**RENOPROTECTIVEEFFECTS OF LYCOPENE IN TOMATO EXTRACTS ON RAT EXPOSED TO CADMIUM**

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Cadmium is a heavy metal widely used in human life. If it enters the body cadmium will bind to metallothionein protein and may impair renal function. The decrease of renal function usually is characterized by an increase of β2-microglobulin, creatinine, ureum and uric acid levels Which possibly might be cured by lycopene, and antioxidant found in tomato. The objectives of the study wastodetermine the effective lycopene dose as renoprotective of Cd exposure. Twenty four Wistar rats were used in this study. They were divided into six groups, with four individuals in each group. Group I was the negative control, Group II was given 5.6 mg/kgBW CdSO4 as a positive control. Group III, IV and V were given 5.6 mg/kgBW CdSO4 and tomato extract at 0.36, 0.72 and 1.08 mg/kgBW/day respectively. Group VI was given CdSO4 and after seven days an additional 0.3mg/kgBW/day of dimercaprol was administered for 14 days as a comparison to tomato. The parameters observed were levels of Cd, creatinine and β2-microglobulin in blood level. Observations were made on day 0 and day 22.The data were analyzed by Anova (F test) followed by Duncan test The results showed all treatments and dimercaprol could reduce the levels of Cd, creatinine, ureum, uric acid and β2-Microglobulin. The optimal decrease was observed in the rats administered with 1.08 mg/kgBW/day. Tomato extract dose 1.08 mg / kgBB is the highest in lowering levels of blood Cd ,β2-M, creatinine, urea and uric acid levels

Keywords: lycopene, cadmium, creatinine, β2-Microglobulin.

**INTRODUCTION**

Industrial waste containing Cd which can pollute the atmosphere, soil and water. Cadmium is a persistent compound in the environment with a half-life of 30-40 years (Satarug *et al*., 2010; Jaques *et al*., 2010), so exposure to both acute and chronic Cd are very harmful to human health, especially to the kidney organ, which is the main target of Cd. Cadmium is also one of the causes of hypertension and human heart disease (atherosclerotic heart disease) (Gallagher and Meliker ,2010.,Messner and Bernhard, 2010; Caciari*et al*., 2013). Cd poisoning occurred in Japan namely Itai-itai disease caused by water pollution in Kumamoto. Cd poisoning in Indonesia occured in Jakarta Bay, respondent who lived in Kaliadem that consume green mussels had a high risk for Cd exposure (RQ>1) with hypertension symptom (Safitri, 2015).The cadmium that enters the body will bind to the metallotioenin protein ( Cho*et al*., 2010; Chen *et al*., 2015). The bonding of Cd and metalotionein is stable and can lead to increased free radicals in the liver and kidneys, resulting in oxidative stress, characterized by decreased of superoxide dismutase (SOD) and Gluthation Peroxidase (GPx), result in decrease of renal function (Hijova*et al*., 2004; Caciari*et al*., 2013). Prevention of Cd toxicity can only be done with antioxidant supplement such as vitamin C, Vitamin E and Selenium. The function of antioxidants is to reduce the absorption of Cd by the kidneys and help eliminate Cd out of the body without damaging the kidneys (Agarwal and Rao., 2000). But antioxidants in the form of food supplements are expensive and can only be obtained by the upper middle class society. Usually treatment of Cd toxicity used a chemical chelation compound namely dimercaprol but it was not recommended using this chemical because it can damage the kidneys and cause hypertension (Caciari *et al*.,2013). To overcome the toxicity of Cd in the body it is necessary to find an alternative by using natural antioxidants that are easily available and cheaper such as tomatoes. Tomato contains an active compound called lycopene. Lycopene is a potential antioxidant because it can reduce the free radical compounds that enter the body (Agarwal and Rao, 2000). Research purpose (1) To know renoprotective effect of lycopene on rat exposed by Cd with measuring β2-M level; (2) To know renoprotective effect of lycopene on rat exposed by Cd with measuring renal fuction creatinin, urea and uric acid level; (3) To know the effective dose of lycopene as renoprotective on rat exposed by Cd in terms of decreasing β2-M, creatinine, urea and uric acid level.

**MATERIAL AND RESEARCH METHODS**

The research material were white male rats Wistar strain, age 2-3 months with weight of 200-220 g, from LPPT IV UGM. Golden tomato from fruit center Kutabawa. AD II feed, CdSO4, commercial kit creatinin, β-2M, urea and uric acid.

**Research Procedure**

A kind of tomato that used in this research is golden jubille or golden tomat. This extract used to be chelating agent for Cd poisoning. To make ethanol tomato extract by Maceration method used 5 kg raw tomato that dissolved with ethanol 96% as a solvent for 3x24 hours (Anonymous,1986). To get thick extract, macerate evaporated by vaccum rotary evaporator. Exctract of tomato produced as much as 0.5 g Administration of tomato extract is 14 days after rat treated by CdSO4. Wistar rats used in these treatment which given AD II pellet for feeding and aquadest for drinking ad libitum. Twenty four were divided into six groups, with four individuals in each group. C1 was the negative control as healthy rat without treated by CdSO4 and tomato extract. C2 was given only 5.6 mg/kgBW CdSO4 for 14 days as a positive control. C3, C4,C5 were given 5.6 mg/kgBW CdSO4 for 7days and given tomato extract at a dose 0.36, 0.72 and 1.08 mg/kgBW/day for 14 days respectively. C6 was given CdSO4 for 7 days and dimercaprol for 14 days at a dose 0.3mg/kgBW. Dose 0.36 mg / kgBW equivalent to lycopene dose 15 mg / kgBW in humans, dose 0.72 mg / kgBB equivalent to 30 mg / kgBW dose in humans and dose 1.08 mg / kgBW equivalent to 45 mg / kgBW. Blood collection was done on day 0 and 15nd .Blood is taken with a hematocrit capillary pipette on the vein orbitalis plexus of rat. Then the blood is collected on Eppendorf tube as much as 3 ml. Amount of 3 ml whole blood is divided to 2 part, 0.5 mL for blood Cd and 2.5 mL for creatinine, uric acid, urea and β2-M. Further blood 2.5 mL, centrifuge for 10 minutes with a speed of 4,000 rpm. Cadmium is measured by AAS machine at 228.6 nm wavelength and a strong current of 3.5mA0. Creatinine level was examined by Jaffe kinetic method and read at spectrophotometer with wavelength 492 nm. Urea and uric acid measured by Dyasis method with wavelength 546 nm. Data of Cd, creatinine, uric acid, urea and β2-M levels were analyzed by Anova test, followed by Duncan test, to find out where the differences in each treatment are.

**RESULT AND DISCUSSION**

The result of parameters cadmium, creatinine,β 2M, urea and uric acid after administrated by lycopene were shown in Table 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Cadmium (ppm) | Creatinin (mg/dL) | β 2M (mg/dL) | Urea (mg/dL) | Uric acid (mg/dL) |
| C1 | 0.2 ± 0.08 a | 0.61±0.09a | 96.21±16.98a | 13.5±1.62a | 2.63±0.12a |
| C2 | 2.08 ± 0.17b | 1.23±0.16b | 188.15±17.91b | 45.38±2.97b | 6.17±0.56b |
| C3 | 1.36±0.04c | 1.00±0.20c | 140.38±28.07c | 28.75±2.01c | 4.67±0.36c |
| C4 | 1.18±0.36c | 0.78±0.11a | 111.05±10.04d | 17.50±1.01d | 2.92±0.20a |
| C5 | 0.84±0.05d | 0.69±0.08a | 84.58±14.48a | 19.70±1.05d | 3.05±0.19d |
| C6 | 1.14±0.40c | 0.82±0.05a | 113.6±15.22d | 22.32±1.15d | 3.23±1.2d |

Explanation: C1(healthy control), C2( CdSO4 with dose of 5.6mg/kg body weight), C3(CdSO4 with lycopene dose of 0.36mg/kg of bodyweight, C4(CdSO4 with lycopene dose of 0.72 mg/kg body weight, C5(CdSO4 with lycopene dose of 1.08 mg/kg body weight, C6(CdSO4 with dimercaprol dose of 0.3 mg/kg body weight).Column followed by the same letter is not significantly different with real level p< 0.05

The highest of Cd level was occur in C2, after rat giving CdSO4. This is because after Cd entering the body will bind into methallothionein (Cd + Mt) and causes increasing of Reactive Oxygen Species (ROS) such as 1O2, O2- and OH- which leads to a lipid peroxidation result in damage of kidney organ especially to renal proximal tubule and Cd accumulate in this organ (Cho *et al*., 2010: Chen *et al*., 2015). The presence of damaging the renal proximal tubules by Cd leading to an increase in blood creatinine, urea,uric acid and β2-M level (Table 1) (Johnson *et al*.,2012;Bernhoft, 2013).

Increasing of β2-M is due to renal dysfunction resulting in inhibition of salt reabsorption, reduction of water reabsorption and consequently an increase in urine volume (polyuria). Normal value of β2-M were 80-150 ng/mL (Li *et al*., 2016). Creatininis a metabolic result of creatine and phosphocreatine which filtered in the glomerulus and reabsorbed in the kidney tubules. Kidney dysfunction caused Glomerular Filtration Rate (GFR) decreases then the ability to filtrate creatinine will decrease,so that serum creatinine will increase (Normal value 0.3-0.9 mg/dL) ( Derelanko, 2000).

After administration of tomato extract and dimercaprol for 14 days resulted in improvements in renal function, characterized by decreased levels of blood Cd, creatinine urea, uric acid and β2-M, which returned to normal. The results of statistical analysis showed very significant differences between control and treatment groups. Lycopene and flavonoids in tomatoes can neutralize free radical of Cd +Mt by giving H+ as an electron donor, result in improvements of kidney organ. Lycopene in tomato fruit may reducing free radicals is 20 times greater than vitamin C and 10 times larger than vitamin E. (Agarwal and Rao, 2000 ;Holzapfel., *et al*., 2013). Lycopene in tomato extract dose 1.08 mg / kgBW is the highest in lowering levels of blood Cd ,β2-M,creatinine, urea and uric acid levels compare to dose of 0.36 mg/kgBW, 0.72 mg/kgBW and dimercaprol dose of 0.3 mg/kgBW.

**CONCLUSION**

Tomato extract dose 1.08 mg / kgBW is the highest in lowering levels of blood Cd, β2-M, creatinine, urea and uric acid levels.

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**REFERENCES**

ACGIH 2000.Thresold Limited value and detection method for chemical substances and biological exposure indices, Cincniati.

Agarwal S and Rao A.V, 2000.Tomato lycopene and its role in human health and chronic diseases. *Journal of Canadian Medical Association*. 163(6):739-744.

Bernhoft, R.A, 2013. Cadmium toxicity and treatment.*The Scientific World Journal* 13: 1-7.

Caciari, T., A. Sancini., M. Fioravanti., A. Cappozella., T. Casale*et al*, 2013. Cadmium and hypertensions in exposed workers : A Meta-analysis. *Int J Occup Med Environ Health* 26 (3) : 440-456.

Chen,L., Jin, T., Huang, B., Norberg G and Norberg, M (2015). Critical exposure level of Cd for elevated urinary methallothionein An occupational population study in China. *Toxicol.Appl. Pharmacol*. pp :93-95.

Cho, M.R., Kang, H.W., Jeon, S.H and Cho, M.H (2010) Time dependent changes of cadmium and methalothionein after short-term exposure to cadmium in rats.*Toxicol. Res*. 26(2) : 131-136.

Derelanko,M.J, 2000. Toxicologist’s Pocket Handbook. CRC Press. Boca Raton London New York Washington, D.C. p : 98.

Gallagher, C.M and J.R. Meliker. 2010. Blood and urine cadmium, blood pressure and hypertension : A systemic review and meta-analysis. *Environ. Health.Perspect.* 118(112) : 1676-1684.

Hijova, E., F. Nistiar and M. Kuchta, 2004. Influence of acute cadmium exposure on plasma antioxidant parameters in rats. *Bulletin of the Veterinary Reseach* Institute Pulawy. 48 (2) : 155-157.

Holzapfel., N.P., B.M. Holzapvel., S. Champ., J. Feldhusen., J. Clement and D.W. Hutmacher, 2013. The potential role of lycopene for the prevention and therapy of prostatcancer : From molecular mechanisms to clinical review.

Jacques, P.F., A. Lyass., J.M. Massaro., R.S. Vasan and R.B. D, Agostino, 2013. Relationship of lycopene in take and consumtion of tomato product to incident CVD.British Journal Nutrition.110 : 545-551.

Johnson, D. W., R.D.J Graham., H.M Timothy., J.L. Marie., P.D. Matthew and P.D. Matthew 2012. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate : new developments and revised recommendations. *Medical Journal of Australia* : 197(4) : 1-5.

Li, L., M. Dong and X.G. Wang, 2016. The implication and significance of Beta-2 Microglobulin: A conservative multifunctional regulator. *Chinese Medical Journal*. 129(4): 448-454.

Messner, B and D. Bernhard 2010.Cadmium and Cardiovasculardisease : cell biology, pathophysiology and epidemiological relevance. *Biometals* 23 (5): 811-822.

Satarug, S and S.H. Garret, 2010. Cadmium, environmental, exposure and health outcome. *Environ .Health Perspect* 118 (2) : 182-190.

Safitri, F.Z, 2015. Level of environmental health effects contents of heavy metal Cadmium (Cd) in greean mussels (*Perna viridis*), which consumed by Kaliadem Muara Angke People, North Jakarta.