400mg/KgBB	155,00±28,87*	460,75±120,98*
IEAF 100mg/KgBB	220,00±98,99*	569,00±209,36*
IEAF 200mg/KgBB	166,00±108,77*	700,00±124,90*#
IEAF 400mg/KgBB	139,17±80,40*	138,00±131,42*#

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

The content of compounds such as brahmoside, brahminoside and other triterpen compounds in insoluble ethyl acetate fraction have possibility role to decline duration time on seizure mice

(Amalia, 2009). In addition, a polar compounds such as flavonoids also have anticonvulsant effect and lowering anxiolytic (Almeida et al., 2008).

Number of Seizures and Mortality

Table II. Number of Seizures and Mortality percetae in Each Group

Group	Number of Seizures	Mortality (%)
NEGATIVE CONTROL	1,71±0,95*	100*
POSITIVE CONTROL	0,00±0,00#	0#
EAF 100mg/KgBB	2,29±0,76*	71*
EAF 200mg/KgBB	2,00±0,58*	57*
EAF 400mg/KgBB	2,00±1,00*	43#
IEAF 100mg/KgBB	2,43±0,79*	100*
IEAF 200mg/KgBB	1,50±0,55*	57*
IEAF 400mg/KgBB	1,57±0,98*	71*

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

From the data showed that none of the giving fraction group can decrease the frequency of seizures. Meanwhile, the group that showed an improvement in the percentage of mortality is ethyl acetate fraction group dose 400mg/KgBW. The certain mechanism of levels of mortality on the fractions giving are different wasn't found. The limited number of mice using in research allows one of many determinant steadiness factor of data.

Liver and Kidney HistopatologyTest

Examination using Hematoxylin-Eosin staining (HE) and the organs are carried out in the laboratory of Pathology Anatomy Faculty of Medicine Gadjah Mada University to made histology preparations of liver and kidney. Liver histopathology test in all groups there were no significant differences at both the negative and positive control.

Table III. Rapairment Percentage of Liver and Kidney

Group	Hepar Histo(%)	Kidney Histo (%)
NEGATIVE CONTROL	0	0*
POSITIVE CONTROL	20	80#
EAF 100mg/KgBB	20	100#
EAF 200mg/KgBB	20	20
EAF 400mg/KgBB	40	40
IEAF 100mg/KgBB	20	40
IEAF 200mg/KgBB	60	80#
IEAF 400mg/KgBB	40	100#

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

Whereas in kidney histopathology test, ethyl acetate fraction group dose 100mg/KgBW and insoluble ethyl acetate fraction dose 400mg/KgBW given a very significant improvement over the negative control group.

Low percentage of liver repairing happent in entire group include the positive control group, it indicate that the induction of PTZ has damage to liver or leaning to hepatotoxic. Several groups of fractions also shows the percentage of bad improvement on the kidney. Besides it hepatotoxic characteristic, PTZ also allows to nefrotoxik on mice kidney.

CONCLUSION

Insoluble ethyl acetate fraction dose 400 mg/KgBW has the ability to reduce duration time however not be able to extend onset time, minimizing frequency of seizures, and decrease the number of mortality. Mortality significantly decreased at dose 400mg/KgBW of ethyl acetate fraction. Ethyl acetate fraction dose 100mg/KgBW and insoluble ethyl acetate faction dose 400mg/KgBW showed improvements against kidney damage induced by PTZ. However, do not indicate a significant improvement of liver damage

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ANTI DIABETIC ACTIVITY OF ETHANOL EXTRACT AND CHLOROFORM EXTRACT *Annona muricata* LEAF IN ALLOXAN INDUCED RATS

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Abstract

Background. *Annona muricata plant is a medicinal plant using by research and drug for human healthy including diabetes mellitus.*

Objective. *This research aimed to determine the effect of ethanol extract and chloroform extract of the sirsak leaf as antidiabetes mellitus.*

Methods. *This research was conducted in 9 groups of male Wistar rats consisting of 5 rats per group, consisting of normal control, alloxan control, glibenclamide control dose of 10 mg/Kg BW, group of ethanol extract sirsak leaf dose of 50 mg/Kg BW; 100 mg/Kg BW; 200 mg/Kg BW, and group of chloroform extract sirsak leaf dose of 50 mg/kg BW; 100 mg/Kg BW; 250 mg/Kg BW. Tests carried out for 2 weeks.*

Outcome measured. I*t also conducted assays of total flavonoids and histopathological tests of pancreatic* β *cells.*

Results. *Results of this research showed that the ethanol extract of sirsak leaves dose of 200 mg/Kg BW has activity in decreasing blood glucose levels better than any other group, with the percentage of total flavonoid 12.5%. Histopathological results of the ethanol extract of sirsak leaf has the same capabilities as compared with glibenclamide in reapair the damage of pancreatic* β *cells.*

Conclusion. *The conclusion of this research is the ethanol extract of sirsak leaf have activity antidiabetic mellitus.*

Keywords : *Annona muricata, antidiabetic, alloxan, blood glucose*

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ISBN : 978-979-18458-6-1

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine and metabolic disorders in the 21st century, and a major threat to health worldwide. Many experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to the formation of excessive free radicals (Ceriello, 2003). Various epidemiological studies have shown a tendency for an increase in the incidence rate and prevalence of DM from year to year. For Indonesia, the WHO predicts rise in the number of patients from 8.4 million in 2000 to be approximately 21.3 million in 2030. Diabetes mellitus is a chronic disease that is characterized by the presence of abnormalities in the metabolism of carbohydrates, lipids, proteins and are associated with insulin deficiency (Suryawanshi *et al.*, 2006). Among the various diseases diabetes, more than 95% of people with diabetes is type 2 diabetes mellitus (T2DM) as well as the type at most disputed. The symptoms of type 2 diabetes mellitus, among others, due to pancreatic dysfunction and increased levels of lipids, fatty acids and cholesterol in the blood (lipemia) (K.A. Wadkar. *et al*, 2008). Annona muricata is a plant of the family Annonaceae. Medicinal plants have been used as a natural remedy for many diseases, one of which is for the treatment of diabetes mellitus (Adeyemi, *et al,* 2009). The bark, roots and leaves of Annona muricata has been reported to be used as an anti-diabetic. Therefore this study was designed to confirm the effects of the ethanol extract and chloroform extract of leaves of Annona muricata on glycemic control in diabetic rats in the alloxan induction, so the results of this study may help in the selection of treatment of diabetes mellitus even better.

METHODS

Materials and Instruments

Materials : the leaves of soursop (*Annona muricata*), ethanol 70%, chloroform, male Wistar rats aged 2-2.5 months with a weight of 190-210 grams, rat pellets, formalin 10%, glibenclamide, alloxan, Na CMC, NaCl 0,9%, aluminum chloride 10%, sodium acetate, coloring Gomori.

Instruments : analytical balance, maceration tools, grinding machines, sieve mesh 40 and mesh 30, separating funnel, rotary evaporator, oral syringes, syringes injection, On Call Plus Blood Glucose Test Strips, glucometer, Spectrophotometer Shimadzu UV-1800, centrifuges, glassware and tools that are commonly used.

Sample Preparation and Anti Diabetes Test

Leaves of soursop (*Annona muricata*) is obtained then do the sorting. Soursop leaves are dried in the powder and sieved to 30 mesh and 40. Extract prepared by maceration using ethanol 70% and chloroform. All maserat collected and evaporated with a rotary evaporator to obtain the viscous extract, weighed and recorded randemen obtained. TLC made to extract the ethanol and chloroform extracts. Stationary phase such as silica gel 254 nm, Butanol: Glacial Acetic: Water = 4: 1: 5 by comparison quercetin. Total flavonoid levels using a spectrophotometer Shimadzu UV -1800 where determination of total flavonoids in accordance with the method of Chang *et al* (2002).

Adaptation of the rats

Adult Wistar rats aged 2-2.5 months with 190-250 gram weight, male sex are maintained in wire cages with room temperature and experience 12-hour cycle of day and night. The rats were fed with pellets and given drink in moderation.

Rats were fasted overnight before intraperitoneal injection of alloxan created using new. Alloxan at a dose of 160mg/kg body weight dissolved in normal saline until dissolved. Fourth day after alloxan injection, blood was taken via the tail vein in the then measured serum fasting blood glucose when blood glucose levels 200-300mg/dl then the rat can be categorized as

diabetic rats. (Dhandapani, 2002; Chougale *et al.*, 2007).

Tests were carried out on 9 test groups each group consisted of 5 rats. Control group consisted of normal, alloxan control, glibenclamide control, ethanol extract group doses of 50mg/kg dose; 100mg/kg; 200mg/Kg, and chloroform extract group doses of 50mg/kg; 100mg/kg; 200mg/Kg. This treatment performed for 2 weeks.

Blood Glucose Measurement and Histopathology

Glucose test performed at baseline before induction of alloxan, after induction of alloxan, week 1 and week 2 to see the comparison. Testing is done by glukometer (On Call Plus Blood Glucose Test Strips). On day 14 control rats glibenclamide, pain control, normal, and ethanol extract group and the chloroform extracts were sacrificed by total anesthetized using inhaled chloroform. The pancreas from each group of rats collected by surgery. Pancreatic cleaned with normal saline and then stored in a tissues fluid-filled pot 10% formalin. Tissue homogenates and then stored for histopathological test-pancreatic β cells by using the method of Gomori.

Statistical analysis

Statistical analysis was done by using Mann-Whitney Results are expressed as the mean ± SD. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSIONS

Total Flavonoid test

Determination of total flavonoid content using UV-Vis spectrophotometry. As standard used quercetin, a flavonoid compound commonly used identifier and quercetin is a flavonoid class of active substances which are biologically very strong. From the calculation of the percentage of total flavonoid levels of ethanol extract was 12.5% and the percentage of total flavonoid levels of chloroform extract was 5.06%. Flavonoids contain a conjugated aromatic system therefore shows a strong absorption band in the ultraviolet and visible area. Determination of flavonoids by using the method of Chang et al (2002), where the addition of a sliding AlCl3 reagent causes a bathochromic shift enables showed in the o-hydroxy group on ring A. While the addition of NaOAc shift reagent will cause bathochromic shift indicating a hydroxyl group (OH) at the C-7 position. Flavonoid easy to detect using UV light, it because of phenyl ring on flavonoid (Andersen and Markham, 2006).

Test of antidiabetic mellitus effects of ethanol and chloroform extract of soursop leaf to decrease blood glucose levels

Table I shows the data of blood glucose rats before and after induction of alloxan. From the the data shows that after alloxan administration for 4 days, blood glucose in mice induced increased.

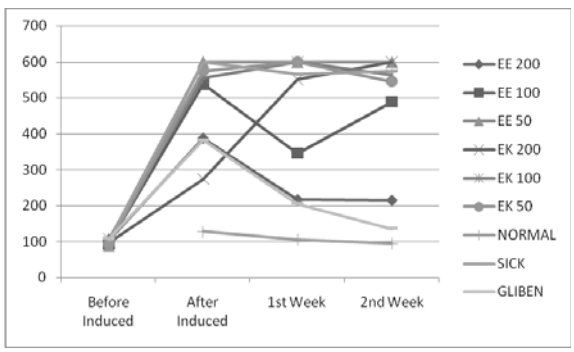
Before the induced with alloxan, fasting blood glucose levels did not differ significantly ($p < 0.05$) between the eight groups of experimental animals. At 24 hours after alloxan administration, blood glucose levels were significantly ($p < 0.05$) higher in the group of animals ethanol extract 50 mg / kg bw and the negative control group which showed blood glucose levels 600 mg / dL. Increased glucose levels in rats that elevated levels 200 mg / dL were categorized as diabetic rats.

ISBN : 978-979-18458-6-1

Table I. Blood Glucose Levels Average During the Treatment

Group	Before induced	After Induced	1st week	2nd week
NORMAL	-	127.6±9,83#	105.5±3,41#	95±9,59#
SICK	102.25±2,06	600±0*	565.4±28,31*	574.8±21,47*
GLIBEN	108.5±9,84	382±57,51*#	205.25±115,79#	135.25±13,69*#
EE 200	104±1,82	386.6±195,18*	216±94,08*#	214±85,14*#
EE 100	94±1,82#	538.25±71,36*	345.4±168,47*#	488.2±153,09*
EE 50	88.75±8,99#	600±0*	600±0*#	600±0*
EK 200	97.25±14,15	275±25*#	551.75±55,72*	600±0*
EK 100	107.25±3,5	556±50,91*	600±0*#	564.5±34,97*
EK 50	92.5±6,45#	575±28,87*	600±0*#	545.67±1,52*#

* p <0.05 significantly different compared to the normal group # p <0.05 significantly different compared with those sick



Picture I Blood Glucose Levels Average Graphic From Each Group

Description :

EE 200 : Ethanol Extract dose 200 mg/Kg BW

EE 100 : Ethanol Extract dose 100 mg/Kg BW

EE 50 : Ethanol Extract dose 50 mg/Kg BW

EK 200 : Choloform extract dose 200 mg/Kg BW

EK 100 : Choloform extract dose 100 mg/Kg BW

EK 50 : Choloform extract dose 50 mg/Kg BW

SICK : Negative controls, alloxan induced group dose 160 mg/Kg BW with blood glucose levels> 200 mg / dL

GLIBEN : Positive controls Glibenklamide dose10 mg/Kg BW

NORMAL : Group Without giving alloxan group, glibenclamide, ethanol extract and chloroform extract

Decrease in glucose levels of instability that occurred in the first week and the second week in which the first week of a decline in glucose levels but after the second week of increased glucose levels back can be seen from the above data. A steady decline in glucose levels only seen in the ethanol extract dose of 200 mg / kg bw.

Data homogeneity tested by ANOVA analysis and show that the data are not distributed homogenous. Data were tested for normality using one-sample analysis of the Kolmogorov-Smirnov test showed that the data were normally distributed for each group except the group after induced where the data is not normal. To see a significant difference from each treatment group can be analyzed using the non-parametric Mann-Whitney test. Said to be significantly different values when the value of significance p <0.05. Alloxan diabetes inducer is a compound commonly used in addition to streptozotocin.

Histopathology of Beta Cell Pancreas

Reading test result pancreatic beta cells can be seen in Table II and Picture II. the result showed that the number of pancreatic beta cells are still normal per surface area of the islets of Langerhans 400x400 micro. Pancreas were taken on day 14 one hour after administration of a preparation. Then the organ was cleaned in a solution of normal saline and immersed in 10% formalin solution to prevent organ damage. Organs are then taken to the Pathology Laboratory of the Faculty of Medicine, University of Gadjah Mada made preparations for pancreatic histology Chromalum Gomori coloring technique.

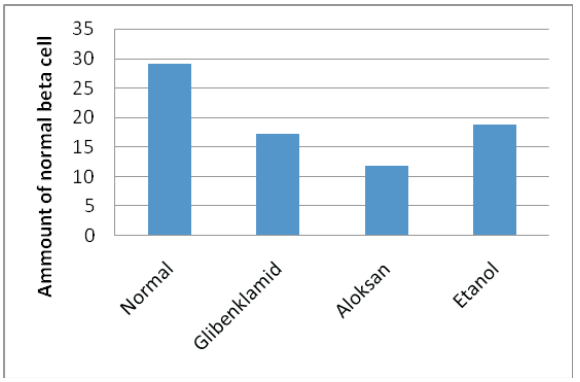
alloxan-induced damage than the islets of Langerhans of normal rats.

Picture III. Pancreas Histology With Gomori Chrome Alum Hematoxylin-Phloxine Staining. Whereas showed alpha cell (A); beta cell (B); vacuolization cell (C); hyperthropy cell (D).

From the picture is known that pancreatic beta cells undergo various damages including vacuolization, necrosis, and pancreatic beta cells hiperthropi. Sick group did not differ significantly with glibenclamide group and the group of ethanol extract dose of 200 mg / kg bw. However, glibenclamide group with a group of ethanol extract dose of 200 mg / kg bw no more

Table II. Histology test of beta cell pancreas with Gomori Chrome Alum Hematoxylin-Phloxine staining method

Group	Beta pankreas
Normal	29,00±7,09
Glibenklamid	17,25±6,95
Aloksan	11,78±3,34
Ethanol Extract 200 mg/Kg BW	18,75±6,27



Picture II. Histopathology Graphic of Beta Cell Pancreas

The data shows that the normal group had a number of healthy beta cells are still more than the other groups. While the alloxan-treated group had a number of less healthy beta cells, this is due to the islets of Langerhans of rats

different than the pain group. It can be concluded that the improvement of pancreatic beta cells by ethanol extract dose of 200 mg / kg bw comparable with glibenclamide administration.

On preparations seen a few different colors, where each color indicates a different cell. In the islets of Langerhans are three types of cells: alpha cells, beta cells and delta cells, and a few cells is not clear that granular (Permata, 2006).

CONCLUSION

Ethanol extract and chloroform extract of leaves of soursop (*Annona muricata*) various doses have not been able to lower blood glucose levels as well as the glibenclamide group.

Decrease in blood glucose levels can decrease and consisten tly better than the other treatment groups was 200mg/Kg BW ethanol extract dose with a significant value compared

with the normal group of post-induction, the first week and the second week in a row is 0.009; 0,020; 0,014. Repair of pancreatic beta cells by ethanol extract doses 200mg/KgBW comparable with glibenclamide administration.

ACKNOWLEDGEMENT

Thanks to Post Graduate Program Faculty of Pharmacy Ahmad Dahlan University Yogyakarta. Pathology and Anatomy Laboratory of Medicine Faculty of Gadjah Mada University, and Pathology Laboratory of Veterinary Faculty of Gadjah Mada University.

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COMPARISON OF SPECTHROPHOTOMETRIC AND TLC-DENSITOMETRIC TECHNIQUE IN DETERMINATION OF PHYTOMELATONIN IN GREEN ALGAE (*Sprirogyra* sp) ETHANOLIC EXTRACT

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Abstract

Background. *Instrumental analysis method give a difference measurement results in determination of content level of active substance in medicinal herb.*

Objective. *The aim of this study is to campare the level content of phytomelatonin in green algae extract determinated by spectrophotometry and TLC-densitometry technique.*

Methods. *The phytomelatonin was extracted from green algae using 96% aethanol. The qualitative Alkaloid screening were done by using Mayer and Dragendorff test. The quantitative determination of phytomelatonin were done spectrophotometrically at 277nm wavelength and using TLC densitometry technique with silica gel GF 254 plate and eluated by BAW (n-buthanol: acetic acid: water=12:3:5 v/v). The spots were scanned at 254nm wavelength*

Outcome measured. *Phytomelatonin level in aethanolic extract of green algae*

Results. *The results showed that the content phytomelonin content of green algae level by spectrophotometric technique was 0.22±0.01 % and 0.88±0.04 % was assayed by TLC-densitometric.*

Conclusion. *The TLC-densitometry technique gave the higher phytomelatonin of green algae level than specthrophotometry technique ($p<0.05$)*

Keywords : *Phytomelatonin, green algae, aethanolic extract, spectrophotometry, TLC-densitometry*

ISBN : 978-979-18458-6-1

INTRODUCTION

Green algae contains melatonin called as phytomelatonin, a substance that wide used for cancer prevention, antioxidant (Veronique *et al,* 2005), surpassing the myocardial damage due to nicotine (Baykan et al., 2008), anti-mouthcancer (Varvares, 2008), prevention of bleeding in the brain (Koh, 2008), inhibited the neurotoxic than arsenic (Lin *et al.*, 2008), prevent kidney damage due to smoking (Ozan *et al*, 2007) and antihypertensive (Xia *et al*, 2008).

Based on it's chemical structure, the phytomelatonin can dissolve in ethanol. Phytomelatonin can be determinated by spectrophotometry and TLC-densitometry technique.

This study aims to campare the phytomelatonin level in ethanolic extract of green algae determinated by spectrophotometry and TLC-densitometry thechnique

MATERIAL AND METHOD

Material

The main material was geen algae (*Spyrogyra sp*). Chemical material : absolute ethanol p.a., petroleum ether, ether, acetic acid p.a, HCl , n-butanol p.a.. purcashed from Merck, Dragendrof dan Meyer reagents, aquadest dan Silica gel GF-254 plate.

Methods

1. Plant identification

Plant identification was done at Laboratorium Ilmu Alam Fakultas MIPA Universitas Ahmad Dahlan..

2. Sample collecting

Green algaes were collected from Rowo Jombor , District Bayat, Klaten regency, Central Java in March of 2012.

3. Aethanolic Exctract preparation

Phytomelatonin was extracted from green algae by Soxhlet apparatus with aethanol then evapotated with rotary evaporator to obtaine thick extract. The water content and the ash content of the aethanolic extract were determinated by gravimetric thecnique.

4. The aethanolic extract Purification

About 15 mL 15% acetic acid was added into the thick aethanolic extract, then filtered using Buchner funnel. Wash the filtrate with petroleum ether. The acetic acid layer were separated and added NH4OH until the pH value was 10. Pour 50 ml of ether into the basic solution. Remove the water layer. Evapoporate the ether layer to obtain the residue. Dissolve the residue using aethanol. The absorbance of the solution were measured at 277nm wavelength.

5. The screening of alkaloid and identification of phytomelatonin in aethanolic extract

The alkaloid screening were done using Dragendorf and Mayer test. The formation of sediment after the addition of the dragendorf or mayer reagent into the acidic sample indicated the presence of alkaloid in the sample.

The qualitative analysis of phytomelatonin in the aethanolic extract was done with TLC thecnique. The similarity Rf value beetween the phytomelatonin standart spot and sample spot indicated the presence of phytomelatonin in the sample.

6. The quantitative analysis of phytomelatonin

a. Spectrophotometry thecnique

The phytomelatonin standart solutions in many various level were prepared. The interval level were between 0.1-0.3mg/ml. The absorbance of various level of phytomelatonin standart solution and the purified aethanolic extract were measured at 277nm wavelength

ISBN : 978-979-18458-6-1

using Pharmaspec UV 1700 (SHIMADZU) spechtophotometer

The linear regression equation between the level of standart phytomelatonin vs absorbance was determinated. This equation was used to calculated the level of phytomelatonin in aethanolic extract.

b. TLC-densitometry thecnique

The silica F254 was used as stationary phase. The phytomelatonin standart (0.2-2.0/mg/ml) and the aethanolic extract were eluated with BAW (12:3:5)v/v. The AUC (area under the curve) values were determinated by scanning the dried spot with TLC scanner 3 (CAMAG) at 284nm wavelength. The linear regreesion equation between the level of phytomelatonin standart vs AUC was determinated. This equation was used to calculated the level of phytomeltonin in the aethanolic extract.

RESULT AND DISCUSSION

The plant identification result showed that the plant used int his study was green algae (Spirogyra sp.) .

About 24.3399 grams aethanolic extract (rendemen 9.74%) was produced from 250 grams green algae The water content of the extract was 8.34% dan 3.06% of ash content

The alkaloid screening, both of Dragendorf and Mayer tests indicated that the aethanolic extract of green algae consist of phytomelatonin.

The qualitative analysis of phytomelatonin in aetaholic extract by TLC thecnique showed the presence of phytomelatonin in both of the purified aethanolic extract and aethanolic extract. (fig.1)

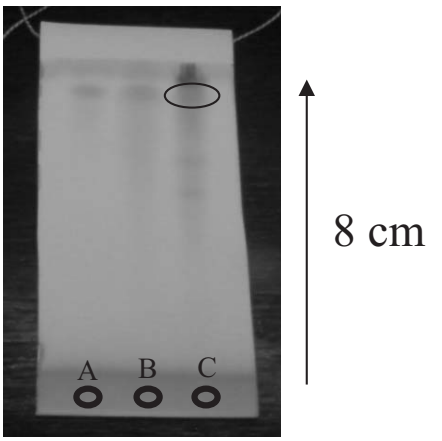

Figure 1. Chromatogram of phytomelatonin strandart (A), purified aethanolic extract (B) and aethanolic extract(C)

The quntitative analysis of phytomelatonin

a. Spectrrophotometry technique

The spectrophotometry technique was based on the ability of the phytomelatonin content in the aethanolic solution to absorb the electromagnetic radiation in Ultra Violet region. The maximum wavelength of the phytomelatonin standart was 277nm. The spectra of purified aethanolic extract showed the mximum wavelength at 275.8nm and the spectra profile showed the similarity between phytomelatonin standart and the purification aethanolic extrct spectra. It's showed that the purified aethanolic extract consit of phytomelatonin.

The absorbance of phytomelatonin standart soution in many various level was available in table I. The graphic fig. 2 showed the corelation between the phytomelatonin standart level Vs the absorbance.($p<0.05$).

ISBN : 978-979-18458-6-1

Table I. The absorbanceof the phytomelatonin standart solution

C (mg/10ml)	Absorbance
0.10	0.286
0.15	0.412
0.20	0.543
0.25	0.688
0.30	0.715

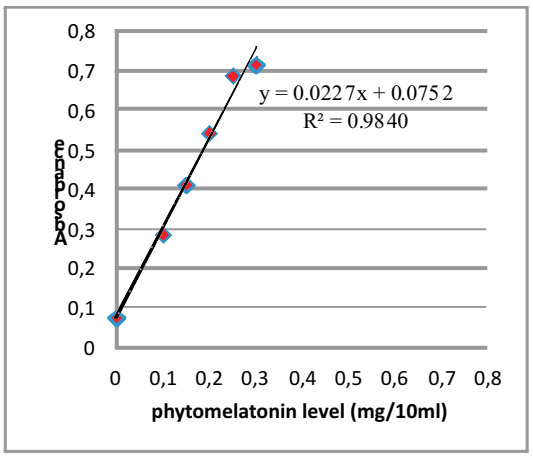

Figure 2. The graphic corelation between phytomelatonin level vs the absorbance

The qualitative parameter of the chromatogram were the Rf value. The Rf value of the phytomelatonin standart was 0.75 and the Rf value of the aethanolic extract was 0.76 . It's indicated that the aethanolic extract consist of phytomelatonin. The quantitative parameter of the TLC-densitometry technique were The AUC values. The AUC values of the phytomeltonin standart were described in table III.

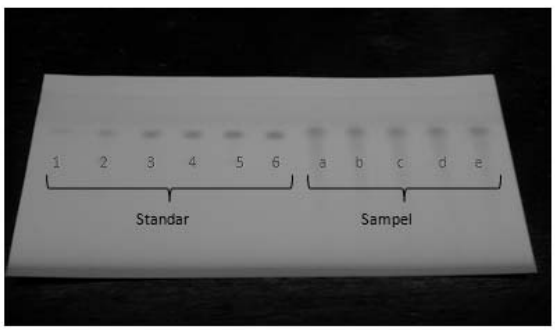

Figure 3. The chromatogram of phytomelatonnin standart (1-6) and aethanolic extract of green algae (a-e) on silica F254 plate after eluated by BAW (12:3:5)v/v under UV detector

Table II. The phytomelatonin level in aethanolic extract of green algae determinated by spectrophotometry technique

Sample weight (mg)	Abs	Phytomelatonin level (%)	$\bar{x}$ (%)	SD	CV (%)	$\bar{x} \pm Le$ (%)
998.6	0.469	0.22				
998.8	0.482	0.22				
998.8	0.489	0.23	.22	5.48×10^{-3}	2.74	0.22 ± 0.01
999.1	0.494	0.23				
998.8	0.475	0.22				

b. The TLC-densitometry technique

The chromatogram result from the TLC technique was desribed at figure3.

ISBN : 978-979-18458-6-1

Table. III. The AUC values of the phytomelatonin standart

No	Phytomelatonin level (mg/ml)	AUC (mV)	Linear regression	R value
1	0.2	9.1528	Y = 12.48 x + 10.63	0.94*
2	0.6	19.5458		
3	1.0	27.2703		
4	1.4	29.7615		
5	1.8	32.9887		
6	2.0	32.3886		

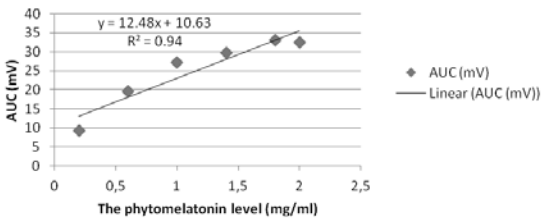

Figure 4. The graphic corelation between the phytomelatonin level Vs AUC

According to The data in the table I showed that the TLC-densitometry technique gave the higher value of the phytomelatonin level in the aethanolic extract of green algae.($p<0.05$) It indicated that the TLC-densitometry technique more sensitive than spectrophotometry technique. Another advantage of TLC-densitometry technique was the selectivity. The TLC-densitometry technique was more selective than spectrophotometry.

CONCLUSION

The TLC-densitometry technique gave the higher phytomelatonin of green algae level than specthrophotometry technique ($p<0.05$)

Tabel IV. The phytomelatonin level in aethanolic extract of green algae determinated by spectrophotometry technique

Sample weight (mg)	AUC (mV)	Phytomelatonin level (%)	$\bar{x}$ (%)	SD	CV (%)	$\bar{x} \pm Le$ (%)
997.8	31.8568	0.85				
995.5	31.6834	0.85	0.88	0.04	4.5	0.88 ± 0.04
1000.2	32.6445	0.88				
999.1	32.1614	0.86				
895.9	31.8769	095				

ACKNOWLEDGEMENT

We would like to thank DP2M dikti and LPP UAD with funding this research

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ACKNOWLEDGMENT

1. Rector of Ahmad Dahlan University
2. Dean of Faculty of Pharmacy, Ahmad Dahlan University
3. Director of Bethesda Hospital Yogyakarta
4. Director of Pharmacy Departement, Bethesda Hospital Yogyakarta
5. Takazadunia
6. JT Baker
7. CV. Ariando Pratama
8. Krisna Utama
9. UD. MultiLab