The Effect of Moringa Oleifera Leaves Plus Royal Jelly Supplement on Cortisol Hormone and Stress Levels on Anemia of Pregnant Women in Takalar Regency

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Abstract
This study aims to determine the effect of Moringa Oleifera plus Royal Jelly leaf extract supplements on stress levels and cortisol levels in anemic pregnant women. This study was a true experimental study with a controlled randomized double blind pretest-post test design. The subjects of this study were 44 pregnant women with anemia, taken based on inclusion and exclusion criteria and divided into two groups, namely the group given moringa capsules (n = 22) and moringa capsules plus royal jelly (n = 22). Data were analyzed using Chi-square test, Paired T-Test, Wilcoxon and Independent T-Test using SPSS. The result of the study shows a significant decrease in the stress level in the royal jelly moringa group from 29.23 ± 10.451 to 17.00 ± 6.325 with a value of P = 0.000 and in the moringa group from 23.59 ± 8.110 to 18.05 ± 4.498 with a value of P = 0.022. Likewise, the cortisol levels decreased significantly in the moringa royal jelly group from 41.17 ± 18.466 to 28.75 ± 15.024 with a P value = 0.002 and the moringa group from 34.54 ± 21.437 to 24.70 ± 18.218 with a P value = 0.009. However, the difference in the magnitude of change did not differ significantly for stress levels and cortisol levels (P> 0.05). It is concluded that the reduction in stress and changes in royal jelly moringa tends to be greater than that of moringa.

Introduction
Anemia is better known to the public as a blood deficiency disease that is prone to all life cycles. Anemia is defined as a condition in which a low concentration of hemoglobin (Hb) or hematocrit based on a threshold value is caused by low production of red blood cells (erythrocytes) and Hb, increased erythrocyte damage (hemolysis) or excessive blood loss (Citrakesumasari, 2012). Anemic pregnant women are prone to stress and increased cortisol levels due to psychological changes (Shapiro et al., 2013)

According to the results of the 2018 Basic Health Research, it shows that the number of pregnant women with anemia in Indonesia has increased from 2013 (37.1%) to (48.9%) (Riskesdas, 2018). Based on the 2013 Basic Health Research, the prevalence of anemia is around 21.7%, and the prevalence of iron deficiency anemia in women aged 15-50 years in
Indonesia is around 33.1% and the percentage of anemia in South Sulawesi Province (48.7%) (Kemenkes, 2013).

In order to overcome anemia, the government has launched a program to provide blood-added tablets to pregnant women and women of childbearing age as outlined in the regulation of the Minister of Health of the Republic of Indonesia number 88 of 2014 concerning the standard of blood added tablets for women of childbearing age and pregnant women.

WHO has introduced Moringa leaves as a plant and alternative type of food to help with nutritional problems in the world. Moringa as a vegetable for modulation of human immunity and its capacity for protection against disease results that moringa can prevent 300 types of diseases and its leaves have been used for preventive and curative purposes and can be made in the form of nutritional supplements in developing countries (Ganguly, 2013). In addition to innovation in the development of moringa plants, in Indonesia the science and art of extending and maintaining health can also be developed by using products obtained from honey bee hives such as honey, bee hives, bee venom, bee pollen, propolis and royal jelly.

Moringa leaf extract supplementation as a natural supplement in addition to iron sulfate can help treat iron deficiency anemia and as an adjunct therapy to increase the average hematocrit (Suzana et al., 2017). Royal Jelly also contains many minerals contained in pollen to stimulate cell regeneration, enzyme systems and hematopoiesis, antioxidants, immunomodulatory, hepatoproactive and anti-anemia (Curr & App, 2018). Royal Jelly can increase corticosterone levels and increase the antioxidant system in stressful conditions (Teixeira et al., 2017). This study wanted to see whether giving moringa royal jelly was better than giving moringa alone.

Methods

This type of research is a true experimental study with a controlled randomized double blind pretest-post test design. This research was conducted in Takalar Regency, precisely in the Work Area of the North Polongbangkeng District Health Center which was conducted on 18 July-18 September 2020. The population in this study were all anemia pregnant women in Polongbangkeng Utara District, Takalar Regency. A sample of 44 anemia pregnant women was selected by purposive sampling that met the inclusion criteria and obtained 44 respondents and divided into two groups of 22 respondents, with the first group being given Moringa capsules plus royal jelly and the second group given Moringa capsules. Data collection was carried out by researchers by conducting characteristic interviews regarding identity, age, parity, occupation, education, food intake (consumption of 1x24 hours recall), stress levels using the Kessler Psychology Distress (K10) questionnaire and measuring the circumference of the upper arm using the LILA tape. Saliva sampling to measure cortisol levels using the ELISA method. 1x24 hour recall consumption data using Nutrisurvey. The data analysis used the SPSS program. The data in this study used the Chi-square test on univariate analysis and paired t-test, Wilcoxon test, and independent t-test on the bivariate analysis.

Results and Discussion

This study was conducted in a region with a high prevalence of anemia in pregnant women. Table 1 shows that all the characteristics of the research respondents did not differ significantly. Most of the respondents who were given Moringa capsule extract plus royal jelly and Moringa capsule extract were aged 20-35 years with the percentages of 81.8% and 72.7%. Primigravida category with a percentage of the Moringa capsules plus royal jelly group of 81.8% and 68.1% of the Moringa capsules, the Education category graduated from
high school and above with a percentage of 77.3% for respondents with royal jelly capsules and 63.6% for respondents who were given capsules Moringa, did not work with a percentage of 77.3% for respondents who were given Moringa capsules plus royal jelly and 90.9% for respondents who were given Moringa capsules. low income or below the minimum wage per month, the percentage, among others, for the group of respondents who were given moringa capsules plus royal jelly was 81.8% while for the group of respondents who were given moringa capsules was 95.5%

Table 1. Frequency Distribution Based on Characteristics

| Characteristics          | Moringa Leaf Extract Plus Royal Jelly (n=22) | Moringa Leaf Extract (n=22) | P-Value |
|--------------------------|---------------------------------------------|-----------------------------|---------|
|                          | n   | %         | n   | %         |         |
| Age 20-35 years          | 18  | 81.8      | 16  | 72.7      | 0.541*  |
| <20 & > 35 years         | 4   | 18.2      | 6   | 27.3      |         |
| Parity Primigravida      | 18  | 81.8      | 15  | 68.1      | 0.263*  |
| Multigravida             | 4   | 18.2      | 7   | 31.8      |         |
| Education ≥ High School  | 17  | 77.3      | 14  | 63.6      | 0.613*  |
| <High School             | 5   | 22.7      | 8   | 36.4      |         |
| Employment Unemployed    | 17  | 77.3      | 20  | 90.9      | 1.000*  |
| Employed                 | 5   | 22.7      | 2   | 9.1       |         |
| Income ≤ 3.100.000       | 18  | 81.8      | 21  | 95.5      | 0.182*  |
| ≥ 3.100.000              | 4   | 18.2      | 1   | 4.5       |         |

*Chisquare Source: Primary data, 2020

Based on table 2, it can be seen that there is no significant difference from the nutritional conditions of the two groups on the intake variable (micro and macro nutrients), HB and upper arm circumference where the value is (P> 0.05).

Table 2. Nutritional conditions of respondents in both groups from intake, HB and upper arm circumference (LILA)

| Respondents intake | Moringa Leaf Extract Plus Royal Jelly (n=22) | Moringa Leaf Extract (n=22) | P-Value |
|--------------------|---------------------------------------------|-----------------------------|---------|
|                    | Mean (±SD)                                  | Mean (±SD)                  |         |
| Macro Nutrients    |                                             |                             |         |
| Energy             | 1268.4±2583.912                             | 1222.30±2448353             | 0.616   |
| Protein            | 49.295±10.3489                              | 48.48±10.775                | 0.792   |
| Carbohydrate       | 198.96±39.885                               | 187.50±61.802               | 0.469   |
| Fat                | 33.17±15.273                                | 31.44±20.742                | 0.753   |
| Micro Nutrients    |                                             |                             |         |
| FE                 | 5.00±1.5493                                 | 5.04±1.728                  | 0.927   |
Bivariate Analysis

Table 3 shows that at the beginning of the intervention there was no difference in the average stress level of the two groups of respondents with a significance value (0.53) or a value (p> 0.05). However, after the intervention was given, there was a decrease in the average stress level of the two groups. The Moringa plus Royal Jelly group experienced a decrease in average from 29.23 ± SD 10.451 to 17.00 ± SD 6.325 with a difference of 12.23±8.395. This means that statistically significant changes in the average stress level of respondents in the Moringa plus royal jelly group have a significant difference, where the value (P = 0.000) or value (P <0.05). The Moringa group also experienced a decrease in the average stress level from 23.59± SD 8.110 to 18.05 ±SD 4498 with a difference of 5.545 10.523 and statistically the stress level in the moringa group had a significant difference where the value (P = 0.022) or value (P> 0.05).

Table 3. The difference between changes in stress levels for pregnant women before and after giving Moringa capsules plus Royal Jelly and Moringa capsules in the working area of the Community, North Polongbangkeng District

| Variable                  | Pre Mean ± SD | Post Mean ± SD | Difference Mean ± SD | P Value |
|---------------------------|---------------|----------------|----------------------|---------|
| Stress level              |               |                |                      |         |
| Moringa Plus Royal Jelly  | 29.23±10.451  | 17.00±6.325    | 12.23±8.395          | 0.000*  |
| Royal Jelly group         |               |                |                      |         |
| Moringa group             | 23.59±8.110   | 18.05±SD 4498  | 5.545±10.523         | 0.022*  |
| P Value                   | 0.53**        | 0.53**         | 0.195**              |         |

*Paired T-Test
**Independent Sampel Test

Table 4 shows that at the beginning of the intervention there was no difference in the mean cortisol levels of the two groups of respondents with a significance value (0.27) or a value (p> 0.05). However, after the intervention was given, there was a decrease in the mean cortisol levels of the two groups. The Moringa plus Royal Jelly group experienced a decrease in average from 41.17 ± SD18.466 to 28.75 ±SD15.024 with a difference of 12.42. This means that statistically significant changes in the mean cortisol level of respondents in the Moringa plus royal jelly group have a significant difference, where the value (P = 0.002) or value (P <0.05). The Moringa group also experienced a decrease in the average cortisol level from 34.54 ±SD21,437 to 24.70 ±SD18,218 with a difference of 10.84. Statistically, the cortisol level in the moringa group had a significant difference in changes where the value (P = 0.009) or value (P> 0.05).

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Table 4. The difference in changes in cortisol levels for pregnant women before and after giving Moringa capsules plus Royal Jelly and Moringa capsules in the working area of the Community Health Center (Puskesmas), Kecamatan Polongbangkeng Utara

| Variable                  | Pre Mean ± SD       | Post Mean ± SD      | Difference Mean±SD | P Value |
|---------------------------|---------------------|---------------------|--------------------|---------|
| Cortisol levels           |                     |                     |                    |         |
| Moringa Plus Royal Jelly  | 41.17±SD18,466      | 28.75±SD15.024      | 12.42±SD16,220     | 0.002*  |
| Group                     | 34.54±SD21.437      | 24.70±SD18.218      | 9.837±SD16,009     | 0.009*  |
| P Value                   | 0.27**              | 0.42**              | 0.582**            |         |

*Paired T Test
*Independent Sample Test

In this study it was reported that there were no differences in characteristics in terms of age, parity, occupation, recent education, and income. According to (Astriana, 2017) aged <20 and > 35 years are at risk of suffering from anemia because they are not biologically ready to get pregnant and experience biological decline. Pregnant women who give birth for the first time usually have difficulty adapting to their pregnancy, which is related to knowledge and experience, which makes pregnant women with high parity less prone to anemia (Xu et al, 2016). High education does not guarantee that respondents have good knowledge about anemia (Nur et al, 2018). Pregnancy with anemia is also still common in women with low socioeconomic conditions and a lack of work (Noviyanti et al, 2019)

This study shows that there is no difference in the intake and upper arm circumference of pregnant women with anemia from the two groups. According to (Yuliasutri, 2014), when a woman is pregnant, she needs more nutrients than women who are not pregnant. Pregnant women also suffer from anemia due to unbalanced consumption patterns, for example consuming more rice and fish than vegetables and fruit (Patimah, 2011).

The government has launched a program to provide blood-added tablets to meet the needs of micro-nutrients for pregnant women and young girls, but WHO itself has introduced Moringa as a solution to prevent anemia on a global level (Ganguly, 2013). Moringa leaves are a local plant that contributes to the prevention of anemia. Apart from moringa, Indonesia also introduces the art of maintaining health from honey and bees and its products, for example Royal Jelly which functions to maintain the health of the human body and contains high polyphenols in suppressing stress.

In this study, consumption of royal jelly combined with moringa had more effect on stress levels and cortisol levels. This is in line with research Morita et al., (2012) which is consumption of royal jelly for six months can improve erythropoiesis, glucose tolerance and mental health. This is in line with the results of research from (Ito et al., 2012) states that Royal Jelly has biological activity on various cells, one of which is this immunomodulator which has effectiveness in improving symptoms of depression and anxiety caused by stress and is one suggestion to be used as an anti-depressant tool.

Moringa plant also has many benefits for anemic pregnant women in relation to stress patterns and increased cortisol. Research results by Muis (2014) stated that stress levels decreased significantly in the group of pregnant women workers in the informal sector who were given Moringa leaf extract. This research is in line with (Hasni, 2018) which states that
there is an effect of giving Moringa leaf flour on stress levels and cortisol levels for pregnant women.

Moringa content is superior to other vegetables because there are high chemical substances from moringa, namely polyphenols which are sedating in nature which can be used to deal with stress during pregnancy. In addition, moringa contains GABA (gamma-aminobutyric acid) which is a non-essential amino acid that helps maintain normal brain function by blocking stress-related impulses and reaching receptors in the central nervous system (Hasni, 2018).

**Conclusion**

There was a significant effect and a decrease in mean levels of cortisol and stress levels before and after the intervention of moringa capsules plus royal jelly and moringa capsules in anemic pregnant women. It was seen that the decrease in the Royal Jelly group was greater than in the group receiving moringa. The provision of royal jelly moringa needs to be investigated further with a larger number of samples in order to see a real difference between the groups receiving moringa royal jelly and moringa alone.

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