BIOEDUKASI: Jurnal Pendidikan Biologi
Volume 12, Nomor 2
Halaman 189-194

p-ISSN: 1693-265X
e-ISSN: 2549-0605
Agustus 2019

The Effects of Kersen Juice (*Mutingia calabura* L.) and Lakum Leaf Extract (*Cayratiatrifolia* L.) on Lipid Profile of White Rats (*Rattus norvegicus* L.) Hyperlipidemia

Didin Sopandi[1*], Tyas Rini Saraswati[2], Enny Yusuf Wachidah Yuniwarti[3]
Biology Magister Program, Diponegoro University, Semarang, Indonesia
*Corresponding authors: didinbio17@gmail.com

Manuscript received: 28-05-2019 Revision accepted: 29-07-2019

ABSTRACT

Kersen fruit and Lakum leaf contain substances that are potential as an antihyperlipidemic. This study aimed to analyze The Effects of Kersen Juice (*Mutingia calabura* L.) with dosage 0.2 ml/200 g BW and Lakum Leaf Extract (*Cayratia trifolia* L.) with dosage 40 mg/200 g BW on Lipid Profile of White Rats (*Rattus norvegicus* L.) Hyperlipidemia. This study used a complete randomized design (CRD). Twenty adult male Wistar rats were divided into five groups, P0 as a control group, P1 as a HFD control group, P2 as a HFD group treated with Kersen juice with dosage 0.2 ml/200 g BW, P3 as a HFD group treated with Lakum leaf extract with dosage 40 mg/200 g BW and P4 as a HFD group treated by simvastatin. The treatments were given orally for 28 days. The parameters of this study were cholesterol level, HDL, LDL, triglyceride, body weight, and food consumption. One way ANOVA test was performed to determine the significant differences $p<0.05$ between groups followed by Duncan test with a 95% significant level. The results showed that compared to Kersen juice with dosage 0.2 ml/200 g BW, administration of Lakum leaf extract with dosage 40 mg/200 g BW was more effective in lowering cholesterol level, triglyceride and increasing HDL level, however, they were not effective in lowering LDL in rats with hyperlipidemia.
.

Keywords: *Cayratia trifolia*, hyperlipidemia rat, lipid profile, *Mutingia calabura*

INTRODUCTION

Hyperlipidemia is a disorder disease characterized by excess on blood lipids which include cholesterol, triglyceride, low-density lipoprotein and a decrease in high-density lipoprotein in the bloodstream (Onwe *et al*., 2015). The elevation of serum total cholesterol and low-density lipoprotein (LDL) has been reported as a primary risk factor for cardiovascular disease (Nirosha *et al*., 2014). The primary reason for hyperlipidemia is a defect in lipid metabolism which is caused by the defect in lipoprotein lipase activity or the absence of the surface Apoprotein C-II (Harikumar *et al*., 2013). Based on the data from WHO in 2015, approximately 17.7 million people died because of cardiovascular disease. In Indonesia, based on data from Riskesdas (2013) shows that coronary heart disease has the highest prevalence among other cardiovascular diseases. They predict that the death rate due to this disease will rise to 23.3 million in 2030.

High lipid diet leading to hyperlipidemia is regarded as an important factor in the development of Ischemic heart disease and the focus so far has been mainly on the systemic and coronary vascular effects of cholesterol (Gazzerro et al., 2012). Antioxidants consisting of vitamin C, minerals, as well as some phytochemicals such as phenol and flavonoid, work by blocking the oxidative stress from free radicals and repair damage to the endothelial tissue, as well as protecting LDL and VLDL especially in the oxidation reaction (Gross, 2004). Endothelial tissue repaired in hyperlipidemia causes a decrease in total cholesterol, triglycerides, and LDL (Kakadiya, 2009).

Kersen (Mutingia calabura) and Lakum (*Cayratia trifolia*) are traditional plants that are less occupied but have high potential to repair the lipid profile of hyperlipidemia (Preethi, 2011). A study showed that Kersen and Lakum contain vitamin C which is quite high at approximately 80.5 mg (Mahmood *et al*., 2014). Besides, they also contain flavonoids, phenols, niacin, and beta–carotene which act as antioxidant agents (Ragasa *et al*., 2015).

Batra et al., (2013) in his research said that the used of Lakum root extract with dosage 200 mg/kg BW and 400 mg/kg BW significantly reduced the level of triglycerides, cholesterol, LDL and increased the level of HDL. However, the research on Lakum leaf has not been reported yet. The research on Kersen fruit has been conducted by Sudargo et al., (2017). The research reported that the administration of Kersen fruit juice with a dosage 0.9 ml/ 200 g BW and 1.8 ml/200 g BW in 14 days can elevate the level of HDL in blood serum, but not significantly reduced the level of triglycerides, cholesterol, and LDL. Therefore, the purpose of this study is to analyze the potentials of Kersen fruit and

DOI: 10.20961/bioedukasi-uns.v%vi%i.34236

Lakum leaf extract on repairing the lipid profiles of rats hyperlipidemia.

METHODS

Standard feed (BR II), High Fat Diet contain of cornstarch 29.67%, casein 14%, fructose 25%, solid oil 21.4%, alpha-cellulose 5%, mineral mix 3.5%, vitamin mix 1%, methionine 0.18% and choline chloride 0.25%, lipid profile kit from diagnostic system (Diasys) were used during study.

The preparation of Kersen juice

Kersen used in this study has the following characters: the color is red, clean and undamaged. The fruit obtained is washed thoroughly and then smoothed using a blender without water addition. The Kersen juice obtained is filtered to separate the juice from the pulp using the filter.

The preparation of Lakum leaf extract

The leafs obtained were washed thoroughly and dried them in the oven with a temperature of 40 ^{0}C until the water level reached 10%. Dried leaves then were blended using a blender. 500 g of blended leaves were soaked in 1000 cc of ethanol 96% and incubated for three days. The suspension obtained was filtered into the sterile glass and filtrate was filtrated into the bottle using filter paper Whatman No.1 until pasta remained. Solution stock of Lakum leaf extract was made by dissolving 1.2 g of pasta into 6 ml equates.

Studies in animals

This study was carried out on December-May 2019 in Animal Laboratory of Biology, Science and Math Faculty, Diponegoro University. During this study, 20 Wistar rats with 2 months old male, the weight of 200 g were obtained from Animal Laboratory, Semarang State University. Before treatment, the rats were adapted for a week by providing a standard diet BR II and drink ad libithum. Rats were individually caged with a controlled temperature room and 12 h lighting for 8 weeks. Ethical clearance of this study was obtained from the Ethics Committee of Faculty of Medicine, Diponegoro University. Once adapted, the ats were divided into 5 groups consisting of 4 mice. Group I was fed with a standard diet without any treatment; Group II with by High Feed Diet without any treatment; Group III fed with High Feed Died and treated by Kersen juice dosage 0.2 ml/200 g BW; Group IV fed with High Feed Diet and treated by Lakum leaf extract dosage 0.2 ml/200 g BW and Group V fed High feed Diet and treated by simvastatin 0.18 ml/200 g BW. The HFD was provisioned for 30 days. The first blood checking was drawn on the 30th day via tail to check whether the rats have already in hyperlipidemia conditions. The treatment was continued by giving Kersen juice and Lakum leaf extract for 28 days. On the 28th day, blood was drawn from the heart for lipid profile analysis. Analysis of cholesterol, triglycerides, LDL and HDL was conducted with CHOD-PAP method using a diagnostic system kit (Diasys). Analysis of the lipid profile follows the protocols contained in the kit.

Data Analysis

The result of the analysis of the lipid profile was expressed as Mean±SD. The differences between treatments were evaluated by One way Anova test and Duncan test was conducted to determine the significant differences between groups with $p<0,05$. SPSS 16 for windows was used to do the statistic operation. Bodyweight was measured every 3 days, while the leftover of the food was measured daily.

RESULTS AND DISCUSSION

Table 1. Lipid Profile of Wistar Male Rats After Treatment for 28 Day**s**

Variables (mg/dL)	Treatment				
	P0	P1	P2	P3	P4
Cholesterol	62.5^{c} ± 5.92	104.75 ± 19.57^{a}	90^{ab} ± 5.29	80^{bc}± 12.19	68.25^{c} ± 9.53
HDL	27.4^{bc} ± 4.51	23.5^{c} ± 3.7	29.5^{ab} ± 2.38	31.5^{ab} ± 4.79	35^{a} ± 3.46
LDL	27.5^{bc} ± 4.51	35^{a} ± 3.46	29.5^{ab} ± 2.38	31.5^{ab} ± 4.79	23.5^{c} ± 3.70
Triglyceride	64.25^{c} ± 8.42	161.5^{a} ± 12.26	92.75^{b} ± 9.74	88^{b} ± 8.16	64.75^{c} ± 10.44

Based on data in table 1, it was found that it is significantly different in the variable of cholesterol between P0 and P1 ($p<0.05$). The increase of cholesterol in the blood is caused by high feed diet administration. Sudargo et al (2017) said that the normal cholesterol level in white rats is between 47- 88 mg/dL with an average of 65 mg/dL. So based on the resulting cholesterol with average >65 mg /dL said to be in hyperlipidemia condition. Cholesterol level in the control group (P0) is found to be significantly different from the groups that have been given Kersen juice (P2). The ability of Kersen juice to lowering cholesterol is allegedly due to the high content of vitamin C and flavonoids (Sudargo et al., 2017). Flavonoids can decrease cholesterol levels by inhibiting the cholesterol absorption in the intestine and can increase the bile formation reaction to being excreted with the feces (Shattat, 2014). Vitamin C is a water-soluble antioxidant that can prevent oxidation. Vitamin C can

decrease the level of cholesterol through activities of hydroxylase 7α-hydroxylation reactions that increase cholesterol to bile acids changes so as to increase the cholesterol in the blood (Fatimah, 2017). Cholesterol level in groups which have been administered by Lakum leaf extract (P3) is not significantly different from the control group. However, it is significantly different from hyperlipidemia rats without any treatment (P1). Regasa et al (2015) said that Lakum leaf has an antioxidant constituent that can inhibit fat accumulation in the blood. The ability of Lakum leaf to decrease cholesterol level is due to the presence of phenol constituent. Phenol can decrease lipoprotein secretion that can cause a decrease of cholesterol ester which is the main component of chylomicron and VLDL (Xenoulis and Steiner, 2010). Hyperlipidemia rats with simvastatin treatment (P4) have no significant difference with the control group. However, there is a significant difference with P1. Simvastatin is clinically proven to reduce cholesterol levels in the blood (Graveline, 2015). Simvastatin effectively inhibits the HMG Co-A reductase so that it prevents cholesterol synthesis in the liver. This enzyme converts 3-hydroxy, 3 methyls. gluteril-CoA into mevalonic acid as the first precursor in cholesterol formation. The decrease of mevalonic acid synthesis can reduce cholesterol levels in the liver, so does in blood circulation (Gazzerro et al., 2012)

Administration of Kersen juice and extract of Lakum leaf in an increasing level of HDL shows significant differences between the control group (P0) and P1 (Table 1.). However, there is no significant difference between hyperlipidemia rats treated by Kersen juice (P2) and extract Lakum leaf (P3). Compared to P1, there is no significant difference in P2, but the difference is significantly seen in P3. The ability of extract Lakum leaf in increasing HDL levels is due to the presence of flavonoid constituents. Flavonoid increases the production of Apo-A1. Apo-A1 used as a cofactor for LCAT (Lechitine-Cholesterol-Acyltransferase) and as a ligan to interact with the lipoprotein receptor in HDL (Nguyen at al., 2008). Vitamin C also has a role in increasing HDL levels. Vitamin C serves as a laxative to increase fecal disposal and lower the reabsorption of bile which can be converted into cholesterol (Sudargo et al., 2017). Hyperlipidemia rats treated with simvastatin (P4) significantly different from the control group (P0). Simvastatin inhibits cholesterol synthesis in the liver so that SREBP (sterol regulatory element-binding protein) in the membrane is broken down and transported to the nucleus. Transcription factors then bind to the LDL receptor. The increase of LDL receptor in hepatocyte membrane reduces the cholesterol level and effects on the decrease of LDL, VLDL, and IDL level, and also elevate the level of HDL (Tripathi, 2013).

excretion of cholesterol. Increased excretion of cholesterol causes a decrease in the amount of

Administration of Kersen juice and extract of Lakum leaf in decreasing the level of LDL shows a significant difference between the control group (P0) and P1 (table 1.), but no significant difference between P2 and P3 compared to P0 and P1. Although none of the treatments can significantly reduce LDL levels, Kersen juice and Lakum leaf contain flavonoid which is potential in lowering LDL levels. Low-Density Lipoprotein (LDL) is a transport protein that carries triglyceride, cholesterol, and phospholipid from the liver to all tissues (Rajalakshmy, 2011). HFD consumption without consuming antihyperlipid agents automatically increases the cholesterol level in the blood, so does the LDL level (Marques *et al*., 2016). Flavonoids can increase the activity of lipoprotein lipase to inhibit cholesterol synthesis enzyme. This inhibition can increase the formation of the LDL receptor in the liver. Flavonoid also inhibits the synthesis of Apo B-48 and Apo B-100. The decrease of Apo B-48 and Apo B-100 can disturb the formation of chylomicron, VLDL, IDL, and LDL (Dashty, 2014). The use of simvastatin significantly reduces the level of LDL. Statistically, it shows that there is a significant difference between hyperlipidemia rats treated by simvastatin (P4) and control group (P0). Simvastatin inhibits HMG Co-A reductase which converts HMG Co-A into mevalonic acid as the precursor for the cholesterol biosynthesis. The inhibition of cholesterol synthesis in the liver can reduce the LDL level in the plasma. Simvastatin also reduces LDL levels by increasing the number of LDL receptors. When cholesterol catabolism increases, it will lower the level of cholesterol and LDL (Graveline, 2015).

Administration of Kersen juice and extract of Lakum leaf in decreasing triglyceride level shows a significant difference between the control group (P0) and P1 (table 1.). The high level of triglycerides in P1 probably due to the consumption of high feed diets without any antihyperlipid agents. Triglycerides have the highest portion of the diet. When food is being digested, more calories needed by muscle cells. High feed diet consumption can increase the number of fat that will be deposited in adipose tissue. Overconsumption of high feed diet will not be used directly by the body but will be stored in adipose tissue as triglycerides. When the cells need energy, triglycerides will be hydrolyzed into free fatty acid and glycerol. This free fatty acid, later on, will be oxidized to produce energy (Kumar *et al*., 2013). Hyperlipidemia rats treated by Kersen juice (P2) and Lakum leaf extract are significantly different from the control group (P0). This result related to the flavonoid present in both Kersen and Lakum leaf. Flavonoid increases the activity

of lipoprotein lipase and can affect on triglyceride level (Gross, 2004). Phenol is another constituent presents in both kersen and lakum leaf, which have an effect on so that it reduces the level of cholesterol ester (Apro, 2015). Cholesterol ester is the main component of the formation of chylomicron and VLDL. Phenol inhibits the synthesis of Apo B-48 and Apo B-100 in the erythrocyte and liver. This inhibition decreases the formation of chylomicron, VLDL, IDL, and LDL and makes the level of triglycerides decrease (Mehta and Bhatt, 2017). In this study, the rates of body weight and food consumption have been investigated.

Table 2. Rate of body weight and food consumption

Variables (g)	Treatment				
	P0	P1	P2	P3	P4
Food Consumption	15.75^{a} ± 0.5	11.25^{b} ± 0.5	10.5^{b} ± 1.29	10.75^{b} ± 0.5	10.25^{b} ± 1.5
Body Weight	290^{a} ± 15.45	251.5^{b} ± 21.14	222.7^{bc} ± 18.62	226.7^{bc} ± 26.32	208^{c} ± 24.28^{c}

Based on data in table 2, it was found that it is significantly different (P<0,05) between the control group (P0) and the hyperlipidemia group (P1, P2, P3, P4). The highest food consumption rates were found to be a control group which is 15.72 g, whereas the food consumption rate for the hyperlipidemia group is 10.25 g. Statistically, there is no significant difference between hyperlipidemia rats treated by Kersen juice, Lakum leaf extract, and simvastatin.

High Feed Diet administered to the rats consists of 21.4 % lipid and 5% of alpha-cellulose. This composition could a reason that makes the food consumption rates of hyperlipidemia rats lower than the control group. Food consists of fibers that increase the viscosity through the formation of an impermeable gel layer along the gastrointestinal tract. The gel formation by fibers, block the contact between food and digestive tract which can affect the secretion of digestive enzymes and makes nutrients less absorption (Dhingra *et al.*, 2012). The low food consumption rates in hyperlipidemia rats can be assumed that a high feed diet has already contained enough calories needed by the cell to performed. When energy needs have been fulfilled, the breaking down of stored energy from protein and fats no longer needed, as a consequence food consumption rates decrease (Wolfenshon and Lloyd, 2013). High Feed Diet administered to the rats also consists of choline chloride 0.25%. Choline chloride is additive substance that can increase the efficiency of energy use both from carbohydrate and lipid catabolism to produce glucose. Choline acts as a methyl group donor in lipid catabolism to produce energy as well as a decreasing triglycerides level (Ragasa *et al*., 2014). Phenol decreases the secretion of lipoprotein in the liver and intestine, reduce cholesterol esterification process precursor in cholesterol and lipoprotein synthesis (Hesti,*et al*., 2015)

Statistical analysis (P<0,05) on the body weight rates (table 2) showed that it is significantly different between the control group (P0) and hyperlipidemia group (P1, P2, P3, P4). However, there is no significant difference between hyperlipidemia groups, before and after treatments. Based on the data, the control group (P0) has the highest body weight rates which are 286.5 g. Meanwhile, hyperlipidemia groups have lower bodyweight rates. The high feed diet administered to the rats contains 20% of fructose and oil 21,4%. Paz-Filho *et al*, (2012) said that a high amount of energy storage in the cell could stimulate leptin hormone secretion by adipose cells which will send signal to the receptor in the hypothalamus to send "full" signal so that the food consumption decrease. Appetite is regulated by the Leptin hormone. Facey at al (2017) said that high-fat diet consumption decreases more of the leptin hormone level than a high carbohydrate diet. The lower level of the leptin hormone will increase the appetite and the bodyweight will increase as well.

CONCLUSION

Administration of Lakum leaf extract significantly decreases the level of cholesterol, triglycerides and increase HDL level, but not significantly decrease the level of HDL. However, the administration of Kersen juice significantly reduces the level of triglycerides but not significantly decrease the level of cholesterol, and not significantly increase the HDL level. These benefits of Lakum leaf and Kersen juice in increasing HDL and lowering cholesterol levels may become a promising alternative therapy solution for hyperlipidemia patients.
.

ACKNOWLEDGMENT

The Authors thank Magister Biology, Maths and Science Faculty, Diponegoro University for the support given to this study. We thank our lectures who provided insight and expertise that greatly assisted the research. We would also like to show our gratitude to all teams who always be supported and make the research done.

REFERENCES

Apro, J. (2015). Studies on cholesterol and lipoprotein metabolism-emphasis on diabetes and sugar. *Karolinska Instituet*, 1-45.

Batra, S., Batra, N., Nagori, B.P. (2013). Preliminary Phytochemical Studies and Evaluation of Antidiabetic Activity of Roots of Cayratia trifolia (L.) Domin in

Alloxan Induced Diabetic Albino Rats. *Journal of Applied Pharmaceutical Science*, 3 (3), 97-100.

Dhingra, D., Michael, M., Rajput, H., Patil, R.T. (2012). Dietary fiber in foods: a review. *Journal of food science and technology*, 49 (3), 255–266.

Facey, A., Dilworth, L., Irving, R. (2017). A Review of the Leptin Hormone and the Association with Obesity and Diabetes Mellitus. *J Diabetes Metab*, 8, 727.

Fatimah, C. (2017). The Effectiveness of Leaf Extract Cherry (*Muntingia calabura* L.) as lowering cholesterol levels in white rats with simvastatin comparison. *Int J Pharm*,7(3), 10-16.

Gazzerro, P., Proto, M.C., Gangemi, G., Malfitano, A.M., Ciaglia, E., Pisanti, S., Santoro, A., Laezza, C., Bifulco, M. (2012). Pharmacological actions of statins: a critical appraisal in the management of cancer. *Pharmacological Reviews*, 64 (1), 102-146.

Graveline, D. (2015). Adverse Effects of Statin Drugs: a Physician Patient's Perspective. *Journal of American Physicians and Surgeons*, 20(1), 7-11.

Gross, M. (2004). Flavonoids and Cardiovascular Disease. *Pharmaceutical Biology*. 42 (1), 21-35.

Harikumar, K., Abdul Althaf, S., Kishorekumar, B., Ramuanik, M., Suvarna, C.H. (2013). A Review on Hyperlipidemic. *International Journal of Novel Trends in Pharmaceutical Sciences*, 3 (4), 59-71.

Hesti, I.S., Subrata, A., Harjanti, D.W. (2015). Pengaruh penambahan kolin klorida pada pakan terhadap kadar kolesterol dan lipoprotein darah sapi perah laktasi. *Jurnal Ilmu-ilmu Peternakan*, 26 (2), 14-23.

Kakadiya, J. (2009). Causes, Symptoms, Pathophysiology, and Diagnosis of Atherosclerosis-A Review, 3,420-442.

Kementerian Kesehatan RI. Riset Kesehatan Dasar. (2013). Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementrian Kesehatan RI.

Kumar, A., Salam, D., Surya, A.S., Tomy, D.V., Carla, B., Sunil, C. (2013). A review of hyperlipidemia and medicinal plants. *Int.J.A.PS.BMS*, 2(4), 219-237.

Mahmood, N.D., Nasir, N. L. M., Rofiee, M. S., Tohid, S. F. M., Ching, S. M., The, L. K., M. Z., Salleh, Z., Zakaria A. (2014). Muntingia calabura: A review of its traditional uses, chemical properties, and pharmacological observations. *Pharmaceutical Biology*. 52 (12) : 1598-1623.

Marques, C., Meireles, M., Noberto, S., Leite, J., Freitas, J., Pestana, D., Faira, A., Calhau, C. (2016). High-Fat-Diet-Induced obesity rat model: a comparison between Wistar and Sprague-Dawley Rat. *ADIPOCYTE*, 5 (1), 11-21.

Mehta, V., Bhatt, K. (2017). Lipids and its metabolism. *Journal Cardiology and Cardiovascular Therapy*, 4(2), 1-6.

Nguyen, P., Leray, V., Diez, M., Serisier, S., Le Bloc'h, J., Siliart, B., Dumon, H. (2008). Liver lipid metabolism. *Journal of Animal Physiology and Animal Nutrition*, 92, 272-283.

Dashty, M. (2014). A Quick Look at Biochemistry: Lipid Metabolism. *J Diabetes Metab*, 1(5), 1-19.

Nirosha, K., Divya, M., Vamsi, S., Sadiq, M. (2014). A review on hyperlipidemia. *International Journal of Novel Trends in Pharmaceutical Sciences*, 4 (5), 81-92.

Onwe, D.E., Folawiyo, M.A., Anyigor-Ogah, C.S., Umahi, G., Okorocha, A.E., Afoke, A.O. (2015). Hyperlipidemia: Etiology and Possible Control, IOSR. *Journal of Dental and Medical* Sciences, 14 (10), 93-100.

Paz-Filho, G., Mastronardi, C., Wong, M., Licino, J. (2012). Leptin Therapy, insulin sensitivity, and glucose homeostasis. *Indian Journal of Endocrinology and Metabolism*, 3 (16), 549-555.

Preethi, K. 2011. *Studies on antioxidant and pharmacological activities of Muntingia calabura Linn (elaeocarpaceae) fruits*. Tesis. Bharatiar University. India.

Ragasa, C.Y., Buluran, A.I., Mandia, E.H., Shen, C.C. (2014). Chemical constituents of Cayratia trifolia. *Der Pharma Chemica*, 6 (6), 418-422.

Ragasa, C.Y., Tan, M.C., Chiong, I.D., Shen, C.C. 2015. Chemical constituent of *Muntingia calabura*. *Der Pharma Chemica*, 7 (5), 136-141.

Rajalakshmy, I., Pydi, R., Kavimani, S. (2011). Cardioprotective Medicinal Plants – Review. *International Journal of Pharmaceutical Invention*, 1(1), 24-41.

Shattat, G.F. (2014). A review article on hyperlipidemia: types, treatments and new drug targets. *Biomedical and Pharmacology Journal*, 7(2), 399-409.

Sudargo, T., Indwiani, A., Eti, N.S., Rasita, A., Laila, M., Risnukhatulistiwi, M., Anandati, P.Y. 2017. The Effect of Kersen Juice on Lipid Pofile of Sprague Dawley Rats : A Randomized Controlled Trial (RCT). *Asian J. Clin. Nutr*, 9, 97-103.

Tripathi, K.D. (2013). *Essentials of Medical Pharmacology*. Edisi Ketujuh. Jaypee: New Delhi

Wolfensohn, S., Lloyd, M., 2013, *Handbook of Laboratory Animal Management and Welfare*, 4th ed., Wiley-Blackwell, West Sussex, 234.

World Health Organization. (2015). Cardiovascular Disease. Jan (Cited 2017, April 22). Retrieved from: http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(CVD)