

THESIS

**EFFECTS OF PROPOLIS ON HISTOLOGY PROFILE
OF KIDNEY IN MALE MICE (*Mus musculus*)**



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**FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA
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ENDORSEMENT FORM

**EFFECTS OF PROPOLIS ON HISTOLOGY PROFILE OF KIDNEY IN
MALE MICE (*Mus musculus*)**

Thesis

Submitted in partial fulfillment of the requirement for the degree of Bachelor of
Veterinary Medicine

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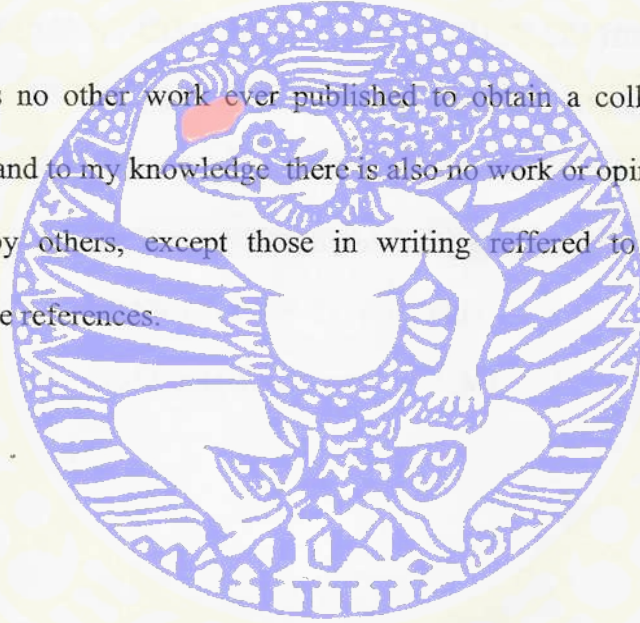
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DECLARATION

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There is no other work ever published to obtain a college degree in a certain college and to my knowledge there is also no work or opinion ever written or published by others, except those in writing referred to this paper and mentioned in the references.



Surabaya 28 October 2015




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ABSTRACT

The research aimed to determine the effect of propolis in The Histology in mice's kidney especially the tubulus and glomerulus. Twenty five male mice 12 weeks old were used as experimental animals were divided into five groups; so each group consisted of five mice. Group T0 served as control group, T1, T2, T3 and T4 respectively were treated orally with propolis ethanolic extract 0.4, 0.8, 1.6 and 3.2 mg each day and sacrificed after 14 days, Histology of tubulus and glomerulus were analyzed microscopically. The data were analyzed by Kruskal-Wallis test. The result showed there was no significant difference amongst group of mice ($p > 0.05$), The conclusion is propolis does not have effect and safe for histological Kidney in male mice.

Key words: Propolis, Kidney , Histology

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I, as the author, acknowledge that this writing is still lack and far from perfection. However, I hope this research will be useful for the advancement of science and may give contributions to veterinary medicine world and society.

Surabaya, October 2015

Author

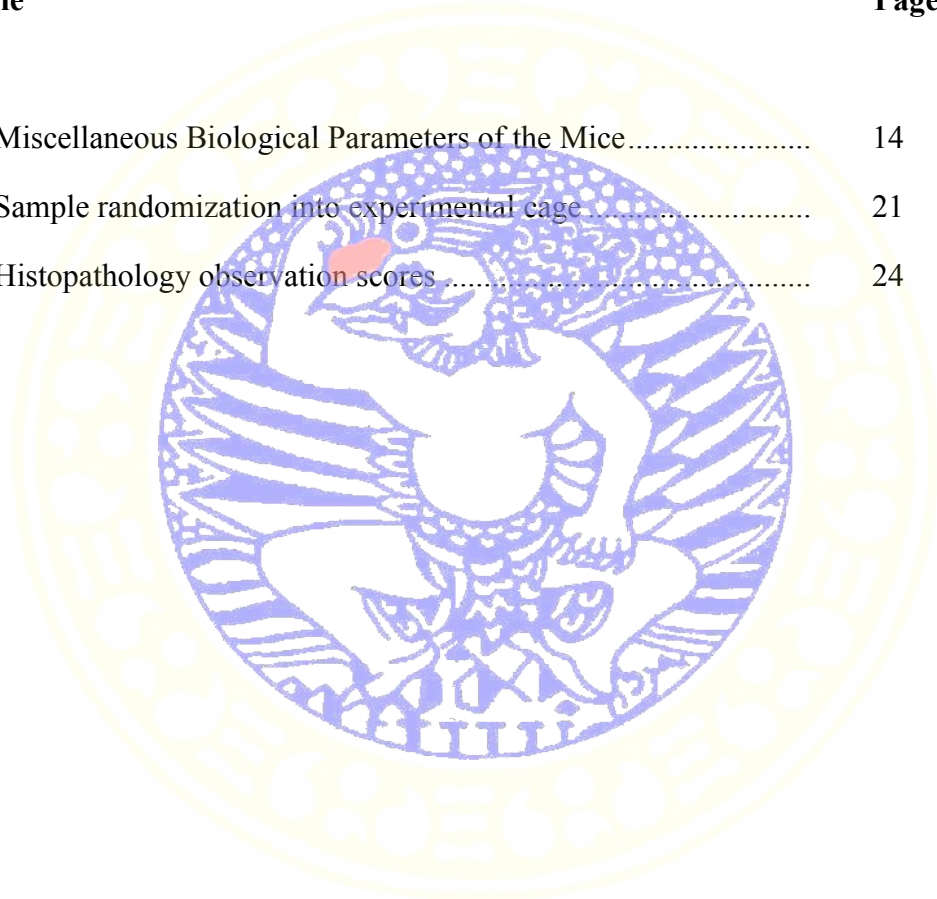
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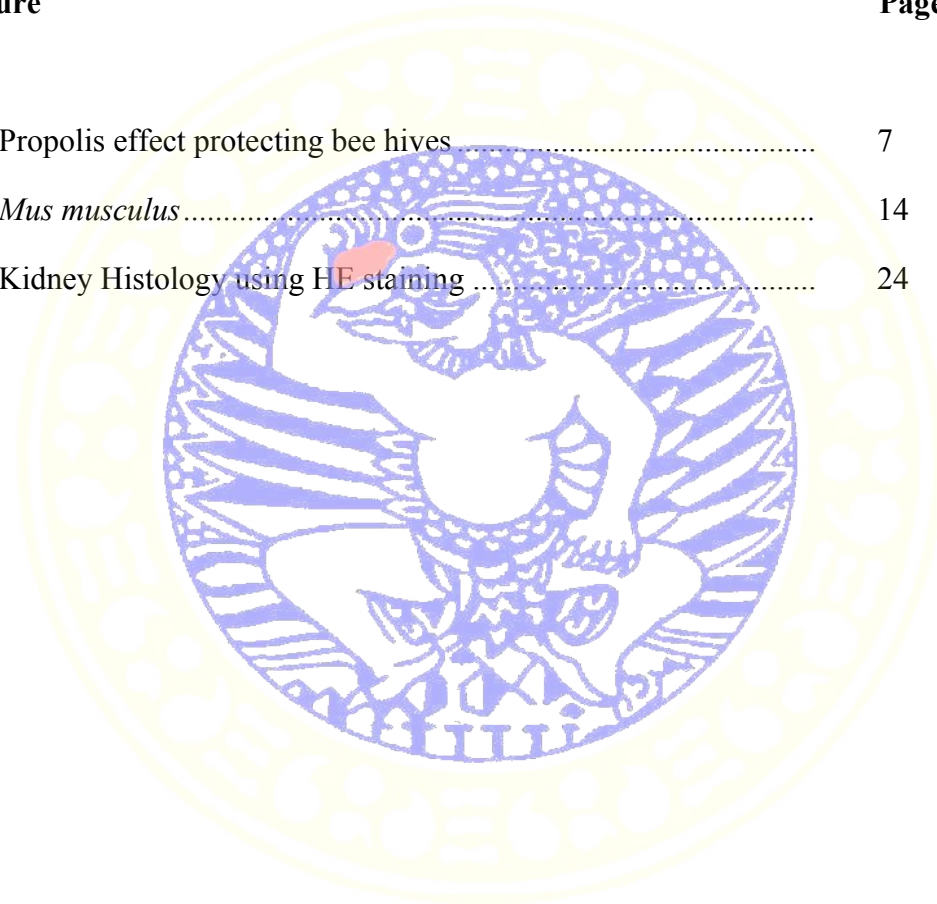
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ABBREVIATION AND SYMBOL INTEPRETATION



| | |
|----------|---|
| cm | : Centimeter |
| CMCNa | : Sodium Carboxyl Methyl Cellulose |
| et al | : et alii |
| FV | : Field of View |
| g | : gram |
| GDC | : Gedung Diagnostic Center |
| HE | : Hematoxilin Eosin |
| Kg/BW | : Kilogram / Body Weight |
| mg | : Miligram |
| ml | : Mililitre |
| NIH | : National Institute of Health |
| Pusvetma | : Pusat Veterinaria Farma |
| SPSS | : Statistical Product and Service Solutions |

CHAPTER 1 INTRODUCTION

1.1 Background

Indonesia is a maritime country, sprinkled with more than 17,500 large and small island. This country right in equatorial region and has more than 42 types of terrestrial and 5 (five) type very unique marine ecosystems, ranging of perennial ice field at the top of the mountain Jaya Wijaya Papua up to the trough of the sea innermost, the uniqueness of this ecosystem has made Indonesia awarded with abundant and diverse natural resources, indonesia is the richest country in the world in terms of biological diversity (biodiversity). World Resource Institut, International Union for Conservation of Nature and National University of Distance Education (1995) describes that Indonesia has up to 25 % various species in the world when wide its land area is only 1.3 % of the total mainland world (Sukara and Tobing, 2008)

This is supported by the condition Indonesian nation which consists of thousands of islands and diverse tribes and availability especially with diverse flora and fauna in Indonesian forests, such as low plains, mountains, swamps, beaches, thats allows more variety of all plants 3000- 4000 species of plants roses in indonesia soil (Wijayakusuma, 2000), not only plants Indonesia also have various and diverse kinds of animals with many benefits that we can utilize, for example honey bee that make honey and propolis.

Propolis is a complex resinous bee product with a physical appearance that varies widely, depending on many factors. The color of propolis may be cream, yellow, green, light or dark brown. Some samples have a friable, hard

texture, while other samples may be elastic and gummy, bees use propolis for diverse purposes, among them to seal openings in the hive. In addition to avoiding the entrance of intruders, this contributes to maintaining the hive inner temperature at around 35°C (Toprakci, 2005)

Propolis is a natural product derived from plant resins collected by honeybees, Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti-ulcer and anti-tumor activities. Therefore, propolis has attracted much attention in recent years as a useful or potential substance that can be used in medicine and cosmetic's products (Lotfy, 2014)

The propolis can be used to improve the pathological condition of the ill individual, works as an antioxidant and antibiotics as well as boosting the immune system both humoral and cellular because it contains flavonoids approximately 15 % (Krell, 2005) The content contained in propolis is very good for the body. One of the largest percentage content of propolis is the resin (flavonoids, terpenoids, polyphenols) that serves as an antibacterial, antiviral, anti-inflammatory effects, antioxidant, antimicrobial and other biological activities (Shuai et al, 2014). Shuai also stated that there some mineral that are considerable as a toxic, Adverse effects which have been reported due to high calcium intakes include the so-called milk-alkali syndrome, the formation of kidney stones in persons with a propensity for nephrolithiasis, hypercalciuria and for hyperabsorption of calcium, and interference with the absorption of other minerals (Whiting and Wood, 1997). The RDAs (Recommended Dietary Allowances) has wide range depend on the age,

gender and the condition of subject ranged from the lowest 200mg/day for 0 – 6 month old infant until 1300mg/day for 14 – 18 years old or lactating woman (NIH, 2013).

The Study of the role of propolis in improving pathological condition of the body parts that damaged, associated with the influence of renal function as a very important organ in maintaining body balance. Kidney function regulate body fluid balance by removing useless or dangerous remnants of metabolism and keep substances that the body needs. This function is very important for the body to maintain homeostasis. The content contained in propolis also be secreted by the kidneys , therefore the authors are interested in doing research on the effect of propolis on kidney histology mice.

The reason why the researcher choose glomerulus and tubulus as the main subject of this research, because both of this part of kidney is the one that might get affected first by the substance used for this research, glomerulus works like a filter allowing only certain ingredients such a vitamin and mineral passed to the tubule, so this two connected part of kidney is the front line for filtration system that might affected first.

1.2 Identification of Problem

Based on the proposed formulation of the problem has been described as follows :

1. Does propolis affect the changes on the of histological view of tubulus and glomerulus in mice kidney ?

2. Does the level of concentration of propolis dosage affect the changes of histological view of tubulus and glomerulus in mice kidney ?

1.3 Theoretical Base

Propolis is a resinous substance collected from various plants by bees. It is used in the construction, and seal the cracks in, the bee hive. For this reason, propolis is often referred to as "bee glue". It is a mixture of resin, essential oils and waxes, and also contains amino acids, minerals, ethanol, vitamin A, B complex, E, and flavonoids (Bruno, 2005)

Kidneys are the organs which have an important role in the body to get rid of metabolic waste and toxins in the form of urine/urine. In addition, the kidneys also play a role in maintaining the balance of water, salts and electrolytes and kidney are no less important endocrine glands secrete at least three hormones.

The kidney is an organ that is susceptible to the influence of chemical substances, since this organ receives 25-30 % of blood circulation to be cleaned, so as filtration organ possibility of very high pathologic changes (Corwin, 2001)

1.4 Research Purposes

1.4.1 Spesific Purposes

1. Knowing the effect of propolis in histological changes of tubulus and glomerulus in mice kidney
2. Knowing the different level of propolis doses in the histological changes of tubulus and glomerulus in mice kidney

1.4.2 General Purposes

This Research aims to find out the safety of propolis in kidney and that propolis can used as herbal medicine for society health purposes

1.5 Researchs Benefits

This research giving us information about the propolis effect on kidney and about the potention in histological changes of mice kidney

The results of this study are expected to provide information to the public that propolis is an alternative medicine that is safe for the kidney and can be used for decreasing of problem that have connection with kidney. Propolis can also be used as a natural remedy for the benefit of public health in general and in particular veterinary medical and science helped contribute to all aspects of medical science and science.

1.6 Research Hypotesis

1. Propolis Treatment may affect Histological changes of tubulus and glomerulus in mice kidney
2. Different Level of propolis dose may affect Histological changes of tubulus and glomerulus in mice kidney

CHAPTER 2 LITERATURE REVIEW

2.1 Propolis

The word propolis originates from Greek, Pro = in front, polis = city. The meaning in front of the city, suits well the protecting role of propolis for the bee colony itself (Bogdanov, 2014)

propolis is a natural product derived from plant resins collected by honeybees. It is used by bees as glue, a general-purpose sealer, and as draught-extruder for beehives. Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti-ulcer and anti-tumor activities. Therefore, propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages with the claim that it can maintain or improve human health. The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sesquiterpene quinones, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The contents depend on the collecting location, time and plant source. Consequently, biological activities of propolis gathered from different phytogeographical areas and time periods vary greatly, the activity of bee propolis will be presented with special emphasis on the antitumor activity in it. (Lotfy, 2006)

Nowadays, propolis use in over-the-counter preparations, “bio”-cosmetics and functional foods. Volatile compounds are found in low concentrations in

propolis, but their scent and significant biological activity make them important for propolis characterisation (Bankova *et al.*, 2014)

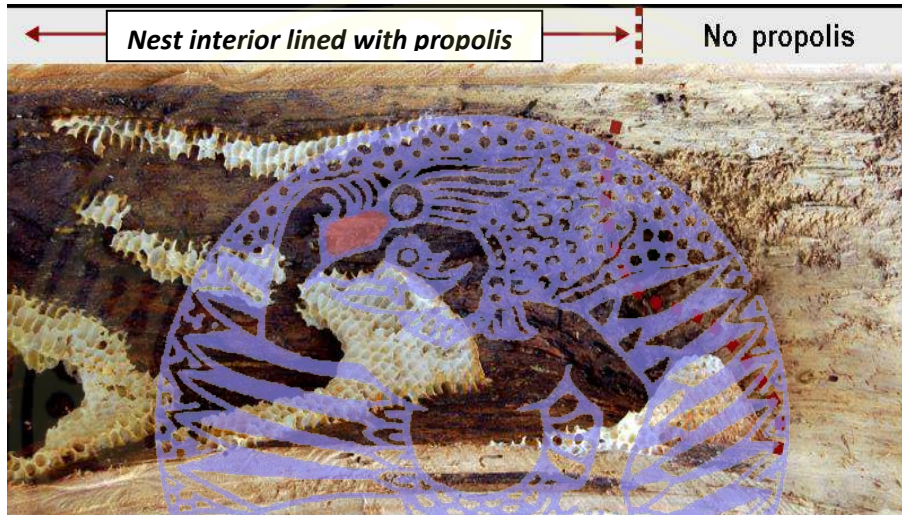


Figure 2.1. Propolis effect protecting bee hives
(Source: Finstrom and Spivak, 2010)

2.1.1 Propolis Characteristic

Propolis is a resin being dark green or brown in color with a pleasant flavor of poplar buds, honey, wax and vanilla but it can also have a bitter taste. When burnt, it exhibits a smell of aromatic resins of great value (Nikolaev. 2008). At temperatures of 25⁰ to 45⁰ C propolis is a soft, pliable and very sticky substance. At less than 15⁰ C, and particularly when frozen or at near freezing, it becomes hard and brittle. It will remain brittle after such treatment even at higher temperatures. Above 45⁰ C it will become increasingly sticky and gummy. Typically propolis will become liquid at 60⁰ to 70⁰ C, but for some samples the melting point may be as high as 100⁰ C. The most common solvents used for commercial extraction are ethanol (ethyl alcohol) ether, glycol and water. For chemical analysis a large variety of solvents may be used in order to extract the

various fractions. Many of the bactericidal components are soluble in water or alcohol. (Krell, 2005)

2.1.2 Propolis Compound

Propolis is composed mainly by the plant resins and exudates that bees gather. Bees add wax, and also some secretions and pollen to it. The composition of propolis depends on its botanical and thus also on its geographical origin (Bogdanov, 2014). Validated spectrophotometric procedures were used to quantify three main groups of bioactive substances (phenolics, flavones/flavonols, flavanones/dihydroflavonols) in 114 samples of poplar-type propolis from different geographic origins. From the results, Moreira *et al.*, (2008) characterized raw poplar propolis in terms of minimum content of its bioactive components (antimicrobial and antioxidant) as follows: 45% resin, 21% total phenolics, 4% total flavones/flavonols; 4% total flavanones/dihydroflavonols. A significant negative correlation was observed between the amount of total phenolics and MIC. The results indicate that measuring the concentrations of groups of active compounds, rather than individual components, is an appropriate approach in developing quality standards for propolis.

Many analytical methods have been used for separation and identification of propolis constituents and the substances identified belong to the following groups of chemically similar compounds: polyphenols, benzoic acids and derivatives, cinnamic alcohol and cinnamic acid and its derivatives, sesquiterpene and triterpene hydrocarbons, benzaldehyde derivatives, other acids and respective derivatives, alcohols, ketones, and heteroaromatic compounds, terpene and

sesquiterpene alcohols and their derivatives, aliphatic hydrocarbons, minerals, sterols and steroid hydrocarbons, sugars and amino acids

2.1.3 Propolis Benefits

Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti-ulcer and anti-tumor activities. Therefore, propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages with the claim that it can maintain or improve human health. The chemical composition of propolis is quite complicated. More than 300 compound such as polyphenols, phenolic aldehydes, sesquiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The contents depend on the collecting location, time and plant source (Lotfy, 2006)

Propolis has bactericidal and fungicidal properties and it is used as an alternative treatment for infections. The wide range of action of propolis on various microorganisms is the result of the combined activities of flavonoids and aromatic compounds (Ivancajij, 2010)

The research that has been conducted by Siti *et al.*, (2011) suggested that propolis as an immunomodulator, potentially increasing the phagocytic index of peritoneal macrophages of mice at doses and for a certain period, and slightly increase the phagocytic index of peritoneal macrophages when given in higher doses. It can be concluded that the nature of propolis as an immunomodulator when given with small doses and in a short period of time, could potentially

increase the average index of phagocytic macrophages, whereas when given in large doses and in the long term it will be as immunosuppressants to the average index of phagocytic macrophages (Takagi *et al.*, 2005).

2.2 The Characteristic of Kidney

The kidneys are a pair of organs that lie outside the peritoneal cavity in the dorsal abdominal wall, each side in the columna vertebral. In each kidney consists of about 1 million units the smallest. Each unit, or nephron consists of a component called the glomerular vascular and tubular components, renal mechanisms in carrying out its functions depends on the relationship between these two components. Tubule lumen wall is covered by a layer of epithelial cells, a different structure and function from first part to the other. The liquid filtrate derived from bag shaped like a balloon, called Bowman's capsule, parallel to the epithelial cells. On the one hand, Bowman's capsule associated with glomerular, and on the other hand, Bowman's capsule opens to the first tubular high coiled, called the proximal convoluted tubules.

The next part of the hollow tubules resembling sharp hairpin, which diesebut loop of Henle. Tubular distal convoluted tubule forming rolls and eventually leads to a straight line, the ductus collecticus. Glomerular until early ductus collecticus, every 1 million tubules perfect apart from its neighbors. Ductus smallest collecticus apart from tubule join into larger ducts, which eventually flows into the large central cavity, the renal pelvis of each kidney. Renal pelvis continues into the ureter, which flows to the bladder, where urine is

stored temporarily before being eliminated. Urine is not changed after passing through the ductus collecticus.

The left kidney of mice little more cranial than the right kidney and is located approximately in the segment costae number eleven.

2.2.1 The Anatomy of Kidney

Kidneys have a fairly diverse formation in animals, in general kidney function as blood filtration. Renal artery and renal vein entrance exit on each renal hilum. Nearly 25% of cardiac output into the kidneys. Blood filtered in the kidney, throwing useless compound and substance, especially urea and nitrogen - containing compounds, and regulate electrolytes extravascular and intravascular volume. Because renal blood flow from the cortex to the medulla and medulla relatively little vascularity, normal oxygen tension in the cord should be lower than other organ than kidney. This causes ischemia in the medulla (Drake *et al.*, 2007)

2.2.2 The Histology of Kidney

In Kidney anatomical divided into two parts, namely the cortex and renal medulla. In the cortex there are millions of nephrons, each consisting of 1-4 million kidney nephrons. whereas in the renal medulla ductus widely available. Nephron is the smallest functional unit of the kidney comprising, proximal convoluted tubules, korpuskulus renal distal convoluted tubule, thin and thick segments loop of Henle, and tubular kolegens.

Blood which carries the remains of the body's metabolism results filtered in the kidney glomeruli and then in the tubulus, some substances are still

needed body undergoes reabsorption and substances undergo metabolic waste results together with water to form urine secretion. Every day not less than 180 liters of body fluids are filtered at the glomerulus and produce 1-2 liters of urine. Urine formed in the nephrons channeled through the pyramid to kidney pelvicalises system to be channeled into the ureter. (Purnomo, 2009)

2.2.3 The Characteristic of Glomerulus

Glomerular is capillary matting, which is a branch of afferent arterioles. After entering the body kidney (renal corpus) corpuscles renal afferent arterioles usually branched into 2-5 main branches, each branch branched off once again into the capillary mesh nets. The hydrostatic pressure of arterial blood contained in this capillaries. glomerulus managed by efferent arterioles. (Eroschenko, 2003)

2.2.4 The Characteristic of Capsula Bowman

Glomerulus capillary surrounded by Bowman 's capsule. Glomerular function as a blood filter. Bowman 's capsule is a double-walled epithelium. The outer layer of the Bowman's capsule consists of flattened epithelial layer, and an inner layer composed of specialized cells called Podosit (cell feet) are located include glomerular capillary. Between the two layers of Bowman's capsule cavity is formed. Podosit cell, the basal membrane and capillary endothelial cells form the lining (membrane) filtration with holes that separates the blood contained in the capillaries with capsular space. Endothelial cells of the glomerular capillary pores having larger cells and more than capillaries in other organs. Results of the glomerular filtration of blood fluid or fluid called ultrafiltrate (primary urine) subsequently accommodated in the cavity of the capsule. (Eroschenko, 2003)

2.2.5 The Characteristic of Renal Corpuscle

Renal Corpuscle nephron is the beginning of each segment. Here, blood is filtered through the glomerular capillaries and the filtrate is collected inside the capsular cavity that lies between the parietal and visceral layer of Bowman capsule. Each korpuskulum renal vascular pole yang have an entry and exit point of the blood vessels of the glomerulus. (Eroschenko, 2003)

2.3 Identification of Mice

2.3.1 Classification of Mice

Mice (*Mus musculus*) is the result of domestication of wild mice is the most commonly animals used as experimental subject. Many of the advantages possessed by mice as experimental animals, which has similarities with human physiology, life cycle is relatively short, rapidly priliferating with large number ofspring each time give birth, its variation in height and easy handling (Moriwaki *et al.*, 1994).

According Ballenger (1999), mice taxonomic classification is as follows :

| | |
|-----------|-----------------------|
| Kingdom | : Animalia |
| Phylum | : Chordata |
| Subphylum | : Vertebrates |
| Class | : Mammalia |
| Order | : Rodentia |
| Suborder | : Sciurognathi |
| Family | : Muridae |
| Subfamily | : Murinae |
| Genus | : Mus |
| Species | : <i>Mus musculus</i> |

2.3.2 Morphology of Mice

Mice has high fertility (reproduction potential), short gestation period, and short lifespan makes this animal used as an animal model for tetralogy, genetic and gerontology lessons. Moreover, because of its small size and inexpensive price, these animals are also used for toxicology and carcinogenicity lessons (Sirois, 2005).

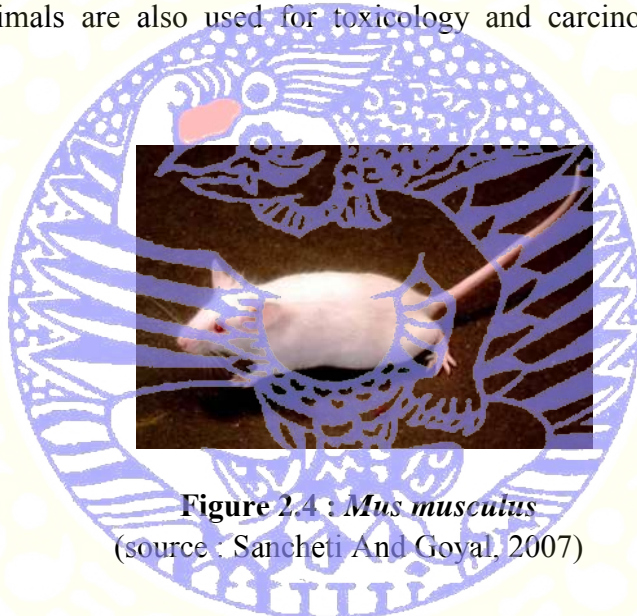


Figure 2.4 : *Mus musculus*
(source : Sancheti And Goyal, 2007)

Table 2.5 Miscellaneous Biological Parameters of the Mice
(Suckow *et al.*, 2001)

| Parameter | Typical Value |
|---------------------------|-------------------------------|
| Diploid chromosome number | 40 |
| Life span | 2–3 years |
| Adult body weight | 20–40 g |
| Body temperature | 36.5–38.0°C (97.5–100.4°F) |
| Metabolic rate | 180–505 kcal/kg/day |
| Food intake | 12–18 g/100 g body weight/day |
| Water intake | 15 ml/100 g body weight/day |
| Respiratory rate | 80–230 breaths/min |
| Heart rate | 500–600 beats/min |

CHAPTER 3 MATERIALS AND METHODS

3.1 Research Location and Date

The research conducted at Laboratory of Experimental Animals, Medical Faculty, Universitas Airlangga, Surabaya for the treatment of the animals and for the preparation of histology specimen conducted at Gedung Diagnostic Center (GDC) Dr. Soetomo Hospital. The extraction of propolis done at Laboratory of Phytochemical, Faculty of Pharmacy, Surabaya University. And for the Histological examination done at Histology Lab, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Implementation of this research held on December 2014 until July 2015.

3.2 Experimental Design

This is an experimental research to determine the effect of propolis on the kidney of mice with Completely Randomized Design. This research is using 5 groups, 1 control group and 4 treatment groups with simple randomized. Each unit of the treatment repeated 5 times. Examination process conducted by observing the changes that occurs in kidney by using post-test control method. Scoring done only during the post-test control, by comparing the results of observation between treatment group and control group, and also between the treatment groups.

3.3 Research Variables

3.3.1 Independent Variable

The independent variable in this study is a dose of propolis that given to mice per orally.

3.3.2 Dependent Variable

Dependent variable in this study is the histological changes of kidney after the treatment.

3.3.3 Controlled Variable

The controlled variables in this study are strain of mice, feed and drink of mice, mice age, mice weight, cages and equipment that used for this research.

3.4 Research Materials

3.4.1 Research Samples

Experimental animals that used are 25 male mice. 12 week with average weight 25-35 gram were obtained from Pusat Veterinaria Farma (Pusvetma), Surabaya, East Java and developed from Laboratory of Experimental Animal, Faculty of Veterinary Medicine, Airlangga University.

3.4.2 Research Equipment

Equipment that used in this research are 1ml sized of gastric probe, water suppliers, feed suppliers, plastic box cages 50cm x 50cm x 40cm, bucket, trash can, husk and anesthetic, digital scales, plastic bags, rough papers, scissor, drip pipette, syringe, bottle, knife, tweezers, scalpel, plastic pot, section tools, object glass and cover glass, microtome, hot plate, microscope.

3.4.3 Research Materials

Materials that used in this research are propolis extract and other chemical materials that necessary to be used in this research.

Propolis that used in this research is pure propolis extracted in Laboratory of Phytochemical, Pharmacy Faculty, Surabaya University. Materials for specimen preparation technique are, 10% of formalin, 70%, 80%, 90% and 96% of alcohol or ethanol, *xylol*, *Hematoxylin Eosin* (HE), *glycerin*, Canada balsamic, and block paraffin (Muntiha, 2001). Mice given feed in the form of pellets, drinking water and husks for the base of cage.

3.5 Research Method

Implementation of this research is conducted in two phases, the first phase is the extraction process of propolis and the second phase is the treatment of experimental animal.

3.5.1 Preparation of Propolis Extract

The basic substances of propolis are a mixture of beeswax, resins and soil that attached to the honeycomb of *Apis mellifera*. Sample material that used is crude propolis of local strain *Apis mellifera* obtained from Lembah Rimba Raya Ranch, Kelurahan Lawang, Kabupaten Malang, East Java. Extraction done using maceration method with 70% ethanol. Extraction process conducted at Laboratory of Phytochemical, Pharmacy Faculty, Surabaya University and the extracted, pure propolis could be given later to experimental animal. The extraction process is contained in Appendix 1.

3.5.2 Treatment of Experimental Animal

The second phase of this research is the treatment in male mice that conducted in experimental cage. This research is using 25 male mice age 12 weeks with a weight of 25-35 grams which is developed at Laboratory of Experimental Animal, Faculty of Medicine, Airlangga University Surabaya randomized by lottery method or randomized table method (Randomized table contained in Table 3.1) and divided into 5 groups, and then be adapted to the environment for 1 week. On the second week, each of experimental animals has treated for 14 days, feed and drink given twice a day *ad libitum*.

3.5.2.1 Dose Determination of Propolis

The dose of propolis solution is based on the conversion of the human dose to mice is 0.0026 (dose conversion table contained in Appendix 3), while the dose of propolis adults (70 kg) according to Krell (2005) is 100 mg/day to obtain a heavy dose of propolis for mice an average of 30 grams is 0.4 mg/day. To determine the effect of the dosage range is best 0.4mg/0.5ml/head/day, 0.8mg/0.5ml/head/day, 1.6mg/0.5ml/head/day and 3.2mg/0.5ml/head/day. Value of 0.5 ml at each dose is dilution, it is necessary to use tween80 as much as 100 μ l and 0.4 ml E - pure in each dose to be easy in giving to the experimental animals, administration of propolis performed with gastric sonde (oral) and the treatment done in the morning.

3.5.2.2 Experimental Design

This research is using Completely Randomized Design with 5 following treatment:

T0: control group, mice are not given with any dose of propolis but only has given distilled water 0.5ml/head/day.

T1: group of mice given 0.4 mg/head/day dose of propolis.

T2: group of mice given 0.8 mg/head/day dose of propolis.

T3: group of mice given 1.6 mg/head/day dose of propolis.

T4: group of mice given 3.2 mg/head/day dose of propolis.

Each treatment is repeated 5 times so there are 25 experimental units. Then conduct randomization in 25 experimental units for placement the experimental animal (Table 3.1). Determination of the number of minimal replication is as follows.

$$t(n-1) \geq 15$$

Explanation :

$$5(n - 1) \geq 15$$

t = number of treatment (1, 2, 3, 4,5)

$$5n - 5 \geq 15$$

n = treatment repeating

$$5n \geq 20$$

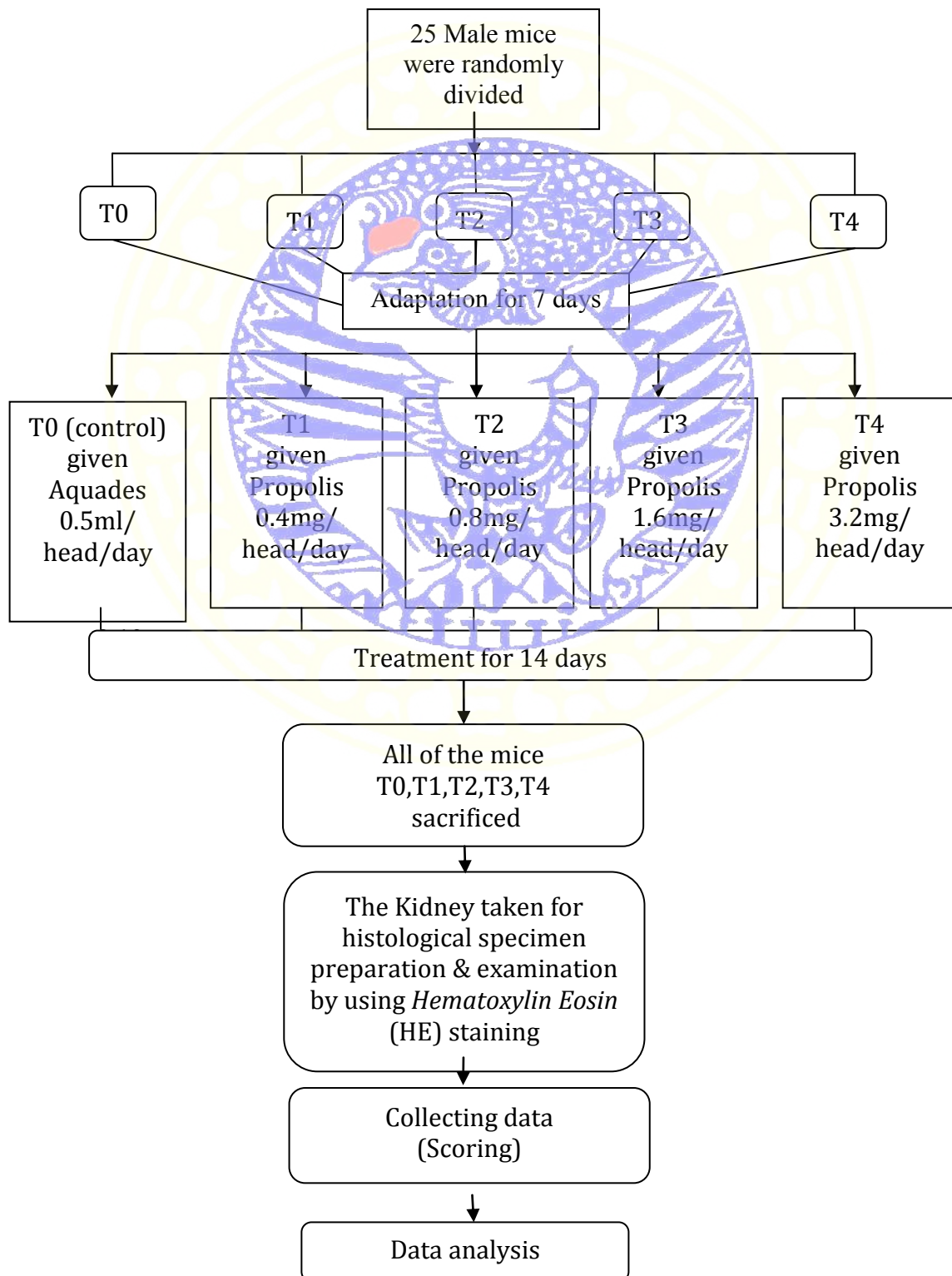
$$n \geq 4 \text{ (Steel and Torrie, 1993)}$$

So the minimum amount of repeating which can be done is greater than or equal to four.

Table 3.1: Sample randomization into experimental cage

| | | | | |
|---------|----------|-----------|----------|---------|
| I. (T0) | II. (T1) | III.(T2) | IV. (T3) | V. (T4) |
| r | n | C | S | G |
| I. (T0) | II. (T1) | III. (T2) | IV. (T3) | V. (T4) |
| h | b | W | x | U |
| I. (T0) | II. (T1) | III. (T2) | IV. (T3) | V. (T4) |
| t | j | Q | a | M |
| I. (T0) | II. (T1) | III. (T2) | IV. (T3) | V. (T4) |
| i | d | E | y | L |
| I. (T0) | II. (T1) | III. (T2) | IV. (T3) | V. (T4) |
| K | O | F | V | P |

3.6 Flowchart of Research



3.7 Kidney Histological Specimen Preparation

The process of histological preparation contained in Appendix 4.

3.8 Kidney Examination

Examination of Kidney especially at glomerulus and tubulus part of the specimen that have been stained by HE examined under the microscope with 100 - 400 times magnification.

Scoring method histological changes in the kidney is determined according to the method Arsad *et al.*,(2014) Light microscopic examination of multiple tissue section from organ in all groups were performed in all groups and images representative of typical histological profile was examined. Changes in the experimental histopathological parameters include granular cast, cellular cast, protein cast, pycnotic cell, hydropic degeneration, for kidney tissues were graded as follows: (0) showing no changes, (1) mild changes (2) moderate changes (3) severe changes, respectively, while the grading was determined by percentage as follows: Changes less than 30% (<30%) showing mild changes, changes less than 30% - 50% (<30% - 50%) indicating moderate changes and changes more than 50% (>50%) showing severe changes

3.9 Data Analysis

The form of data obtained stated in scores of histological changes level in the kidney of mice that arranged in table for later statistically analyzed using the Kurskal-Wallis test. If there is real difference, then the analysing continue using Mann-Whitney test. The whole process of analysis done with SPSS 21 for Windows.

CHAPTER 4 RESULT OF RESEARCH

Results of the effect of propolis on histology of kidney in male mice (*Mus musculus*) are as follows:

4.1 Effect of propolis on the histological change in kidney

Histological changes in the kidney as a result of giving a variation of various doses of propolis, as control or T0 (distilled water + mCMCNa 0.5 ml), T1 (propolis dose of 0.4 mg/KgBW/day), T2 (propolis dose of 0.8 mg/KgBW/day), T3 (propolis at a dose of 1.6 mg/KgBW/day) and T4 (propolis at a dose of 3.2 mg/KgBW/day) in this study assessed semiquantitatively (scoring) according to the method Arshad (2014). Forms lesions on all parts of the nephron which is considered as the deciding factor (determinant factors) and lead to impaired renal function as well as the basis for assessment of change histological kidney.

In this study, the data analysis of multi-factor histological changes in the kidney using the Kruskal-Wallis test showed that there were no significant differences ($p > 0.05$) between treatments, as presented in Table 4.1 on SPSS version 21 for Windows.

Table 4.1 Histopathology observation scores

| Histopathology Parameters | Group | | | | | Significance |
|---------------------------|-------|---|---|---|---|--------------|
| | A | B | C | D | E | |
| Granular cast | 0 | 0 | 0 | 0 | 0 | 1.00 |
| Cellular cast | 0 | 0 | 0 | 0 | 0 | 1.00 |
| Protein cast | 0 | 0 | 0 | 0 | 0 | 1.00 |
| Pycnotic cell | 0 | 0 | 0 | 0 | 0 | 1.00 |
| Hydropic degeneration | 0 | 0 | 0 | 0 | 0 | 1.00 |
| Mean Score | 0 | 0 | 0 | 0 | 0 | 1.00 |

Group A: T0 (Control); Group B: T1(Propolis 0.4 mg/0.5ml/day); Group C: T2(Propolis 0.8 mg/0.5ml/day); Group D: T3(Propolis 1.6 mg/0.5ml/day); Group E: T4(Propolis 3.2 mg/0.5ml/day)

From the data result of statistic test of Histoology observation after given propolis in various dose, using Kruskal-Wallis test showed that there were no significant differences ($p>0.05$) Among the treatments, as we can see in the figure 4.1.

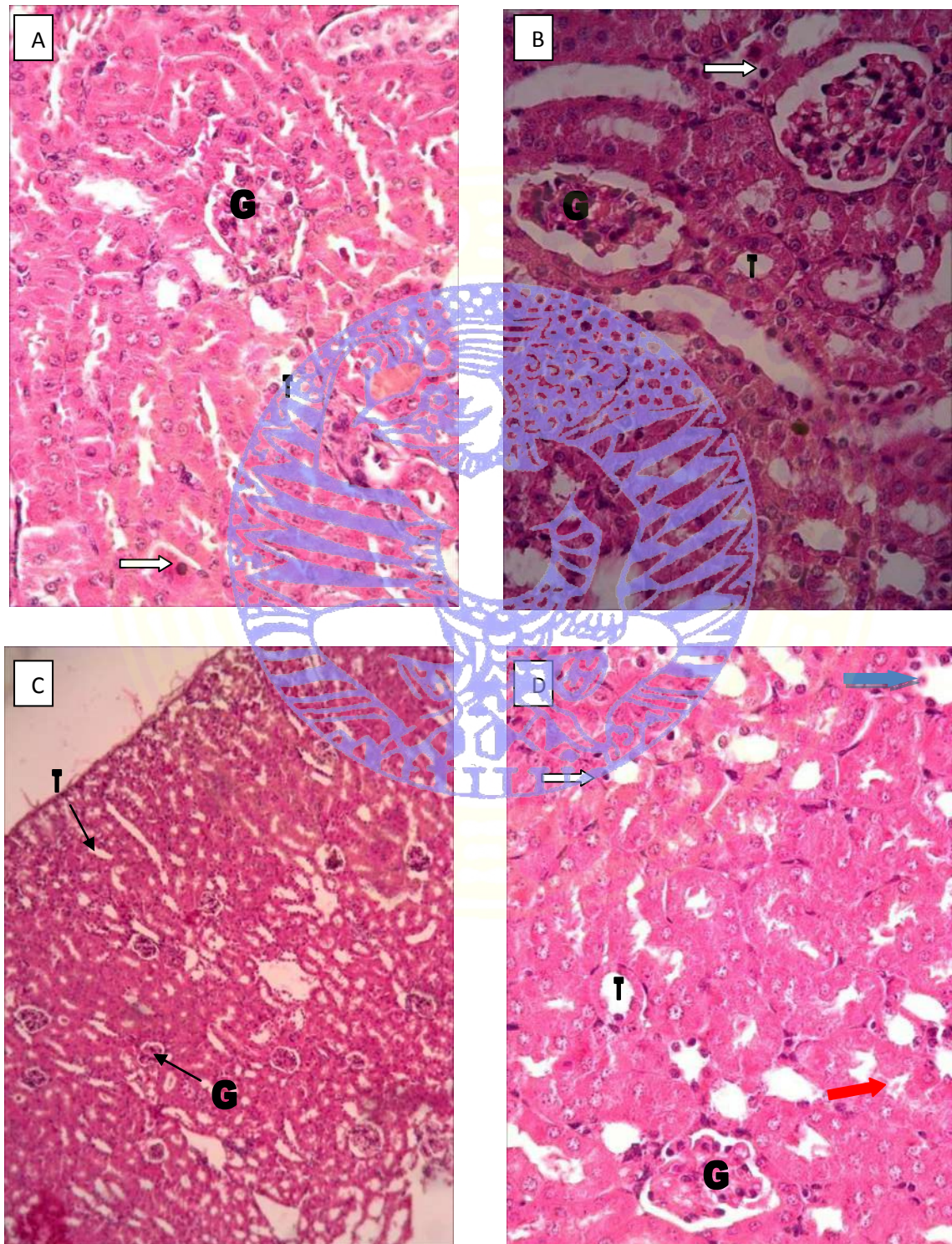


Figure 4.1. Kidney Histology using HE staining

A, B, D (T0, T2, T4, 400x magnification), C (T3, 100x magnification)

Figures shows histological view of mice that used in the research, (⇐⇒) shows pycnotic, (➡) shows Cellular cast, (➡) shows Protein cast, (G) shows glomerulus, and (T) shows Tubulus

CHAPTER 5 DISCUSSION

Microscopic observation of the histological changes in tubulus and glomerulus at mice kidney was used to determine the effect of propolis on histology of tubulus and glomerulus of kidney in male mice (*Mus musculus*). The main target of observations is histological view of tubulus and glomerulus.

The result based on statistical analysis shows that the administration of propolis does not have effect on histological changes of kidney in male mice both on the lowest dose group (0.4mg/head/day) until the highest dose group (3.2mg/head/days) and even when compared with the control group, the results show that both tubulus and glomerulus is normal, while we might find a bit of necrosis in tubulus that mainly pycnotic, both of that result might have affected by the compound that propolis contain in this chase all the vitamin and mineral to help maintain the organ health but Shuai (2014) also stated that there some mineral that are considerable as a toxic, propolis contain resins such as flavonoids (Jaya *et al.*, 2005) and other substances that include vitamins such as vitamin A, B1, B2, B6, C, D, E and trace minerals such as calcium, magnesium, iron, copper, zinc (Farooqui and Farooqui, 2010).

The author made this research to know the safety of propolis for kidney and what other benefits from propolis in the kidney, from the result that shows that propolis does not change the histological profile in both in tubulus and the glomerulus and any other histopathological parameters, and a little bit of pycnotic visible in the histological profile, and Cormack (2001) in his book explain that,

pycnotic seen at sites where there is evidence of inflammation generally indicates accidental cell death caused by some extrinsic hazard, Such cell death termed necrosis. Cells can also perish under physiologically normal condition, for example, when they become senescent, so in this chase pycnotic that visible might not because the effect of propolis itself, instead its naturally occurred, the author make the conclusion that propolis is safe for the kidney and even can be beneficial supplement as the study Lahouel *et al.*, (2010), says that the renal oxidative stress caused by doxorubicin can be prevented by the effect of flavonoid content in propolis, and as we all know propolis have a lot of beneficial to our body and can be use for supplement, Supplementation is important for the treatment of certain health problems but there is little evidence of benefit when used by those who are otherwise healthy (Fortmann *et al.*, 2013). Therefore, treatment of propolis to healthy mice does not affect the kidney.

CHAPTER 6 CONCLUSION AND SUGGESTIONS

6.1 Conclusion

Based on the present research that have been conducted, it shows:

1. Propolis extract does not affect Histological change on tubulus and glomerulus in male mice.
2. Different Level of propolis dose does not affect Histological changes of tubulus and glomerulus in male mice kidney

6.2 Suggestions

Based on the research results, the author suggest:

1. Propolis is safe for the kidney in this range of dose
2. Propolis can be used for supplementation
3. Necessary to conduct a research on the effect of propolis extract in other animal model.
4. Necessary to conduct a research using unhealthy animal.

SUMMARY

Hadi Muhammad Hadi. Effect of Propolis on Histology Profile of Kidney in Male Mice (*Mus musculus*). The present research was conducted under the guidance of Prof. Romziah Sidik, drh., PhD. as the supervisor and Prof. Lucia Tri Suwanti, drh., MP as the co-supervisor.

Propolis is a resinous mixture collected from trees by the *Apis mellifera* bee. Bees collect this material from the leaflet, bark, and from other parts of the plant. Propolis is a plant-derived product: its chemical composition depends on the local flora at the site collection, thus it offers a significant chemical diversity. Propolis consisted of resin (50%), wax (30%), essential oil (10%), pollen (5%), and organic compounds (5%). Resin contains flavonoid, phenol and various form of acid. Flavonoid as one of the phenolic compounds found in many plant tissues that can act as an antioxidant. Propolis presents plenty of biological and pharmacological properties, such as immunomodulatory, antitumor, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, antiparasite activities.

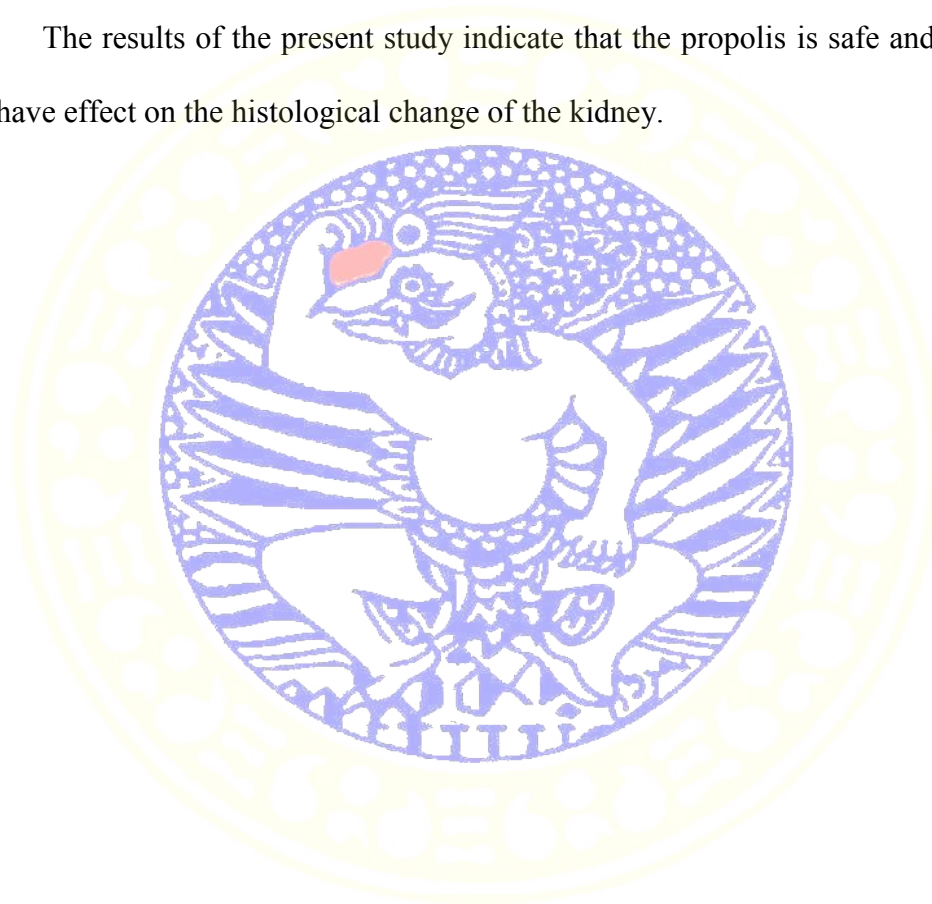
The kidneys are a pair of organs that lie outside the peritoneal cavity in the dorsal abdominal wall, each side in the columna vertebral. In each kidney consists of about 1 million units the smallest. Each unit, or nephron consists of a component called the glomerular vascular and tubular components, renal mechanisms in carrying out its functions depends on the relationship between these two components. Tubule lumen wall is covered by a layer of epithelial cells,

a different structure and function from first part to the other. Kidneys have a fairly diverse formation in animals, in general kidney function as blood filtration. Renal artery and renal vein entrance exit on each renal hilum. Nearly 25% of cardiac output into the kidneys. Blood filtered in the kidney, throwing useless compound and substance, especially urea and nitrogen -containing compounds, and regulate electrolytes extravascular and intravascular volume.

This study consisted of two stages, the propolis extraction process and propolis treatment to experimental animals. Basic material used was raw propolis from Rimba Raya Lawang bees ranch, as much as 500g and extracted using ethanol 70%. Propolis extraction was conducted in the Phytochemistry Laboratory Faculty of Pharmacy, Universitas Surabaya in November to December 2014. The second stage is propolis extract administration to experimental animals using five treatments with five replications. The treatment given to the inclusion criteria of male mice, aged 12 weeks, weigh 25-35g strain Balb/c. Provision of propolis was held for 14 days preceded by a period of adaptation for seven days. Experimental were using 25 mice (*Mus musculus*). Plastic enclosure cages were used for maintenance of experimental animals at Experimental Animal Laboratory, Medical Faculty, Universitas Airlangga in December 2014 to July 2015. The observed subject in this research are the histology profile of the kidney itself using various histopathology parameters in tubulus and glomerulus to know the safety of the propolis. Experimental animals grouping of the present study used Completely Randomized Design. Data were analyzed with Kruskal-Wallis. The

results show that there were not significant difference in the histology of the kidney.

The results of the present study indicate that the propolis is safe and does not have effect on the histological change of the kidney.



REFERENCES

- Arsad, S.S., N. M. Esa. And H. Hamzah. 2014. Histopathologic Changes in Liver and Kidney Tissues from Male Sprague Dawlet Rats Treated with *Rhaphidophora Decursiva* (Roxb.) Schoot Extract. J.Cytol Histol.S4:1-6
- Ballenger, L. 1999. "*Mus musculus*" (On-line), Animal Diversity Web. http://animaldiversity/Mus_musculus.html. April 2007
- Bankova, V., M. Popova and B. Trusheva. 2014. Propolis volatile compounds: chemical diversity and biological activity: a review. Chemistry Central Journal. Sofia.
- Bogdanov, S. 2014. Propolis: Composition, Health, Medicine. A Review. Bee Product Science. www.bee-hexagon.net
- Bruno, G. 2005. Bee Pollen, Propolis & Royal Jelly. Huntington College of Health Sciences., West Virginia.
- Cormack, D.W. 2001. Essential Histology Second Edition. Lipincot William & Wilkins. Pennsylvania. Philadelphia. 52.
- Corwin, E.J. 2001. Buku Saku Patofisiologi. Alih Bahasa Brahm U. Pendit. Penerbit Buku Kedokteran EGC., Jakarta.
- Drake, R. L., W. Vogt. And A. W. Mitchell., 2004. Gray's Anatomy for Students. 19th ed. London., Chruchill Livingstone.
- Eroschenko, V.P. 2003. Atlas histology di fiore dengan korelasi fungsional. Edisi 9. Jakarta. 249-261.
- Farooqui, T and A.A, Farooqui. 2010. Molecular Mechanism Underlying the Therapeutic Activities of Propolis : A Critical Review. Current Nutrition and Food Science. 6(3):1-15.
- Finstrom, S.M and M. Spivak.2010.Propolis and Bee Health: The Natural History and Significance of Resin Use by Honey Bees. Apidologie 41 (2010) 295–311
- Fortmann, S. P., Burda, B. U., Senger, C. A., Lin, J. S., Whitlock, E. P. 2013. Vitamin and Mineral Supplement in the Primary Prevention of Cardiovascular Disease and Cancer: An Updated Systematic Evidence Review for the U.S. Preveptive Services Task Force. Annals of Internal Medicine. 159(12): 824-834.

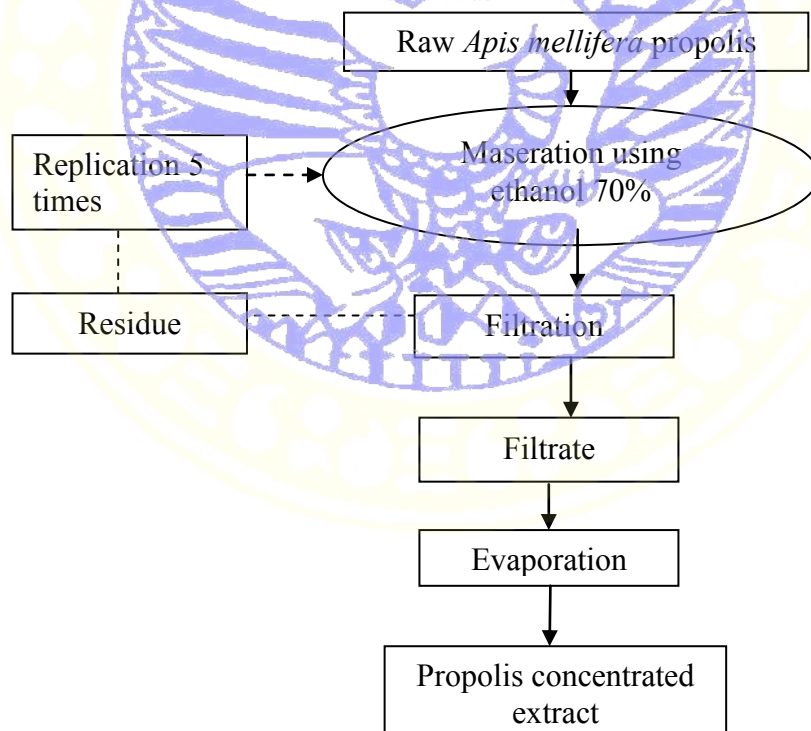
- Ivancajic, S., I. Mileusnic, D. Cenic-Milosevic. In vitro antibacterial activity of propolis extracts on 12 different bacteria in condition of 3 various pH values. Arch Biol Sci 2010;62(4):915-34
- Jaya, F., K.U. Al Awwaly., U. Kalsum dan L.E. Radiati. 2005. Pengaruh Pemberian Ekstrak Propolis terhadap Sistem Kekebalan Seluler pada Tikus Putih (*Rattus norvegicus*) Strain Wistar. Fakultas Peternakan Universitas Brawijaya. Malang.
- Krell, R. 2005. Propolis. Value Added Products From Beekeeping. FAO Agricultural Service Bulletin. Food and Agricultural Organization of The United Nation number 124. P. 157-194.
- Lahouel, M., K. Boutabet., W. Kebsa and M. Alyane. 2010. Polyphenolic Fraction of Algerian Propolis Reverses Doxorubicin induced Acute Renal Oxidative Stress. African Journal of Pharmacy and Pharmacology. 4(10):712-720.
- Lofty, M., 2006. Biological Activity of Bee Propolis in Health and Disease. Asian Pacific Journal of Cancer Prevention, P. 22-31
- Moreira, L., L. G. Dias, J. A. Pereira, L. Estevinho. 2008. Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. Elsevier. Bragança
- Moriwaki, K, T. Shiroishi, H. Yonekawa. 1994. Genetic in Wild Mice. Its Application to Biomedical Research. Tokyo: Japan Scientific Societies Press. Karger. Penerbit Buku Kedokteran. Jakarta. Hal 247-261.
- Muntiha, M. 2001. Teknik Pembuatan Preparat Histopatologi Dari Jaringan Hewan Dengan Pewarnaan Hematoksilin Dan Eosin (H&E). Temu Teknis Fungsional Non Peneliti 1001. Bogor.
- NIH. 2013. Calcium fact sheet. <https://ods.od.nih.gov/factsheets/Calcium-Consumer/> [26 October 2015]
- Nikolaev, A.B. 1978. Defending the bee town. In Remarkable, hive product: Propolis. Scientific data and suggestions concerning its composition, properties and possible use in therapeutics. APIMONDIA standing commission on beekeeping technology and equipment, Bucharest.
- Purnomo, B. 2009. Dasar-dasar urologi edisi kedua. FK Univ. Brawijaya Malang. Malang.

- Sancheti, G. and P. K., Goyal. 2007. Prevention Of Radiation Induced Hematological Alterations By Medicinal Plant *Rosmarinus Officinalis*, In Mice Afr. J. Trad. Cam. 4 (2): 165-172.
- Shuai, H., Z. Cui-Ping., W. Kai., Q.L. George and H. Fu Liang. 2014. Recent Advances in the Chemical Composition of Propolis. *Molecules*. 19:19610-19632.
- Sirois, M. 2005. *Laboratory Animal Medicine*. USA :Mosby, Inc. P:89-91.
- Siti, E. M., F., Dina, Y., Iwang. 2011. Indeks Daya Fagosit Makrofag Peritoneum setelah pemberian propolis pada mencit "*Mus musculus*". *Sains medika*. 3(2).121-128.
- Steel, R.G.D. and J.H.Torrie 1993. *Prinsip dan Prosedur Statistika, Suatu Pendekatan Biometrik*. P.T Gramedia Pustaka.Jakarta. 168-205.
- Suckow, M. A., P., Danneman, and C., Brayton. 2001. *The Laboratory Mouse*. CRC Press LLC: London.
- Sukara, E dan I. Tobing. 2008. *Industri Berbasis Keanekaragaman Hayati, Masa Depan Indonesia*. Fakultas Biologi Universitas Nasional. Jakarta.
- Takagi, Y., Choi, I., Yamashita, T., Nakamura, T., Suzuki, I., hasegawa, T., Oshima, M., Gu, Y., 2005. Immune Activation and Radioprotection by Propolis. *The American Journal of Chinese Medicine*. 33(3): 231-240.
- Toprakci, M.B.S. 2005. *Origin and Chemical Variation of Brazilian Propolis*. Department of Botany. Brazil: Institute of Biosciences University of São Paulo. Brazil.
- Whiting, S.J and R.J. Wood. 1997. Adverse effects of high-calcium diets in humans. *Nutr Rev* 55: 1-9.
- Wijayakusuma, H . 2000. *Potensi Tumbuhan Obat asli indonesia sebagai produk kesehatan*. Naju. South Korea.

Appendix 1

Propolis Extraction Process

Alcohol extraction of propolis typically using ethanol as a solvent, ethanol is the best solvent for the extraction of propolis, whereas propolis can be used for identification of other solvents such as ethyl esters, water, methanol and chloroform (Marcucci *et al.*, 1998). Here is a flowchart of making up of the propolis extract can be concentrated extract of propolis :



300 grams of raw propolis macerated using 70% ethanol. Extraction is done by soaking 300 grams of raw propolis using 850 ml of 70% ethanol for 4 days, with shaking for 1 hour and conducted a three-day immersion, the filtrate was decanted, the remaining residue was extracted again with 850 ml of 70% ethanol, shaken 1 hour at 120 rpm, and the filtrate was decanted. Extraction of the residue was repeated up to five times, for a total of 4250 ml of solvent used, and the total time of maceration 7 days. The filtrate is collected in a container, the

filtrate was concentrated using a rotary evaporator, the extract forms a paste that is ready for further testing (EEP: ethanol extract of propolis).

Propolis maceration result is a dark red filtrate (red-brown). The amount of yield obtained is closely related to the intensity of the color of the solution extract. Propolis extract solution with a darker color, indicating a higher yield obtained compared with a brighter color. Dark color is due to the high content of flavonoids it contains. Removal of the solvent using a freeze dryer is done to minimize heating. Evaporation of the solvent using a vacuum evaporator, still requires heating to a temperature of about 60°C. Removal of the solvent using a spray dryer also need heating. Heat kept to a minimum in the extraction of propolis, as it can alter or damage the structure of the main bioactive namely propolis bioflavonoids.

Appendix 2

1) Human and Animals Dosage Conversion Table

| | Mice 20 gr | Rat 200 gr | Guinea Pig 400 gr | Rabbit 1,5 kg | Cat 2 kg | Ape 4 kg | Dog 12 kg | Human 70 kg |
|-------------------------|---------------|---------------|-------------------------|------------------|-------------|-------------|--------------|----------------|
| Mice 20 gr | 1,0 | 7,0 | 12,25 | 27,8 | 29,7 | 64,1 | 124,2 | 387,9 |
| Rat 200 gr | 0,14 | 1,0 | 1,74 | 3,9 | 4,2 | 9,2 | 17,8 | 56,0 |
| Guinea Pig 400 gr | 0,08 | 0,57 | 1,0 | 2,25 | 2,4 | 5,2 | 10,2 | 31,5 |
| Rabbit 1,5 kg | 0,04 | 0,25 | 0,44 | 1,0 | 1,08 | 2,4 | 4,5 | 14,2 |
| Cat 2 kg | 0,03 | 0,23 | 0,41 | 0,92 | 1,0 | 2,2 | 4,1 | 13,0 |
| Ape 4 kg | 0,016 | 0,11 | 0,19 | 0,42 | 0,45 | 1,0 | 1,9 | 6,1 |
| Dog 12 kg | 0,008 | 0,06 | 0,10 | 0,22 | 0,24 | 0,52 | 1,0 | 3,1 |
| Human 70 kg | 0,0026 | 0,018 | 0,031 | 0,07 | 0,076 | 0,16 | 0,32 | 1,0 |

(Suhardjono, D. 1995).

2) Table of Maximum Volume List of Test Preparation Solution should be Given in Various Animals

| Experimental Animals Type | Maximum Volume (ml) according the pathway | | | | |
|---------------------------|---|------|-------|--------|-------|
| | i.v. | i.m. | i.p. | s.c. | p.o. |
| Mice (20-30 gr) | 0,5 | 0,05 | 1,0 | 0,5-10 | 1,0 |
| Rat (100 gr) | 1,0 | 0,1 | 2,5 | 2,5 | 5,0 |
| Hamster (50 gr) | - | 0,1 | 1-2 | 2,5 | 2,5 |
| Guinea Pig (250 gr) | - | 0,25 | 2-5 | 5,0 | 10,0 |
| Dove (300 gr) | 2,0 | 0,5 | 2,0 | 2,0 | 10,0 |
| Rabbit (2,5 kg) | 5-10 | 0,5 | 10-20 | 5-10 | 20,0 |
| Cat (3 kg) | 5-10 | 1,0 | 10-20 | 5-10 | 50,0 |
| Dog (5 kg) | 10-20 | 5,0 | 20-50 | 10,0 | 100,0 |

(Suhardjono ,D. 1995).

Explanation

- i.v. : intravein
- i.m. : intramuscular
- i.p. : intraperitoneal
- s.c. : subcutan
- p.o. : peroral

Appendix 3

Dosage Calculation

Propolis Doses for Human = 100 mg (Krell, 1966).

Dosage Convection from human (70 kg) to mice (20 g) in Appendix 2 tabel is 0,0026

- Dosage of Propolis Extract for 20 g weight mice
 - = $100 \text{ mg} \times 0.0026$
 - = 0,26 mg
- Dosage for 30 g weight mice
 - = $30 \text{ g} \times 0,26 \text{ mg}$
 - 20 g
 - = 0,39 mg ~ (integration to be 0,4)

- Consider the dose range of propolis extract for mice are 0,4 mg for minimum dosage and 16 mg for maximum dosage.
- The formulation to determine the dosage constanta

$$X = \sqrt[n-1]{\frac{\text{Maximum dose range}}{\text{Minimum dose range}}}$$

Explanation : n = treatment (in this calculation the control group are not include, so there are 4 propolis dose treatments)

$$X = \sqrt[4-1]{\frac{16 \text{ mg}}{0,4 \text{ mg}}}$$

$$X = \sqrt[3]{\frac{16}{0,4}}$$

$$X = \sqrt[3]{10}$$

$$X = 2,15 \sim (\text{constanta integration to be } 2)$$

Therefore, the dosage calculation is

$$P1 = 0,4 \text{ mg (minimum dose)}$$

$$P2 = 0,4 X = 0,4 \times 2 = 0,8 \text{ mg}$$

$$P3 = 0,4 X^2 = 0,4 \times 2^2 = 0,4 \times 4 = 1,6 \text{ mg}$$

$$P4 = 0,4 X^3 = 0,4 \times 2^3 = 0,4 \times 8 = 3,2 \text{ mg}$$

- Maximum dose volume that can be given to the mice per oral is 1 ml. In this research the maximum dose which used is 0.5 ml

- Dosage dilution for treatments

| Treatment | Treatment Dosage mg/day | Tween 80 (μ l) | E-pure (ml) | Total volume (ml) |
|-----------|-------------------------|---------------------|-------------|-------------------|
| P1 | 0,4 | 100 | 0.4 | 0.5 |
| P2 | 0,8 | 100 | 0.4 | 0.5 |
| P3 | 1,6 | 100 | 0.4 | 0.5 |
| P4 | 3,2 | 100 | 0.4 | 0.5 |

(Radiati, L., dkk., 2011).

Therefore the final dose after dilution are

P0 = 0.5 ml Aquadest/day

P1 = 1.6 mg/0.5ml/day

P2 = 3.2 mg/0.5ml/day

P3 = 6.4 mg/0.5 ml/day

P4 = 12.8 mg/0.5ml/day

Appendix 4

Procedure of Histological Specimen Preparation

1. Organ Cutting

The first thing to do after the experimental animals euthanized is tissue immersion in preservative agent. Preservatives which usually used are Neutral Buffered Formalin solution (BNF) 10% with a pH range between 6.5 - 7.5. The ideal pH is 7.0. Comparison between the organ and the solution is 1: 10 so that the fixation of tissue with the solution is complete, while the length of fixation of at least 2 days.

2. Dehydration and Clearing

Drained the organ using filter then cut it using a scalpel blade with a thickness of 0.3 - 0.5 mm and arranged into a tissue cassette, then a tissue cassette is inserted into a special basket. Basket that contains the organ

tissue are placed in an automatic processor machine. Furthermore, the network experienced a gradual dehydration process with a lap time as follows: ethanol 70% (2 hours) ethanol 80% (2 hours), 90% ethanol (2 hours) 96% absolute ethanol (2 hours) xylol (2 hours) of liquid paraffin (2 hours). Furthermore, the basket which contain tissue cassette is issued to do the next process.

3. Vacuum

Dehydration process carried out, followed by removal of air from the tissue using a vacuum machine that includes the tube to store basket filled with liquid paraffin with temperature (59-60 ° C) in a vacuum for 30 minutes. Basket removed, the tissue cassette removed and stored at 60 ° C for a while before printing done with liquid paraffin.

4. Paraffin Block Production

Prepare some iron mold that has been smeared with glycerin in order to prevent the paraffin sticking inside the mold, then a tissue that has been cut included with tweezers and wait until the paraffin solidified. Paraffin blocks removed from the mold and stored in a freezer (-20 ° C) before cutting. Paraffin block containing the tissue will be cut using a microtome machine with thicknesses ranging from 4-6 µm.

5. Tissue Block Slicing

The tissue blocks pieces are carefully placed on the surface of water in a water bath at temperature 46 ° C. On this occasion the tissue sliced orderly then placed on a microscope slide that smeared with ewith, which

serves as an adhesive. Slide with tissue on it are arranged in a special rack and put in an incubator at 60 ° C until the specimen is ready for staining process.

6. Hematoxilin – Eosin Staining

Specimen which will be stained are placed in a special rack then dipped in a series into some solutions with time as follows

1. Xylol 3 minutes
2. Xylol 3 minutes
3. Absolute ethanol 3 minutes
4. Absolute ethanol 3 minutes
5. Ethanol 90% 3 minutes
6. Ethanol 80% 3 minutes
7. Rinse with tap water 1 minute
8. Hematoxilin solution 7 minutes
9. Rinse with tap water 1 minute
10. Blue solution 1 minute
11. Tap water 1 minute
12. Eosin solution 5 minutes
13. Rinse with tap water 1 minute
14. Ethanol 80% 10 times dip
15. Ethanol 90% 10 times dip
16. Absolute ethanol 10 times dip
17. Absolute ethanol 1 minute

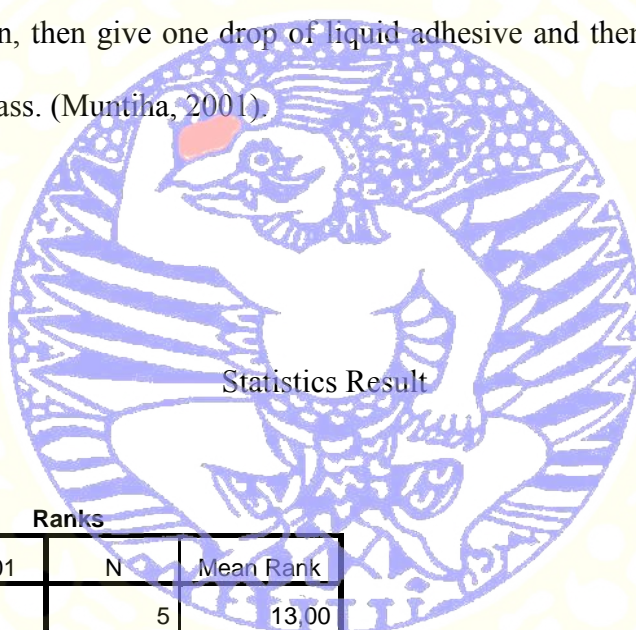
18. Xylol 3 minute

19. Xylol 3 minute

20. Xylol 3 minute

Specimens removed one by one from xylol solution in wet condition, then give one drop of liquid adhesive and then covered it with cover glass. (Muntaha, 2001).

Appendix 5



Statistics Result

Ranks

| | VAR00001 | N | Mean Rank |
|-------|----------|----|-----------|
| Total | p0 | 5 | 13,00 |
| | p1 | 5 | 13,00 |
| | p2 | 5 | 13,00 |
| | p3 | 5 | 13,00 |
| | p4 | 5 | 13,00 |
| | Total | 25 | |

Test Statistics^{a,b}

| | Total |
|-------------|-------|
| Chi-Square | ,000 |
| df | 4 |
| Asymp. Sig. | 1,000 |

a. Kruskal Wallis Test

b. Grouping Variable:

VAR00001

Descriptives

Total

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|-------|----|-------|----------------|------------|----------------------------------|-------------|---------|---------|
| | | | | | Lower Bound | Upper Bound | | |
| p0 | 5 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |
| p1 | 5 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |
| p2 | 5 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |
| p3 | 5 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |
| p4 | 5 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |
| Total | 25 | ,0000 | ,00000 | ,00000 | ,0000 | ,0000 | ,00 | ,00 |

Appendix 6

Research Documentation



Picture 1: Propolis Extract



Picture 2: Propolis dilution



Picture 3: Propolis extract after dilution



Picture 4: Propolis final dose



Picture 5: Mice cages



Picture 7: Mice necropsy