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The Future of Medicinal Plants: From Plant to Medicine

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WIDYA MANDALA CATHOLIC UNIVERSITY

in collaboration with
National Working Group on Indonesian Medicinal Plants
and German Academic exchange Service
PREFACE

Earth is perfectly made by God for His people to live. It consists of different bodies of land and water where thousands of species of plants and animals can be found. The human race is called to explore this order, to examine it with due care and to make use of it for the benefits of human being. Since very early in human history, people have relied on medicinal plants to cure them of their various ills. This can be partly attributed to the simple yet highly effective forms of traditional medicine. Knowledge of medicinal plants is a part of the Indonesian national heritage known as jamu. To facilitate networking, collaboration, exchange of information, experiences and and knowledge in the key issues of medicinal plants development, the Faculty of Pharmacy of Widya Mandala Catholic University Surabaya in collaboration with National Working Group on Indonesian Medicinal Plants (POKJANAS TOI) and German Academic Exchange Service (DAAD) held the International Conference on Medicinal Plants on 21-22 July 2010 in Surabaya. The conference provided a evaluation in pharmacology, pharmacognosy, ethnobotany, standardization, cultivation, cell culture and chemistry for medicinal and aromatic plant species. There were over 250 participants, 8 plenary speakers, 101 contributed speakers in oral presentation, and 101 posters presented.

The papers contained in the first volume of the proceeding report the submitted papers on ‘The Future of Medicinal Plants: From Plant to Medicine’. Keynote speakers and authors of selected contributed oral and poster presentations were given the opportunity to submit a manuscript for publication.

The conference organizers gratefully acknowledge the financial and other support from the following:

National Working Group on Indonesian Medicinal Plants (POKJANAS TOI)
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I hope that this publication will raise international awareness of the value of medicinal plants in Indonesia and hence makes a contribution towards promoting the proper use of medicinal plants.

Dr.phil.nat. Elisabeth Catherina Widjajakusuma
Conference Chairman
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ANALYSIS OF PLASMA MALONDIALDEHIDE (MDA) CONCENTRATION BY GIVING SHALLOT’S EXTORTION WATER (Allium ascalonicum L.) ON HYPERCHOLESTEROLEMIC MICE

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Abstract : Malondialdehyde, a product of lipid peroxidation, was often used as an indicator to detect oxidative stress damage in the body. Lipid peroxidation is associated with progression of atherosclerosis. It had been known that onion (Allium ascalonicum L.) can be used as an antioxidant and hypolipidemic agent, but whether onion can decrease MDA plasma in hypercholesterolemia mice induced by yolk is still unknown. The aim of this research was to investigate effect of onion’s extortion water on hypercholesterolemia mice MDA plasma level. This was an experimental laboratory research with post test only design. The measured parameters were cholesterol and MDA level on tested groups. Groups were divided into yolks group; yolks group plus addition of 80% shallot extortion water, simvastatin and aquadest groups were separately used as positive and negative controls. The result showed that there was significant difference between high fat feed group which added 80% onion’s extortion water and high fat feed group only. Addition of 80% onion’s extortion water in high fat feed groups has decreased MDA plasma level equal to normal feed group. As the conclusion, this research proves that onion’s extortion water has an effect to decrease mice MDA plasma level by using 80% onion at the best.

Keywords : malondialdehyde, onion (Allium ascalonicum L.), cholesterol

INTRODUCTION

Indonesia’s predominant case of mortality has shifted from infectious diseases to degenerative diseases, named circulation system diseases. These consist of heart and circulatory diseases. Based on ICD X, there are more than 10 diseases in circulation system. Some of them are chronic rheumatic heart disease, ischemic heart disease and cerebral blood vessel disease (Jamal, 2004).

Circulation system diseases have several risk factors such as; smoking habit, diabetes mellitus, hypercholesterolemia, hypertension and obesity. Over past decades, there are indicators of increasing risk factors prevalence. In 1993-1998, MONICA Survey reported the prevalence of hypercholesterolemia have been increased from 13.6% to 16.5% in men and from 16% to 17% in women. Obesity has been increased from 2.3% to 3.7% in men and from 16% to 17% in women (Jamal, 2004).

Most common cause of circulation system diseases was generated from atherosclerosis effects. The process have been began in childhood and continued as clinical manifestations in middle ages and elderly. It mainly hit medium-sized arteries, coronary artery, carotid, basilar, vertebral, iliac, femoral and so forth. Large arteries, such as aortic, usually have aneurism as a complication. Commonly, the most heavily affected is coronary artery (Pratanu, 1995).

There are several theories about pathogenesis of atherosclerosis. The most popular is aterogenesis can be stimulated from arterial endothelial damage. It can caused by mechanical, viral infection (herpes virus and citomegal), and increasing of specific
metabolites substances, such as glucose and cholesterol (Pratanu, 1995; Halliwell, 1999). Research on experimental animal showed that given high–cholesterol diet would lead to atherosclerosis (Wissler, 1990).

Many research present that oxidative stress play a rule in the occurrence of atherosclerosis (Stocker, 2004). Oxidative stress was caused by imbalance of free radical and antioxidants. Free radicals were defined as atoms or molecules which has un-paired electron in outermost layer and has high capability to react with surrounding molecules (Halliwell, 1999). Oxidized LDL will be phagocitized by macrophages to form foam cells. Phagocytosis process by neutrophil cells, monocytes and limfosit also produce free radical that would increase the condition of oxidative stress (Murray, 2000; Sargowo, 1997).

If free radicals attack, the lipid might be oxidized and formed lipid peroxidation. Lipid peroxidations proceed through several stages, which called as initiation, propagation and termination (Thomas, 1998). End of these reactions is transformation of the fatty acid chain into—various compounds that are toxic to cells, such as malondialdehyde (MDA), 9-hydroxy-nonenal and various hydrocarbons such as ethane and pentane (Sukmawati, 2005). Cholesterol lowering drug, named simvastatin, can decrease plasma MDA concentration of mice proportional to increasing doses (Sadikin, 2003).

Research on experimental animal showed that antioxidants can inhibit the occurrence of atherosclerosis (Mustafa, 2005). Therefore, research on material that contain antioxidants and its effect in preventing atherosclerosis needs to be done. One of the natural ingredients that contain antioxidants is onion. Antioxidants ability of onion can be seen from reduced number of liver lipid peroxidation of mice protected by onion before given CCl₄ and improved number of hepatic glutathione (Harahap, 1995). Besides its function as antioxidant—result of research show that onion can decrease the amount of cholesterol of rabbits which were feed with sucrose in significant amounts (Sadikin, 2005).

Until now there has been no research on the antioxidant effects of onion to experimental animals in hypercholesterolemia condition. So, this research addressed to investigate the antioxidant effect of onion by onion extortion were given to mice with high-cholesterol diet fed and then analyzed plasma MDA concentration of mice.

METHODS AND MATERIALS
This was experimental research using post test only with control design. This study used 30 male white mice with approximately 4 months age. All of these experimental animals were randomly divided into six groups (each group consisted of five mice). The groups are:
P1: Group of mice were given normal diet fed + drinkable water as negative control
P2: Group of mice were given egg yolk in addition to normal fed + drinkable water
P3: Group of mice were given egg yolk in addition to normal fed + onion extortion with 80% concentration.
P4: Group of mice were given egg yolk in addition to normal fed + simvastatin at 0.0026 mg/g BB which had been converted from human dose as positive control, in the form of solutions in distilled water as much as 1 ml per oral.
Drinkable water as negative control, egg yolk, onion extortion, and simvastatin are given through gastric sonde. Type of egg yolk is raw egg yolk that derived from races chicken in 1 ml dose. Egg yolk were carried out everyday for 5 weeks, in condition three weeks before onion extortion fed and two weeks with onion extortion fed. Onion extortion obtained from Bima Brebes Onion variety an average age 2-2.5month with 80% of concentration. Onion extortion fed done for two weeks period and at the same time also given 1 ml egg yolk (1:10 dillution). But, both of fed has interval time in given approximately 1 hour. Simvastatin is given in the form of tablet dissolved with distilled water.
water with a dose at 0.0026 mg/g BB which had been converted from human doses. Simvastatin conducted for two weeks period and at the same time also given 1 ml egg yolk (1:10 dilution). But, both of fed has interval time in given approximately 1 hour. At the appointed time, mice were ethically sacrificed and blood were taken from heart. Total cholesterol measurement made in accordance Enzimatic Colorimeter Method CHOD PAP Test. MDA measurement was based on Wills method.

RESULT AND DISCUSSION
Cholesterol Concentration
Plasma cholesterol concentration of each group (P1, P2, P3, P4) can be seen in Table 1. The data presented that P2 has higher cholesterol concentration than P1. Test statistical (Table 2) showed significant difference (p <0.005). This test showed that addition of egg yolk to the mice treated on 5 weeks will increase total cholesterol level of mice plasma with an average value of 149.16 mg/dL. The same research result was also obtained in studies conducted by by Anggraini CD that proved egg yolk given at 6.25 gr/kgBB/hari for 28 days period to white mice can cause hypercholesterolemia with cholesterol levels on average 134.83 mg/dL. This result test supported by theory which state egg yolks can raise levels of total cholesterol in the blood because the fat content contained in. The mechanism is the egg yolk is a source of exogenous cholesterol in the body that can increase level of total cholesterol in the blood (Anggraeni, 2009).

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<td>59.72</td>
<td>151.38</td>
<td>61.72</td>
<td>35.80</td>
</tr>
<tr>
<td>4</td>
<td>59.72</td>
<td>155.55</td>
<td>62.96</td>
<td>38.27</td>
</tr>
<tr>
<td>5</td>
<td>56.94</td>
<td>156.94</td>
<td>74.07</td>
<td>33.33</td>
</tr>
<tr>
<td>Total</td>
<td>308.32</td>
<td>745.8</td>
<td>346.88</td>
<td>185.18</td>
</tr>
<tr>
<td>Mean</td>
<td>61.66</td>
<td>149.16*</td>
<td>69.38**</td>
<td>37.04***</td>
</tr>
</tbody>
</table>

Plasma MDA Concentration
Plasma MDA concentration of each group (P1, P2, P3, P4) can be seen in Table 2. The data presented that P2 show the increase of MDA concentration compared to P1.
Statistical test (Table 2) showed significant difference (p<0.05). Thus, egg yolk can increase the MDA’s concentration. This proves that the addition of egg yolk to mice treated on 5 weeks can cause the increasing of MDA level with an average rate 0.1224 nmol/mL. The increasing of concentration MDA’s plasma occurs through LDL oxidation caused by hypercholesterolemia condition in giving high-diet cholesterol. High-fat diet can increase blood triglyceride levels and lipid peroxidations, decrease of thiols levels (Wilson 2007).

<table>
<thead>
<tr>
<th>MICE GROUPS</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.039</td>
<td>0.283</td>
<td>0.048</td>
<td>0.033</td>
</tr>
<tr>
<td>2</td>
<td>0.042</td>
<td>0.031</td>
<td>0.059</td>
<td>0.009</td>
</tr>
<tr>
<td>3</td>
<td>0.037</td>
<td>0.145</td>
<td>0.047</td>
<td>0.036</td>
</tr>
<tr>
<td>4</td>
<td>0.044</td>
<td>0.063</td>
<td>0.024</td>
<td>0.027</td>
</tr>
<tr>
<td>5</td>
<td>0.028</td>
<td>0.089</td>
<td>0.053</td>
<td>0.015</td>
</tr>
<tr>
<td>Total</td>
<td>0.190</td>
<td>0.612</td>
<td>0.231</td>
<td>0.120</td>
</tr>
<tr>
<td>Mean</td>
<td>0.038</td>
<td>0.122*</td>
<td>0.046**</td>
<td>0.024***</td>
</tr>
</tbody>
</table>

*p<0.05 vs P1; **p<0.05 vs P2; ***p>0.05 vs P3.

The oxidation process initiated when free radical attack hydrogen atom PUFA in LDL. Catalyzed by Fe$^{2+}$, peroxide sulfide formed radical hydroxyl which can generate chain reaction. Finally, fat degradation occurred and formed a variety of product, such as MDA, ethane and pentane. MDA appears in the blood and urine. Its can use as an indicator of free radical damages (Widowati, 2007). P3 presented significant decrease of plasma MDA compared to P2 (Table 2). Based on result test, onion extortion is significant can decrease concentration of mice plasma MDA. It can be related with antioxidant activity in onion, defined quersetin which include in flavonoid classification (Stajner, 2003; O’Reilly, 2001). Another research found that quersetin can decrease plasma concentration on gastric homogenates in condition ulcers suffering by etanol induced effect in mice (Coskun, 2004).

Quersetin works with by breaking perhidrosil radical chain, eliminating metal ions reactive power and capture reactive oxigen compounds directly, such as OH, ONOOH and HOCL. In-vitro experimental presented that radical quersetin can reduced back into quersetin by vitamin C (Halliwell, 1999). Onion has both of two component, named quersetin and vitamin C, therefore can increase antioxidant activity.

Onion also has another component which can inhibit lipid peroxidation, named sulfur. Onion has high antioxidant activity if compared to yellow onion and Welsh onion (Stajner, 2003). Flavonoid compounds where contained in onion also stable during boiling process, so this process does not reduce the amount of flavonoids (Aoyama, 2007).

The relationship between plasma MDA concentration and Mice plasma cholesterol concentration

Several researchs presented that free radical play a role in occurrence of atherosclerosis. Cholesterol carried in lipoprotein of plasma and the largest proportion of cholesterol contained in LDL (Murray, 2004). Lipids, which contained in LDL lipoprotein susceptible to free radical. Oxidized LDL will be photogocitized by macrophages to form foam cells. Foam cell is an early sign of atherosclerosis. Research on mice DM show that MDA can use as predictor of the occurrence of atherosclerosis (Musthafa, 2000).

On this research, decreasing of MDA plasma concentration is related with decreasing of mice plasma cholesterol concentration. Pearson Correlation test between
MDA plasma concentration and mice plasma cholesterol concentration show very strength positive correlation with coefficient 0.625. Although the study was not conducted measuring LDL concentration, but decreased the total cholesterol concentration has strong possibility that will reduce the concentration of LDL, so that oxidation of LDL decrease and MDA level decreased. The result of this study also support other research which found that giving simvastatin also reduce MDA concentration and its reduce similar with increasing simvastatin doses. Therefore, this research proves that onion can reduce MDA concentration in mice by hypercholesterolemia induced.

CONCLUSION
Extortion onion with 80% concentration can decrease mice plasma MDA level by hypercholesterolemia induced. There is strong positive correlation between plasma MDA concentration and plasma cholesterol concentration.

ACKNOWLEDGMENTS
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REFERENCES


