

**ACUTE TOXICITY STUDY OF BUTTERFLY PEA LEAF
EXTRACTS ON EXPERIMENTAL MICE**

By
Agatha Tika Pangestuti
14310050

A Thesis submitted to the Faculty of
LIFE SCIENCES

In partial fulfillment of the requirements for the
BACHELOR'S DEGREE
In
BIOMEDICAL ENGINEERING



SWISS GERMAN UNIVERSITY
EduTown BSD City
Tangerang 15339
Indonesia

August 2014

Revision after the Theses Defense on 19th July, 2014

STATEMENT BY THE AUTHOR

I hereby declare that this submission is my own work and to the best of my knowledge, it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at any educational institution, except where due acknowledgement is made in the thesis.

Agatha Tika Pangestuti

Student

Date

Approved by:

Dr. rer. nat Maruli Pandjaitan

Thesis Advisor

Date

Dr. Min Rahminiwati

Thesis Co-Advisor

Date

Irvan Kartawiria, M.Sc.

Dean

Date

Agatha Tika Pangestuti

ABSTRACT

ACUTE TOXICITY STUDY OF BUTTERFLY PEA LEAF
EXTRACTS ON EXPERIMENTAL MICE

By

Agatha Tika Pangestuti

Dr. rer. nat. Maruli Pandjaitan, Advisor

Dr. Min Rahminiwati, Co-Advisor

SWISS GERMAN UNIVERSITY

The extracts of butterfly pea leaves have been suggested to be an effective cure for diabetes. This study has the objective to test the acute oral toxicity of the extracts. Powder form of the extracts was initially prepared through freeze-drying process and was orally administered in high concentration to thirty male experimental mice (*Mus musculus*). The test method used in the study was based on OECD guidelines no. 423. Six dose concentrations of 2500mg/kg bw, 5000mg/kg bw, 10000mg/kg bw, 20000mg/kg bw, 40000mg/kg bw and 80000mg/kg bw were evaluated. Signs of toxicity and mortality rate were recorded and evaluated during a 4-hour critical observation and 7 days daily observation. The 3 first lower doses have 0% mortality rate while the highest dose have 100% mortality rate. Using probit analysis, the LD₅₀ value of the extracts was revealed to be 19380.692mg/kg bw and classified as practically non-toxic.

Keywords: Clitoria ternatea, Herbal, Medicine, Toxicology, Acute Toxicity, LD₅₀, Histopathology, Mus musculus.

© Copyright 2014
by Agatha Tika Pangestuti
All rights reserved

DEDICATION

I dedicate this work to the research development of medicinal plant cultivations, especially those that are vastly growing in Indonesia.

ACKNOWLEDGEMENT

My first and foremost gratitude goes to Jesus Christ always, for every accomplishment I've ever achieved is not possible without His grace and His blessings.

Next, I wish to express my gratitude to my thesis advisor, Dr. Maruli Pandjaitan for his constant guidance and support. His immense knowledge and positive energy has been a true inspiration not just for me, I believe, but also for all the students who have had the fortune to be taught by him.

I would also like to sincerely thank my Co-Advisor, Dr. Min Rahminiwati, who has given me her time even in between her busy schedules.

Furthermore, I would also like to thank my lecturer Muhammad Fathony, Ph.D. for helping me with my statistics and also Tabligh Permana, S. Si for his truly helpful input and constant consultations whenever needed, not just for me but also for all students in Life Sciences.

I would also like to thank Pak Rudy and Ibu Diana from Puspitek and also to Pak Choer from IPB, for their assistance in handling lab procedures.

My sincere gratitude also goes to my closest friends Nabila, Nurul, Erika, Andara and to all my biomedical engineering classmates, all of whom I have spent the majority of these last 4 years with and have given me inside jokes that would sure last for years and years to come.

Last but not least, I wish to express my love and never ending gratitude to my mother, my father, my three sisters and Kenji Junardy, who, even without their presence, never failed to give me their unconditional love and support.

TABLE OF CONTENTS

STATEMENT BY THE AUTHOR.....	2
ABSTRACT.....	3
DEDICATION.....	5
ACKNOWLEDGEMENT	6
TABLE OF CONTENTS.....	7
LIST OF FIGURES	10
LIST OF TABLES.....	10
1 INTRODUCTION	11
1.1 Background	11
1.2 Research Problem.....	12
1.3 Research Objectives	12
1.4 Significance of Study	12
1.5 Research Questions.....	13
1.6 Hypothesis.....	13
2 LITERATURE REVIEW.....	14
2.1 Butterfly Pea	14
2.2 Butterfly Pea Leaf	15
2.2.1 Effects on Diabetes Mellitus (Anti-hyperglycemic Activity).....	17
2.3 Toxicology	19
2.3.1 Acute Toxicity	20
2.3.2 Lethal Dose 50 (LD ₅₀) Determination	22
2.3.3 Clinical Examination.....	24
2.3.4 Target Organs	26
2.3.5 Histopathology Examination.....	27
2.4 Freeze Drying	29
2.5 Experimental Mice (Mus Muculus) as Animal Model.....	32
3 METHODOLOGY	33

3.1	Venue and Time	33
3.2	Materials and Equipment.....	33
3.2.1	Raw Materials.....	33
3.2.2	Chemical and Drugs.....	33
3.2.3	Equipment	33
3.3	Preliminary Research	34
3.4	Design of Experiment	35
3.5	Preparation Phase.....	37
3.5.1	Preparation of Butterfly Pea Leaves Extract	37
3.5.2	Freeze-drying	37
3.5.3	Content Analysis	38
3.5.4	Preparation of Animal Model.....	40
3.6	Primary Research: Acute Toxicity Test	41
3.6.1	Dose Preparation and Administration	41
3.6.2	Clinical Observation	43
3.6.3	Histopathology Examination.....	44
3.6.4	Data Analysis	44
4	RESULT AND EVALUATION.....	45
4.1	Content Analysis.....	45
4.2	Acute Toxicity Result	45
4.2.1	LD ₅₀ Determination with Probit Analysis	46
4.3	Clinical Observation.....	49
4.3.1	Signs of Toxicity Revealed during Critical Observation	49
4.3.2	Daily Observation of Body Weight and Food Consumption	57
4.4	Histopathology Examination	60
5	CONCLUSION AND RECOMMENDATIONS	62
5.1	Conclusion.....	62
5.2	Recommendations.....	62
	GLOSSARY	63

REFERENCE	64
APPENDIX.....	67
Appendix 1. Methodology Process.....	67
Appendix 2. Gallic Acid Standard Curve	70
Appendix 3. Quercetin Standard Curve	71
Appendix 4. Data Results of Content Analysis.....	72
Appendix 5. Dose Administration Tables	75
Appendix 6. Probit Analysis Result	77
Appendix 7. Data of Average Body Weight and Food Consumption for 7 Days	80
Appendix 8. Individual Regression Analysis of Each Dose Groups	82
Appendix 9. ANOVA Result of Body Weight Average in 7 Days.....	85
Appendix 10. ANOVA Result of Food Intake Average in 7 Days	86
Appendix 11. Necropsies	87
CURRICULUM VITAE	89

LIST OF FIGURES

Figure 2.1 Butterfly Pea Plant (Surya, 2012) & Butterfly Pea (Kumar, 2003) .	14
Figure 2.2 Butterfly Pea Leaf (Surya, 2012)	15
Figure 2.3 Dose-Effect Curve (Patient Ed. Institute, 2010).....	22
Figure 2.4 Freeze-drying Steps (How Stuff Works, 2002)	31
Figure 4.1 Plotted Graph of Log-dose vs Probit Transformed Response	49
Figure 4.2 Toxicity Signs in Mice of Dose Group 2500mg/kg bw	51
Figure 4.6 Toxicity Signs in Mice of Dose Group 40000mg/kg bw and 80000mg/kg bw	55
Figure 4.7 Graph of Body Weight Gain Percentage for 7 Days	58
Figure 4.8 Graph of Food Consumption Average in 7 Days	58

LIST OF TABLES

Table 2.2 Phytochemicals Constituent of Butterfly Pea Leaf (Nahar, 2010)...	16
Table 2.2 Subclasses of Flavonoid (Bhagwa et al., 2012).....	18
Table 2.3. Toxicity Ratings (Lu, 2002)	23
Table 2.4. Relationship Between Toxic Signs and Body Organs or Systems (McNamara, 1976).....	25
Table 3.1. Concentrated Extract Administration for Dose 2.5gr/kg.....	43
Table 4.1 Mortality Rate of Mice Exposed to Different Dose Groups	46
Table 4.2 Correction of Mortality Rate of Animal Exposed to Dose.....	47
Table 4.3 Log-Transformed Dose and Probit Values of Mortality	48
Table 4.4. ANOVA Result of Dose and Body Weight of 7 days.....	59
Table 4.5. ANOVA Result of Dose and Food Consumption for 7 days	60
Table 4.6. Summary Table of Correlation between Body Weight and Food Consumption for 7 Days	60
Table 4.7 Histopathology Result of The Liver.....	61
Table 4.8 Histopathology Result of The Kidney.....	61