The combined effect of zinc and honey to increase hemoglobin and albumin levels in white rats induced by low protein diet

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ABSTRACT

Undernutrition is a type of malnutrition. Malnutrition can endanger human life, particularly that of children. Honey, which has a high nutritional value, is one of the components that can be utilized to alleviate malnutrition. Zinc tablets, in addition to honey, are thought to boost hemoglobin (Hb) and albumin levels. Zinc, as an antioxidant and catalyst for biochemical events, aids in organ healing, particularly by restoring the role of enzymes in the process of food metabolism in the body. This study was conducted as an experimental study with a pretest-posttest design and a control group. Thirty Wistar rats were randomly distributed into six groups of five rats. The average Hb and albumin levels in the group of rats differ significantly, both in the single treatment and the combination of normal rats and malnutrition control. The groups that were given zinc and honey once a day had the greatest rise in hemoglobin, which was 0.5 g/dl (3.10%). The groups who received zinc and honey twice a day had the greatest rise in albumin (1.99 g/dl) (163.11%).

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1. INTRODUCTION

Malnutrition is a malnourished condition consisting of undernutrition and overnutrition. United Nations International Children's Emergency Fund (UNICEF) [1]–[3] reported that malnutrition causes a threat to human life, especially in children. Wasted or thin children prevalence is 7.5% or 50.5 million children under 5 all over the world. In Indonesia, the mortality case of toddlers because of malnutrition massively occurs in the distribution area that is nearly prevalent all over the nation. The result of basic health research (Riskesdas) showed that there was a national-wide increase in undernutrition and malnutrition in toddlers, where the prevalence of under-mal in 2018 was 17.7%, consisting of 3.9% of undernutrition and 13.8% of malnutrition. When compared to the national prevalence in 2010 (17.9%) and in 2013 (19.6%), it showed a decrease. The change was, especially in the undernutrition prevalence of 4.9% in 2010, 5.7% in 2013, as well as 3.9% in 2018. However, undernutrition prevalence experienced a decrease of as much as 0.1% from 13.9% in 2013 to 13.8% in 2018 [4].

Various problems caused by malnutrition are the high birth rates with babies who have low birth weight (LBW) caused when pregnant mothers suffer from protein energy undernutrition (PEU) condition [5]–[9]. Pregnant mothers with PEU conditions will likely have three times bigger risks of suffering from anemia than those who have a good nutritional status [10]. Biochemically, malnutrition condition can be marked by the high number of oxidants in the body, while the natural antioxidant in the body can no longer

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among the groups. If data was not normally distributed, an alternative test the Kruskal-Wallis test was used. This research bears a meaning if the value is p < 0.05.

3. RESULTS AND DISCUSSION
3.1. Result
Malnutrition is a pathological condition, caused by low protein intake, in malnourished animals, one of which is marked by a decrease in body weight compared to normal [26]. Malnutrition is the main cause of disruption of metabolism in the body, including the absorption of food intake of macro and micro minerals such as ferro, zinc, and cobalt, will be disturbed. In addition to changes in metabolic enzymes, given the limited protein intake given to rats.

Pathological changes caused by malnutrition include the digestive tract, body fluid composition, tissue protein, liver and kidney function, hormones, and immune changes [27]. In this study, an analysis of the histopathological structure of the intestinal villi that affected changes in macro and micro food absorption was carried out, and some differences between normal rats; can be seen in Figure 1 and Figure 2. The results of histopathological analysis of normal rats and malnourished (low protein) rats’ liver is shown in Figure 1. PAS staining, objective lens 20, nucleus clearly visible, glycogen vacuole deposits, sinusoids still clearly visible. PAS staining, objective lens 20, nucleus, glycogen stores have decreased, vacuole, sinusoids are not clearly visible as shown in Figure 2.

![Figure 1. Histopathological analysis of normal rats’ liver](image1)

![Figure 2. Histopathological analysis of malnourished (low protein) rats’ liver](image2)
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Analysis of the pathological structure of the liver was carried out on malnourished rats compared to normal rats, which can be seen in Figure 3 and Figure 4. The results of histopathological analysis of intestinal villi of normal rats and malnourished (low protein) rats in Figure 3 and Figure 4. Hematoxylin and eosin (HE) staining, objective lens 20, Histological features of the intestinal villi preparations have not degenerated as shown in Figure 3. HE staining, objective lens 20, histological description of the preparation intestinal villi have degenerated to necrosis as shown in Figure 4.

Figure 3. The results of histopathological analysis of intestinal villi of normal rats

Figure 4. The results of histopathological analysis of intestinal villi of malnourished (low protein) rats

Changes in intestinal villi will interfere with the absorption of macro and micro food intake, including honey, and zinc, which will affect Hb, and albumin as can be seen in Table 1. The result of the research including body weight, Hb level, and albumin level can be completely viewed in Table 1.
Table 1. Bodyweight, Hb, and albumin level during research

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group (n=5)</th>
<th>p1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Bodyweight of malnutrition (g)</td>
<td>172.4±13.35a</td>
<td>99.5±10.16b</td>
</tr>
<tr>
<td>Bodyweight of post-treatment (g)</td>
<td>181.2±12.15a</td>
<td>92.5±5.35b</td>
</tr>
<tr>
<td>Increase%</td>
<td>5.08</td>
<td>-</td>
</tr>
<tr>
<td>Hb level of malnutrition (g/dl)</td>
<td>15.4±0.52</td>
<td>15.52±0.67</td>
</tr>
<tr>
<td>Increase%</td>
<td>4.40</td>
<td>-</td>
</tr>
<tr>
<td>Albumin level of malnutrition (g/dl)</td>
<td>2.78±0.09b</td>
<td>1.34±0.08b</td>
</tr>
<tr>
<td>Increase%</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.855</td>
<td>0.082</td>
</tr>
</tbody>
</table>

Description: Result presented in average ± SD, n = number of the rats, p1 = analysis result of one-way ANOVA among groups and Kruskal-Wallis, p2 = analysis result of pre and post paired t-test and Wilcoxon, a and b = different notation and the row showed a meaningful difference of p < 0.05. G1 = group of normal rats, G2 = group of malnourished control rats without treatment, G3 = group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc, G4 = group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey, G5 = group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day, G6 = group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day.

Data normality test using Shapiro-Wilk showed abnormal distributing data which p-value is p = 0.001 < 0.05 for malnutrition bodyweight, while p-value of p = 0.030 < 0.05 was gained for the bodyweight after treatment. The difference in the bodyweight average, Hb level, and albumin level was tested by using a one-way test of ANOVA and Kruskal-Wallis.

Test results of one-way ANOVA post hoc Mann Whitney showed an average meaningful difference in the weight of the malnourished rats, and after the treatment to the group of normal rats (G1), the group of malnourished rats treated with 0.99 mg/kg of rats body weight dose of zinc (G3), group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) while the amount of the five groups is p < 0.05. However, the weight of the malnourished rats and the post-treatment on the group of malnourished control rats without treatment (K2) did not have a meaningful difference (p > 0.05).

The analysis result showed an average meaningful difference in the Hb level of the group of malnourished rats, and after the treatment to the group of normal rats (G1), group of malnourished control rats without treatment (G2), group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) while the five groups are with p < 0.05. This result provides an image that honey and zinc treatment has an effect on the improvement of Hb levels. The Hb level of the group of malnourished rats pre and post-honey treatment with a dose of 12.5 ml/kg of rat’s body weight (G4) did not have a meaningful difference (p > 0.05).

The statistical test results showed an average meaningful difference in the albumin level of the group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), the group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) with p < 0.05. This result provides an image that honey and zinc treatment can improve the albumin level. In the group of malnourished control rats without treatment (G2), the average of albumin level did not have a meaningful difference (p > 0.05).

Statistical test results of one-way ANOVA and Kruskal-Wallis showed no meaningful difference of Hb level of the pre-treated rats among the groups to the group of normal rats (G1), group of malnourished control rats without treatment (G2), group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) with p > 0.05.
The statistical test results showed a meaningful difference in the data of the Hb level among the group of post-treatment; normal rats (G1), group of malnourished control rats without treatment (G2), group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) with p < 0.05.

There was a statistically-wise meaningful difference in the albumin level among the group of post-treatment; normal rats (G1), the group of malnourished control rats without treatment (G2), the group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), the group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) with p < 0.05.

The analysis result showed that there was a meaningful difference in post-treatment albumin levels among a group of post-treatment; normal rats (G1), the group of malnourished control rats without treatment (G2), the group of malnourished rats treated with 0.99 mg/kg of rat’s body weight dose of zinc (G3), the group of malnourished rats treated with 12.5 ml/kg of rat’s body weight dose of honey (G4), group of malnourished rats treated with 50% dose of zinc and honey, 1 x a day (G5), and a group of malnourished rats treated with 50% dose of zinc and honey, 2 x a day (G6) with p < 0.05.

Statistical test of two-sample Wilcoxon rank-sum post hoc Mann Whitney between the control group and treatment group showed a significant difference in the Hb level average of the group of normal control (G1) compared to the malnourished group (G2) with p = 0.008. The Hb level average between the group of normal control (G1) and the group of treatment with zinc supplement administration (G3) does not have a meaningful difference (p = 0.169). A meaningful difference in Hb level was found between the group of normal control (G1) and the group of treatment with honey administration (G4) with p = 0.027. The Hb level average between the group of normal control (K1) and the group of treatment with the combination of zinc and honey once-a-day administration (G5) does not have a meaningful difference (p = 0.342). A meaningful difference in Hb level was found between the group of normal control (K1) and the group of treatment with the combination of zinc and honey two times a day administration (G6) with (p = 0.035).

Statistical test of two-sample Wilcoxon rank-sum post hoc Mann Whitney between the malnourished group and treatment group showed a meaningful difference in the Hb level of the group of malnutrition (G2) and the group of treatment with zinc administration (G3) with (p = 0.008). A meaningful difference in the Hb level was found between the group with malnutrition (G2) and the group treated with honey administration (G4) with (p = 0.008). A meaningful difference in the Hb level was found between the group of malnutrition (G2) and the group treated with a combination of zinc and honey supplementation administration once a day (G5) with p = 0.008. A meaningful difference in the Hb level between the group of malnutrition (G2) and the group treated with a combination of zinc and honey supplementation twice a day (G6) with (p = 0.046).

Statistical test of two-sample Wilcoxon rank-sum post hoc Mann Whitney between the group of treatment with zinc administration and other groups showed an average of Hb level between the group with zinc administration (G3) and the group of treatment with honey administration (G4) did not have a meaningful difference (p = 0.402). The Hb level average between the group of zinc (G3) and the group of treatment with the combination of zinc and honey administration (G5), the once-a-day administration did not have a meaningful difference (p = 1.117). The Hb level average between the group of zinc (G3) and the group of treatment with the combination of zinc and honey administration (G6), the twice-a-day administration did not have a meaningful difference (p = 0.402).

Statistical test of two-sample Wilcoxon rank-sum post hoc Mann Whitney between the group of treatment with honey administration and other groups showed a meaningful difference in Hb level between the group of treatment with honey administration (G4) and the group of treatment with the combination of zinc and honey (G5), the administration is 1 x a day with (p = 0.028). The meaningful difference in Hb level between the group treatment with honey administration (G4) the group treatment with the combination of zinc and honey (G6), and the twice-a-day administration is with (p = 0.832). A meaningful difference in Hb level was found between the group of treatment with the combination of zinc and honey (G5), with 1 x administration, and the group of treatment with the combination of zinc and honey (G6) twice-a-day administration with (p = 0.036).

The statistical test result of Mann Whitney proved that there was an average meaningful difference of Hb level between the control group (G1), the malnourished group (G2), and the group that got a treatment of zinc supplementation administration (G3), the group of honey supplementation treatment (G4), the group of treatment with honey and zinc combination 1 x a day (G5), and the group of treatment with honey and zinc combination 2 x a day (G6) with p = 0.02.
Statistical test of one way ANOVA Bonferroni test between the control group and treatment group showed the result that there was an average meaningful difference of the albumin level between the group of normal control (G1) in comparison with the malnutrition group (G2) with \( p = 0.000 \). The albumin level difference was meaningfully found between the group of normal control (G1) and the group of treatment with zinc administration (G3) with \( p = 0.001 \). The albumin level difference was meaningfully found between the group of normal control (G1) and the group of treatment with honey administration (G4) with \( p = < 0.001 \). The albumin level average between the group of normal control (G1) and the group of treatment with the combination of zinc and honey administration 1 x a day (G5), did not have a meaningful difference \( (p > 0.05) \). The albumin level difference was meaningfully found between the group of normal control (G1) and the group of treatment with the combination of zinc and honey administration 2 x a day (G6) with \( p = 0.001 \).

Statistical test of one way ANOVA Bonferroni test between the malnourished group and treatment group showed a result of a meaningful difference in the average of the albumin level in the group of malnutrition (G2) in comparison with the group of treatment with zinc administration (G3) with \( p = 0.000 \). The albumin level difference was meaningfully found between the group of malnutrition (G2) and the group treated with honey administration (G4) with \( p = 0.000 \). The albumin level difference was meaningfully found between the group of malnutrition (G2) and the group of treatment with the combination of zinc and honey administration 1 x a day (G5) with \( p = 0.000 \). The albumin level difference was meaningfully found between the group of malnutrition (G2) and the group of treatment with the combination of zinc and honey administration 2 x a day (G6) with \( p = 0.000 \).

Statistical test of one way ANOVA Bonferroni test between the group of treatment with zinc administration and the other treatment groups showed a result that there was no meaningful difference in the albumin level average between the group with zinc administration (G3) and the group of treatment with honey administration (G4) with \( p = 0.001 \). The albumin level difference was meaningfully found between the group with zinc administration (G3) and the group of treatment with the combination of zinc and honey (G5) administration 1 x a day with \( p = 0.017 \). The albumin level difference was meaningfully found between the group with zinc administration (G3) and the group of treatment with the combination of zinc and honey (G6) administration 2 x a day with \( p = 0.000 \).

Statistical test of one way ANOVA Bonferroni test between the group of treatment with honey administration and the other treatment groups showed a result that there was no meaningful difference in the albumin level between the group of treatment with honey administration (G4) and the group of treatment with the combination of zinc and honey (G5) administration 1 x a day with \( p = 0.077 \). The albumin level difference was meaningfully found between the group treated with honey administration (G4) and the group treated with the combination of zinc and honey (G6) administration 2 x a day with \( p = 0.000 \). The albumin level difference was meaningfully found between the group of treatment with the combination of zinc and honey (G5) administration 1 x a day and the group of treatment with the combination of zinc and honey (G6) administration 2 x a day with \( p = 0.000 \).

3.2. Discussion

Malnutrition conditions greatly affect metabolism, some of the criteria for malnutrition observed in this study are histopathological features of the liver and intestinal villi. Under conditions of malnutrition, there has been a difference in worsening compared to normal conditions.

3.2.1. Effect of treatment on the change of Hb level

Hb is the main component of the erythrocyte and it is a type of protein that contains a lot of zinc, it plays an important role in carrying oxygen from the lungs into all parts of the body’s system [28]. The synthesis of Hb is influenced not only by the availability of zinc substance; but also, by the sufficiency of protein. Protein deficiency in daily food intake can cause synthesis problems in the Hb [27]. The result of the Hb level measurement; showed that there are various improvements in the Hb level among the groups of the administration treatment of zinc, honey, the combination between zinc and honey, and the control group.

After 21 days of malnutrition treatment, the rat’s condition showed an average increase in the Hb level of each group, either on the treatment group with honey administration; treatment group with zinc administration; treatment group with a combination of honey and zinc administration 1 x a day; treatment group with a combination of honey and zinc administration 2 x a day, and control group. The group with the highest average of Hb improvement is the group treatment with a combination of honey and zinc administration 1 x a day, which improves to 0.5 gr/dl (3.10%). On the other hand, the group with the lowest average of Hb level improvement is the group of treatment with honey administration (2.7%).

The Hb level change that was not really different was probably caused by a difference in the metabolism speed of each tested animal to recover, either on malnutrition condition or treatment condition of
pre-malnutrition. The level of immunity and adaptation of the tested animals of one and also a contribution to the recovery of their condition, including consuming the provided food and drink intake. Furthermore, the honey used had a lower acidity level than Indonesian National Standard (SNI) honey and other researchers’ honey composition [28].

At the administration of zinc and honey combination 2 x a day, the improvement of Hb was lower than the administration of zinc and honey combination 1 x a day or than the administration of single zinc without honey. This was probably because the zinc absorption, administered at the same time with a more dose of honey (2 x a day) will affect the zinc absorption, considering that absorption is influenced by acidity. Honey in this research is acid (1.86) compared to SNI honey (3.9). It will influence the process of zinc substance absorption which is administered at the same time [29], [30]. However, zinc absorption will also be disrupted with iron (Fe++) obtained in the honey, both competing with each other chaining with metallothionein [31]. Zinc absorption in the intestine is also influenced by transferrin. The similarity of a transporter between iron and zinc causes the absorption between iron and zinc affects one another [32].

The combination of Fe in the administered honey and zinc will depend on the availability of transferrin, consequently, it hinders zinc absorption [33]. Furthermore, the total content of sugar within honey in this research was (76.15%), which is considered high, belonging to the same group as apples and pears. The high amount of total sugar can cause zinc absorption problems, thus the Hb level did not improve with the combination of zinc and honey administration 2 x a day [34].

3.2.2. Effect of treatment on albumin level

Albumin level in the plasma is closely related to protein saving in the body and the consumed food intake. Albumin is synthesized in the liver; the amount is around 52%–65% of the total protein in the blood plasma, consequently, a decline of albumin level within the plasma can be an indicator of protein deficiency in the body. One of the factors that can cause the change in albumin levels is malnutrition condition [27].

The albumin level measurement result; shows the albumin level among the groups of zinc administration treatment, honey, zinc, and honey combination, and the control group experiences various improvements. After 21 days of treatment, it shows an average improvement of albumin level in each rat group, either in the honey administration treatment group, zinc administration group, the combination of zinc and honey administration 1 x a day group, and a group of treatment with the combination of zinc and honey administration 2 x a day, as well as a control group. However, the group with the highest improvement in the albumin level is the group treatment with zinc and honey administration 2 x a day as much as 1.99 gr/dl (163.11%).

The reality showed that the administration of zinc and honey combination was not better than that of single honey to improve the albumin level. It will rapidly improve the administration of zinc and honey that was administered 2 x a day. This was in accordance with the literature that less than 40% of albumin is in the blood circulation; the remaining is in the systems of muscles, skin, and intestines. Albumin plays a role in managing body liquid distribution and has a characteristic of a means of transportation throughout the body. The 2 times a day frequency of zinc and honey administration will influence the albumin level improvement, along with food administered during the treatment. AIN-93 growth that is rich with protein (20%). Tsutsumi [22] stated that the improvement of protein level is on the same level as that of the albumin level. Albumin is zinc’s main means of transportation. Zinc absorption will decline if the value of blood albumin declines. In this research, zinc was administered at a 2 x a day dose that would optimally help albumin improvement.

4. CONCLUSION

The administration of zinc and honey to the malnourished rats could improve their Hb level. Group administered a combination of 50% zinc from 0.99 mg/kg rat's bodyweight and 50% honey from 12.5 ml/kg rat's bodyweight 1 x a day had the highest Hb improvement of 0.5 g/dl (3.10%). The administration of zinc and honey to the malnourished rats could improve their Hb level. Group administered a combination of 50% zinc from 0.99 mg/kg rat's bodyweight and 50% honey from 12.5 ml/kg rat's bodyweight 2 x a day had the highest Hb improvement of 1.99 g/dl (163.11%). To increase Hb, it can be given through food intake, but it is necessary to pay attention to the presence of other compounds and minerals that can inhibit the absorption of ferrous. That can inhibit the absorption of ferrous. The combination of zinc and honey has an effect on increasing Hb which is proportional to the increase in serum albumin levels.

REFERENCES

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