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Renoprotective Effects of Lycopene in Tomato Extracts on Rat Exposed to Cadmium

Hernayanti[1], S Santoso, S Lestari, L Prayogo, Kamsinah, and Rochmatino

Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia

E-mail : hernayantioentoro@ymail.com

Abstract. Cadmium is a heavy metal widely used in human life. When it enters the body, cadmium might bind to metallothionein protein and impair renal function. The renal function decrease usually is characterized by raise of β2-microglobulin, creatinine, ureum and uric acid levels, which possibly be cured by lycopene, and antioxidant found in tomato. The objective of the study was to determine the effective lycopene dose as a renoprotective of Cd exposure. Twenty four Wistar rats used in this study were divided into six groups (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 of 0.36, 0.72 and 1.08 mg/kgBW/day respectively. Group VI exposed to CdSO4, and after seven days an additional 0.3 mg/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 the Duncan test. The results showed that 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/kgBW was the highest in lowering levels of blood Cd, β2-M, creatinine, urea, and uric acid levels.

1. Introduction

Industrial waste containing Cd can pollute the atmosphere, soil, and water. Cadmium is a persistent compound in an environment with a half-life of 30-40 years [1, 2]. Consequently, exposure to both acute and chronic Cd is very harmful to human health, especially to the kidney, which is the main target of Cd. Cadmium is also one of the causes of hypertension and human heart disease (atherosclerotic heart disease) [3, 4, 5]. Poisoning of Cd, Itai-Itai disease, occurring in Japan was caused by water pollution in Kumamoto. In Indonesia, it happened in Jakarta Bay. A Kaliadem resident who consumed green mussels had a high risk for Cd exposure (RQ > 1) with hypertension symptom [6]. In the body, cadmium binds to the metallothionein protein [7, 8]. This bonding is stable and can lead to increase free radicals in the liver and kidneys, resulting in oxidative stress characterized by decreased of superoxide dismutase (SOD) and Glutathione Peroxidase (GPx), leading to a decrease of renal function [3, 9]. An antioxidant supplement such as vitamin C, Vitamin E, and Selenium prevents Cd toxicity. They reduce Cd absorption by the kidneys and help to eliminate Cd out of the body without damaging the kidneys [10]. However, antioxidants in the form of food supplements are expensive and only the upper-middle-class society that might afford it. Usually, Cd toxicity treatment involves application of a chemical chelation compound, dimercaprol, but it is not

recommended because it can damage the kidneys and cause hypertension [3]. It is necessary to find an alternative treatment of easily available and cheap natural antioxidants such as tomatoes to overcome Cd toxicity. Tomato contains an active compound called lycopene, a potent antioxidant because of its capacity to reduce free radical compounds in the body [10]. This research aimed to understand renoprotective effects of lycopene on rat exposed by Cd with measuring of their β2-M level, and renal function creatinine, urea, and uric acid level, as well as to find the effective dose of lycopene as renoprotective on rat exposed by Cd in terms of decreasing β2-M, creatinine, urea, and uric acid level.

2. Methods

We selected a golden jubilee or golden tomato. The ethanol tomato extract (by maceration method) was used as a chelating agent for Cd poisoning. Five kg of raw tomato was dissolved in 96%-ethanol for 3x24 hours, and to thicken the extract the macerate was evaporated by vacuum rotary evaporator to produce 0.5 g extract. Tomato extract was administered 14 days after rat exposures to CdSO4. Wistar rats were fed with AD II pellets and distilled water for drinking ad libitum. They (24) were divided into six groups, C1: negative control (non-CdSO4, non-tomato extract), C2: positive control (exposed to 5.6 mg/kgBW CdSO4 for 14 days), C3, C4, C5 were exposed to 5.6 mg/kgBW CdSO4 for seven days and given tomato extract at a dose of 0.36, 0.72 and 1.08 mg/kgBW/day for 14 days, respectively. C6 was exposed to CdSO4 for seven days and dimercaprol for 14 days at a dose of 0.3 mg/kgBW. Dose of 0.36 mg/kgBW equals to lycopene dose of 15 mg/kgBW in humans, 0.72 mg/kgBB correspond to 30 mg/kgBW in human, and 1.08 mg/kgBW was to 45 mg/kgBW. The blood was collected on day 0 and 15, using a hematocrit capillary pipette on the vein orbitalis plexus of rat, then collected in Eppendorf tube (3 ml). The blood was divided into two parts, i.e., 0.5 ml for blood Cd analysis, and 2.5 ml for measurements of creatinine, uric acid, urea, and β2-M. The 2.5 ml blood was centrifuged for 10 minutes (4,000 rpm). Cadmium was measured by AAS at 228.6 nm wavelength and a strong current of 3.5 mA0. Creatinine level was examined by Jaffe kinetic method and read at spectrophotometer (492 nm). Urea and uric acid were measured by Dyasis method with a wavelength of 546 nm. Data of Cd, creatinine, uric acid, urea, and β2-M levels were analyzed by ANOVA, followed by Duncan test, to find out the differences in the treatments.

3. Results

The levels of cadmium, creatinine, β 2M, urea and uric acid after administrated by lycopene is presented in Table 1.

Table 1. Cadmium, creatinine,β 2M, urea and uric acid levels after lycopene administered (C1: healthy control, C2: $CdSO_4$ + 5.6 mg/kgBW lycopene, C3: $CdSO_4$ + 0.36 mg/kgBW lycopene, C4: $CdSO_4$ + 0.72 mg/kgBW lycopene, C5: $CdSO_4$ + 1.08 mg/kgBW lycopene, C6: $CdSO_4$ + 0.3 mg/kgBW dimercaprol. Column followed by the same letter is not significantly different with $p < 0.05$.)

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 ± 0.09^{a}	96.21 ± 16.98^{a}	13.5 ± 1.62^{a}	2.63 ± 0.12^{a}
C2	2.08 ± 0.17^{b}	1.23 ± 0.16^{b}	188.15 ± 17.91^{b}	45.38 ± 2.97^{b}	6.17 ± 0.56^{b}
C3	1.36 ± 0.04^{c}	1.00 ± 0.20^{c}	140.38 ± 28.07^{c}	28.75 ± 2.01^{c}	4.67 ± 0.36^{c}
C4	1.18 ± 0.36^{c}	0.78 ± 0.11^{a}	111.05 ± 10.04^{d}	17.50 ± 1.01^{d}	2.92 ± 0.20^{a}
C5	0.84 ± 0.05^{d}	0.69 ± 0.08^{a}	84.58 ± 14.48^{a}	19.70 ± 1.05^{d}	3.05 ± 0.19^{d}

Treatment	Cadmium (ppm)	Creatinin (mg/dl)	β 2M (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
C6	1.14 ± 0.40^{c}	0.82 ± 0.05^{a}	113.6 ± 15.22^{d}	22.32 ± 1.15^{d}	3.23 ± 1.2^{d}

4. Discussion

The highest Cd level was in C2 after giving the rat with CdSO4. It is because Cd in the body binds to metallothionein (Cd + Mt) and increase Reactive Oxygen Species (ROS) such as $1O_2$, O_2- and OH- which leads to lipid peroxidation that accumulates and damages the kidney especially renal proximal tubule [7, 8]. The damage of proximal renal tubules by Cd leads to an increase in blood creatinine, urea, uric acid, and β2-M level (Table 1) [11, 12].

The β2-M increase in the rats (normal value 80-150 ng/ml) is due to renal dysfunction and inhibits salt reabsorption, reduction of water reabsorption, and consequently an increase in urine volume (polyuria) [13]. Creatinine is a creatine and phosphocreatine metabolites which are filtered in the glomerulus and reabsorbed in the kidney tubules. Kidney dysfunction causes Glomerular Filtration Rate (GFR) to decrease, followed by decrease ability to filtrate creatinine, and increase of serum creatinine (normal value 0.3-0.9 mg/dl) [14].

After administering the tomato extract and dimercaprol for 14 days, improvements were shown in renal function characterized by decreased levels of blood Cd, creatinine urea, uric acid, and β2-M, which returned to normal levels. The analysis showed highly 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 reduce free radicals 20 times greater than vitamin C, and 10 times larger than vitamin E [10, 15]. Lycopene in tomato extract dose of 1.08 mg/kgBW was the best in lowering levels of blood Cd, β2-M, creatinine, urea, and uric acid levels compared to dose of 0.36 mg/kgBW, 0.72 mg/kgBW, and dimercaprol dose of 0.3 mg/kgBW.

5. Conclusion

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

6. Acknowledgment

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RENOPROTECTIVE EFFECTS OF LYCOPENE IN TOMATO EXTRACTS ON RAT EXPOSED TO CADMIUM

Hernayanti[1] Slamet Santoso[1] Sri Lestari [1] Lucky Prayogo[1] Kamsinah[1] Rochmatino[1]

[1] Faculty of Biology, Jenderal Soedirman University

Correspondence author: Hernayanti, email : hernayantioentoro@ymail.com

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 was todetermine 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 $CdSO_4$ as a positive control. Group III, IV and V were given 5.6 mg/kgBW $CdSO_4$ and tomato extract at 0.36, 0.72 and 1.08 mg/kgBW/day respectively. Group VI was given $CdSO_4$ 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.

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

INTRODUCTION

Industrial waste containing Cd when released into the environment can pollute the atmosphere, soil and water. In addition Cd can also enter into the human body through the inhalation path of exposure to dust produced in industrial processes. 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 (Gallagher and Meliker,2010; Messner and Bernhard, 2010). Cadmium is also one of the causes of hypertension and human heart disease (atherosclerotic heart disease) (Messner and Bernhard, 2010; Caciari *et al*., 2013). The cadmium that enters

kidneys, resulting in oxidative stress, characterized by decreased of superoxide dismutase (SOD) and Gluthation Peroxidase (GPx),result in decreaseof 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 classsociety.Treatment of Cd toxicity is not recommended using a chemical chelation compound 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 (Agarwaland Rao, 2000).

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

Making tomato extract by Maceration method with ethanol 96% as a solvent (Anonymous,1986). Twenty fourWistar rats were divided into six groups, with four individuals in each group. C1 was the negative control. C2 was given 5.6 mg/kgBW $CdSO_4$ as a positive control. C3, C4,C5 were given 5.6 mg/kgBW$CdSO_4$ and tomato extract at a dose 0.36, 0.72 and 1.08 mg/kgBW/day respectively. C6 was given $CdSO_4$ anddimercaprol at a dose 0.3mg/kgBWDose 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 15^{nd} .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 3.5mA^0. 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.

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±0.09^a	96.21±16.98^a	13.5±1.62^a	2.63±0.12^a
C2	2.08 ± 0.17^b	1.23±0.16^b	188.15±17.91^b	45.38±2.97^b	6.17±0.56^b
C3	1.36±0.04^c	1.00±0.20^c	140.38±28.07^c	28.75±2.01^c	4.67±0.36^c
C4	1.18±0.36^c	0.78±0.11^a	111.05±10.04^d	17.50±1.01^d	2.92±0.20^a
C5	0.84±0.05^d	0.69±0.08^a	84.58±14.48^a	19.70±1.05^d	3.05±0.19^d
C6	1.14±0.40^c	0.82±0.05^a	113.6±15.22^d	22.32±1.15^d	3.23±1.2^d

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 wasoccur 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 $^1O_2$, O_2^- 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 dysfunctioncaused Glomerular Filtration Rate (GFR) decreases then the ability to filtratecreatinine 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. (Agarwaland Rao,2000;Holzapfel., *et al.*, 2013).

CONCLUSION

Lycopene in tomato extracts all doses can function as Cd anti-nephrotoxicin white rat exposed byCd SO4 in terms of decreased blood Cd levels, β-2 microglobulin, urea, uric acid and creatinine levels. Tomato extract dose 1.08 mg / kgBB is the highestin lowering levels of blood Cd ,β2-M,creatinine, urea and uric acid levels.

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