

The Level of Zinc Serum after Oral Zinc on Mice with *Escherichia Coli* LPS- Induced Diarrhea

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Abstract: The level of zinc requirements will increase due to infection, for formation of immune function and new cells. The advantage of Zinc is maintain the integrity of the intestinal mucosa through its function in cell regeneration and membrane stability. The aim of this study is to determine the level of zinc serum after oral zinc on mice diarrheal induce E.Coli-LPS. This study used a controlled trial experimental design in the laboratory. Sample of 20 mice were randomly divided into 4 groups:1). Control group was given standard foods, 2). Trial group was given E.Coli-LPS 2.5 mg/kg/oral once on day-1, 3). Preventive group was given E.Coli-LPS once 2.5 mg/kg/oral once on day-1 + 30 mg/kg/oral of zinc once daily for 12 days. 4). Therapy group was given E.Coli-LPS 2.5 mg/kg/oral once on day-1, if diarrheal was given 30 mg/kg/oral of zinc once daily for 12 days. Blood samples of mice were taken through the orbital sinus on the 0, 5th, 10thhour, 4th, 8th and 12thday. Data are presented in tables and graphic. We found that higher levels of zinc in the preventive and therapy group especially on 4th, 8th and 12th day. I conclusion, oral administration of zinc increase serum zinc levels, especially in control and experimental groups.

1 INTRODUCTION

Zinc deficiency is still common, especially in developing countries. This can be related to lack of intake, increasing needs, and the amount of zinc loss from the body due to diseases, especially infections. There is associated between infection and zinc deficiency which influence each other. Zinc requirements of the body will increase during infection, formation of immune functions and new cells. Zinc deficiency can cause suppression of immune function, making it easier for infection to occur (Baqui, 2006; King, 2003).

Zinc plays a role in maintaining the integrity of the intestinal mucosa through its function in cell regeneration and membrane stability. Zinc has a direct impact on intestinal villi, disaccharidase brush border activity and intestinal water and electrolyte transport. Zinc also plays a role in T cell function and enhances immunity thereby reducing the severity of diarrhea (Roy, 1992).

The risk of zinc deficiency in Indonesia is estimated being greater because of Indonesian community menu, especially in the low socio-economic group, with lower animal protein consumption even though this type of protein contains a lot of zinc. Moreover, the menu of Indonesian society is relatively high in phytate and fiber in inhibits zinc absorption, such as the habit of drinking tea every day, even on certain social groups that consume thick tea. Moreover, they also consume lots of beans and cereal, including the processed products. This food contains a lot of phytate or tannin so that the potential lack of zinc in Indonesian society is quite higher because the absorption of zinc will be disrupted (Nona, 2010).

Escherichia coli is the main occupant of healthy colon flora, but also cause various diseases such as diarrhea by releasing endotoxins, which triggers the release of pro-inflammatory mediators. Lipopolysaccharide (LPS) is the main component of the cell wall of gram-negative bacteria which is also called endotoxin and is known to be the trigger of

several types of inflammatory or infectious reactions in macrophage cells and other cells that have CD14 receptors. In fact, endotoxin (LPS) of bacteria that binds to TLR (Toll Like Receptor) in DC (Dendritic Cells) can stimulate monocyte and macrophage cells to secrete nitric oxide (NO) and inflammatory substances (cytokines) such as Tumor Necrosis Factor-alpha (TNF- α) and interleukin-1-beta (IL 1 - β), IL-6 and IL-8 (Rahman, et al., 2007). As a result of the release of excessive pro-inflammatory cytokines can cause symptoms of decreased blood pressure, fever and diarrhea (Baratawidjaya, 2009).

In experimental studies, zinc deficiency has a direct effect on the digestive tract, in the form of villous atrophy, a decrease of disaccharidase enzyme activity in brush borders, and impaired intestinal transport. However, the exact mechanism that connects the pathophysiology of diarrhea with zinc deficiency has not been agreed yet. However, the incidence of persistent diarrhea has been reduced by zinc supplementation, and administration of ORS (oral rehydration solutions) with zinc has substantially reduced the duration and severity of diarrhea in children with acute and persistent diarrhea (Bhandari et al., 2002). Although the mechanism of zinc supplementation reduces diarrhea is unknown, zinc therapy in diarrhea patients has shown better absorption of water and electrolytes by the intestine, faster regeneration of intestinal epithelial cells, increased levels of enzymes from brush border, and increased immune response so that it can eliminate pathogens in the intestine (Fenwick et al., 2004).

The purpose of this study was to determine serum zinc levels after administration of oral zinc in mice diarrheal induced E. coli-LPS.

2 METHODS

This study is designed with Controlled Trial Design, and done in The Laboratory of Nutrition and Food Centre (PSPG) in GadjahMada University Yogyakarta. Sample consist of 20 white mice *Sprague Dawley* which is randomly chosen and divided into 4 groups randomly and each group consist of 5 mice. 1). Control Group (G1), was given standard nutrition, 2). Trial Group (G2) was given LPS E.Coli 2,5 mg/kg/oral in day 1-3, 3). Preventive Group (G3) was given LPS E.Coli 2,5 mg/kg/oral in day 1 + zinc 30 mg/kg/oral since day 1 until 12 days, 4). Therapy Group (G4) was given LPS E.Coli 2,5 mg/kg/oral in day 1, if diarrhea occur, this group will be given zinc 30 mg/kg/oral

since day 1 until 12 days. Sample of the mice blood was taken from sinus orbitalin 0, 5th, 10th hour, 4th, 8th, and 12th day to measure zinc level in serum (Madiono, et, al., 2002).

3 RESULTS

The characteristics of subjects in this study include weight and Hemoglobin level (Hb) presented in Table 1.

Table 1. The Characteristics of subjects.

Group	Mean of Weight(gram)	Standard of Deviation	p-value*
G1	73,4	8,02	0,04
G2	88,4	10,92	
G3	93,8	15,06	
G4	89,6	7,335	
Group	Mean of Hb(g%)	Standard of Deviation	p-value*
G1	11,88	0,362	0,138
G2	11,76	0,295	
G3	11,36	0,268	
G4	11,57	0,441	

* ANOVA, p < 0,05

G1= Control group G3=Preventive groupG2= Trial groupG4= Therapy group

3.1 The Level of Zinc based on Treatment Time

The results of zinc level can be seen in Table 2 and Picture 1. In control group (G1) there is no difference of zinc level based on treatment time in 5 mice that has been evaluated, the level range is 1,14 until 1,2. However, zinc level in trial group (G2) seems to be lower compared to group G1, G3 and G4 which can be seen obviously in Picture 1, since treatment time in 4th, 8th and 12th day in group G2, mice 3rd, 4th and 5th experience diarrhea.

The difference also can be seen in preventive group (G3), zinc level in 5th hour and 10th hour after treatment with LPS is 0,94 μ g/dl and 0,77 μ g/dl, meanwhile in 4th, 8th and 12th day, zinc level is higher compared to group G2 and can be seen obviously in Picture 1, this event occur in group G3 mice 3rd, 4th and 5th. In preventive group all mice were given zinc everyday.

Table 2: The level of zinc ($\mu\text{g}/\text{dl}$) based on treatment time.

Treatment time	G1 ($\mu\text{g}/\text{dl}$)	G2 ($\mu\text{g}/\text{dl}$)	G3 ($\mu\text{g}/\text{dl}$)	G4 ($\mu\text{g}/\text{dl}$)
5 th hour	1,14	0,95	0,94	0,93
10 th hour	1,19	0,83	0,77	0,52
4 th day	1,16	0,51	1,17	1,05
8 th day	1,18	0,5	1,18	1,09
12 th day	1,2	0,6	1,2	1,07

It is the same in therapy group (G4) zinc level is higher in mice 3rd, 4th and 5th compared to mice in group G2 in 4th, 8th and 12th day. Actually zinc level is higher in mice 3 in the group G3 3 days after receiving zinc, it is also the same for mice 4 in group G4, zinc level is higher in day 6 after receiving zinc, mean while in mice 5 in group G4, zinc level is higher after 10 days receiving zinc, can be seen in Table 2 and Figure 1.

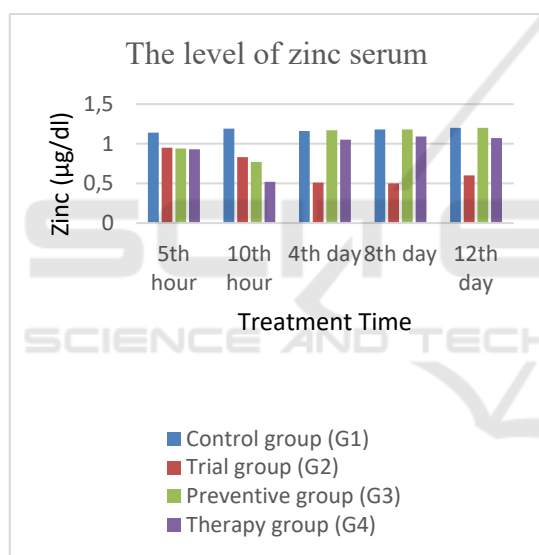


Figure 1. Diagram of zinc level in serum on control group (G1), Trial group (G2), Preventive Group (G3) and Therapy group (G4).

The result of zinc level in trial group (G2) is lower than control group (G1) since 5th hour after treatment time of LPS and lower in 10th hour, 4th, 8th and 12th day can be seen in Table 2 and Picture 1. The zinc level is lower in control group (G2) compared to preventive group (G3) and therapy group (G4) especially in 4th, 8th and 12th day. Mice in preventive and therapy group (G4) is given zinc everyday, meanwhile in trial group (G2) is not given zinc.

4 DISCUSSION

In Figure 1, generally we can see zinc level in control group (G2) is lower in mice experienced diarrhea and zinc level is higher in group received zinc, preventive group (G3) and therapy group (G4). In this study, mice in trial group (G2) were not given zinc and experienced diarrhea since 3rd day until 12th day, in which zinc level is the lowest since 4th, 8th and 12th day. Zinc associated with gut villi regeneration and function, therefore it will influence formation of disaccharide enzyme such as lactase, sucrose and maltase. Therefore, zinc can influence process of osmotic diarrhea, most of which caused by malabsorption and maldigestion. In diarrhea, there is excessive loss of zinc. Prolonged diarrhea cause decreased the serum level of zinc. There is a circulation between diarrhea, zinc deficiency, duration of diarrhea, and malnutrition. Administration of micro zinc in oral can replace loss of zinc in diarrhea (Artana et al., 2005).

Some studies show the role of zinc associated with red blood cell formation. In biosynthesis of heme, enzyme δ ALA dehydratase which is depend on zinc plays an important role. There are lots of studies show lower level of zinc can distract synthesis of Heme. Meanwhile, the higher supplement of zinc can also distract the absorption of copper and iron. This can distract the immune system response which lead to anemia (Olivares et al., 2007). Another mechanism which shows by another researchers were animal study with zinc deficiency shows that decrease of erythrocyte precursor in bone marrow and erythropoietin level in plasma in which experiment were mouse and rat (King et al., 2001; King et al., 2005; Konomi, 2005).

Another opinion shows that deficiency of mineral can reduce the red blood cell lifetime because zinc is a cofactor of Superoxide Dismutase (SOD) in red blood cell (RBC-SOD) which contribute in protecting from oxidative stress and cell integrity (Powell, 2000; O'Dell, 2000).

According to the theory which developed by Shankar & Prasad that zinc deficiency make less productivity and activity of SOD enzyme and can reduced free radical activity, which lead to excessive fat peroxidation. The free radical in intestinal mucosa cause atrophy of intestinal mucosa through cell apoptosis. The atrophy intestinal mucosa that caused by zinc deficiency can make decreasing productivity and activity of SOD enzyme in intestinal mucosa cell, therefore free radical activity increase which can lead to fragmentation of DNS and also can lead to cell apoptosis. The apoptosis of

cell make atrophy of gut villus. Cumulative effect of intestinal atrophy and broken of *tight junction* caused the permeability of membrane increase and disturb intestinal absorption and lead to diarrhea (Shankar, 1998).

Patel et al in their meta-analysis found that zinc supplementation has a modest beneficial association (9% reduction) with incidence of diarrhea, a stronger beneficial association (19% reduction) with prevalence of diarrhoea and occurrence of multiple diarrheal episodes (28% reduction) (Kalavakuri, 2017).

Zinc is usually given as zinc sulphate, zinc acetate, or zinc gluconate, which are all water-soluble compounds. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend 10 mg to 20 mg of zinc per day for children with diarrhoea. There are several mechanism of action of zinc on acute diarrhoea, some of which are specific to the gastrointestinal system: zinc restores mucosal barrier integrity and enterocyte brush-border enzyme activity, it promotes the production of antibodies and circulating lymphocytes against intestinal pathogens, and has a direct effect on ion channels, acting as a potassium channel blocker of adenosine 3-5-cyclic monophosphate-mediated chlorine secretion (Lazzerini, 2016).

The conclusion of this study are the level serum of zinc can increase in preventive group (G3) and therapy group (G4) after receiving oral zinc and lower in trial control (G2) which did not received oral zinc in mice with diarrhea induced by LPS from *E. coli*.

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