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Research Article

The Effect of Ethanolic Extract of Cashew Fruit Peel on The Liver Histological Structure in Rat (*Rattus norvegicus* Berkenhout, 1769)

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ABSTRACT
Cashew fruit peel is a waste produced from the cashew nut industry, and it has not been utilized optimally yet. Cashew peel extract has the potential to be used as a contraceptive agent, which capable of reducing reproductive capacity. However, its side effects on other tissue and organ such as liver not clearly studied yet. This study aims to determine the effect of ethanolic extracts of cashew peel on the histological structure of the white rat liver. In this study, 21 female white rats were used and be grouped for control (6 mice) which were treated with CMCMa 0.5% and 15 mice were treated with peel extract of 500 mg/kg body every day for one month. Liver for examination was collected sequentially at 3rd, 5th, 8th, 11th, and 14th of the estrous cycle. The liver was processed for histological observation and stained with Hematoxylin Eosin and Mallory Acid Fuchsin staining solution. The liver hepatocyte was observed for it abnormality and be scored to calculate the number of cell damage or abnormality. The result showed that peel extract-treated mouse liver was similar to control ones; we did not witness any evidence of fibrosis, pyknosis and cellular necrosis on either control or treated mouse. Statistical analysis by SPSS showed that the p-value between the control and treatment groups was 0.078 (> 0.05) so there was no significant difference between control and treatment. It could be concluded that ethanolic extracts of cashew nuts peel with a concentration of 500 mg/kg body weight caused no effect on the mouse liver histological structure. application with reduced-dosages of NPK fertilizers were arranged in a random block design with three replicates. The results show that large quantities of silica bodies attached to the surface of EFB fibers and amounting to 0.44% soluble Si. The FFB data indicated that the application of 75% NPK + 500 kg composted EFB + 2 L BioSilAc/ha/year on a five-year-old plant resulted in higher yield than that obtained from 100% standard dosage of NPK. The study also revealed that the application of EFB compost reduced 50% of BioSilAc dosage.

INTRODUCTION
The potential of cashew cultivation in Indonesia is promising, especially in the eastern part of Indonesia. Production of cashew from year to year has increased, as in 1999, nuts cashew production reached to 88,658 tons, and increased to 94,439 tons in 2002 (BPS, 2002). Cashew fruit peel is a waste produced from the cashew nut industry, and it has not been utilized optimally yet. Cashew nut fruit peel (shell) contain oil known as Cashew Nut Shell Liquid (CNSL). The main components of CNSL are anacardate acid, cardanol and cardol (Patel, 2016).

According to Harlita (2016), cashew peel extract has an antifertility effect by changing the uterine structure of albino rats. In addition, Herlina's (2013) also shown that cashew fruit extracts (*Anacardium occidentale* L.) had cytotoxic and estrogenic activity, decreased body weight and also testosterone levels on white mice.

The liver is the main organ that responsible for detoxification of hazard or harmful compounds that enter the body and also protecting the body against the accumulation of harmful substances from outside and inside. The liver also the site where

drugs and other toxic substances are metabolised (Sativani, 2010). The presence of hazard compounds inside the body could cause an adverse effect on the histological structure and physiological function of the liver. Research on the effect of ethanolic extracts of cashew (*Anacardium occidentale*) peel on liver organ histopathology has never been done, therefore this study is important to know the effect of giving ethanolic extract of cashew (*Anacardium occidentale*) peel on the histological structure of the liver in Wistar mouse as a model animal (*Rattus norvegicus* Berkenhout, 1769).

MATERIALS AND METHODS

Materials

This research was conducted from February to July 2018 at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) UGM and at the Histology Laboratory Faculty of Biology, Universitas Gadjah Mada. The material used in this study was cashew nut (*Anacardium occidentale* L.) which was dried and then extracted using ethanol 96% distillate, white rat (*Rattus norvegicus* Berkenhout 1769) 21-month old female Wistar strain, picric acid, Sodium solution Carboxyl Methyl Cellulose (CMCNa) 0.5%, ketamine, and physiological saline solution. The material for histology preparations was distilled water, Bouin solution, ethanol, toluene, 0.1% Acid Fuchsin solution, PMA solution, Mallory solution, paraffin, xylene, Meyer` s albumin, Ehrlich's hematoxylin, and Eosin-Y 1-2%.

Methods

Cashew nut seed extraction

Cashew seed peel was washed and dried in an oven at 30ºC for 72 hours and then ground to powder. The powder was soaked in 96% ethanol solution, stirred for 30 minutes, allowed to stand for 24 hours, and filtered. This step was repeated three times. The extract solution was separated and distilled at 70 ºC, and the oil was separated from the solvent using a vacuum rotary evaporator and a water bath heater. The thick extract was heated with a water bath while stirring it.

Treatment

Twenty one female white rats were divided into two groups: 6 mice as control and 15 mice for extract treatment, respectively. The control group were divided into two, which were untreated control and placebo control mouse, which were fed with 0.5% CMCNa. The treated group were fed with 500 mg extract/kg BW. Termination of the treatment was carried out during the 3rd (K3) , 5th (K4), 8th (K5), 11th (K6) and 14th (K7) estrous cycles. The mouse liver organs were collected and processed for histological preparation.

Treatment termination and organ collection

Mouse were euthanized with an overdose of ketamine, dissected, and the liver organ was taken and then was with a salt buffer for debris cleaning and post-mortem reaction delay.

Histology preparation for liver organ

The liver organs were collected, washed with salt buffer, fixed with Bouin's solution for 12 hours, and processed following paraffin standard method. The histological slides were stained with Haematoxylin-eosin and Mallory Acid Fuchsin staining.

Qualitative data were acquired from the descriptive analysis of treatment and control groups liver histological structure, using a microscope with a magnification of 10x10 and 10x100, respectively. Moreover, quantitative data was obtained from the number of defect cells, based on three layout view, which was analyzed with One Way ANOVA for social sciences (SPSS).

RESULTS AND DISCUSSION

Microscopic observation on Hematoxylin-Eosin stained liver showed that hepatocytes were in radial arrangement, with rounded-purple stained of nuclear cell positioned on the center of the cells. Sinusoid was radially arranged, which extends from the sinus area to the central vein at the center of the liver lobule. The extract-treated group of K3, K5, and K6 showed the sign of hepatocyte defect or degeneration, which were characterized by the occurrence of cytoplasmic vacuolization. It was also observed the appearance of some pyknotic cells, which were characterized by denser nuclear cell and the darker image of cell cytoplasmic content compared to control or normal cells (Figure 1). The pyknotic cells are one of the symptoms of the occurrence of cell damage. The nucleus of a pyknotic cell is smaller because of the irreversible chromatin and nucleus condensation, which is called the pyknosis process. Pyknosis usually occur in cells that experience apoptosis or necrosis (Hou et al., 2016).

The result showed that the percentage of the pyknotic cell on the control and placebo control group was 6% and 5.47%, respectively. Meanwhile, extract-treated mouse showed a relatively higher percentage of pyknotic cells, which the highest percentage of the pyknotic cell occurs at the group of K3 mouse of 8.93% (Figure 2).

Based on statistical analysis, the extract does not affect the percentage of rat liver cell damage

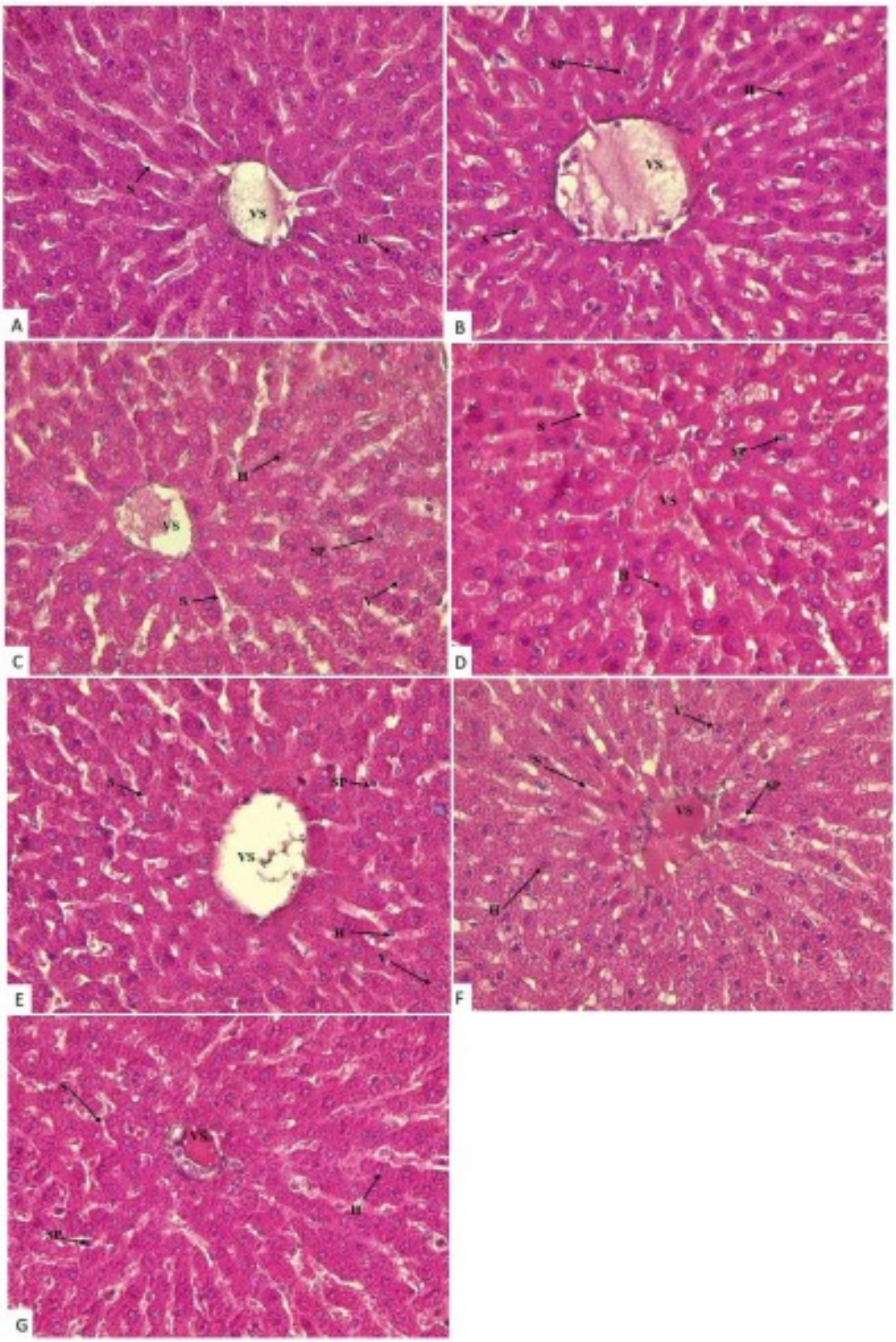

Figure 1. Histological structure of mouse liver group of A). control, B). placebo control, C). K3, D). K4, E). K5, F). K6 and G). K7. Central veins (VS), hepatocytes (H), pyknotic cells (SP), and sinusoids (S). K1= Control; K2= Placebo control; K3= termination at 3rd estrous cycle; K4= Termination at 5th estrous cycle; K5= termination at 8th estrous cycle; K6= termination at 11th estrous cycle; K7=termination at 14th estrous cycle. HE stained, 10x40 magnification.

significantly compared to control (P=0,05). Cell vacuolization was observed in several groups of the extract-treated mouse of K3, K5, and K6. Cell vacuolization is one of the characteristics of cell degeneration. The cells degeneration usually occurs as a response to cell injury or stress, which is reversible. If the toxic exposure is removed from the mouse, therefore the cells could be restored to normal and could resume its normal physiological condition. Cell degeneration is characterised microscopically by the presence of cell vacuolization, which is found in clear spaces in the cell cytoplasm (Carlton and McGavine, 1995). This condition is caused by metabolic disorders in the liver organ. Damage to the cell membrane causes leakage of the membrane which disrupts the activity of the K + transport that comes out of the cell, and the entry of Ca2 +, Na +, and water into the cell. Excessive

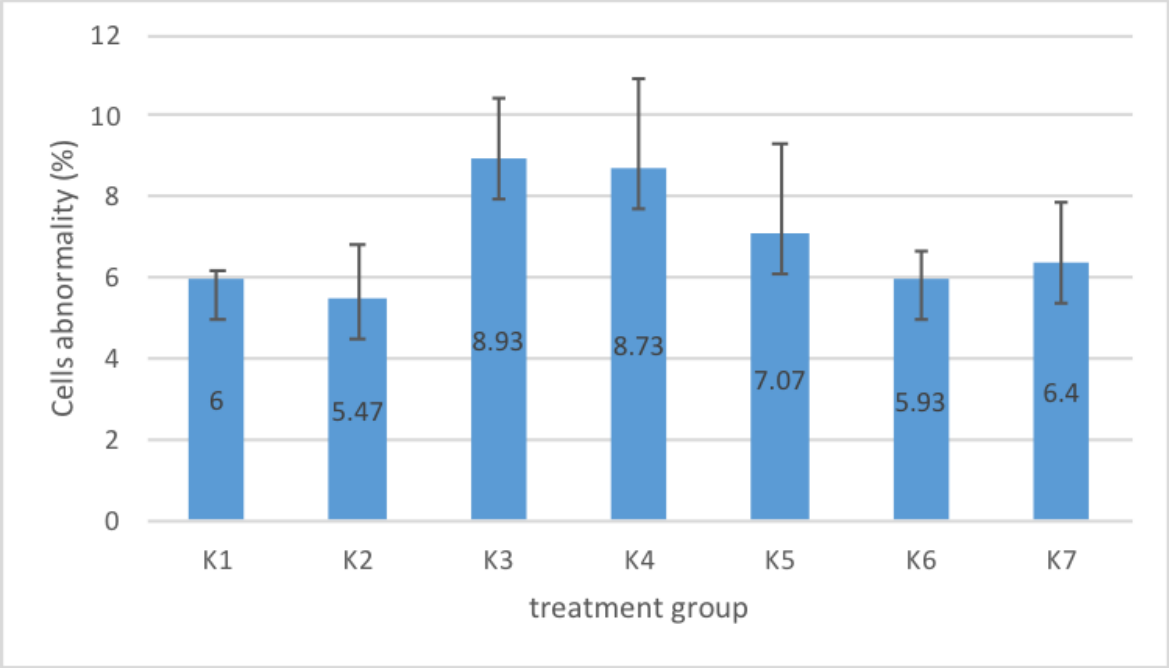

Figure 2. Percentage of pyknotic cells on the liver of control and extract treated mouse. K1= Control; K2= Placebo control; K3= termination at 3rd estrous cycle; K4= Termination at 5th estrous cycle; K5= termination at 8th estrous cycle; K6= termination at 11th estrous cycle; K7=termination at 14th estrous cycle

extracellular fluid influx causes cytoplasmic inflating, mitochondria, and coarse endoplasmic reticulum (King and Joseph, 1996).

The liver is the main organ that functions as detoxification of harmful substances in the body. The detoxification of harmful substances in the liver consists of phase I and phase II processes. Generally harmful substances that are not needed by the body, are easily soluble in lipids (lipophilic) naturally and difficult to pass through the cell membrane in the process of excretion. The liver is able to change the chemical components of harmful substances through several reactions such as oxidation and conjugation. This reaction will produce compounds that are more polar and water-soluble so that easily to be excreted through urine or bile. In phase I detoxification there is an oxidation reaction involving the cytochrome P450 enzyme. Whereas, phase II detoxification, is a conjugation reaction where small polar molecules are added to the chemical substance so that the substance is water-soluble (Chiang, 2014).

Microscopic observation of the liver was also carried out with Mallory-Acid Fuchsin staining to observe the presence of fibrosis in the liver connective tissue. Liver fibrosis usually occurs in the form of accumulation of extracellular matrix in response to various stimuli and causes various liver function disorders and blood flow. When fibrosis occurs in the tissue, normal tissue will be replaced by non-functional connective tissue which can further reduce the physiological function of the liver. Fibrosis is divided into several scales, consisting of F0, F1, F2, F3, and F4. F0 is the smallest scale where fibrosis is not found, or there is no enlarged portal tract and septa. F1 is characterized by portal fibrosis without septa. F2 is characterized by portal fibrosis with little septa or fibrous tissue around the portal tract. The F3 scale is indicated by the presence of portal fibrosis with many septa or fibrous tissue that connects the portal tract with the portal tract or with a central vein or bridging fibrosis, and the F4 scale is characterized by cirrhosis (Bedossa, 1994).

Observations result on liver tissue, which stained with Mallory-Acid Fuchsin, showed the presence of connective tissue in the form of collagen, be shown in blue color (Figure 3). The collagen fiber can be found at the area around the central vein, and there was no difference in collagen composition of both control and treatment groups. Moreover, It was also evidenced by the absence of fibrosis symptoms in the portals vein, nor the formation of septa between one lobe and the other (Figure 3). The observation results leading to the conclusion that the extract does not affect the connective tissue damage in the histological structure of mouse liver.

CONCLUSIONS

It can be concluded that ethanolic extracts of cashew peel did not affect the histological structure of liver of mouse (*Rattus norvegicus* Berkenhout, 1769), which was characterized by no significantly different between the control and treatment group on cell damage and fibrotic liver tissue of the mouse.

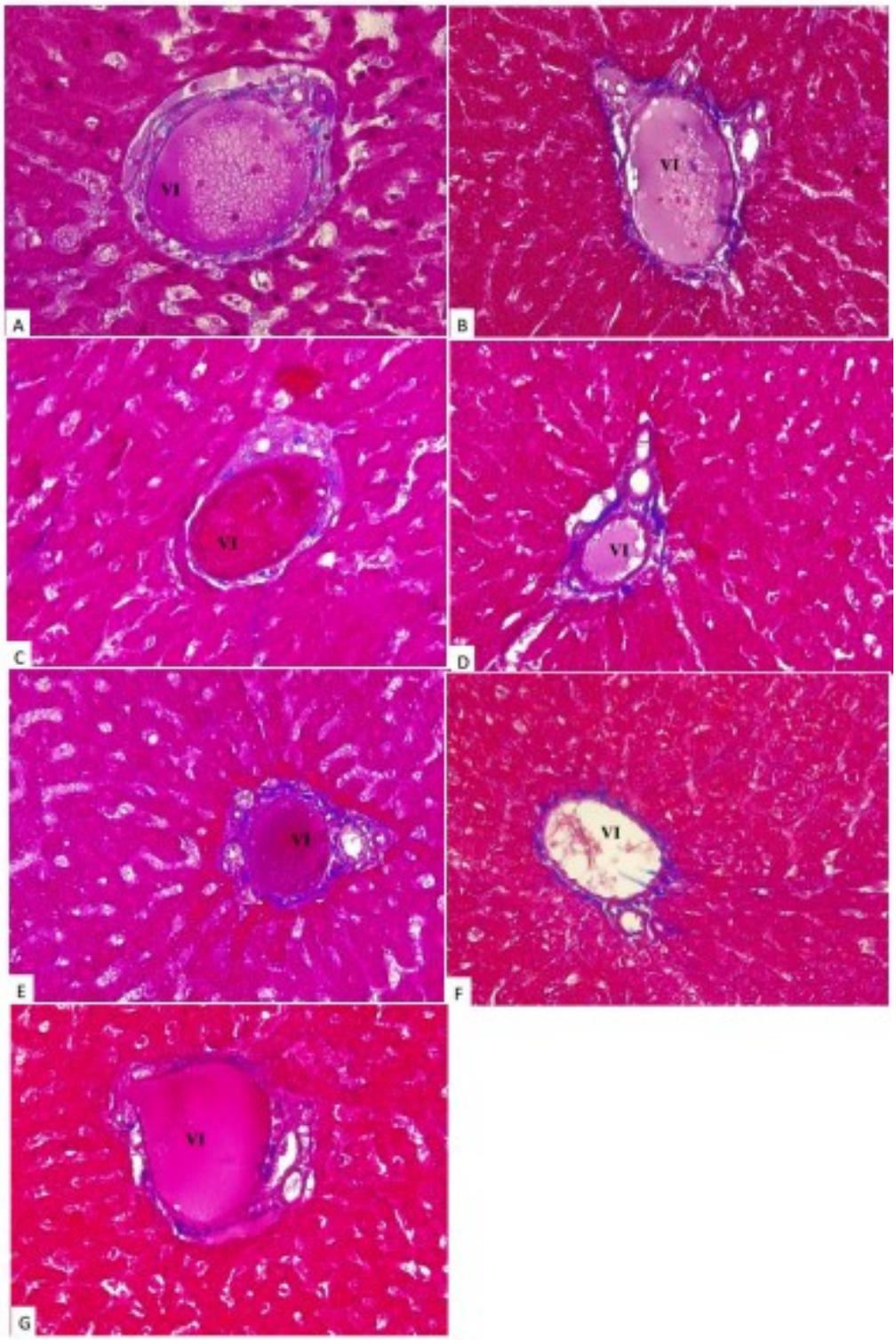

Figure 3. Histological structure of liver group A) control, B) placebo control, C) K3, D) K4, E) K5, F) K6, and G) K7. Showing Portal veins (VP) structure. MAF stained, 10x40 magnification

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REFERENCES

Carlton, W.W. & McGavin, M.D., 1995, *Special Veterinary Pathology*, 2nd ed., Mosby, United States of America.

Chiang, J., 2014, 'Liver Physiology: Metabolism and Detoxification', in L.M. McManus & R.N.. Mitchell (eds), *Pathobiology of Human Disease,* pp. 1770-1782, Elsevier, San Diego.

Bedossa, P., 1994, Intraobserver & Interobserver Variations in Liver Biopsy Interpretation in Patients with Chronic Hepatitis C, *Hepatology* 20(1), 15-20.

BPS, 2002, Statistik Perkebunan, Jakarta.

Harlita, 2016, Potensi Ekstrak Kulit Biji Mete (*Anacardium occidentale* L.) Sebagai Agen Penurun Kapasitas Reproduksi Tikus Putih (*Rattus norvegicus* Berkenhout 1769) betina [Potential of Cashew Seed Extract (*Anacardium occidentale L.*) As a Reducing Reproductive Capacity of White Rats (*Rattus norvegicus* Berkenhout 1769) female], *Doctoral thesis*, pp. 2-14, Universitas Gadjah Mada, Yogyakarta.

Herlina, 2013, Potensi Biotoksikan Ekstrak Etanolik Kulit Biji Mete (*Anacardium occidentale* L.) Pada Sistem Reproduksi Jantan Tikus Putih (*Rattus norvegicus* Berkenhout 1769) Galur Wistar [Biotoxic Potential of Ethanolic Extract of Cashew Seed Skin (*Anacardium occidentale L.*) in Male Reproduction System of White Rat (*Rattus norvegicus* Berkenhout 1769) Wistar Strain] *Master thesis,* pp. 122-132, Universitas Gadjah Mada, Yogyakarta.

Hou, L., Kai, L., Yuhong, L., Shuang, M., Xunming, J. & Lei, L., 2016, Necrotic Pyknosis is a Morphologically and Biochemically Distinct Event from Apoptotic Pyknosis, *Journal of Cell Science* 129, 3084-3090.

King, N.W. & Joseph, A., 1996, 'Intracellular and Extracellular Deposition; Degenerations', in T.C. Jones, R.D. Hunt & N.W. King (eds.) *Veterinary Pathology* 6th ed., Blackwell Publishing Professional, USA.

Patel, S., 2016, Emerging Adjuvant Therapy for Cancer; Propolis and Its Constituents, *J Diet Suppl.* 13:245-268

Sativani I., 2010, Pengaruh Pemberian Deksametason Dosis Bertingkat Per Oral 30 Hari Terhadap Kerusakan Sel Hepar Tikus Wistar [The Effect of Giving Dexamethasone 30-Day Oral Dose to Wistar Rat Cell Damage], *Undergraduate thesis,* Universitas Diponegoro, Semarang.