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The effect of pumpkin seed flour (*Cucurbita moschata* Durch) on zinc serum levels in malnourished Wistar rats☆

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Abstract
Objective: This study aims to determine the right dose of zinc in the administration of pumpkin seed flour to increase zinc levels in malnutrition Wistar rats.
Methods: This study was a true experiment, by designing pre and post-controlled group designs. Twenty-eight male Wistar rats were malnourished. Each group was given feed in the form of corn every day for 14 days of intervention. Data were analyzed using Paired *t*-test and ANOVA.
Results: The results showed significant body weight gain for all groups when comparing baseline ($p < 0.0001$, $p = 0.024$, $p = 0.035$, and $p = 0.008$, respectively, for C, PSF1, PSF2, and PSF3). However, there was no difference in body weight at the end of the intervention for all groups ($p > 0.05$).
Conclusion: This study concludes that pumpkin flour in malnourished Wistar rats increasing body weight and serum zinc levels. Further research is needed on effective doses which significantly increase serum zinc levels.

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Introduction

The prevalence of zinc deficiency varies per major region in developing countries. The regions that have the greatest risk of experiencing zinc deficiency are Africa, South Asia and Southeast Asia.[1] The prevalence of zinc deficiency especially in Africa and Asia Region ranges from 25.6% to 43.8%.[2] In Indonesia, studies from 12 provinces show the prevalence of zinc deficiency in infants reaching 36.1%.[3] Recently data based on Indonesia Basic Health Research show the prevalence of Stunting in Indonesia reaching 30.8%.[4] Stunting population can be used as an indirect indicator to assess the status of zinc in the population.[5,6]

Zinc deficiency is closely related to impaired growth and development of children, the possibility of impaired intellectual development, and an increased risk of diarrhea and pneumonia[7] and decreased immune function. Pumpkin seeds (*Cucurbita pepo*) have received considerable attention in recent years because of the nutritional and health values of pumpkin seeds. Yellow pumpkin is a plant that is easily found in Indonesia. However, the use of pumpkin seeds in the community is still minimal[8] and only uses fruit for food production.[9]

Studies on the administration of pumpkin seed flour to zinc deficiency are still very limited so we try to develop and utilize agricultural waste, namely pumpkin seeds into functional food which is expected to overcome nutritional problems. This study aims to determine the right dose of zinc in the administration of pumpkin seed flour to increase zinc levels in malnutrition Wistar rats.

Methods

Location and design of research

Pumpkin Seed Flour Making is carried out at the Food and Nutrition Laboratory of the Research Activity Center Hasanuddin University. This study was conducted in the Biopharmacy Laboratory of the Faculty of Pharmacy Hasanuddin University and examination of serum zinc levels of experimental animals was conducted at the Makassar Health Laboratory Center. This study was included in the type of true experimental design with pre- and post-controlled group.

Population and sample

The population in this study were Wistar rats. The sample in this study used male Wistar rats obtained from PT Abadi Jaya, Yogyakarta. The sample of the study was selected by simple random sampling amounting to 28 Wistar rats which were divided into 4 groups and each group consisted of 7 Wistar rats. The inclusion criteria in this study were male Wistar rats, 8 weeks old, healthy during the adaptation period (non-dull hair, fall, bald and active) and obtained from the same breeding place, the same feed. The exclusion criteria in this study are pain (appearance of dull hair, loss or bald and less active or inactive activity) and die during treatment.

Method of collecting data

Acclimatization period

The maintenance of experimental animals was carried out in the experimental animals in the Biopharmacy Laboratory of the Faculty of Pharmacy Unhas. Acclimatization of animals was carried out for 7 days to familiarize animals in experimental conditions and was given a standard 10% feed of body weight of rats and drinks in the form of clear water ad libitum. Each animal was placed in a cage consisting of 3–4 rats each.

Treatment of experimental animals

Prior to treatment, rats were fasted for 3 days to get malnutrition rats. This is based on a preliminary study conducted where mice after 3 days fasted, showed an average reduction in body weight of 20 g with serum zinc levels <0.01 μg/dL. Then the weight and initial blood sampling are performed after the malnutrition process to assess zinc levels. The dose given is a conversion dose from humans 5 mg/day, 10 mg/day, and 15 mg/day, namely a zinc dose of 0.09 mg/rats, 0.18 mg/rats, and 0.27 mg/rats. Of the three doses the best effect on increasing zinc levels in male Wistar rats was compared and compared with the control group.

Data analysis

Data processing is done using the SPSS program for windows with a significance level of $p < 0.05$. Data were tested for normality using the Shapiro–Wilk test. To see the effect before and after the treatment, a paired test was used. Data that were normally distributed were paired *t*-test while those that were not normally distributed were Wilcoxon Tests. To analyze the difference in dose effect from the four treatment groups, Independent *t*-test was performed. If the data are normally distributed, the one-way ANOVA test is performed. While those with abnormal distribution of data were tested using Kruskal–Wallis.

Research ethics

This study was conducted after obtaining recommendations for approval from the Ethics Committee of the Faculty of Public Health, Hasanuddin University with the number of letter: 6400/UN.4.14.8/TP.02.02/2019 dated July 29, 2019 and protocol number: 15071945017.

Results

Table 1 shows that the mean body weight of Wistar rats for all groups after being fasted (H-0) experienced weight loss compared to body weight before fasting. Significant weight gain was seen for all groups after treatment (day 15) compared to baseline (day 0) ($p < 0.0001$, $p = 0.024$, $p = 0.035$, $p = 0.008$, respectively, for K, PSF1, PSF2, and PSF3), where the PSF1 group with a Zn dose of 0.09 mg had the highest body weight of 193.45 g compared to other groups.

Based on ANOVA test results, it can be concluded that there were no differences in body weight between groups ranging from H-0 to H-15 ($p > 0.05$). Table 2 shows the mean

Table 1 Body weight before and after intervention in pumpkin seed flour or control group (gram).

Weight	C	PSF1	PSF2	PSF3	*p*-Value
Before fasting	179.47 ± 13.90	186.68 ± 16.71	198.50 ± 21.37	184.55 ± 18.60	0.264[a,b]
After fasting					
Day 0	156.14 ± 9.75	166.23 ± 13.45	171.17 ± 20.49	158.37 ± 16.09	0.270[b]
Day 3	166.71 ± 12.43	181.50 ± 15.21	180.71 ± 21.56	171.42 ± 16.04	0.313[b]
Day 6	182.42 ± 16.29	187.91 ± 18.52	192.14 ± 23.73	176.85 ± 16.52	0.484[b]
Day 9	184.42 ± 13.40	187.83 ± 17.38	191.71 ± 24.70	175.87 ± 15.07	0.431[b]
Day 12	177.28 ± 16.65	192.01 ± 23.09	185.15 ± 24.62	171.10 ± 12.24	0.265[b]
Day 15	185.80 ± 15.09	193.45 ± 25.85	186.64 ± 25.72	171.82 ± 11.94	0.291[b]
p-Value, days 0–15	0.000[a]	0.024[a]	0.035[a]	0.008[a]	

C: control, PSF1: Zn with doses 0.09 mg/kg BW, PSF2: Zn with doses 0.18 mg/kg BW, PSF3: Zn with doses 0.27 mg/kg BW.
[a] Paired *t*-test.
[b] One-way ANOVA.

Table 2 Zinc serum level baseline and endline in pumpkin seed flour or control group (mg/L).

Zinc	Baseline	Endline	*p*-Value	Δ Pre–post
C	8.06 ± 5.53	4.72 ± 2.14	0.085[a]	↓ 3.33 ± 4.29
PSF1	4.72 ± 3.04	6.77 ± 5.08	0.463[b]	↑ 2.04 ± 6.06
PSF2	6.65 ± 5.69	9.05 ± 11.45	0.499[b]	↑ 2.39 ± 10.99
PSF3	3.20 ± 2.37	6.56 ± 5.31	0.176[b]	↑ 3.35 ± 6.26
p-Value	0.220[c]	0.863[d]		0.133[d]

C: control, PSF1: Zn with doses 0.09 mg/kg BW, PSF2: Zn with doses 0.18 mg/kg BW, PSF3: Zn with doses 0.27 mg/kg BW.
[a] Paired *t*-test.
[b] Wilcoxon.
[c] One-way ANOVA.
[d] Kruskal–Wallis.

values of the highest serum zinc levels in the baseline group were in the K group which was 8.06 ± 5.53 mg/L and the lowest in the PSF3 group was 3.20 ± 2.37 mg/L.

There were no differences in serum zinc levels in the four groups after the intervention (endline) ($p > 0.05$). Meanwhile, based on statistical results for each intervention group (PSF1, PSF2, and PSF3) and control there were no significant differences ($p > 0.05$) between serum zinc levels at baseline and endline. In addition, there was a decrease in serum zinc levels from baseline to endline for control group, amounting to 3.33 mg/L, and an increase in serum zinc levels for all intervention groups, where the highest increase came from the PSF3 group which amounted to 3.35 mg/L and there were no differences in the mean serum zinc levels in all groups ($p > 0.05$).

Discussion

In this study it was found that the intervention of pumpkin seed flour in malnutrition Wistar rats with various dose variants showed a significant increase in body weight for all intervention groups. Meanwhile, the mean serum zinc levels in all treatment groups increased before and after treatment but did not show any significant difference for the three dose groups.

Zinc plays an important role in improving nutritional status. Zinc supplementation can increase BMI, body weight.[10] Weight gain in all groups resulted from increased appetite and increased IGF-1. Zinc generally plays a role in growth by combating growth hormone synthesis, the synthesis of Adenosine Dinucleosides (DNA), and Adenosine Ribonucleosides (RNA).[11] The results of this study indicate a significant weight gain before and after treatment both the intervention and control groups ($p < 0.05$). Other studies also support the results of this study which show that zinc supplementation significantly increases growth, reduces stunting and wasting.

The results of the study showed that the highest average weight gain was at a dose of 5 mg (0.09 mg rats) and the lowest at a dose of 15 mg (0.27 mg/rats). This is presumably because the group of rats at a dose of 15 mg (0.27 mg/rats) had the lowest serum zinc level at baseline compared to other treatment groups. Zinc is known to affect carbohydrate metabolism. Administration of pumpkin seed flour for 14 days statistically has not shown significant results. Administration of zinc sulfate for 14 days in mice (2.5 g zinc/kg) in vitro, showing a significant difference in serum zinc levels compared with controls.[12] Provision of zinc in the short and long term showed a positive effect on zinc levels in the body. Another study showed that the fortification group of zinc supplementation at a dose of 15 mg on the 15th day increased plasma zinc levels compared to placebo and the non-zinc fortification group. However, at the end of the study that was on the 29th day there was no difference in plasma zinc levels between all groups.[13]

Some factors inhibiting zinc absorption such as calcium, iron, fiber and phytate.[14] Phytate is a strong mineral inhibitor, including zinc, because phytate cannot be absorbed in human digestion, the bond cannot be absorbed because in the digestive tract there is no phytase enzyme.[15] In addition, the intake of micronutrients with valence 2, such as iron and calcium, is thought to inhibit zinc absorption. Calcium can inhibit zinc when it is in tandem with phytate. Calcium has a tendency to form complexes with phytate and zinc and will become an insoluble form, causing inhibition of zinc absorption.

The results of the serum zinc levels of the three treatment groups did not show any difference, possibly due to the high fiber content in pumpkin seeds and yellow corn that could interfere with zinc absorption. However, there was a tendency for an increase in serum zinc compared to controls. Besides being due to low intake of zinc sources of food and perhaps also low bioavailability can be a major cause of low serum zinc levels. Phytate molar ratio: high zinc can cause disruption of zinc absorption in the body.[15]

Based on the results of the study showed that the highest increase in serum zinc levels after treatment was in the PSF3 group (Zn dose 0.27 mg/rats from the conversion of 15 mg Zn), and the lowest in the PSF1 group (Zn dose 0.09 mg rats from the results conversion of 5 mg Zn). The PSF3 group had the lowest zinc levels at baseline. Zinc status is influenced by zinc absorption, where low zinc levels will absorb zinc more efficiently than high zinc levels. If more zinc is needed, more zinc is absorbed.[14]

Conclusions and suggestion

We conclude that administration of pumpkin seed flour to malnutrition Wistar rats can significantly increase body weight. In other hand, administration of pumpkin seed flour increases serum zinc levels, although it is not significant compared to the beginning of the study. Further research needs to be done on effective doses that can significantly increase serum zinc levels.

Conflict of interest

The authors declare no conflict of interest.

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