Indonesian Journal of Biomedical Sciences Volume 8, Number 1, January-June 2014: 4-7
Print-ISSN: 2085-4773, E-ISSN: 2302-2906.

ANTI-HIPERCHOLESTEROLEMIA OF ANREDERA CORDIFOLIA IN HYPERCHOLESTEROLEMIA RAT WISTAR THROUGH DECREASE OF MALONDIALDEHYDE AND 8-HYDROXY-DIGUANOSINE

Sri Wahjuni

Chemistry Department Faculty of Math and Science Udayana University Bali-Indonesia

ABSTRACT

High cholesterol diet leads to increase of plasma cholesterol and subsequently will end up with hypercholesterolemia. Ignorance of healthy food and lacks of activity are the trend of modern life style. These conditions exacerbated the incidence of hypercholesterolemia in either developed or developing countries. This research aims to determine potential of anredera cordifolia leafs extracts as an anti-hypercholesterolemia by lowering of malondialdehyde (MDA) and 8-hydroxy-diguanosine (8-OHdG) blood levels in hypercholesterolemia Wistar Rat. This is an experimental study applied post only control group design. A number of 15 male wistar rats were used in this study. Rats were randomly divided into 3 groups, i.e. negative control group (C1), positive control group (C2) and anredera cordifolia leaf extract at dose of 100 mg/kg BW group (T). MDA was determined based on malondialdehyde assay (NWLSS^{TM}). 8-OHdG was determined based on oxidative DNA Damage (Cell Biolabs, Inc). The mean difference between control and treatment was assessed by applying ANOVA and consider significant at $p < 0.05$. In this study, we observed that there were a significant of MDA and 8-OHdG levels between group C1 and T; C2 and T within $p < 0.05$. This result indicates anredera cordifolia leafs extract has an ability to inhibit hypercholesterolemia in wistar rat fed with high cholesterol diet.

Keywords: Anredera cordifolia, hypercholesterolemia, MDA, and 8-OHdG.

INTRODUCTION

High cholesterol diet and less activity were becoming a trend in this modern life style. Diet with high protein and carbohydrate and less fiber were also more attractive to this modern age. All of this will results in hypercholesterolemia and will end up with atherosclerosis. Ignorance towards healthy food and exercise were also triggering the incidence of hypercholesterolemia and stimulating many diseases, such as cancer, diabetes, hypertension and coronary heart disease.[1]

Constant of hypercholesterolemia stimulates high oxidative stress and produce high reactive oxygen species (ROS). ROS promotes oxidation of membrane cells producing an aldehyde known as Malondialdehyde (MDA). This is the end product of lipid peroxydation and could in the form of free compound or bind to the tissue. In addition, MDA could be regarded as a decomposition of amino acids, complexes carbohydrate, pentose and hexose. High plasma MDA can be used as a measure of increase free radical activity and decrease of superoxide dismutase (SOD).

Correspondence: Sri Wahjuni
Address: Chemistry Department, Faculty of Math and Science, Udayana University, Bali-Indonesia
Email: wahjunimanuaba@gmail.com

Antioxidant and oxidant were balanced in the body. Disturbing this balancing leads to decrease of antioxidant and stimulating oxidative stress.[2] Continues of oxidative stress will also trigger DNA damage. The DNA damage produces 8-OHdG that can be detected by applying DNA damage ELISA kit.[3] To kip oxidant and anti-oxidant balanced in the body, the body itself has already producing antioxidant known as endogenous antioxidant. In some cases, an antioxidant could also be obtained from outside the body, such antioxidant known as exogenous antioxidant.

Anredera cordifolia leaf is one of the source of exogenous antioxidant. This plant was initially from Southern America. China and Korea have take advantages of this leaf since thousand year ago for curing many diseases.[4] The chemicals content of this plant are saponin, alkaloid, polifenol, flavonoid and polysacaride, including L-arabinosa, D-galaktosa and L-rhamnosa.[5] In addition, this plant has also activity as an antibacterial similar to antibiotic of penisilin and tetrasiklin with a wide target spectra. This leaf has also antioxidant activity and anti-inflammation.[6]

This study aims to determine the potential activity of Anredera cordifolia leaf as an anti-hypercholesterolemia in hypercholesterolemia rat wistar through decrease of MDA and 8-OHdG blood levels.

METHODE

This is an experimental study with posttest only control group design. A number of 15 wistar rats were used in this study. Those rats were grouped into three groups, i.e. negative control group (C1), positive control group (C2), and Anredera cordifolia leaf extract at a dose of 100 mg/kg BW (T). In C1 group, rats were fed with normal diet, in C2 group rats were fed with a mixture of normal and high cholesterol diet. For treatment group, rats were fed with mixture of normal and high cholesterol diet and treated with Anredera cordifolia leaf extract at a dose of 100 mg/Kg BW. Normal diet for rat was purchased from poultry store with the name of pellet 511. High cholesterol diet was made by a mixture of 18 % fatty fork, 30 % quail eggs and 52 % of pellet 511. Treatment in all groups was carried out during 35 d. Wistar rats were provided by UPT. Laboratorium Analitik Universitas Udayana, Bali-Indonesia.

MDA was assayed by applying NWLSS™ MDA assay based on reaction of MDA with thiobarbituric acid (TBA) forming an MDA-TBA adduct that absorbs strongly at 532 nm. Assay was carried out corresponding to assay procedure as indicate in the manual kit. MDA assay kit was purchased from Northwest Life Science Specialties, LLC.

8-OHdG was assayed using OxiSelect™ Oxidative DNA Damage ELISA Kit. Assay was carried out based on manual on the Kit.

Extract of Anredera cordifolia leaf was obtained through maceration of the leaf using ethanol. Filtration and vaporization of the crude extract were carried out to gain the leaf extract.

Data were analyzed statistically by applying SPSS for windows. ANOVA was performed to obtain the mean different of MDA and 8-OHdG in all groups. Results were considered significant at $p < 0.05$.

RESULTS

In this study GC-MS was also performed to obtain rough data of chemical content in Anredera cordifolia leaf extract. The chromatogram of GC-MS was presented in Figure 1.

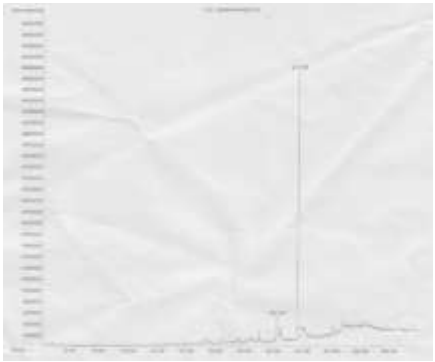

Figure 1
Chromatogram of Anredera cordifolia leaf extract

Two peaks were observed from the GC-MS chromatogram of Anredera cordifolia leaf extract. The retention time of these two peaks were listed in Table 1. Based on bank data of the GC-MS these two peaks were identified as two compounds as indicates on the same table.

Table 1
Retention Time, Percent Area and Proposed Compounds Observed of Anredera cordifolia leaf extract

Peaks number	Retention time (t_R)	% Area of the peak	Proposed compound
1	21.66 minute	73.82	Phytol
2	20.22 minute	26.18	n-hexadecanoic acid

Data of rat body weight, MDA and 8-OHdG were listed in Table 2.

Table 2
Characteristic of Rat Observed

Parameter	Groups of Wistar Rat		
	C1	C2	T
Body weight (g)	250.35±1.06	259.44±1.76	255.64±1.88
MDA (ng/mL)	1.71±0.51	2.69±0.88	1.66±0.72
8-OHdG (μg/mL)	0.32±0.05	0.81±0.03	0.34±0.04

C1 = control negative (rat fed only with normal diet)
C2 = control positive (rat fed with high cholesterol diet)
T = treatment (rat fed with high cholesterol and Anredera cordifolia leaf extract at a dose of 100 mg/bw.

All MDA and 8-OHdG data obtained were normally distributed ($p > 0.05$) and their variances were also homogenous ($p > 0.05$). Based on ANOVA analysis data, we obtained that there are significant means different of MDA between control negative group (C1) and control positive group (C2) within $p < 0.05$. There was also a significant of MDA means different between C2 and treatment group, however, no different observed between C1 and treatment group. A similar trend was also observed for 8-OHdG data.

DISCUSSION

Regardless of Figure 1, it can be seen that there were two peaks observed from GC-MS of Anredera cordifolia leaf extract. Based on GC-MS data, there were two main compounds identified, i.e. phytol and n-hexadekanoic acid. The n-hexadekanoic molecule has a similar structure to tetrametil-2-hexadekanoic. This compound has a similar number of C atoms, 20 C atoms, therefore, is a little bit difficult to obtain its

fragmentation. On the other hand, phytol compound with a formula of ($C_{20}H_{40}O$) is a diterpen alcohol and function as a vitamin E and K precursor in animal that can be converted further to phytanic acid and can be found on animal fatty tissue.[7]

In this study, it was obtained that Anredera cordifolia leaf extract has an ability to inhibit hypercholesterolemia in Wistar Rat fed with high cholesterol diet. Compare to negative control group in which no hypercholesterolemia occurred as indicates by MDA levels of 1.71±0.51 ng/mL. This value is comparable to the MDA levels for treatment group. This is probably due to antioxidant properties of the leaf extract that muffle the effect of free radical during the high cholesterol diet on Wistar Rat.

In term of cell damage due to constant high choleterol diet without the leaf extract will induce the demage, however, the leaf extract was potent to inhibit hypercholesterolemia. This indicates by the marker of cell damage, 8-OHdG which was similar to wistar rat that fed with normal diet.

As reported by Packer et al (1999), that antioxidant system in the body protects towards free radical were inter connected each others to attempt balancing of oxidation reaction. Input of antioxidant exogen from Anredera cordifolia leaf extract will protect reaction to free radical, therefore, no further lipid peroxidation occurred and that will decrease the formation of MDA.[7]

Fatty tissue membrane lipid bilayer consists of adipose cells regulate lipid metabolism and store triglyceride for energy reserve sources. In addition, this fatty tissue has also a role in liver, adipose and extra hepatic tissue metabolism regulation.

Trigliceride was synthezised from asil-CoA activated from fatty acids. These fatty acids derive fro fatty acid in triglyceride synthezised in liver transported in VLDL (Lipoprotein) and from lipogenesis in fatty tissue that come from glikolisis. Therefore, there are always two path ways contribute in this condition. The first path way is lipolisis resulted in fatty acid and gliserol. This fatty acid activated and re-esterified to form triglyceride. Gliserol as a results of lipolisis could not be used appropriately in lack of gliserokinase. The second path way is esterification due to too much triglyceride accumulated in fatty tissue that leads to obesity. In some cases, lipolisis is higher then easterification that will lead to fatty acids accumulated on blood fatty tissue. Accumulation of fatty acid on blood will be transported as a complex of fatty acid and albumin. This complex compound will affect metabolism process in every tissue.[8]

Compound of n-hexadecanoic acid is a saturated fatty acid similar to palmitic acid, therefore, it is not affecting hypercholesterolemia process due to not affecting the lipolisis process.[9] On the other hand, phytol content in Anredera cordifolia leaf extract has an activity as a hypocholesterolimea that decrease LDL cholesterol.[10-12] However, the mechanism of this activity has not been well understood. It is probably due to cholesterol micellisation inhibition by the leaf extract during degestion in small intestine. Therefore, this will decrease the cholesterol occurent to be absorbed into enterocyte cells. Anredera cordifolia leaf extract was probably also inhibited cholesterol absorbtion from micel and inhibit reabsorbtion of bile acid and synthesis cholesterol. This is as a results of saponin interaction to bile cid that form a big mixture of micel that could not be absorbed to small intestine and to be excreted through feces.[10-12]

Inhibition of bile acid reabsorbtion from small intestine trigering cholesterol metabolism on liver and converted to bile acid. Penghambatan penyerapan kembali asam empedu dari usus memacu metabolisme kolesterol pada hati kemudian mengkonversinya menjadi asam empedu.[13]

In general, phytol inhibits cholesterol absorption in either direct or indirect ways. Direct inhibition absorption inhibition occurs in small intestine and indirect absorption take places through inhibition of re-absorption of bile acid through enterohepatic circulation.[11-12]

CONCLUSION

In this study we obtained that Anredera cordifolia leaf extract potent as an anti-hypercholesterolemia in hypercholesterolemia wistar rat. This ability as an anti-hypercholesterolemia was determined on the basis of decrease MDA and 8-OHdG blood levels of the rats. The leaf extract was found to have a phytol compound that was believed play a role in the inhibition of hypercholesterolemia on rats.

AKNOWLEDGMENT

The author would like to thanks Prof. I. B. Putra Manuaba for aids and access to UPT Laboratorium Analitik Udayana University for MDA and 8-OHdG assays.

REFERENCES

1. Kopin, L. and Lowesntein C. 2010. *In The Dyslipidemia*. American College of physicians.
2. Konig, D., and Berg, A. 2002. *Exercise and Oxidative Stress: is there a need for additional antioxidant*. Osterreichisches J Fur Sportmedizin 3: 6-15.
3. Braunwald, E. 2005. *Approach to the Patient With Cardiovascular Disease. In :Kasper D.L, Longo, S.L, Hauser (editors*). *Harrison's Principles InternalMedicine.*Volume 2. 16 th Ed. New York, McGrawHill Medical Publishing Division, Hal. 208, 1301-4
4. Manoi F. 2009. *Binahong (Anredera cordifolia) Sebagai Obat Penelitian dan Pengembangan Tanaman Industri. Badan Penelitian dan*

Pengembangan Pertanian. Bulletin Warta Volume 15, Number 1, April 2009. Pusat Penelitian dan Pengembangan Perkebunan, Indonesia.

5. Rachmawati S. 2008. *Study Macroscopic, dan Skrining Fitokimia Daun Anredera cordifolia (Ten) Steenis[skripsi].* Airlangga University. Indonesia.
6. Hammond G.B. 2006*. In Vivo Wound-Healing Activity of Oleanolic Acid Derived from the Acid Hydrolysis of Anredera diffuse*. The Guardian, America.
7. Packer L, Colman C. 1999. *The antioxidant miracle : your complete plan for total health and healing.* John Wiley & Sons, Inc. New York.
8. Sheyla Leite Matos, Heberth de Paula, Maria Lúcia Pedrosa, Rinaldo Cardoso dos Santos, Eduardo Luiz de Oliveira, Deoclécio Alves Chianca Júnior, and Marcelo Eustáquio Silva. 2005. Dietary models for inducing hypercholesterolemia in rats. Braz. arch. biol. technol. vol.48 no.2 Curitiba.
9. Sarkas Mohammad*.* 2000. *Chemical Constitutions and Hemolitic Activity of Macrotyloma Uniflorum Lin*. International Journal of National and Engineering Science 3(1): 69 – 72., Bangladesh.
10. Zhao, H.L., Sim, J-S., Shim, S.H., Ha, Y.W., Kang, S.S. and Kim, Y.S. 2005. Antiobese and hypolipidemic effects of platycodin saponins in diet-induced obese rats; evidences for lipase inhibition and calorie intake restriction. International Journal of obesity,29: 983-90.
11. Lee Sun-Ok, Simon A.L., Murphy P.A., and Hendrich S. 2005. *Soyasaponin Lowered Plasma cholesterol and increased Fecal bile Acids in Female Golden Syrian hamsters.* Experimental Biology and Medicine, 230:472-78.
12. Lakshmi, V., Mahdi, A.A., agarwal, S. K . and Khanna, A. K. 2012. Steroidal saponin from Chlorophytum nimonii (Grah) with lipid-lowering and antioxidant activity. Original article, 3:227-32.
13. Shin, D.H., Heo, H. J. Lee, Y. J. and Kim, H. K. 2004. Amaranthsqualene is reduced serum and liver lipid levels in been fed acholesterol diet," British Journal of Biomedical Science, 61: 11–14.
14. Kristanti, Novi A. 2008. *Buku Ajar Fitokimia.* Laboratorium Kimia Organik Jurusan Kimia, Fakultas MIPA Universitas Airlangga. Surabaya
15. Ngatijan. 2006. *Metode Laboratorium dalam Toksikologi*. Bagian farmakologi dan toksikologi fakultas kedokteran UGM, Yogyakarta
16. Nurul. 2007. *Uji Aktivitas antibakteri Ekstrak air daun Binahong (Anredera scandens(L) Mor) terhadap bakteri Staphylococcus aureus and Escherichia coli beserta Skrining Fitokimia dengan uji tabung*. Skripsi Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta
17. Annisa. 2007. *Uji Aktivitas antibakteri Ekstrak air daun Binahong (Anredera scandens(L) Mor) terhadap bakteri Klebsiella pnemonie dan Bacillus subtilis ATCC6633 beserta Skrining Fitokimia dengan Uji tabung*. Skripsi Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta