

I INTRODUCTION

The Presidential Regulation Number 8 Year 2012 on Indonesian Qualification Framework (IQF) asserts that graduates of master programs should have the ability to develop science, technology, and/or arts in their field of study or professional practices through research so that they are able to produce innovative and tested works. Likewise, the graduates of doctoral programs should be able to invent or create science and/or technology in their field of expertise through inter, multiple, or transdisciplinary research. Therefore, it can be concluded that the two programs are research-based educations.

At the end of study period, students are required to report the research conducted during their study in the form of scientific writing called thesis for master program and dissertation for doctoral program. Writing a scientific work either in the form of a thesis or a dissertation is not easy. There are stipulations and requirements that should be met so that a scientific writing can be “feasibly” called a thesis or a dissertation. In this context, a written handbook that regulates how thesis and dissertation are written and provides guideline for both students and advisor is required.

Thesis advisor committee is responsible for the advisee’s thesis or dissertation both in terms of its scientific legitimacy and the writing format. The committee has to assist and direct students both in conducting the research and writing the report; right from the point of the research title selection, proposal writing, the research implementation, research report writing, scientific publication writing, to thesis or dissertation examination. The end of the lengthy process is signified with the signatures of all members of advisor committee on the validation page of the thesis or dissertation. Although every education institution may formulate its own format of thesis and dissertation writing handbook, basically there are established formats and stipulations that are nearly identical in all institutions.

II PARTS OF THESIS OR DISSERTATION

Thesis or dissertation is divided into three parts: opening, main part, closing.

II.1 Opening Part

The opening consists of:

- Cover
- Title page
- Validation page
- Statement of Originality page
- Identity of Examiner Committee page
- Statement of Scientific Publication and Communication page
- Dedication page (optional)
- Acknowledgement page
- Ringkasan
- Summary page (in English)
- Table of Content page
- List of Tables page
- List of Figures page
- List of Appendixes page
- List of Symbols and Abbreviation page

II.2 Main Part

II.2.1 Thesis

Chapter 1 Introduction
 Chapter 2 Literature Review/Theoretical Framework
 Chapter 3 Conceptual Framework
 Chapter 4 Methods
 Chapter 5 Findings and Data Analysis
 Chapter 6 Discussion
 Chapter 7 Conclusions and Suggestions
 List of References

II.2.2 Dissertation

Chapter 1 General Introduction
 Chapter 2 General Literature Review/Theoretical Framework
 Chapter 3 General Conceptual Framework
 Chapter 4 General methods
 Chapter 5 Research I consists of:
 5.1 Introduction (specific)
 5.2 Methods (specific)
 5.3 Findings and Data Analysis (specific)
 5.4 Discussion
 5.5 Conclusion
 5.6 Suggestion
 Chapter 6 Research II consists of (identical to Chapter 5)
 Chapter 7 Research III (if any) consisting of (identical to Chapter 5)
 Chapter 8 General Discussion
 Chapter 9 Conclusion and Suggestion
 List of References

Notes:

- For dissertation research consisting of 1 research only, the format of the main part follows that of thesis.
- For dissertation research that has been published in an international or a nationally accredited journal, the published journal may replace Chapter 5, 6 and 7.

II.3 Closing Part

The closing part of a thesis or dissertation includes appendixes (where applicable) that may consists of:

- Specific Procedure/Methods
- Statement of Originality of Material/Sample (Material data sheet)
- Worksheet including patient status, question list/questionnaire
- Ethical Clearance
- Raw Data and Statistical Analysis
- A copy of a published/communicated journal manuscript that is related to the dissertation
- Curriculum Vitae

III WRITING GUIDELINES

III.1 Paper

The paper used is A4-sized HVS of 80 gram. The reproduction of thesis or dissertation manuscript is done by photocopying.

III.2 Format

Thesis or dissertation is computer-typed using Arial font of 11 cpi (character per inch) or 28-30 lines per page in two spaces. The typing margin is 4 cm on the left, 3 cm on the right, top, and bottom of the paper. At the beginning of a new paragraph, the first word should be indented by 5 strokes (1 tab). The sentences should meet standard, correct, and good grammar (both for Bahasa Indonesia and English). Every sentence should have a good subject, predicate, and object/complement. The object should not be too long. In addition, there should not be any repetition of words in a sentence. Too-long sentences (run-on sentences) that hinder comprehension should be cut into shorter sentences that are more easily understood. The cutting of a word in a sentence should also follow the standard and correct rules of Indonesian or English. Punctuation in front of or behind a word should be attached to the marked word and separated from words that are not related to the punctuation.

A new chapter should be written on a new page. The chapter title should be capitalized and put at the center of the top of the page. Every new chapter and sub-chapter should be numbered in accordance with the order of the said chapter and sub-chapter, in relation with other chapters and sub-chapters. Sub-chapter title is typed on the left side of the page in lowercase; However, the initial letters of each word is capitalized (Capitalize each word).

Table and figure title is typed in bold Arial font of 11 cpi. The numbering of a certain table or a figure is in accordance with the number of its respective chapter. Notes of a table/figure is typed in regular, not bold, arial of 10 cpi. Table title, figure title, sub-chapter, and summary are typed in one space. Table title is typed above the table, while figure title is typed below the figure. A figure is printed without frame.

A tabel is made by using "header row" as the upper border and a horizontal line as the lower borders. Vertical lines indicating columns are made invisible. See appendix 21 for a standard format for table. When a table/figure is quoted or taken from other sources, information regarding the source from which the table/figure is taken should be typed below the table/figure.

III.3 Page Number

The page number of the opening part of thesis/dissertation uses small roman numerals (i, ii, iii, etc.). The page number is typed on the right bottom part of the page. The page number of the main and closing parts uses arabic numerals which is typed also on the right bottom part of the page.

IV THE OPENING PART OF THESIS OR DISSERTATION

IV.1 Cover

The cover of a thesis or dissertation consists of two parts: the outer cover (hard cover) is in green, while the inner cover is in white HVS paper. The title of the thesis/dissertation is typed in capital letters on both covers. "THESIS" or "DISSERTATION" is typed in capital letters below the title. Below "THESIS" OR "DISSERTATION", is "to fulfill the requirements for the Degree of Master/Doctor in ..." followed by the name of the study program, the logo of FMBU, the complete name of the writer (without title), the student id number, Postgraduate Programs of Faculty of Medicine Universitas Brawijaya Malang, and the year in which the thesis/dissertation is written respectively. An example of thesis cover can be seen on **appendix 1** and an example of dissertation cover can be seen on **appendix 2**.

The name of the writer, thesis/dissertation title, and the year of graduation are also typed on the spine of the thesis/dissertation. How the information on the spine is written can be seen on **appendix 3**.

IV.2 Title Page

The title page presents the same information as the cover page, but it is printed on white HVS paper. The title of thesis/dissertation is ideally formulated in a maximum of 20 words and represents the problems and objectives of the research. If a title is longer than 20 words (for example, when the title contains further essential information), the title can be broken down into the main title and its sub-title. An example of a thesis title page is presented on **appendix 4** and dissertation on **appendix 5**.

IV.3 Validation Page

Thesis validation page presents thesis title, name of the writer, validation statement, thesis advisor committee, thesis examiners and the validation signatures of the thesis advisor committee and thesis examiners. Dissertation validation page presents dissertation title, name of the writer, validation statement, promotor committee, and the validation signatures of the promotors. The name of the promotors is typed in chronological order; the head of the promotor committee first and followed by the members of the promotor committee. Thesis or dissertation is pronounced "legitimate" if the thesis/dissertation has been signed by the Dean of the Faculty of Medicine **(Validation Page both for thesis and dissertation is available at Postgraduate Programs – FMUB). (see Appendix 6)**

IV.4 Statement of Originality Page

Statement of originality page presents the statement of the writer which asserts the originality of the thesis/dissertation. The statement also establishes that the thesis/dissertation is not a product of plagiarism **(statement of originality page of thesis/dissertation is available at Postgraduate Programs – FMUB). (see Appendix 7)**

IV.5 Identity of Examiners Committee page for Thesis and Dissertation

Identity of Examiner Committee page for thesis is printed on white HVS paper. It presents thesis title, the identity of the writer, name of advisor committee, name of examiner committee, name of evaluation monitoring member, date of thesis examination and the decree number of the appointment

of thesis advisor and examiner. Identity of Examiner Committee page for dissertation is also printed on white HVS paper. It presents thesis title, the identity of the writer, name of advisor committee, name of examiners, the signatures of both promotor and examiner committee, date of dissertation examination and the decree number of the appointment of promotors and examiners. See **appendix 8 and 9** for examples of Identity of Examiner Committee page for thesis and dissertation

IV.6 Statement of Scientific Publication and Communication page

Statement of Scientific Publication and Communication page presents the statement of the writer and a list of researches that have been published/communicated in scientific forum. A copy of published writings should be enclosed. See **appendix 10** for an example of Statement of Scientific Publication and Communication page

IV.7 Dedication Page

The page is optional and quite personal since it expresses to whom the thesis/dissertation is dedicated to. An example of a dedication page is on **appendix 11**.

IV.8 Acknowledgement Page

On the page, the writer elucidates the objective and the content of the thesis/dissertation and expresses his/her appreciation and gratitude to those that have provided assistance to or contributed to the process of thesis/dissertation writing. Here, the writer also states his/her wish for further research and the research significance. The maximum length of foreword page is three pages. An example of a foreword page is on **appendix 12**.

IV.9 Summary Page

A thesis/dissertation summary is written both in Indonesian and English. The title of the summary is identical to that of the thesis/dissertation. The title of the summary is typed in capital letters on the center top of the page. Summary covers the problems of the research, the objective of the research, the methods of the research, and key findings of the research. There should be no quotations from any references in a summary, so it is purely an authentic account of the research. The summary content should reflect what the theis/dissertation is about. It ideally allows anyone to understand the thesis/dissertation without actually refering to the full manuscript. An ideal length of a summary is between 600 to 800 words (1.5 - 2 pages). A summary is typed in 10 cpi at one space. Examples of summaries are on appendix **13 & 14**

IV.10 Table of Content Page

Table of Content page is typed on a new page. The title, "TABLE OF CONTENT", is typed in capital letters, put in the middle of the upper part of the page without period. Table of content lists all parts of a thesis/dissertation manuscript including list of tables, list of figures, list of abbreviations, list of terminologies, list of references and appendixes. Chapter titles are capitalized, while sub-chapter titles are typed in small letter except for the initial letter of the title (capitalized). Both chapter and sub-chapter titles are not ended with a period. The number of both chapters and sub-chapters are in arabic numerals. The the space between one chapter title to another is two spaces, while sub-chapter titles are typed in one spaces. Example of a Table of Content page is on **appendix 15**

IV.11 List of Tables Page

List of Table page is typed on a new page. The words “List of Table” is typed on the center top part of the page in capital letters and is not ended with a period. The page lists all tables included in the manuscript and its appendixes. The number of the table is typed using arabic numerals. Table title taking up more than one line is typed in one space, while the space between table titles is two spaces. Table titles listed on the page should be identical to those in the manuscript and its appendixes. An example of List of Table page is on **appendix 16**.

IV.12 List of Figures page

List of Figure page is typed on a new page. The page presents a list of figures used in the manuscript and its appendixes, the number of the figures, and the number of the page on which they are printed. Figure titles on the page should be identical to those in the manuscript and its appendixes. An example of a List of Tables page is on **appendix 17**.

IV.13 List of Appendixes Page

List of Appendixes page is typed on a new page. The words “ List of Appendixes” is typed on the center top part of the page in capital letters. The page lists the title of appendixes, their numbers, and the page number on which they are presented. The title of appendixes listed on the page should be identical to those in the manuscript. Appendixes cover supporting documents including raw data of the research, statistical analysis and computation, worksheets, list of questions/questionnaire, laboratory test procedure, map, statement of ethical clearance, an so forth. An example of a List of Appendixes page is on **appendix 18**

IV.14 List of Symbols and Abbreviation Pages

Both the List of Symbols and Abbreviations are typed on new pages. Words “List of Symbols” and “List of Abbreviations” are typed on the center upper part of the page in capital letters. The pages list symbols/terminologies and abbreviations (including besaran/satuan) in table format. These two pages are present only when applicable (optional). The detail of how the pages are typed in column as follow:

- Symbols/abbreviations are typed on the first column
- notes on what the symbols represent or unit of measure or what the abbreviations stand for are given on the second column.
- The list of abbreviation is presented in chronological order
- if the symbols are in Greek, they are also presented in chronological order.
- the explanation of abbreviations given on the second column is typed in small letters with capitalized initial letter of each word (capitalized each word). An example of List of Abbreviation page is on **appendix 19**

V MAIN PART OF A THESIS OR DISSERTATION

The main part of a thesis or dissertation consists of several chapters. The number of chapters is not rigidly set as it should accommodate the difference in nature and coverage of the researches. The main part commonly comprises introduction, literature review/underlying theoretical framework, conceptual framework, methods, findings, discussion, conclusions and suggestions, and list of references. The way how information is presented in a thesis should be precise, efficient, self-explanatory, sharp, relevant, and consistent.

Chapter 1. Introduction

The chapter covers background of the research, research problems, research objectives, and significances of the research.

- a. Background of the Research: presents relevant statements or facts to the topic or coverage of the research, including empirical and technical arguments corroborating the significance of the research being conducted. These arguments should be written in such a coherent order that the relationship between the facts and empirical data is undeniably obvious. This will result in a discrepancy or ke-tidak sesuaian between the facts and the empirical data presented. The discrepancy serves as the starting point for the formulation of research problems.
- b. Research Problems refer to statements representing the discrepancy between scientific knowledge or technology that is being researched and the existing knowledge. The discrepancy is then formulated into researchable problems. Research problems are stated in the form of questions and can be elaborated into sub-problems. For dissertation, sub-problems should indicate the different stages of the research to be conducted by the writer.
- c. Research Objectives refer to statements that specifically indicate the objectives to be achieved by the research. Research objectives should be implied in the thesis/dissertation title and can be stated in general objective and few specific objectives. Both general and specific objectives should be relevant with research problems and sub-problems.
- d. Research Significances refer to expected benefits of the research findings both theoretical (for the development of scientific knowledge) and practical.

Notes

Mistakes that frequently occur in the writing of introduction are :

- too long (the ideal length of an introduction is approximately 8% of the total length of the thesis/dissertation. For example, if a thesis is 10 or 15 or 20 pages long, the introduction should be about 1 or 1.25 or 1.5 page long
- too detail, so that what is being researched becomes vague
- difficulty to differentiate an introduction from a summary. An introduction brings the readers to the topic of the thesis/dissertation, while a summary presents an account of main ideas of a thesis/dissertation.

Chapter 2. Literature Review or Theoretical Framework

Literature Review is set after the formulation of research problems, research objectives, and significances, so that all referred literatures focus on the issues being discussed and written integrately. The chapter systematically and analytically presents results of analysis on theories, concepts, propositions, and so forth, or findings of earlier research that are relevant with problems and objective of the research. Therefore, a literature review is not a mere organized account of “cut and pasted” quotations of theories, concepts, propositions, and paradigms which are taken from various refereces. A literature review is the result of combination, comparison, and dialog processes between theories, concepts, propositions, and paradigms, from the clasical ones right up to the most current. From here, a red line can be drawn by the researcher so that he/she comes up with new theoretical ideas. Plagiarism in any forms are strongly prohibited in the chapter. Literature sources should be from scientific journals that are relevant with the research and most current (not older than 5 years than the year in which the research is conducted).

Chapter 3. Conceptual Framework of the research

Concept is basically an understanding about a phenomenon. Concept is the underlying element of thingking process. Conceptual Framework covers: a) theoretical framework, b) hypothesis. The framework may be a summary of literature review that supports or is against theories related to research problems. In the chapter, discrepancy between findings of different researches may also be highlighted since it serves as the argument of why the current research need to be conducted. Conceptual framework usually leads to a hypothesis/hypotheses and can be presented in the form of naration accompanied by a flow chart.

Hypothesis is a statement of presumption or temporary answer for research problems that is going to be tested using empirical data through research that is conducted based on theoretical analysis, or analysis on previous literatures or researches.

Chapter 4. Research Methods

Research Methods need to be written in accordance with research problems being proved. Research methods in a thesis/dissertation should explicitly state the definition and theoretical framework of research methods being used, and the level of its rigor/thoroughness as well as its limitations. Therefore research methods is much more than a detailed description of research procedure or how the research is conducted.

It is imperative that in writing a thesis/dissertation be able to answer such questions as “How is the research conducted?” and “Why is the research conducted that way?”. Hence, the chapter should also cover the justification of selecting certain research methods and data analysis, including the selection of research site (for example why the research is conducted in general hospital or teaching hospital), research subjects, and data collection and analysis. Illustration in the forms of figure, scheme, or flow chart can be added to make the description clearer (for example: timeline indicating the order of various stages of the research). Likewise, the research methods should be approved by a nationally accredited ethical committee.

Thesis on science and engineering should also provide justification for the selection of materials, methods, and procedures as well as description on details (characteristics) of the materials, instruments/tools, and procedures so that the research can be accurately replicated by other researcher. This includes information on technical problems faced by the research, why they occured and

how the problems are solved. The detailed procedure of the research should be put in passive sentences instead of imperative ones.

If a thesis is written in a classical standard way consisting of introduction, methods, findings, and discussion, research methods is exclusively discussed in “Methods” chapter. However, if a research consists of a series of experiments or studies, the chapters are written in accordance with the series of experiments/studies (each experiment requires a chapter on its methods)

In Indonesia, a chapter on research methods commonly presents a detailed description of the following:

a. Research Type and Design

Information on research type and design that are adopted should be provided. For example:

- laboratory experimental research with *pre-post test control group design* or *post test only control group design*
- laboratory experimental research with *completely randomized design*
- Clinical test with *Double Blind Randomized Clinical Trial design*

b. Time and Site of Research

The site where the research is conducted should be clearly stated, whether it is in the field or in the laboratory. Elaboration on research conducted in the field may cover the administrative area (village, sub-district, regency, or province) or the institution. If the research is conducted in a laboratory, the name of the laboratory and the name of the institution that the laboratory belongs to should be stated.

“Time of the research” covers information on when the research begins, starting from the preparation stage, and when it ends (month and year and, when applicable, season).

c. Materials and Instruments

Information on research materials should cover the detailed specification of the materials, where the materials are from, how the materials are prepared, how the materials are selected including the inclusive and exclusive criteria used in the sample selection, the age of the sample (when applicable), physical characteristics, and chemical substance being used (brand name and the country where it is from).

Information on instruments used in the research should also cover the detailed specification of the instruments since the validity of the research is also indicated by the instruments. Such detailed information will also allow the research to be accurately replicable.

For example:

The research used 40 healthy female rats of Balb/c strain. The rats, weighing between 20 to 30 gram are of 12 weeks of age. The inbred rats were obtained from Pusvetma Wonocolo Surabaya.

The animal subjects were infected by *Plasmodium berghei* of ANKA strain which was obtained from NAMRU-2 Jakarta. The measurement of parasitemia level was done using Olympus binokuler microscope

d. Operational Definition and Variable Observation/Measurement

Types of Variables that are to be observed/measured either qualitatively or quantitatively should be elaborated. In the sub-chapter, the method of data

collection covering the procedure of how the data were collected or how the data were measured, instruments used in the process, unit (either chemical, organoleptic, physical or biological), and statistical and/or mathematical methods and model of data analysis should be elaborated as well.

e. Research Methods

Detailed and complete account of the research procedure should be provided. This covers steps in conducting the research which are described in the form of a flow chart. Explanation in the chapter may vary depending on the coverage and complexity of the research. In a dissertation, which consists of more than one stage or type of research, the research method of each stage/type of research should be separately elaborated. If the method used is standard or refers to an established and available method, a reference concerning the source of the method should be provided. If the method being used is an innovation of the researcher himself/herself, a reference is not needed.

Chapter 5. Research Findings and Data Analysis

The presentation of findings in the chapter begins with a general introduction on what the chapter is about and a description on the sample characteristics. Then, it is followed by a detailed presentation of the research findings.

Research findings or observation result should include data required to support research findings or observation result. The data can cover the main data as well as supporting and complementary data. Research findings can be presented using texts, tables, figures, graphs, and pictures.

The tables, figure, or graphs is not to be thoroughly described, nor justified (the why and the how). What should be provided, by means of a narration, are what the data represent (what the data mean) and highlights on significant or controversial features of research findings.

The chapter should be able to present findings for every research objective by means of statistical analysis. The result of statistical analysis can be presented in the form of tables, but the table should be included as an appendix, not part of the chapter on research findings.

Research findings for dissertation may be presented in more than one chapter depending on the nature of the research. Titles given for the chapters in a dissertation is in accordance with the topic of the chapters.

To write research findings well, the followings are required:

1. The structure or the order of research findings presentation should be in accordance with the order of hypotheses or research problems. This will allow the readers ease in understanding the research findings. The presentation of research findings should begin with an outline of every hypothesis or problem and followed by the result of statistical analysis (if any)
2. The result of data analysis begins with a description of statistical analysis used to compare different conditions (significance value) or hypothesis test (if any)

Chapter 6 Discussion

The main principle applied in this chapter is to discuss the findings by referring to the problems being questioned in hypothesis, and later compare the findings to the other research findings that are conducted by other researchers using either the same or different methods. This chapter should provide explanations why phenomena being observed happen, either the expected or unexpected ones. This chapter also covers the impacts of the research findings both theoretical and practical/clinical.

Below are several techniques or ways to discuss research results.

1. Based on the problems and objectives
This technique is frequently applied and considered good since researcher can directly discuss research problems and provide direct and immediate answers to the problems.
2. Based on the proposed hypotheses
The researcher can directly answer the proposed hypotheses.
3. Based on the obtained result
This technique is the easiest, most frequently used, and most effective since the coverage of the discussion is focused. In the process of the research, it is possible that there are other findings besides the mainly researched ones. These other findings may serve as a starting point for further research. Another advantage of the technique is that a discussion that is based on the order of the findings will minimize the probability of overlooking certain points of the research findings.

Every paragraph contains at least 3 main elements:

Main idea or main sentence;

- 1) Main idea or main sentence: a sentence that conveys the main idea. This can be directly from the main idea or refer to the results which have been presented, such as sub chapter, certain figures or tables.
- 2) supporting sentence(s); one or some sentences whose ideas refer to the results of the previous research, or opinions taken from literatures which are relevant or related to the main idea. They might support or contradict with the main idea.
- 3) Conclusion or closing: sentences that state the summary, explanation or opinion which is the result of researcher's interpretation of various opinions stated in the supporting sentences.

For Example:

From table 5.1 it can be seen that the feeding of leaf "X" extract to the research animals caused a insignificant decrease in the level of leptin serum ($p > 0,05$, ANOVA). This is different from the previous research by Anu (2005) indicating that feeding with "Y" leaf containing alkaloid the same as "X" leaf decreases leptin level significantly ($p < 0.05$). Badu (2007) conducted the similar experiment using "z" leaf extract which is suspected to contain the same active ingredient, and it was found that it caused an insignificant leptin level. A significant decline was found in the group of research animal fed with stem extract of "Z" plant. This facts indicate that the three plants of "X", "Y" and "Z" are actually contain active ingredients that can decrease leptin level. The "X" leaf

extract turns out give result that is in line with the "Z" stem extract. To find out the difference and similarities of the results, a further research is encouraged.

Chapter 7 Conclusions and Suggestions

Conclusion is not a repetition or summary of the research result, instead it contains a conclusion derived from the whole research activities. A conclusion should be relevant and able to answer questions stated as the research problems and hypotheses. A conclusion should also explain whether the hypothesis is proven or not, and include the main reasons. A conclusion should also indicate other phenomena apart from the hypothesis and might become a new topic for research.

Suggestion contain researcher's opinion that can be done or must be done to continue or clarify the results and conclusion resulted from the research, both for own research or study by other reaserchers.

VI CLOSING PART OF THESIS OR DISSERTATION

The final section of thesis and dissertation include list of references and appendixes. List of references contains the literature that have been taken as references in writing the thesis or dissertation. List of literature must be relevant with the citations in the text. The method to cite the literature and the writing system is elaborated separately.

The appendixes contain data or other information which function to support the elaboration presented in the main section of thesis and dissertation. Appendixes can be in the form of work sheet, status, calculation method, questionnaire, elaboration of analysis, picture, photo, map, supporting data, inform consent etc. Basically, appendixes are additional explanation which are useful but not directly discussed in the text for, if they are presented in the text, they will disturb the context of the discussion.

Curriculum Vitae/Resume

The resume contains the name of the writer, place and date of birth, name of parents, education and work summary as well as the outstanding achievements. It is written in 1 space, (Appendix 20)

VII CITATION AND REFERENCES

This chapter discusses the citation and referencing method. In writing academic work, honesty and consistency are basic principles that need to be underscored. Every statement written in the text, from the introduction section to the discussion, must be able to be justified scientifically. If the idea or the written statement quoted or derived from the writings of others, the source should be written clearly. Otherwise, it is not allowed to write the source or name of a writer that are not mentioned in the text. Taking idea, statement, picture or table, the whole or in part, without citing the source is a violation of writing ethic which is called as PLAGIARISM, and this can result in illegitimacy of the scientific work.

There two methods of citation (1) citations of sentences presented in the body of text, and (2) citation presented as a footnote.

VII.1 Citation Presented in the text

The Post Graduate Program of FMUB has set way of writing literature in the text by writing the name and year. The author's name is written in the text and only the family name and year written in parentheses.

Example:

Kader (1991) reported that

Based on the research by Tarwiyanto (1990) it was found out that

The quality standards for syrup commodity used in this research was.....

(Sentono, 1994).

If the reference is written by two persons, both names must be written.

Example:

Brown and Belding (1976) expound that

If the number of authors is more than one, the name of the first writer is written, followed by an abbreviation of *et al.* For example: Kader, et.al (1991). The writer can cite the result or opinion of the researcher listed in the literature of the other researcher. The maximum number of citation is five. The technique of citing the other writer's opinion presented in the other's work.

Example:

Biale (1984) in Asrofi (1986) states that ...

Marketing model (Biale, 1984 in Asrofi, 1986)

If a series of ideas obtained from a number of different sources, for example : (Cai *et al.*, 1999; Like *et al.*, 2004). Citing a series of different sources in the same year, they are written in alphabetical order e.g Chen *et al.*, 2000; Like *et al.*, 2000).

VII.2 Citation Presented as Footnote

There are two types of footnote; based on (1) content and (2) literature. Footnote written based on the content holds important information, but it is too long or may disturb the flow of the idea if presented in the body of the text. For footnote which is based on the literature, the text and footnote are separated by a line from the left margin of the page.

A footnote is written in paragraph. Sentences in a footnote is typed in one space, while two footnotes are separated by 2 spaces. A footnote must be presented on the same page as the text that cites the footnote. *Ibid* is used when a certain footnote refers to a previously used footnote. In a footnote, the title of the book is typed in italic.

An example of footnote from textbook :

³R.M. Dowben, "*Cell Biology*", Harper and Row, Publishers, New York, Evanston, San Francisco and London, 1971, pp.40-50.

⁴*Ibid.* p. 95.

op.cit is use if the cited footnote is interspersed with another footnote

For example :

⁵P.D. Pages, SEARCA Bulletin 2:102 (1971).

⁶E.B. Pantastico, UP College of Agriculture Monthly Bulletin, 36(8):3.

⁷Pages, *op. cit.* p.4.

⁸Pantastico, *op. cit.* p. 364.

VII.3 Writing List of References

List of references is presented on a new page, typed in capital letters, and put on the left top part of the page.

List of references is specifically intended for references cited in a thesis or dissertation. The references are presented in alphabetical order. Variation in the way a reference is written may result from the difference in the types of references used, for example textbooks, scientific journal articles, and thesis or dissertation.

VII.3.1 The writing of author's name of list of references

The writing of author's name of list of references is as follow:

1. For Indonesian names, if it consists of more than one word, the last name or a name wellknown in scientific publication is written first.
For example: Muhammad Sudomo is written Sudomo, M.; Franciscus G. Winarno s written d Winarno, F.G.
2. For foreigner name, the family name is written first.
For example:James Stewart is written Stewart, J.
3. For Chinese name, if it consists of three separate, the first word indicates the family name.
For example: Gan Koen Han is written Gan, K.H.
4. If a Chinese name consists of three words, but the first two words are connected by a dash, the connected words are given name (not a family name)
For example: Hwa-wee Lee is written Lee, H.

5. Avoid anonymous or general references (Wikipedia, Kompas, dictionary, etc), unless it is used to highlight common problems or issues such as an epidemi outbreak, natural disaster, president's instruction, etc.

VII.3.2 Writing Title in List of References

List of references catalogs all references cited by the writer in his/her thesis/dissertation. The writing of a reference begins with the name of the author (the family name first followed by a comma, then the initial letters of the author's given and middle name followed by a period), year of publication (followed by a period), title of the reference (followed by a period), name of journal (if the reference is taken from a journal) followed by a period, name of publisher (if the reference is a textbook) followed by a period, the city where the book is published (if the reference is a textbook) followed by a period, and page number followed by a period.

The followings are general rules in writing a reference.

- Title of textbook is typed in regular letters (not in italic). The initial letter of each word is capitalized (except prepositions and conjunctions).
- Title of journal articles is also typed in regular letters (not in italic). However, only the initial letter of the title is capitalized.
- Title of thesis or dissertation is typed in italic and only the initial letter of the title is in capital letter.
- Name of journal from which the journal article is taken is typed in italic. The volume number is typed in bold. The issue number is given in parentheses. The page number is provided after colon (:). For example: *Journal of Immunology and Infectious Diseases* **15**(4): 112-122.
- Page numbers for textbooks are not provided, while the page number for an article taken from a textbook is provided after the name of the editor (Ed) or "editor" (eds) for journals.
- Any second lines in a list of references page is typed in 1.5 cm indentation.

Examples of how a reference is written are provided below:

- a. Reference in the form of journals (Indonesian or English or other foreign languages): Name of author, year of publication, title of journal article, name of the journal, volume number, issue number, page numbers of the article.

An example of reference from a journal article:

Lecomte, N.B., Zayas J.F., and Kastner C.L., 1993. Soya proteins: Functional and Sensory Characteristics Improved in Comminuted Meats, *J. Food Sci.* **58** (3): 464 - 466.

- b. Reference in the form of textbooks: name of author, year of publication, book title, edition, publisher, name of the city in which the book is published, page number.

An example of a reference from a textbook:

Salunke, D.K. and Desai BB. 1984. *Post Harvest Biotechnology of Fruit and Vegetables*. First edition. CRC Press. Inc. Cleveland Ohio. p. 44-128.

- c. Reference in the form of proceedings: Name of author, year of publication, title of the paper, name of the editor(s), title of the proceeding, publisher, city in which the proceeding is published, page number from which the reference is cited.

An example of reference from a proceeding :

Zagory, D. D. and Kader A.A., 1989. Long term Storage of Early Gold and Shinko Asian Pears in Low Oxygen Atmospheres in J.K., Fellman (ed.), *Proc. Fifth Intl. Controlled Atmospheres Res. Conf.*, Wenatchee, Wash. p. 44-47.

- d. Reference in the form of abstracts
Citing from a reference in the internet is allowed as long as it is from a reliable and authorized source (name of author, name of journal and/or publisher).

For example:

Ohmiya, Y., T. Hirano, M. Ohashi. 1996. The Structural Origin of the Color Differences in the Bioluminescence of Firefly Luciferase. *Abstracts FEBS Letters* 381 (1): 83-86.

- e. Reference in the form of translated textbooks: Both versions (original language and the translated one are mentioned (see point b: reference for textbooks). The page number from which the reference is cited is also provided.

For example:

Fukuoka, M. 1991. *The One Straw Revolution. An Introduction to Natural Farming*, L. Korn. (editor), 1978. First Edition Rodale Press. Inc. H. Soedarwono (penterjemah). 1991. *Revolusi Sebatang Jerami. Pengantar Menuju Pertanian Alami. Edisi Pertama*. Yayasan Obor Indonesia. Jakarta. p. 44-47.

- f. Reference in the form of bulletins: name of institution publishing the bulletin. Page number is not provided.

For example:

UNEP, 1993. *United National Environment Program: Environmental Data Report, 1993-1994*. Blackwell Publishers, Oxford, UK. n.p.

- g. Reference cited from the internet (non-journal)
If year of publication is not provided, the date from which the article is downloaded should be mentioned.

For example:

Witherspoon, A.M. and R. Pearce. 1982. Nutrient and multispecies criteria standard for the Chowan River, North Carolina. Report No. 187. www.2.ncsu.edu/wrri/reports/report187.html. downloaded on May, 28, 2004.

- h. Reference in the form of journal article taken from the internet is written the same way as point 7.4. a.

Some journals have standard abbreviations for their name. few examples are:

1. **Parasitol Int** : *Parasitology International*

For example:

Huy NT, Trang DT, Kariu T, Sasai M, Saida K, Harada S, Kamei K, 2005.
Lekocyte Activation by Malarial Pigment. Parasitol Int. 55(1):75-81.

2. **AJTMH** -- *American Journal of Tropical Medicine and Hygiene*

For example:

Lyke KE, Diallo DA, Dicko A, Kone A, Coulibaly D, Guindo A, Cissoko Y, Sangare L, Coulibaly S, Dakouo B, Taylor TE, Doumbo OK, Plowe CV. 2003.
Association of intraleukocytic Plasmodium falciparum malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria. AJTMH. 69 (3): 253-9.

3. **AJRCCM** : *American Journal of. Respiratory and. Critical Care Medicine*

For example:

Ortolani, O., Conti, A., Gaudio, A.R.D., Moraldi, E., Cantini, Q., & Novelli, G. 2000. The effect of glutathione and N-acetylcysteine on lipoperoxidative damage in patients with early septic shock. *AJRCCM.* 161:1907–1911

4. *Australian Journal of Exp. Agric. Animal Husbandry*

For example:

Russell, J.S.1963. Nitrogen content of wheat grain as an indication of potential yield response to nitrogen fertilizer. *Aust. J. Exp. Agric. Anim. Husb.* 4: 345-351.

VIII WRITING TABLES, FIGURES, SYMBOLS, UNIT OF MEASURES, ABBREVIATIONS, AND ITALICS

VIII.1 Table

A table should be fully presented in one page. It should not be presented partly on one page and continued on another. A table should be followed by a narration about what the table represents or significant features in the table, not about the description of the table. In certain condition, the size of the letters (fonts) inside the table can be made smaller. The tables that are presented in the thesis/dissertation's main part are those that are directly discussed in the part. If a table is not discussed in the part, but required for reference, it should be included as an appendix. Table should be given an identity in the form of arabic numerals indicating (1) the chapter number in which the table is presented and (2) the order of appearance of the table in the said chapter and sub chapter. The identity of a table is typed using capital "t", for example: Table 1.1 for the first table which is presented in chapter 1: Table 5.2.2 for the second table which is presented in chapter 5, and sub-chapter 2, and so forth.

Information in table title and the table itself should be presented in ways that allow easy and independent understanding, without any other references. Therefore, the use of codes and symbols in tables describing variables or treatments used in a research is strongly discouraged. If the use of codes or symbols cannot be avoided, notes on the meanings of the codes or symbols should be provided below the table.

For a table which is taken from a reference, name of author and year of publication should also be provided in parentheses.

Table number and title are provided above the table. The space between table number and title and the table itself is two spaces. Table title is typed at one space and not ended with a period. An example of a table is provided in **appendix 21**

VIII.2 Figures

Figures cover graphs, diagrams, monogram, photographs, and maps. It is strongly suggested that both graphs and monogram be made using computer program with symbols whose meanings are clearly defined. Graphs presented in the latest scientific journals serves as references in the making of a graph. Ideally, a graph should be able to present data or research findings independently, without any other supporting texts. Similar to tables, the identity of a figure is given in the form of arabic numerals indicating (1) the chapter number in which the figure is presented and (2) the order of appearance of the figure in the said chapter and sub-chapter. The identity of a figure is typed using capital "f". Figure number and figure title are typed below the figure. The space between the identity of the figure and the figure is two spaces. Figure title is typed at one space.

A photograph should be presented in such a way that allow clear understanding of what the photograph is about. For maximum effect, it is recommended that the background of the photograph be in contrast with the object of the picture. When the photograph is taken, a ruler should be put beside the object to give reader an idea of the real length/diameter of the object. An alternative is by the provision of a scale of the object, for example a scale of 1:100.

VIII.3 Symbols, Unit of Measurement, and Abbreviations

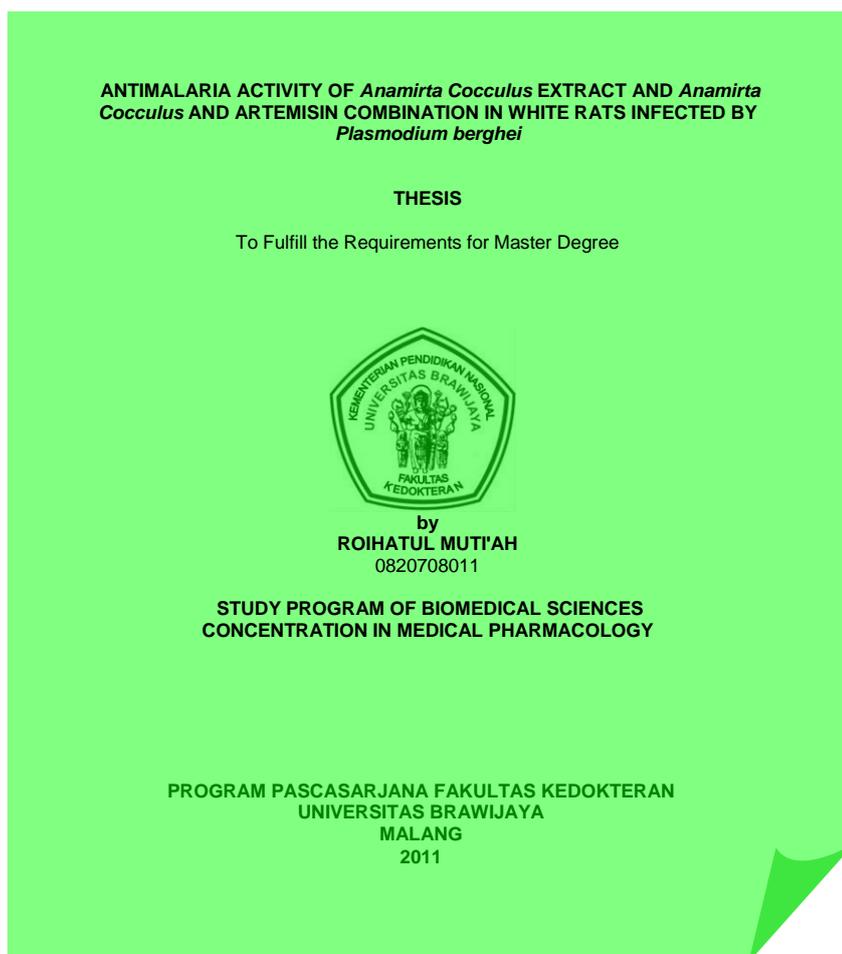
Symbols for research variables is used to make the writing of the variables in formulas and other algebra equations easier. The writing of symbols should be done using symbols readily provided by software programs such as *Wordstar* or *Microsoft Word*. Symbols to be used should be those that are commonly used in certain academic discipline. A formula should be completely presented in one line. If it is not possible, the formula should be typed in such a way that will not hinder comprehension. Symbols are typed using Roman and Greek characters.

The followings are some examples of it of measurements to be used should be those that are common in a certain discipline. The followings are some examples of the use of unit of measurement: 25°C; g; mg; 10 g ml⁻¹ or 10 g/ml; 50%; 10 ppm; 1.5 N of H₂SO₄; L; kg; ton; kw; °Brix; °Baume; mg O₂/kg/hour; atau mg O₂ Kg⁻¹ hour⁻¹.

VIII.4 Words in Italic

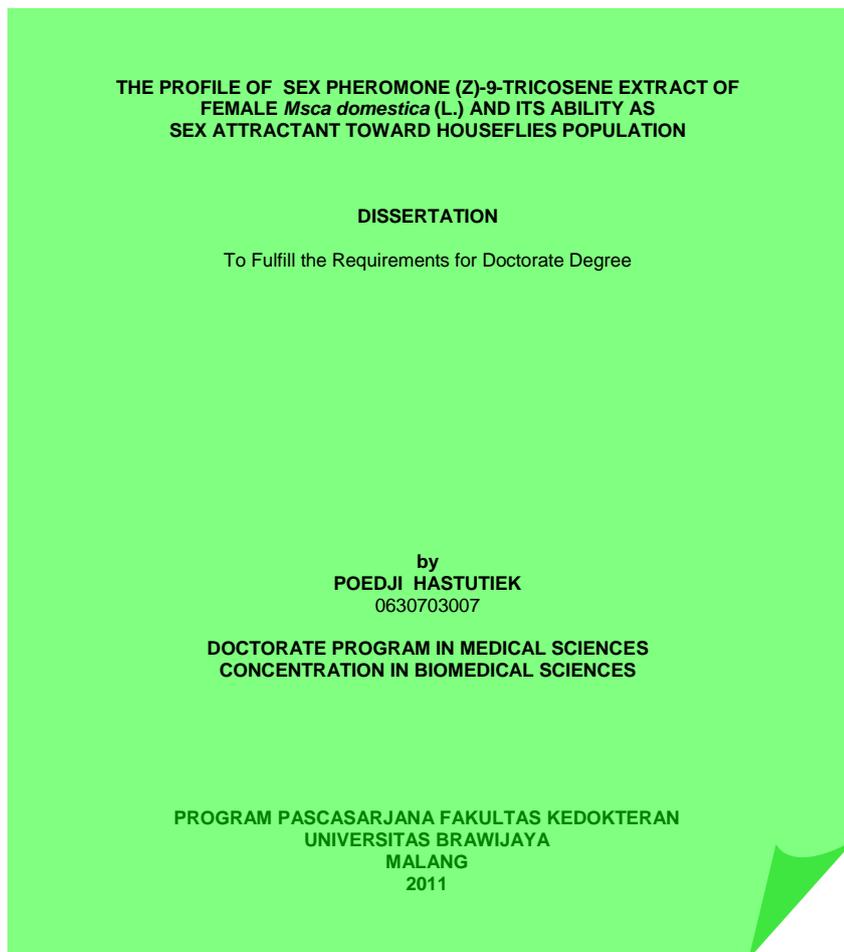
Words which are put in italic are foreign words or terminologies (such as English or Latin) for examples: *et al.*; *ibid*; *op. cit.*; *curing*; *starter*; *trimming*; *dummy*. For the latin name for plants or animals, only the species name is in italic (*Rhizopus oryzae*). The genus/family name is in regular font (non-italic).

Appendix 1. Front Cover of Thesis
(cover color: light green D8 or Linen, letters: black)¹



¹ for thesis/dissertation examination, the manuscript is bound using soft cover. After the thesis/dissertation is validated, the manuscript is bonded using hard cover

Appendix 2. Front Cover for Dissertation
(cover color: light green D8 or Linen, letters: black)¹



¹ for thesis/dissertation examination, the manuscript is bound using soft cover. After the thesis/dissertation is validated, the manuscript is bound using hard cover

Appendix 3. The Writing of Book Spine



Set 5 cm (from the bottom) aside for the symbol of UB library

Appendix 4. Inner Cover for Thesis
(white A4 paper, black letters)

**ANTIMALARIA ACTIVITY OF *Anamirta Cocculus* EXTRACT AND
Anamirta Cocculus AND ARTEMISIN COMBINATION IN WHITE
RATS INFECTED BY *Plasmodium berghei***

THESIS

To Fulfill the Requirements for Master Degree



oleh
ROIHATUL MUTIAH
0820708011

**STUDY PROGRAM OF BIOMEDICAL SCIENCES
CONCENTRATION IN MEDICAL PHARMACOLOGY**

**POSTGRADUATE PROGRAMS FAKULTY OF MEDICINE
UNIVERSITAS BRAWIJAYA
MALANG
2011**

Appendix 5. Inner Cover for Dissertation
(white A4 paper, black letters)

**THE PROFILE OF SEX PHEROMONE (Z)-9-TRICOSENE EXTRACT
OF FEMALE *Musca domestica* (L.) AND ITS ABILITY AS
SEX ATTRACTANT TOWARD HOUSEFLIES POPULATION**

DISSERTATION

To fulfil the requirements for
The degree of doctor

by
POEDJI HASTUTIEK
0630703007

**DOCTORATE PROGRAM IN MEDICAL SCIENCE
CONCENTRATION IN BIOMEDICAL SCIENCE**

**POSTGRADUATE PROGRAMS FAKULTY OF MEDICINE
UNIVERSITAS BRAWIJAYA
MALANG
2011**

DISERTASI

**FIBROSIS GINJAL NEFRITIS LUPUS DIPENGARUHI POLIMORFISME
GEN T869C, PERUBAHAN PEPTIDA SINYAL DAN EKSPRESI PROTEIN
DARI *TRANSFORMING GROWTH FACTOR-β1***

Oleh:

HANI SUSIANTI

Dipertahankan di depan penguji
Pada Tanggal : 6 Nopember 2014
Dan dinyatakan memenuhi syarat

Komisi Pembimbing,

Prof.Dr.dr. Handono Kalim, SpPD.,KR.
Promotor

Prof.Dr.dr. Kusworini, M.Kes.,SpPK.
Ko-Promotor 1

Dr.dr. Basuki B. Purnomo, SpU.
Ko-Promotor 2

Prof.Dr.dr. Edi Widjajanto, MS.,SpPK.(K)
Penguji 1

Widodo, S.Si.,M.Si.,Ph.D.Med.Sc.
Penguji 2

Prof.dr. Moch. Thaha, PhD.,SpPD-KGH,FINASIM,FACP,FASN.
Penguji Luar

Malang,

Universitas Brawijaya
Fakultas Kedokteran
Dekan,

Dr.dr. Karyono Mintaroem, SpPA.
NIP. 19501116 198002 1 001

Appendix 7 Statement of Originality Page for Thesis and Dissertation

STATEMENT OF ORIGINALITY

I truly state that to the best of my knowledge there is not a single part of the dissertation manuscript which belongs to other's scientific works as part of the requirements to obtain academic title from a higher education institution, and that there is no other's written or published works or opinions stated in dissertation except those that are properly cited in the manuscript.

If it is revealed that the dissertation manuscript contains, partly or wholly, elements of PLAGIARISM, I readily accept that the dissertation is invalidated, the academic title that I have been granted revoked, and I will be subject to legal processes in accordance with the current legislation

Malang, 17th March, 2010

Student,

Revenue stamp of 6000 rupiahs

Name : Dorta Simamora
SIN : 0530300005
SP : Medical Sciences
Prog. : Postgraduate
Faculty : Medicine UB

STATEMENT OF ORIGINALITY

I truly state that to the best of my knowledge there is not a single part of the thesis manuscript which belongs to other's scientific works as part of the requirements to obtain academic title from a higher education institution, and that there is no other's written or published works or opinions stated in the thesis except those that are properly cited in the manuscript.

If it is revealed that the thesis manuscript contains, partly or wholly, elements of PLAGIARISM, I readily accept that the thesis is invalidated, the academic title that I have been granted revoked, and I will be subject to legal processes in accordance with the current legislation

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Student,

Revenue stamp of 6000 rupiahs

Name : Dorta Simamora
SIN : 0530300005
SP : Medical Sciences
Prog. : Postgraduate
Faculty : Medicine UB

Appendix 8. Identity of Examination Committee for Thesis

THESIS TITLE:

Name : -----
Identity Number : -----
Study Program : -----
Interest : -----

ADVISOR COMMITTEE:

Chairman : -----
Member 1 : -----
Member 2 : -----

EXAMINER COMMITTEE:

Examiner 1 : -----
Examiner 2 : -----

MONEV COMMITTEE -----

Date of Examination : -----
Examiner Decree : -----

Appendix 9. Identity of Examiner Committee for Dissertation

DISSERTATION TITLE:

Name : -----
Identity Number : -----
Study Program : -----
Interest : -----

PROMOTOR COMMITTEE:

Promotor : -----
Co-promotor 1 : -----
Co-promotor 2 : -----

EXAMINER COMMITTEE:

Examiner 1 : -----
Examiner 2 : -----
Examiner 3 : -----

Date of Closed Examination-----
Date of Public Examination----- :
Examiner Decree : -----

SCIENTIFIC COMMUNICATION AND PUBLICATION

- Dorta Simamora**, Sutiman B. Sumitro, Loeki Enggar Fitri. Intervensi *Cytoadherence* Sebagai peluang Pencegahan dan Terapi Malaria *falciparum* : Jurnal Kedokteran Brawijaya Vol. XXII No 2 Agustus 2006.
- Dorta Simamora**, Sutiman B. Sumitro, Loeki Enggar Fitri Resistensi Obat malaria : Mekanisme dan Peran Kombinasi obat Antimalaria Untuk Mencegahnya : Jurnal Kedokteran Brawijaya Vol. XXIII no 2 Agustus 2007.
- Dorta Simamora**, Sutiman B. Sumitro, Loeki Enggar Fitri. Epidemiologi Malaria : Majalah Kedokteran Tropis Indonesia Vol. 18 No 1 Maret 2007.
- Loeki Enggar Fitri, **Dorta Simamora**, Fitri Armania, Setyawati Karyono, Sumarno. Efek *N-Acetyl Cysteine (NAC)* dengan Artemisinin terhadap kadar GSH, GSSG serta rasio GSH/GSSG pada eritrosit mencit Balb/C terinfeksi *Plasmodium berghei*. Jurnal Ilmu-Ilmu Hayati (Life Science). 2008 Volume 20 No 2: 182-192.
- Dorta Simamora**, Nur Permatasari, Teguh Wahyu Sardjono, Loeki Enggar Fitri. Therapeutic Effect of Combination of Artemisinin and Acetyl Cystein to the Malondialdehyde (MDA) and Radical Peroxide (H₂O₂) level of Erythrocyte Balb/C Infected with *Plasmodium berghei*. Presentation pada Seminar International Molecular and clinical Aspect of HIV-AIDS, Tuberculosis and Malaria. 16-18 Pebruari 2009. Fakultas Kedokteran Universitas Brawijaya Malang.
- Loeki Enggar Fitri, **Dorta Simamora**, Fitri Armania, Setyawati Karyono, Sumarno. Effect N Acetyl Cysteine, Combined with artemisinin on erythrocyte Antioxidant enzymes in *Plasmodium berghei* infected mice. 5th Malasya Indonesia Brunei Medical Science Conference 2009. Jakarta 23-25 July 2009
- Dorta Simamora**, Teguh Wahyu Sardjono, Loeki Enggar Fitri. Effect of N-acetyl Cysteine on Caspase-3 and ROI Expression of HUVECS Exposed with serum Malaria *falciparum* patient. Disampaikan, pada Seminar dan Workshop Pertemuan Ilmiah Berkala Parasitologi Klinik III (PDS PARKI) 23-24 Oktober 2009 di FK Universitas Indonesia. Jakarta.
- Dorta Simamora**, Loeki Enggar Fitri. Antioksidan Pada Infeksi Malaria. Jurnal Moluca Medica. 2009 Oktober Volume 1 No 2,
- Dorta Simamora**, Wahyuda Ngatiril Lady, Wibi Riawan, Sumarno, Setyawati SK Teguh Wahyu Sardjono Loeki Enggar Fitri. N-Acetyl Cysteine Reduces Intercellular Adhesion Molecule-1 Expression of HUVECs Exposed with Serum of Malaria *Falciparum* Patient. Disampaikan pada Seminar Internasional. The Fourth Asean Congress of Tropical Medicine and Parasitology di Singapore 2-4 Juni 2010.
- Loeki Enggar Fitri, Teguh Wahyu Sardjono, **Dorta Simamora**, Sumarno, Setyawati SK. High Dose of N-Acetyl Cysteine Increase H₂O₂ and MDA Level and Decrease GSH Level of HUVECs Exposed with Malaria Serum. Disampaikan pada Seminar Internasional. The Fourth Asean Congress of Tropical Medicine and Parasitology di Singapore 2-4 Juni 2010.

Appendix 11. Dedication Page



*The thesis is dedicated to
my beloved Mother and Father,
Husband/Wife, My Children
xxx dan xzy*

Appendix 12. Acknowledgement Page for Thesis or Dissertation

ACKNOWLEDGEMENT

Praise to Almighty Allah SWT, for His blessing that enables the writer to complete this thesis/dissertation entitled:

.....

Main points presented in the thesis/dissertation covers

.....

With the completion of the thesis/dissertation, the writer would like to express his/her deepest gratitude to:

1.....

2.....

Due to his/her limitation and shortcoming, the writer realizes that this thesis/dissertation is far from perfection; therefore, any suggestions and constructive criticisms are greatly appreciated so that the thesis/dissertation is of use for those who need it.

Malang,

The writer

RINGKASAN

Dorta Simamora, NIM. 0530300005. Program Pascasarjana Fakultas Kedokteran Universitas Brawijaya Malang, 17 Maret 2010. Efek *N-Acetyl Cystein* (NAC) Terhadap Kadar Reaktif Oksigen Spesies (ROS) dan Antioksidan Selama Infeksi Malaria : (Studi In Vivo pada Mencit Terinfeksi *Plasmodium berghei* dan In Vitro pada Kultur HUVECs Dipapar Serum Penderita Malaria *falciparum*) Komisi Pembimbing Ketua: Soemarno, Anggota : Loeki Enggar Fitri, Setyawati Suharto, Teguh Wahyu Sardjono.

Malaria *falciparum* merupakan jenis malaria yang dapat menyebabkan timbulnya komplikasi pada berbagai organ, seperti malaria otak yang menjadi penyebab kematian terbanyak. Salah satu patomekanisme terjadinya komplikasi tersebut berhubungan dengan produksi *Reactive Oxygen Species* (ROS) yang berlebihan sehingga mengakibatkan terjadinya stres oksidatif. Respon imun terhadap malaria melibatkan imunitas selular maupun humoral dan dihasilkan berbagai macam sitokin terutama *Tumour Necrosis Factor alpha* (TNF- α) dan *Interferon gamma* (IFN- γ) yang dapat menyebabkan terjadinya sitoadheren dan terbentuknya radikal bebas yang berlebihan.

Artemisinin merupakan obat pilihan pertama yang direkomendasikan WHO untuk mengatasi kegawatan dan resistensi obat malaria. Artemisinin bekerja melalui mekanisme stres oksidatif, dan penghambatan ATPase untuk membunuh parasit malaria. Artemisinin juga menyebabkan penurunan deformabilitas eritrosit dan hemolisis eritrosit terinfeksi secara prematur. Penambahan *N-Acetyl Cystein* (NAC) sebagai antioksidan, imunostimulator, dan penghambat produksi TNF α yang berlebihan diharapkan mampu menurunkan pembentukan kadar H₂O₂ dan MDA eritrosit mencit Balb/C terinfeksi *Plasmodium berghei* (*P.berghei*).

Penelitian dilakukan 2 tahap yaitu secara in vivo pada mencit Balb/C terinfeksi *Plasmodium berghei* dan in vitro pada sel endotel HUVECs yang dipapar dengan serum penderita malaria *falciparum*. Tujuan penelitian Tahap I (in vivo) adalah untuk mengetahui perbedaan derajat parasitemia, kadar H₂O₂ dan MDA, kadar GSH, GSSG maupun rasio GSH/GSSG eritrosit mencit Balb/C terinfeksi *P. berghei*, antara kelompok yang diberi kombinasi artemisinin dan NAC dibandingkan dengan yang diberi artemisinin monoterapi.

Metode penelitian tahap I adalah "*True Experimental Design*" dengan desain "*The post test- only control group*". Sampel terdiri dari mencit normal (kontrol negatif), mencit yang diinfeksi *P. berghei* (kontrol positif), mencit yang diinfeksi *P. berghei* dan diberi artemisinin saja, mencit yang diinfeksi *P. berghei* dan diberi artemisinin ditambah NAC dosis konstan, dan mencit yang diinfeksi *P. berghei* dan diberi artemisinin ditambah NAC dosis *tapering*. Pada uji *Univariant Analysis of Variance* dengan tingkat kepercayaan 0,05 didapatkan hasil bahwa terjadi perbedaan yang signifikan pada kadar MDA dan H₂O₂ eritrosit mencit galur Balb/c di antara kelompok yang diberikan artemisinin mono-terapi 0,0364 mg/gBB mencit dan kelompok yang diberikan artemisinin 0,0364 mg/gBB mencit dan NAC dosis konstan dan dosis *tapering* pada hari ke-3, ke-5, dan ke-7.

Artemisinin monoterapi menurunkan derajat parasitemia yang sama efektifnya dengan kombinasi artemisinin dan NAC. Kadar MDA dan H₂O₂ eritrosit terendah ditemukan pada kelompok terapi kombinasi artemisinin dan NAC dosis *tapering*. Kadar GSH dan rasio GSH/GSSG eritrosit tertinggi ditemukan pada kelompok terapi kombinasi artemisinin dan NAC dengan dosis konstan.

Tujuan penelitian Tahap II (in vitro) adalah untuk mengetahui efek *N-acetyl cystein* terhadap produksi radikal bebas H_2O_2 , MDA, GSH, GSSG, rasio GSH/GSSG, ekspresi Caspase 3, ICAM-1 dan produksi ROI sel endotel yang dipapar dengan serum penderita malaria falciparum berat. Kultur HUVEC's yang dipapar dengan serum penderita malaria falciparum berat secara bersamaan diberi NAC dosis 2 μM , 4 μM 8 μM . Kadar H_2O_2 adalah total kadar H_2O_2 yang diukur atas dasar reaksinya dengan horseradish peroksidase (HRP) sehingga menghasilkan produk oksidase yang berfluorosensi merah yaitu resorufin (*NWLSSTTM Hydrogen Peroxide Assay Product NWK-HYP01*). Malondialdehyde (MDA) adalah metabolite peroksidasi lipid yang diukur dengan reaksi *Tio Barbituric Acid reaction (TBARs)*. Konsentrasi GSH diukur menggunakan spektrofotometer dengan absorbansi 412. nm, menggunakan kit (*OxisResearchTM A Division of OXIS Health Products, Inc. BIOXYTECH[®] GSH/GSSG-412TM (Catalog Number 21040)*). Konsentrasi GSSG dibentuk dari disulfide melalui gabungan reaksi antara GSH GS-TNB yang dengan segera tereduksi menjadi GSH diukur menggunakan kit (*OxisResearchTM A Division of OXIS Health Products, Inc. BIOXYTECH[®] GSH/GSSG-412TM. Catalog Number 21040*). Rasio GSH/GSSG menggambarkan presentasi GSH tereduksi dihitung setelah nilai GSH dan nilai GSSG diperoleh. Dengan menggunakan metode *Nitro Blue Tetrazolium (NBT)-reduction assays* (secara *bioassay*) diukur produksi *reactive oxygen intermediate (ROI)* sel endotel dan dihitung skor ROI. Apoptosis sel endotel ditunjukkan dengan imunositokimia menggunakan antibodi monoklonal terhadap caspase 3 (Catalog Number: AF-835 NeoMarkers For Lab Vision Corporation). Persentase keberadaan ICAM-1 di membran sel tiap 100 endotel pada kultur HUVECs, diukur secara semikuantitatif menggunakan teknik imunositokimia modifikasi *Avidin Biotin Peroxidase Complex* menggunakan antibodi ICAM-1, (US Patent 5879712).

Analisis hasil penelitian menggunakan uji BNT dengan tingkat kepercayaan 0.05 menunjukkan bahwa *N-Acetyl Cystein* dosis 4 μM dapat menurunkan kadar H_2O_2 pada HUVECs. Kadar MDA HUVECs menurun dengan pemberian NAC dosis 2 μM . Kadar GSH tertinggi terdapat pada HUVECs yang diterapi dengan NAC dosis 4 μM . Rasio GSH/GSSG tertinggi ditemukan pada HUVECs yang diterapi dengan NAC dosis 2 μM . Ekspresi Caspase-3 dan ROI terendah ditemukan pada HUVECs yang diterapi dengan NAC dosis 8 μM . Ekspresi ICAM-1 terendah pencapaian yang paling baik terdapat pada HUVECs yang diterapi dengan NAC dosis 4 μM .

Dapat disimpulkan bahwa NAC dapat menetralsir stres oksidatif pada infeksi malaria falciparum in vitro dengan menurunkan kadar H_2O_2 dan MDA pada sel endotel. Pemberian NAC juga mampu meningkatkan kadar GSH dan rasio GSH/GSSG sel endotel. Pemberian NAC juga dapat menurunkan produksi ROI sel endotel, menurunkan ekspresi ICAM-1 dan menghambat apoptosis sel endotel.

SUMMARY

Dorta Simamora, NIM. 0530300005. Postgraduate Programs Faculty of Medicine Universitas Brawijaya Malang, March 17th 2010. The Effect of *N-Acetyl Cystein* (NAC) Level of Reactive Oxygen Species (ROS) and Antioxidants During Malaria Infection (In Vivo Studies on *Plasmodium berghei* Infected Mice and In Vitro Culture HUVECs Exposed to Serum falciparum Malaria) Supervisor Chairman: Sumarno, Members: Loeki Enggar Fitri, Setyawati Suharto, Teguh Wahyu Sardjono.

Malaria falciparum is a type of malaria that can cause complications in various organs, such as cerebral malaria. One of the pathophysiology of this complication is related to the presence of high Reactive Oxygen Species (ROS) production on brain due to oxidative stress conditions. Immune response to malaria include cellular and humoral immunity and produce a variety of cytokines, especially *Tumour Necrosis Factor alpha* (TNF- α) and *Interferon gamma* (IFN- γ) which can cause cytoadherence and the formation of excessive free radicals.

Artemisinin is the first choice drug recommended by WHO to overcome the emergency in severe malaria and malaria drug resistance cases. Artemisinin worked through oxidative stress mechanisms and inhibit ATP-ases to kill the malaria parasite. Artemisinin also cause a decrease in deformability of erythrocytes and hemolysis of infected erythrocytes. Addition of NAC as an antioxidant, immunostimulator, and inhibitor of excess production of TNF α is expected to reduce the formation of H₂O₂ and MDA levels of erythrocytes of mice Balb/C infected with *Plasmodium berghei* (*P.berghei*).

This research was done in 2 stages, the first stage is in Balb/C mice infected with *P.berghei* (in vivo) and the second in Human Umbilical Vascular Endothelial Cells (HUVECs) exposed with serum falciparum malaria (in vitro). The objective of stage 1 is to determine the difference of the parasitemia degree, the H₂O₂, MDA, GSH and GSSG level as well as the ratio of GSH/GSSG of erythrocytes of Balb/C mice infected with *P.berghei* between group that treated with artemisinin-NAC combination and artemisinin monotherapy. The method of the first stage of this research is "True Experimental Design" with "The post test-only control group". The samples consisted of normal mice (negative control), mice infected with *P.berghei* (positive control), mice infected with *P.berghei* and treated with artemisinin monotherapy, mice infected with *P.berghei* and treated with artemisinin plus NAC in constant dose, and mice infected *P.berghei* and treated with artemisinin plus NAC in tapering dose. Univariate test analysis of variance with a 0.05 level of confidence results showed that there were a significant differences in levels of erythrocytes MDA and H₂O₂ among the group treated with artemisinin mono-therapy 0.0364 mg/BW (body weight) mice and the group treated with artemisinin 0.0364 mg/BW mice and NAC constant dose and tapering doses on day 3, 5, and 7. Artemisinin monotherapy reduced parasitemia as effective as the combination of artemisinin and NAC. The lowest levels of erythrocytes MDA and H₂O₂ was found in group that treated with artemisinin combination therapy, with tapering doses of NAC. The highest level of GSH and the ratio GSH/GSSG was found in group of artemisinin combination therapy with constant dose of NAC.

The objective of stage 2 is to determine the effect of N-acetyl cystein on free radical production such as H₂O₂, MDA, GSH, GSSG level, ratio of GSH / GSSG, the expression of Caspase 3, ICAM-1 level and production Reactive Oxygen Intermediates (ROI) of HUVECs exposed with serum of severe falciparum malaria patient and treated with NAC 2 μ M, 4 μ M 8 μ M, respectively.

Total level of H_2O_2 was measured on the basis of reaction with horseradish peroxidase (HRP) to produce oxidase products that fluorosens oxidase of resorufin red (NWLSTm Hydrogen Peroxide Assay Product NWK-HYP01). Malondialdehyde (MDA) is a lipid metabolite peroksidasi measured using a spectrophotometer in absorbance 412. Concentration of the disulfide GSSG formed through a combination of the reaction between GSH GS-TNB, which immediately reduced to GSH. Using the kit OxisResearch™ A Division of Oxis Health Products, Inc. BIOXYTECH® GSH/GSSG-412TM. (Catalog Number 21040). The ratio of GSH/GSSG was calculated after the value of GSH and GSSG values obtained. Using the method of Nitro Blue Tetrazolium (NBT)-reduction assays (bioassay) the production of ROI could be measured and calculated for ROI score. Endothelial cell apoptosis determined by imunocytochemistry staining using monoclonal antibodies against caspase 3 (Catalog Number: AF-835 NeoMarkers For Lab Vision Corporation). The percentage of ICAM-1 presence in the membrane per 100 endothelial cells in culture endothelial cells (HUVECs), measured using modification of immunocytochemistry technique Peroxidase Avidin Biotin Complex with ICAM-1 antibody (US Patent 5879712)

Analysis study using BNT test with 0.05 confidence level indicates that the administration of N-Acetyl Cystein with 4 μ M dose can reduce the levels of H_2O_2 of HUVECs. MDA levels of HUVECs decreased by administration a 2 μ M of NAC. The highest level of GSH was found on HUVECs that treated with NAC 4 μ M. The highest ratio of GSH/GSSG was found in HUVECs that treated with NAC 2 μ M. The lowest Caspase-3 expression, and ROI production was found in the HUVECs treated with NAC 8 μ M. The lowest achieving ICAM-1 expression was found in HUVECs that treated with NAC 4 μ M.

It can be concluded that NAC can neutralize oxidative stress on falciparum malaria infection in vitro by decreasing the levels of H_2O_2 and MDA on endothelial cells. Provision of NAC was also able to increase the levels of GSH and the ratio of GSH/GSSG of endothelial cells. Administration NAC can also reduce the production of ROI, decreasing the expression of ICAM-1 and inhibits endothelial cell apoptosis.

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Appendix 19. List of Abbreviation Page for Thesis/Dissertation

LIST OF ABBREVIATIONS

ADCC	:	<i>Antibody Dependent Cell Cytotoxicity</i>
APC	:	<i>Antigen Presenting Cell</i>
ATPase	:	<i>Adenosine Tri Phosphat-ase</i>
BBB	:	<i>Blood-brain barrier</i>
BSO	:	<i>L-buthionine-S,R-sulfoximine</i>
COPD	:	<i>Chronic Obstructive Pulmonary Disease</i>
CM	:	<i>Cerebral Malaria</i>
DAB	:	<i>Diaminobenzidine Tetrahydrochloride</i>
DCs	:	<i>Dendritic Cells</i>
Depkes	:	<i>Departemen Kesehatan</i>
DNA	:	<i>Deoxyribosa Nucleid Acid</i>
ELAM-1	:	<i>Endothelial Leucocyte Adhesion Molecule-1</i>
eNOS	:	<i>endothelial Nitric Oxide synthase</i>
Fe ²⁺	:	<i>ferrous iron</i>
Fe ³⁺	:	<i>ferric iron</i>
FP IX	:	<i>ferri/ferrotoporphyrin IX</i>
GCL	:	<i>Glutamate Cysteine Ligase</i>
GCS	:	<i>Glutamylcysteine Synthetase</i>
G6PD	:	<i>Glucose 6-Phosphatase Deficiency</i>
GPX	:	<i>Glutathione Peroxidase</i>
GRD	:	<i>Glutathione reductase</i>
Grx	:	<i>Glutaredoxin</i>
GS	:	<i>GSH synthetase</i>
GSH	:	<i>Glutathione</i>
GSSG	:	<i>Glutathione Disulphide</i>
H ₂ O ₂	:	<i>Hidrogen Peroksida</i>
Hb C	:	<i>Hemoglobin C</i>
Hb E	:	<i>Hemoglobin E</i>
HBMECs	:	<i>Human Bone Marrow Endothelial Cells</i>
Hb S	:	<i>Hemoglobin S</i>
HBSS	:	<i>Hank's Balance Salt Solution</i>
HLECs	:	<i>Human Lung Endothelial cells</i>
HPLC	:	<i>High performance Liquid Chromatography</i>
HRP	:	<i>Horseradish Peroxidase</i>
HRP2	:	<i>Histidin Rich protein 2</i>

RIWAYAT HIDUP

Roihatul Muti'ah, born in Malang, Februari 3rd 1980 is a daughter of Mr. Abdul Kholiq and Mrs Siti Romelah. She graduated from MI Nasrul Ulum in 1992, from SMP Islam in 1995 and from MAN Malang I in 1998. In 1998, she pursued further study in Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, and graduated in 2002. At the same year, she took a professional degree program in pharmacy. In 2008 she got her master in biomedical sciences from the Faculty of Medicine, Universitas Brawijaya, Malang. Starting from 2009 until now, she is a teaching staff of Science and Technology of Universitas Islam Negeri Malang.

Malang, August 2010

Appendix 21. Example of Table

Tabel 5.5. Percentage of Parasite in *Batang Talikuning* Extract Using Hemozoin Test

Treatment Group	Percentage					
	Hour 0		Hour 20		Hour 25	
	(mean ±SD) (%)	p value	(mean ±SD) (%)	P value	(mean ±SD) (%)	p value
K (-)	100,00 ± 0,00 ^a		100,00 ± 0,00 ^a		100,00 ± 0,00 ^a	
KI	102,04 ± 8,42 ^a	1,000	51,26 ± 10,55 ^b	0,012*	31,92 ± 1,99 ^b	0,002*
ETk1	92,01 ± 15,73 ^a	0,962	85,28 ± 9,13 ^{ab}	0,494	59,31 ± 10,41 ^b	0,018*
ETk2	101,82 ± 19,54 ^a	1,000	82,70 ± 2,79 ^{ab}	0,369	53,92 ± 8,31 ^b	0,010*
ETk3	104,43 ± 9,57 ^a	0,996	67,68 ± 12,67 ^{ab}	0,061	57,86 ± 11,11 ^b	0,015*

The same letter indicating insignificance difference (p>0.05)

*) p < 0,05 = significant difference compared to control group

Notes :

K(-) = Culture of *P.falciparum* 3D7 without any addition of drug/extract,

KI = Culture of *P.falciparum* 3D7 + chloroquine dose 10⁻²mg/ml

ETk1 = Culture of *P.falciparum* 3D7 + *batang talikuning* extract dose 10⁻³mg/ml

ETk2 = Culture of *P.falciparum* 3D7 + *batang talikuning* extract dose 10⁻²mg/ml.

Etk 3 = Culture of *P.falciparum* 3D7 + *batang talikuning* extract dose 10⁻¹mg/ml.

Appendix 22. Example of Graph

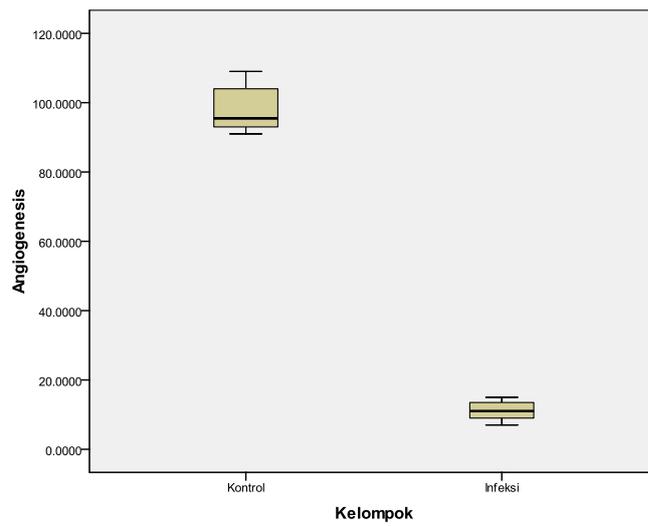


Figure 5.2.2 Data of VEGF expression in treatment and control group using boxplot. Data distribution between control and treatment group is normal. There is a significant difference between the two groups. In the control group, the data range is wide and its median is higher than the maximum value of the treatment groups.

Comment [A1]: nilai

Appendix 23 Example of Photographs

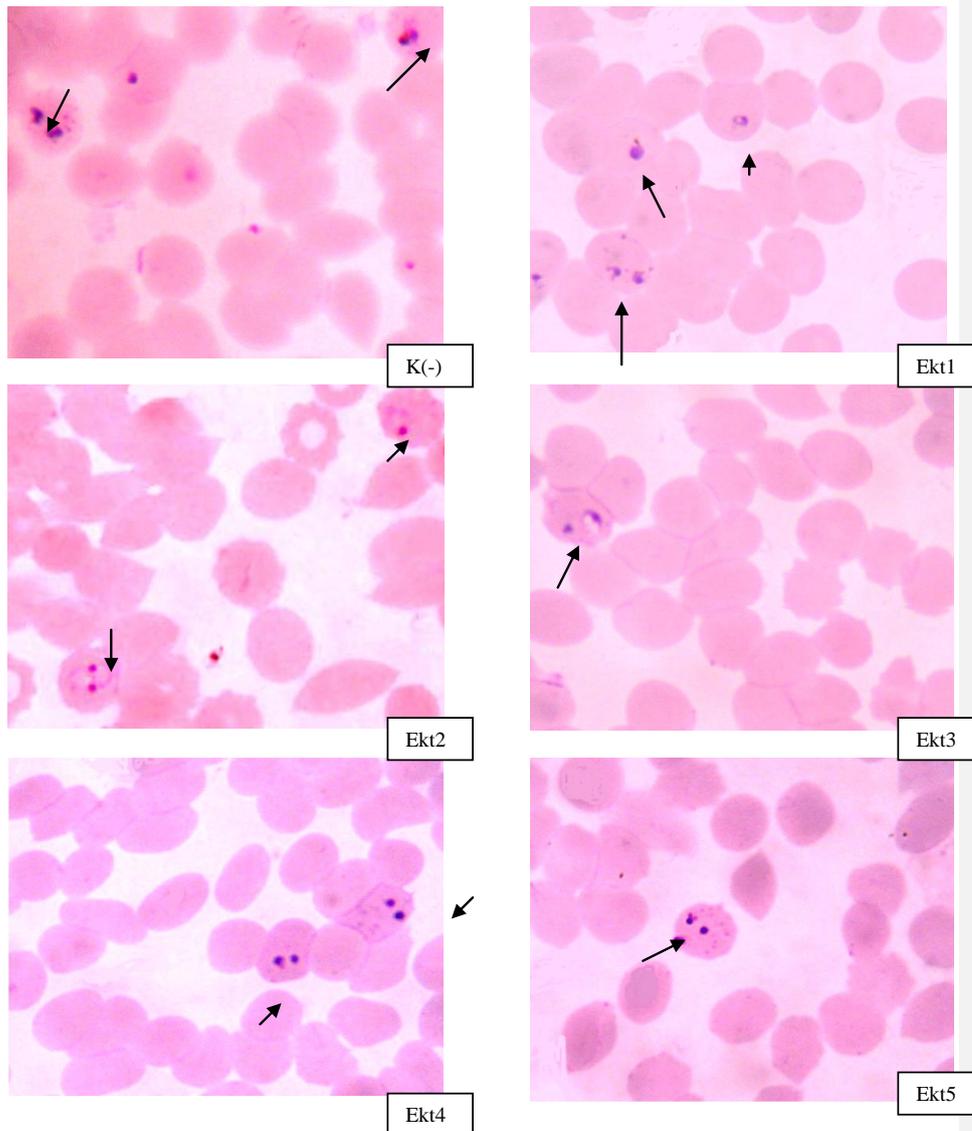


Figure 5.2.3.a. Thin blood smear of red blood cells infected by *P. falciparum* 3D7 which have been treated by batang Talikuning extract after incubation period of 48 hours.

Notes : **K(-)** is negative control in which *Plasmodium falciparum* 3D7 culture is given no treatment. The successive pictures are *Plasmodium falciparum* 3D7 cultures which are exposed to batang talikuning extract of dose 10^{-6} mg/ml (**Ekt1**), 10^{-5} mg/ml (**Ekt2**), 10^{-4} mg/ml (**Ekt3**), 10^{-3} mg/ml (**Ekt4**) and 10^{-2} mg/ml (**Ekt5**). The photographs are observation results of thin blood smear under a microscope of 1000 magnification. Infected red blood cells are indicated by the arrows.

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