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The Effect of Biscuits Made From Pumpkin Seeds Flour on Serum Zinc Levels and Weight in Malnutrition Wistar Rats

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Abstract

BACKGROUND: Serum zinc levels are found to be low in children of protein energy malnutrition globally. Pumpkin seed flour has a high zinc content of 6.88 mg/100 g, has been formulated biscuits made from pumpkin seed flour with a zinc content of 1.52 mg/100 g.

AIM: This study aims to determine the effect of giving biscuits made from pumpkin seed flour to serum zinc levels and body weight in malnutrition Wistar rats.

METHODS: This research is a true experiment with pre-post-test with control group design. Subjects were 28 male Wistar strain rats induced by fasting malnutrition for 3 days and then given biscuits with a zinc dose of 0.027 mg; 0.054 mg; and 0.081 mg/BW/day and in the control group without biscuits for 2 weeks. Serum zinc levels were examined using the atomic absorption spectrophotometry method. Data were analyzed using paired t-test and one-way ANOVA test.

RESULTS: Paired t-test results on serum zinc levels obtained $p > 0.05$ and on body weight obtained $p < 0.05$. One-way ANOVA test results on serum zinc levels after the intervention obtained $p > 0.05$ and at body weight obtained $p < 0.05$ followed by post hoc least significant difference test found that there were significant differences between the control group and the P1 group, a dose of 0.027 mg ($p = 0.015$) and P2 groups, doses of 0.054 mg ($p = 0.012$). Greater weight gain was found in the P1 group with an increase of 38.85%.

CONCLUSION: The provision of biscuits made from pumpkin seed flour has an effect on increasing body weight of malnutrition Wistar rats and has no effect on serum zinc levels, so further research is expected to use different doses.

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Introduction

The prevalence of global malnutrition in children aged 6–12 years shows the average prevalence of stunting between 20% and 30% in all regions except Latin America. The average prevalence of underweight is 17%, wasting 35%, overweight 16% and 8% are obese [1]. In Indonesia, based on data from the Basic Health Research (Riskesdas) 2018, the prevalence of wasting, overweight/ obesity, and stunting was relatively high (9.2%, 20.0%, and 23.6%, respectively) [2].

Malnutrition can be an energy deficit (protein energy malnutrition-PEM) or micronutrient deficiency [3]. Insulin like growth factor 1 is a growth hormone mediator that acts as a growth promoting factor in the growth process. Decrease in IGF-1 concentration is caused not only by a lack of protein energy but also zinc deficiency [4]. Serum zinc levels are found to be low in children of protein energy malnutrition globally. In PEM children often zinc deficiency occur which can cause decreased appetite, growth failure, skin injury, diarrhea, healing old wounds and lack of immune response, and inhibit the recovery

stage in PEM children. Zinc supplementation during the rehabilitation phase of malnutrition has been linked to rapid weight gain [5]. The previous studies in severely malnourished children demonstrated an increased weight after administering zinc supplementation for two weeks [6].

Mineral zinc (Zn) is one of the important nutrients needed by the body in maintaining health. This mineral plays a role in a variety of enzyme activities, cell growth, and differentiation, and plays an important role in optimizing the function of the immune system [7]. Based on the IZINCG technique, the global prevalence of zinc deficiency is estimated at 31%, starting at 73% in the sub regional. In developing countries, zinc deficiency is common in infants and children who cause growth retardation, along with a high incidence of severe infectious diseases such as diarrhea, pneumonia, and malaria [8].

One strategy for increasing the production and/or intake of zinc-rich foods in populations at risk of zinc deficiency is by modifying/diversifying the diet [9]. Zinc source foods such as meat and liver tend to be expensive and have low consumption levels. A new innovation is needed by utilizing other local food ingredients that contain high amounts of zinc for

supplementation so that children's zinc needs can be met [10], [11].

At present, pumpkin seeds have received considerable attention, because they contain beneficial nutrients, such as protein, fiber, minerals, fatty acids, and phytosterols. Based on the USDA National nutrition database, zinc content of 100 g of pumpkin seeds is 7.81 mg. Several studies have developed the addition of pumpkin seed flour as an economical nutritious food with a very acceptable taste. Sensory characteristics in formulas made from pumpkin seed flour mixed with bread, biscuits, and cake products are highly accepted [12], [13]. Results of the analysis on 100 g of pumpkin seed flour obtained 6.8 mg zinc content [14]. The use of pumpkin seed flour in additional food porridge significantly increases zinc content without reducing the nutritional value and quality of the local complementary food [15].

The previous study has been formulated biscuits made from pumpkin seeds with organoleptic test results obtained formula I with a comparison of pumpkin seed flour and wheat flour (20%: 80%). Based on the analysis of nutrient content in 100 g of biscuits made from pumpkin seed flour produces about 534.7 kcal of total calories. The composition of macronutrients is 48.16 g of carbohydrates, 33.05 g of fat, and 11.20 g of protein. Content of micronutrients is around 8.22 mg of Vitamin A, 0.27 mg of Vitamin C, 6.08 mg of calcium, 36.77 mg of potassium, 46.23 mg of chlorine, 0.5 mg of molybdenum, and zinc content of 1.52 mg [16].

Considerations affecting decisions regarding the method of supplying zinc supplementation are solubility, bioavailability, taste, side effects, and frequency of doses administered [9]. Before it is applied to humans, a series of experiments using animal models need to be carried out. It has been reported that zinc is very important for mouse growth, where the mouse growth is very low in the ration without additional zinc given [17]. Wistar rats are one of the most widely used experimental animals as models in biomedical research. Serum or plasma zinc is the best biomarker available to assess the risk of zinc deficiency [18].

This study aims to determine the effect of giving biscuits made from pumpkin seed flour to serum zinc levels and body weight in malnutrition Wistar rats.

Materials and Methods

Location and research design

This research was conducted in June 2019–July 2019. The location of biscuits is substituted for pumpkin seeds production at the Faculty of Public Health - Culinary Laboratory of Hasanuddin University.

The process of research and weighing in experimental animals is carried out in the Laboratory Biopharmacy Laboratory of the Faculty of Pharmacy, Hasanuddin University. Examination of serum zinc levels at the Makassar Health Laboratory Center. Measurement of serum zinc levels using the atomic absorption spectrophotometry method, body weight measurements using Henherr digital scales (g). This research is a true experimental with a pre-post-test with control group design.

Population and samples

This study used experimental animals of male white rats Wistar strain (*Rattus Norvegicus* strain Wistar) aged 8 weeks induced malnutrition by fasting for 3 days by providing drinking water *ad libitum*. A total of 28 rats were divided into four groups, control (C) group, P1 group receiving a zinc dose of 0.027 mg/1.8 g of biscuits, P2 group receiving a dose of zinc 0.054 mg/3.6 g of biscuits, and P3 group receiving a zinc dose of 0.081 mg/5.4 g of biscuits/BW/day. The intervention has been given for 14 days with a standard feed, 2 times per day. Determination of multilevel zinc dose based on the results of human conversion to mice (0.018) from biscuits made from pumpkin seed flour with zinc content of 1.5 mg, 3 mg, and 4.5 mg or 30%, 60%, and 90% of RDA for child, respectively.

Each group used two cages, meaning that one cage was filled with four rats and another cage was filled with three rats. In the end of the study, the rats were not terminated, but donated to the biopharmacy laboratory for further investigations. This study received ethics approval from the Health Research Ethics Committee, School of Public Health, Hasanuddin University (No. 15071945017).

Data analysis

Normality and homogeneity analysis were performed using SPSS v.21 (IBM Corp). Paired t-test and one-way ANOVA test were used to see differences in serum zinc levels and body weight between intervention groups and *post hoc* test analysis with the least significant difference (LSD) method to calculate the values of different groups. Interpretation of test results on groups with a significance value of $p < 0.05$ indicates there is a significant difference.

Results

Table 1 shows an increase in serum zinc levels in P2 group (0.054 mg dose) with mean zinc levels before intervention at 4.74 mg/L and after intervention increasing to 5.13 mg/L but not statistically significant.

After performing paired t-test analysis, we found no significant differences of zinc serum change in all intervention and control groups ($p > 0.05$). Based on the results of the one-way ANOVA test, the mean zinc levels between groups after intervention showed no significant difference where the $p = 0.481 > 0.05$.

Table 1: Analysis of zinc levels in Wistar rats before and after the intervention

Group	n	Serum zinc (mg/L)		Δ Mean	Change %	p*
		Pre-test Mean \pm SD	Post-test Mean \pm SD			
C (Control)	7	8.06 \pm 5.53	4.72 \pm 2.14	-3.33	41.31	0.085
P1 (Dose 0.027 mg)	6	10.25 \pm 9.18	4.73 \pm 1.18	-5.52	53.85	0.201
P2 (Dose 0.054 mg)	7	4.74 \pm 2.46	5.13 \pm 1.97	0.387	8.01	0.779
P3 (Dose 0.081 mg)	6	8.19 \pm 3.86	6.43 \pm 3.00	-1.76	21.48	0.510
		0.391	0.481			

*Paired t-test. **One-way ANOVA test.

Table 2 shows that there was an increase in body weight after intervention in each group. Based on paired t-test in each group, there was a significant difference between body weight before and after intervention with $p < 0.05$. Greater weight gain occurred in the P1 group (zinc dose 0.027 mg/1.8 g of biscuits) with an increase of 38.85%. Based on the results of the one-way ANOVA test, the average weight between groups after intervention showed a significant difference with $p = 0.040 < 0.05$.

Table 2: Analysis of weight in Wistar rats before and after the intervention

Group	n	Body weight (g)		Δ Mean	Change %	p*
		Pre-test Mean \pm SD	Post-test Mean \pm SD			
C (Control)	7	156 \pm 9.75	185 \pm 14.9	29	18.58	0.000
P1 (Dose 0.027 mg)	6	157 \pm 8.88	218 \pm 27.9	61	38.85	0.002
P2 (Dose 0.054 mg)	7	160 \pm 17.7	218 \pm 23.0	58	36.25	0.000
P3 (Dose 0.081 mg)	6	154 \pm 9.23	206 \pm 21.1	52	33.76	0.000
		0.865	0.040			

*Paired t-test. **One-way ANOVA test.

Table 3 shows in the *post hoc* LSD test analysis that there were significant differences in body weight between the control group and the P1 group (dose 0.027 mg) and P2 (dose 0.054 mg) with $p = 0.015$ and $p = 0.012$, while the other intervention groups did not differ significantly $p > 0.05$.

Table 3: Analysis of weight with *post hoc* tests LSD

Group	K	P1	P2	P3
C (Control)		0.015	0.012	0.105
P1 (Dose 0.027 mg)	0.015		0.998	0.369
P2 (Dose 0.054 mg)	0.012	0.998		0.351
P3 (Dose 0.081 mg)	0.105	0.369	0.351	

LSD: Least significant difference.

Discussion

This study showed that there was no effect of giving biscuits made from pumpkin seed flour to serum zinc levels in malnourished Wistar rats. However, this zinc-sourced biscuit could significantly increase weight of Wistar rats. Serum zinc levels are found to be low in children of protein energy malnutrition globally. Zinc supplementation during the rehabilitation phase of malnutrition has been linked to rapid weight gain [5].

This study found that there were no significant changes of zinc serum due to intervention. However, there was an increase in serum zinc levels in the P2 group who were given a zinc dose of 0.054 mg after the intervention. The zinc dose P2 group had the lowest average initial serum zinc level compared to the other groups. Zinc status is influenced by zinc absorption where low zinc levels will absorb zinc more efficiently than high zinc levels [19]. Zinc absorption is regulated by metallothionein which is synthesized in cells of the digestive wall. If zinc consumption is high, cells in the gastrointestinal wall are partially converted to metallothionein as a deposit and absorption is reduced. This form of storage will be removed with cells of the small intestine wall that is 2–5 days old [20].

In this study, Wistar was induced by fasting/not being fed for 3 days. Wistar rat that was malnourished before and after intervention showed that rat did not experience zinc deficiency. Deficiency is said if serum zinc level is $< 9.9 \mu\text{mol/L}$ or 0.65 mg/L [6]. During fasting, serum zinc concentrations increase due to release by muscles during catabolism; after eating, serum zinc levels decrease progressively due to hormonal changes and absorption of nutrient tissue induced by fuel metabolism [21].

Giving biscuits made from pumpkin seed flour for 14 days did not show significant results, but there was a tendency for an increase in the mean serum zinc level compared to the control group. Similar to Vakili *et al.* [22] study which showed that there was no significant difference in serum zinc levels for 6 months in primary school children, but there was a positive effect of 10 mg zinc supplementation on serum zinc levels compared with placebo.

Zinc absorption varies greatly and depends not only on the zinc content in food but also on the bioavailability of zinc. In this study, the feed given to the four groups was yellow corn which contained 2 g of dietary fiber and 0.46 mg/100 g of zinc. In the intervention group biscuits were added, containing 6.08 mg of calcium and 1.52 mg/100 g of zinc. Pumpkin seed flour used contains high iron which is about 10.43 mg/100 g.

Calcium, iron, fiber, and phytate can inhibit zinc absorption [19]. Phytate with zinc cation will form a strong and insoluble complex because the digestive tract is very deficient in phytase enzyme activity, zinc and phytate bonds will be removed through feces. Calcium has a tendency to form complexes with phytate and zinc and will be an insoluble form, causing inhibition of zinc absorption. In addition, intake of micronutrients with valence two such as iron can inhibit zinc absorption. Zinc and iron interactions first occur in the intestine. Interaction type between two is in the form of absorption pathway, iron transport protein on apical side of enterocytes is also known to be a transport protein for zinc, meaning that if the content of

one element is high it will affect the absorption of other elements [23].

Zinc minerals are included in trace minerals, meaning that relatively little is needed by the body. The body's need for zinc depends on adequate food regulation so that it can provide zinc for the purposes of various metabolic processes in the body. Dose used in this study 1.5 mg and 4.5 mg converted to mice was relatively lower than needed to improve zinc status, which might explain the smaller effect on increasing serum zinc levels than studies using doses that were greater than. The results of Akbar *et al.* [24] study showed that administration of 2.5 g of zinc sulfate for 14 days could increase serum zinc levels in rats.

Changes in plasma zinc concentration in the zinc supplementation group were significantly associated with age, with younger children having greater changes in plasma zinc concentration than older children. Giving a dose of 5 mg for 2 weeks at under 2 years significantly increased zinc plasma levels better than placebo [25]. Giving a dose of 10 mg/day for 3 months in children showed that there was a significant increase in serum zinc in the intervention group [26].

In normal circumstances, growth of rat at 5 g/day. Growth speed depends on the species, sex, age, and balance of nutrients in the ration. Statistical analysis showed that there were significant differences in weight gain in malnutrition Wistar rats before and after the intervention in each group. One of the factors that influence mouse growth is the quality of feed given. Weight gain is mainly caused by the balance of energy and protein and other nutrients contained in the feed [27].

After comparing with the control group, it was found that there was a significant difference in giving biscuits made from pumpkin seed flour for 14 days to the increase in body weight of malnutrition Wistar rats. Based on the results of the *post hoc* LSD test, there was a significant difference in body weight of malnutrition Wistar rats between the control group and group P1 (dose 0.027 mg) and P2 (dose 0.054 mg). Weight gain was greater in the P1 group with an increase of 38.85% compared to the P2 group with an increase of 36.28%. Similar to the research of Maukina *et al.* [28], there was an effect of giving 1.25 mg zinc syrup for 3 months on weight changes in infants with moderate PEM.

Greater weight gain in the P1 and P2 intervention groups resulted from biscuit supplemental food and increased appetite. Zinc supplementation stimulates increased consumption of some micronutrients and basal serum zinc and increases plasma alkaline phosphatase levels. An increase in appetite will increase the amount of calorie and protein intake so that with regular consumption the intake will increase compared to the irregular. Excess calories will be stored in the form of fat and protein tissue to increase muscle mass and minerals as enzymes for

growth [29], [30]. Research by Candra [31], on the effect of zinc supplementation on growth shows that giving zinc supplementation 10 mg/day for 3 months can improve appetite and nutritional status of weight-for-age in children.

In the process of growth, zinc plays a role in protein synthesis needed for new tissue formation, growth, and normal bone development [32]. Zinc induces neuropeptides in hypothalamus, GH secretion, endogenous GH sensitivity, GH bioactivity, and GH receptors. Zinc stimulates GHRH which in turn stimulates the pituitary somatotrophic area which secretes GH. Zinc is responsible for longitudinal bone growth especially in chondrocyte cells in epiphyses. Thus, zinc affects all mechanisms of cell and bone growth [29].

Zinc has many functions in the body and is very important for all types of animals, because it is involved in the function of various enzymes that have to do with carbohydrate metabolism, energy, degradation, and synthesis of proteins and nucleic acids [33]. Zinc can help the metabolic process and help the work of enzymes in body one of which will increase the function of enzymes in body. If the function of enzymes in body goes well, then intake of food enters the body can be easily absorbed and function optimally in helping growth process [20]. Based on the results of research Alves *et al.* [34] showed that zinc administration can increase energy, protein intake, carbohydrates, calcium, iron, and zinc.

Pumpkin seed flour biscuits are rich in nutrients, besides containing zinc, the nutrient content of 100 g of pumpkin seed biscuits made with about 534.7 kcal total calories, 48.16 g carbohydrates, 33.05 g fat, and 11, 20 g of protein. In group P1 received an energy intake of around 9.5 kcal/1.8 g of biscuit/3 ml, group P2 around 19 kcal/3.6 g of biscuit/6 ml, and group P3 around 28.5 kcal/5.4 g of biscuit/9 ml. The amount of food intake affects the amount of energy intake which is then stored as fat and ultimately has implications for weight gain from experimental animals. Consumption of a diet that is rich in carbohydrates and fats will cause an increase in the amount of fat deposited in adipose tissue, especially those under the skin and in the abdominal cavity. Any excessive and indirect amount of fat diet and carbohydrate used will be stored in adipose tissue in the form of triglycerides [35].

In this study, the high of dose in the P3 group did not differ significantly from control group; the results were similar to the study of Tsalissavri *et al.* [35] that the high-carbohydrate diet group did not differ significantly from the control group. High glucose will stimulate the satiety center located in the ventromedial nucleus in the hypothalamus. In addition, the results of Doherty *et al.* [36] showed that higher zinc doses were not associated with significant changes in anthropometric measurements, but were associated with significantly greater mortality in children who received high zinc doses (6.0 mg/kg) compared to

those who received low-dose zinc supplementation (1.5 mg/kg). From these findings, therefore, a high dose of zinc supplementation can probably exacerbate deficiency of other minerals by decreasing intestinal absorption. Zinc inhibits copper absorption and copper deficiency occurs in severe PEM.

Conclusions

The provision of biscuits made from pumpkin seed flour did not affect to increase in serum zinc levels in malnutrition Wistar rats so further research is expected to use different zinc doses. Meanwhile, the provision of biscuits made from pumpkin seed flour influenced increase in body weight of malnutrition Wistar rats. The highest weight gain was found in the P1 group by giving biscuits by 1.8 g in malnutrition Wistar rats with an average increase of 38.85%. For further research is worth investigating the possible to see the effect of giving biscuits made from pumpkin seed flour to weight gain in humans.

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