

## Oral Administration of 250 Mg Vitamin C Reduces the Blood Level of Lead in Female Street Sweepers of Denpasar City

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### ABSTRACT

The marked increase in number of motor vehicles contributes to air pollution. The lead content of vehicle emissions is prone to cause both lung and blood toxicity if continuously inhaled. Lead exerts its hematotoxicity effect by means of inhibiting the enzymes of heme biosynthesis, leading to the decrease of both erythrocyte and hemoglobin count in individuals who were exposed to it. Vitamin C inhibits lead uptake thus decreasing its cytotoxicity by means of decreasing the absorption rate of lead in small intestine. This study aimed to know whether vitamin C administration could decrease the blood level of lead, as well as increasing both erythrocytes and hemoglobin count of street sweepers. Pre test and post test control group design was used for this study with 30 street sweepers as the subjects. Subjects were randomly grouped into either control or treatment groups, where 15 control subjects were given placebos and 15 other subjects were given 250mg of vitamin C for 30 days as the treatment group. The blood level of lead before and after administration of vitamin C was tested by *Independent t test*, while the hemoglobin and erythrocyte count were tested by *Mann-Whitney Test*. The mean level of lead content in control and treatment group was  $1.413 \pm 0.348$  and  $1.033 \pm 0.324$ , respectively ( $p= 0.034$ ). The mean erythrocyte count in control and treatment group was  $4.511 \pm 0.349$  and  $4.744 \pm 0.363$ , respectively ( $p= 0.765$ ), while the mean hemoglobin level in control and treatment group was  $13.355 \pm 0.605$  and  $13.352 \pm 0.340$ , respectively ( $p= 0.665$ ). The results showed that there was significant decrease of blood lead level after administration of vitamin C ( $p<0.05$ ), while no significant increase of either erythrocyte count nor hemoglobin level was found ( $p>0.05$ ). It is concluded that administration of 250 mg vitamin C per day reduced the blood lead level. The result of this study is expected to be used for preventing lead toxicity in street sweepers.

**Keywords:** lead, vitamin C, erythrocyte, hemoglobin, street sweepers.

### INTRODUCTION

The marked increase in number of motor vehicles that contributes to air pollution is due to the incomplete combustion of fuel. The size of lead particles contained in vehicle emissions is 0.02-1.00 $\mu$ m, with mean duration of exposure of 4-40 days. These properties enable them to be easily inhaled into the lungs, which will accumulate in blood leading to toxicity. Even though human body

absorbs lead in small number, the posed effect is remarkably dangerous.<sup>1</sup> The first apparent effect of chronic lead toxicity before reaching its organ target is the disturbance of heme biosynthesis. This will further leads to the disorders of nervous system, kidneys, reproductive system, digestive system, and also anemia.<sup>2</sup> The risk of lead toxicity increases in individuals who are frequently exposed by it, such as drivers, traffic police officers, road workers, and those who live alongside the road. The highest level

of toxicity was found in street children, as high as 600-680 µg/l of blood, with normal value of 400 µg/l of blood.<sup>3</sup>

The lead absorbed by body interferes with the synthesis of erythrocytes by binding to functional group of amino levulinic acid dehydratase (ALAD) which functions in this process.<sup>4</sup> This enzyme works at the early stage of synthesis and also during the circulation of erythrocytes, hence in individuals who are continuously exposed to lead, there could be decrease of erythrocyte count and also the level of hemoglobin. Lead interferes with the blood circulation system in several ways, including (a) by delaying the normal maturation of erythrocytes in bone marrow which leads to anemia, (b) decreasing the life span of erythrocytes, and (c) interfering the biosynthesis of hemoglobin by inhibiting the activity of both  $\delta$ -ALAD and ferrochelatase enzyme.<sup>5</sup>

Vitamin C is a water-soluble vitamin that is required for several metabolism processes including the prevention of tissue oxidative stress.<sup>1</sup> Vitamin C could also inhibit lead uptake thus decreasing its toxicity, and has been shown to effectively acts as kidney protector and reducing nephrotoxicity.<sup>6,10</sup> A study showed that heme production was improved after administration of vitamin C as much as 100 mg/kg body weight.<sup>7</sup> Vitamin C could reduce the blood lead level by means of inhibiting its absorption in the small intestine. Street sweepers work for several hours each day, which poses them as individuals with high risk of lead exposure from vehicle emissions. This study aimed to know whether vitamin C administration could decrease the blood level of lead, as well as increasing both erythrocytes and hemoglobin count of street sweepers.

## MATERIALS AND METHOD

This study was conducted in Denpasar city, starting from March until September 2014 with street sweepers as the subjects. The criteria of inclusion was females of 20-45 years old willing to participate in the study, while the criteria of exclusion were having signs of infections 1 week before and during study, has been routinely consuming vitamin C or other antioxidants for at least 1 week before study, and also those who were having chronic gastritis. *Drop out* criteria includes resigning from the

study and inability to attend the final measurement. Subjects were chosen by means of simple random sampling. The minimum number of samples for each study group was obtained by using p value of <0.05, power value of 80%, and mean difference of blood lead level of 0.086 according to previous studies. The control group was assigned to placebo capsules while treatment group was assigned to capsules containing 250 mg vitamin C for 30 days.

The variables used in this study are classified as follows: the dose of vitamin C as the independent variable; the hemoglobin level, erythrocyte count, and blood lead level as the dependent variable; while age and sex as the controlled variables. Street sweepers are defined as individuals who work as street sweepers daily, with certain work hours, and are members of Environmental Service Agency of Denpasar city. The level of hemoglobin is the result of measuring the level of hemoglobin of street sweepers by using hematology analyzer. The erythrocyte count is the result of measuring the number of erythrocytes in the blood samples of street sweepers by means of hematology analyzer. The blood lead level was measured by means of spectrophotometer. Vitamin C was administered in form of capsules containing 250 mg vitamin C for 30 days, once daily. Placebo is defined as low-calorie sugar packed into capsules with the same color as the vitamin C capsules.

The baseline data of subjects such as age, symptoms, duration of work, and associated diseases was obtained by filling out questionnaires during interview. The blood lead level, hemoglobin level,

**Table 1: The Characteristics of Female Street Sweepers According to Age and Duration of Work**

Characteristics	Treatment group (n)	Control group (n)	Total (n)
Age			
< 30 years old	3	2	5
≥ 30 years old	12	13	25
Duration of work			
< 5 tahun	2	1	3
≥ 5 tahun	13	14	27

and erythrocyte count of all subjects were measured before and after treatment was administered. Data was then collected and presented in narration and tables. The comparability test between control and treatment group was conducted by *Independent t test* for normally distributed data and *Mann-Whitney test* for not-normally distributed data with  $\alpha < 0.05$ . The ethical clearance for this study has issued by Unit of Research and Development, Faculty of Medicine, Udayana University/Sanglah General Hospital Denpasar with issue No: 184/UN. 14.2/Litbang/2014.

## RESULTS

There are a total of 30 subjects participating in this study which were grouped into 2 groups consisting of 15 subjects each, namely the control group and treatment group. There were no subjects dropped out of this study. The characteristics of subjects are presented in Table 1.

The blood lead level was analyzed by *Independent t test* due to its normally distributed data, as presented in Table 2:

With p value of 0.034 ( $p < 0.05$ ), it means that the blood lead level was reduced significantly after treatment.

Both the level of hemoglobin and erythrocyte count was not normally distributed, thus comparability test was conducted by using Mann-Whitney test (table 3 and table 4).

From the table, it is shown that the Z value of hemoglobin level was 0.433 dengan significance level of 0.665 ( $p > 0.05$ ), which means that there was no significant difference resulting from the treatment in term of level of hemoglobin.

The significance level of erythrocyte count was 0.765 ( $p > 0.05$ ), which means that there was

**Table 2: The Comparability Test Result after Treatment**

Variable	Lead Level ( $\mu\text{g/dl}$ )		F	P
	TG	CG		
Pre test	1.622 $\pm$ 0.507	1.570 $\pm$ 0.392	0.266	0.034
Post test	1,033 $\pm$ 0.324	1.413 $\pm$ 0.348		

TG= Treatment group  
CG= Control group

**Table 3: The Comparability Test Result of Hemoglobin Level in Both Groups after Treatment**

Variable	Level of Hb (g/dl)		F	P
	TG	CG		
Pre test	13.83 $\pm$ 0.975	13.52 $\pm$ 0.734	0.443	0.665
Post test	13.35 $\pm$ 0.340	13.36 $\pm$ 0.605		

**Table 4: The Comparability Test Result of Erythrocyte Count after Treatment**

Variable	Erythrocyte Count ( $\times 10^6/\mu\text{l}$ )		F	P
	TG	CG		
Pre test	4.933 $\pm$ 0.289	4.713 $\pm$ 0.381	0.474	0.765
Post test	4.744 $\pm$ 0.363	4.511 $\pm$ 0.349		

no significant difference resulting from the given treatment.

### DISCUSSIONS

After the administration of treatment, a decrease in the mean level of lead, hemoglobin, and erythrocytes was observed. Due to the comparability result that had been performed between the control and treatment group, the decrease of mean blood lead level was considered significant ( $p < 0.05$ ), while the decrease of hemoglobin level and erythrocyte count was not considered as significant ( $p > 0.05$ ).

Vitamin C is a powerful natural antioxidant, but its property is not enzymatic. Its main function is to prevent the chain reactions caused by harmful free radicals. Antioxidants are substances that neutralize free radicals, protecting the body from harmful effects of excessive oxidation. As a free radical neutralizer, antioxidants are further classified as enzymes or micronutrients.<sup>8</sup> Antioxidants with enzymatic properties are generated within the body (such as superoxide dismutase (SOD), glutathione peroxidase, catalase, and glutathione reductase). There are three main antioxidants those are acting as micronutrients, namely betacarotene, vitamin C dan vitamin E.<sup>9</sup> Betacarotene is a single oxygen scavenger, while vitamin C scavenge superoxides and other free radical agents. Vitamin E acts by breaking the peroxide chains of membrane lipids.<sup>8</sup>

Vitamin C is a water-soluble vitamin that is required for several metabolism processes including the prevention of tissue oxidative stress. Vitamin C could also inhibit lead uptake thus decreasing its toxicity, and has been shown to effectively acts as kidney protector and reducing nephrotoxicity.<sup>6</sup> A study showed that the toxic effect of lead on heme biosynthesis in rats was reduced after administration of 2 g/L vitamin C incorporated to the drinking

water exposed to 300 mg/L lead.<sup>7</sup> This is due to the ability of vitamin C to reduce the lead uptake in small intestines. Since it is relatively low-cost and could be obtained easily, vitamin C could be consumed daily as a mean of self-protection against lead toxicity. Hence, vitamin C administration will reduce the blood lead level without any significant change in hemoglobin level and erythrocyte count. Some possibilities that might be the cause of this insignificant result are (1) duration of treatment that is too short, where significant results might not be apparent yet due to the 120 days life span of hemoglobin and erythrocytes, and (2) the dose of vitamin C that is too low.

The weakness of this study is that it is not yet able to provide information on how many days the vitamin C treatment should be administered. Another thing is that this study distributed the subjects into two groups only (placebo group and treatment group). Further studies should consider another study design where the treatment group is further subclassified into subgroups that differ in terms of the dose of administered vitamin C.

### CONCLUSION

From our study, it is concluded that the administration of 250 mg vitamin C in street sweepers reduces the blood lead level significantly, but was not shown to increase neither the erythrocyte count nor the level of hemoglobin.

### Suggestions

Further studies are required to obtain the exact dose of vitamin C needed to effectively prevent and reduce lead toxicity in street sweepers.

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### REFERENCES

1. Gurer, H and Ercal, N. Review Article : Can Antioxidants Be Beneficial In The Treatment Of Lead Poisoning? *Free Radical Biology & Medicine.* ; **29**(10): 927–45 (2000).
2. Lyn Patrick, ND. Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity. *Alternative Medicine Review;*

- 11**(2): 114-27 (2006).
3. Anonymous. Peraturan Pemerintah No.41 Tahun 1999 tentang Pengendalian Pencemaran Udara. Kementrian Lingkungan Hidup. (2000).
  4. Palar H. *Pencemaran dan Toksikologi Logam Berat*. Penerbit Rineka Cipta. 1994.
  5. WHO. *Lead Environmental Health*. Published Under The Joint Organization Geneva, 3<sup>rd</sup> Ed. 1997.
  6. Kathuria, P., Jadav, P. and Marsoni, N. Lead Nephropathy. 2004. (Accessed on 3<sup>rd</sup> of August 2014) downloaded from <http://www.emedicine.com/med/topic1267.html>.
  7. Eshginia, S and Marjani, A. The Effect of Vitamin C on the Erythrocyte Antioxidant Enzymes in Intoxicated-Lead Rat Offsprings. *Journal of Clinical and Diagnostic Research*; **7**: 1078-81 (2013).
  8. Jassim, HM and Hassan, AA. Changes In Some Blood Parameters In Lactating Female Rats And Their Pups Exposed To Lead: Effects Of Vitamins C And E. *Iraqi Journal of Veterinary Sciences*. **25**(1): 1-7 (2011).
  9. Shahidi, F. *Natural Antioxidant, Chemistry, Health Effect and Application*, AOCS Press, Illinois. 1997.
  10. Markoviæ SD, Ðaèiæ DS, Cvetkoviæ, DM, Obradoviæ AD, Žižiæ JB, Ognjanoviæ BI, *et al.* Effects Of Acute Treatment Of Vitamin C On Redox And Antioxidative Metabolism In Plasma And Red Blood Cells Of Rats. *Kragujevac J. Sci.* **32**: 109-16 (2010).