EFFECT OF ROSELLE (HIBISCUS SABDARIFFA) ON CHANGES IN HEMOGLOBIN LEVELS IN PREGNANT WOMEN WITH ANEMIA TAKING IRON SUPPLEMENT

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Abstract
Background: Anemia during pregnancy is one of the most common disorders in pregnant women in Indonesia. The Government has made efforts to overcome this problem, however, the rate of anemic mothers remains high. Rosella (Hibiscus Sabdariffa) is considered able to increase the hemoglobin levels in pregnant mothers.

Objective: To analyze the effect of Rosella flower extract (Hibiscus Sabdariffa) on the increase of Hemoglobin level in pregnant women with anemia receiving Fe tablet.

Methods: This study was a quasi-experiment with pretest-posttest control group design conducted in November - December 2016 in the working area of Tlogosari Wetan Community Health Center. Forty-two participants were selected using accidental sampling, which 21 assigned in the experiment and control group. All samples were pregnant women in the second trimester suffering from anemia and receiving iron tablets. Hemoglobin levels were measured using hematology analyzer in laboratory. Independent t-test and paired t-test were used for data analysis.

Results: Paired t-test obtained p-value 0.00 (<0.05) indicated that there was an increase of hemoglobin levels in both experiment and control group. The mean increase of hemoglobin levels in the control group was 0.61 gr/dl and in the experiment group was 1.08 gr/dl. The hemoglobin levels in the experiment group were higher than the levels in the control group. Independent t-test obtained p-value 0.000 (<0.05) indicated that there was a significant difference of mean of hemoglobin levels between the control group and the treatment group.

Conclusion: The consumption of rosella extract combined with Fe tablet showed a significant increase of hemoglobin levels compared with the consumption of Fe tablet alone. Therefore, it is suggested for midwife to use the result of this research as evidence practice through counseling for pregnant mother about utilization of rosella extract that can increase hemoglobin level in pregnant woman with anemia.

Keywords: Rosella extract; hemoglobin level; pregnant mother; anemia; Fe tablet

INTRODUCTION
Anemia is one of the most common disorders during pregnancy. Anemia in pregnancy is a condition of blood hemoglobin levels < 11 g/dL in the first and third trimester, and <10.5 g/dL in the second trimester (Cunningham, Leveno, Bloom, Spong, & Dashe, 2014). Physiologically, maternal blood circulation during pregnancy will have an increase of blood volume, which the amount of blood serum is greater than blood cell growth, resulting in blood thinning (hemodilution) beginning at 16 weeks of gestation and peak at 32-36 weeks of gestation (Cunningham et al., 2014).

The most common anemia in pregnancy is due to iron deficiency and acute hemorrhage, which is often related each other. The consequences of anemia in pregnancy vary, from very mild complaints to the occurrence of impaired pregnancy severity (abortion, immature part/premature), labor impairment...
(uterine inertia, uterine atony, old partus), puerperal disorders (uterine subclinical, resistance to infection and low milk production), and impairment of the fetus (abortion, dysmaturity, microsomy, low birth weight, perinatal death, etc.) (Cunningham et al., 2014; Manuaba, 2010).

According to the World Health Organization (WHO), the prevalence of anemia in pregnant women was 41.8% in the world, and Asia ranks second in the world with percentage of prevalence of anemia of 48.2% of pregnancies (WHO, 2012). Based on data of Riskesdas, the prevalence of anemia in pregnant women in Indonesia amounted to 37.1%, and in Central Java Province was 57.7%; while in the City of Semarang the prevalence of anemia in 2013 was 20.01%, in 2014 was 18.86% and in 2015 was 18.34%. It tells that the prevalence of anemia has decreased slightly in the last three years (Kementerian Kesehatan, 2013). Data from January to March 2016, the incidence of anemia in pregnant women in Semarang city was highest in the Public Health Center of Tlogosari Wetan with 174 cases, followed by the Public Health Center of Bandarharjo with 144 cases and Public Health Center of Mijen with 115 cases of anemia (Dinkes, 2014).

The government has made efforts to overcome the problem of anemia in Indonesia by launching the distribution of Fe tablet to health service to be distributed to all pregnant women for free. The distribution includes one of the achievement targets in the Antenatal Care (ANC). Four time of ANC visits is considered sufficient, with one-time per trimester and twice in the last trimester (Manuaba, 2010). Provision of at least 90 iron tablets during pregnancy is also one of the operational applications of minimum standard of "7T" for antenatal care (Depkes, 2007).

The government program in regarding to the distribution of Fe tablets for pregnant women is almost reaching to the national target, and the compliance of pregnant women in consuming Fe tablets is good enough. The coverage of 90 iron tablets in pregnant women in Semarang city from 2013 until 2015 has reached the expected target (96%), which was 96.36% in 2013, 97.23% in 2014, and 97.05% in 2015 (Dinkes, 2014).

In addition, the efforts made by the Government of Semarang city to decrease the incidence of anemia are through the formation of team work in Maternal-Child Health, local regulation of safety of mother and child, cooperation with universities in high risk pregnant women, recruitment of Health Surveillance (GASURKES) for data collection and mentoring pregnant women, as well as activities of assisting pregnant women until childbirth by health cadres (Dinkes, 2014). However, these efforts did not give a significant decrease of anemia incidence in Indonesia, particularly in Central Java Province.

Inhibition factors of iron absorption are influenced by substances that are mostly found in plant foods. The strongest inhibitors are polyphenol compounds such as tannins present in tea. Tea can reduce absorption to 80% as a result of the formation of iron-tannate complex. This is one of the factors causing the coverage of Fe tablets has met the target but the incidence rate of anemia in pregnant women is still quite high (Arief, 2008; Varney, Kriebs, & Gegor, 2007).

Rosella (Hibiscus Sabdariffa) is one of the easiest growing herbs in many regions. Generally, this plant is known to have benefits as antihypertensive drugs, diabetes, and antimitosis. This plant also has the highest mineral (Fe) and vitamin C content among other plants, such as Spinach (Amarthus Janjeticus), Cassava leaf (Manihot esculenta), and Katuk leaf (Sauropus androgynous). The content in 100gr of Rosella petals has iron as much as 8.98 mg and vitamin C as much as 244.4 mg (Kustyawati & Ramli, 2008).

The role of vitamin C in the process of iron absorption is to help in reduction of iron ferri (Fe3 +) to ferro (Fe2 +) in the small intestine. The reduction process will be greater when the pH in the stomach is increasingly acid. Vitamin C can also increase the acidity so it can increase the absorption of iron up to 30%. Vitamin C inhibits the formation of hemosiderin, which is difficult to mobilize to free iron when necessary. The absorption of iron in nonheme increases fourfold when
there is vitamin C. Vitamin C also plays a role in transferring iron from transferrin in plasma to liver ferritin (Almatsier, 2002). Previous study showed that Rosella calyx extract gave an effect to the increase of erythrocytes and hemoglobin (Hb) level in the blood of white rat. The most optimal dosage of Rosella's extract to increase the amount of erythrocytes and hemoglobin (Hb) levels in the blood of white rats was 0.72 g/head/day, followed by doses of 0.36 and 0.18 g/head/day (Munawaroh, 2009). The purpose of this study was to prove the effect of Rosella flower extract (Hibiscus Sabdariffa) on changes in hemoglobin level in pregnant women with anemia who got Fe tablet.

METHODS
Research design
This study was a quasi-experiment with pretest-posttest control group design to know the effect of Rosella flower extract on changes in hemoglobin level in pregnant women with anemia who got iron tablet supplementation. This study aimed to reveal the possibility of a causal relationship between variables without the manipulation of a variable. This research was conducted in November - December 2016 in the working area of Tlogosari Wetan Community Health Center.

Sample
Forty-two participants were selected using accidental sampling, which 21 assigned in the experiment and control group. All samples were pregnant women in the second trimester who had anemia and received iron tablets.

Intervention
The experiment group was given the extract of Rosella petals with doses of 115.2 mg/kg BW/day for 10 days together with iron tablets, which was consumed at night; while for the control group was only given iron tablets.

Instrument
Hemoglobin levels were measured using hematology analyzer in laboratory. An observation sheet was used for checking the time for consuming the extract, and food recall sheet was used as a tool to record the type and amount of food that had been consumed within 24 hours.

Ethical consideration
Ethical clearance was obtained from the Ethics Commission of POLTEKKES Semarang. The researchers have confirmed that all participants have signed informed consent prior to data collection.

Data analysis
Univariate analysis was used to explain or describe the distribution and percentage of each variable; and bivariate analysis used Independent t-test and paired t-test. Paired t-test was used to compare the average of two sets of data (data before and after intervention) pairwise. In this study two sets of data were hemoglobin levels before and after treatment in each sample group, at 95% of confidence level (α 0.05); while Independent t-test was to identify differences in control and experiment group. Prior to bivariate analysis, the data normality test was performed by Shapiro-Wilk test, resulting on normal data distribution.

RESULTS
Characteristics of respondents showed that all respondents were not in high-risk category in both control or treatment group, which was 21 people (100%). The majority of respondents had a middle education (47.6%), working a private employee in control group (47.6%) and a housewife (28.6%) in experiment group; and the characteristics of respondents based on parity showed that most of the respondents in the control group were multigravida (60%) and in the experiment group were primigravida (64.7%).

Table 2 shows that the mean of hemoglobin levels in the experiment group before given the intervention of Rosella extract and Fe tablet were 9.31 g/dl with the lowest hemoglobin level of 7.8 g/dl and the highest was 10.3 g/dl; while the mean of hemoglobin levels after given intervention were 10.39 g/dl with the lowest hemoglobin level of 9 g/dl and the highest of 11.8 g/dl.

The mean of hemoglobin level in the control group before given the intervention of giving
Fe tablets as much as 9.09 gr / dl with the lowest hemoglobin level of 7.3 gr / dl and the highest was 10.4 gr / dl; while the average hemoglobin levels after the intervention of giving Fe tablet were 9.705 gr/dl with the lowest hemoglobin level was 8 gr / dl and the highest was 11 gr / dl.

Table 1 Characteristics of respondents

| Characteristic | Control group | Experiment group | p-value |
|----------------|---------------|------------------|---------|
| Age (year)     | f %           | f %              |         |
| <20            | 0 0           | 0 0              |         |
| 20-35          | 21 100        | 21 100           |         |
| >35            | 0 0           | 0 0              |         |
| Education      | f %           | f %              |         |
| Elementary     | 3 28.6        | 6 14.3           | 1.692   |
| Middle         | 10 47.6       | 10 47.6          |         |
| High           | 8 23.8        | 5 38.1           |         |
| Occupation     | f %           | f %              |         |
| Housewife      | 6 47.6        | 10 28.6          |         |
| Entrepreneur   | 3 9.5         | 2 14.3           | 1.674   |
| Private employee | 11 38.1    | 8 52.4           |         |
| Government employee | 1 4.8     | 1 4.8            |         |
| Parity         | f %           | f %              |         |
| Primigravida   | 6 52.4        | 11 28.6          | 2.471   |
| Multigravida   | 15 47.6       | 10 71.4          |         |

Table 2 Hemoglobin level before and after intervention in the experiment and control group

| Hemoglobin levels | Control group | Experiment group |
|-------------------|---------------|------------------|
|                   | Before | After | Before | After |
| Mean              | 9.090  | 9.705 | 9.310  | 10.395|
| Minimum           | 7.3    | 8.0   | 7.8    | 9.0   |
| Maximum           | 10.4   | 11.0  | 10.3   | 11.8  |

Table 3 Mean difference of hemoglobin levels before and after intervention in the experiment and control group using Paired t-test

| Group             | Mean | SD  | Std. Error | Mean | 95% CI | t   | df  | Sig. (2-tailed) |
|-------------------|------|-----|------------|------|--------|-----|-----|-----------------|
|                   |      |     |            |      | 95% CI |     |     |                 |
|                   |      |     |            |      | Lower  | Upper|     |                 |
|                   |      |     |            |      | 95% CI |     |     |                 |
|                   |      |     |            |      | Lower  | Upper|     |                 |
| Control            |      |     |            |      |        |      |     |                 |
| Pretest-posttest   | -.6143| .4385| .0957| -.8139| -.4147| -6.420| 20  | .000            |
| Experiment         |      |     |            |      |        |      |     |                 |
| Pretest-posttest   | -1.0857| .3554| .0775| -1.2475| -.9240| -14.001| 20  | .000            |

Paired t-test as shown in the table 3 obtained p-value 0.00 (<0.05), which indicated that there was an increase of hemoglobin levels in both experiment and control group. The mean increase of hemoglobin levels in the control group was 0.61 gr and in the experiment group was 1.08. The hemoglobin levels in the experiment group were higher than the levels in the control group.
Table 4 Mean difference of hemoglobin levels in the experiment and control group

|                                | Levene's Test | t-test for Equality of Means | 95% CI of the Difference Lower | 95% CI of the Difference Upper |
|--------------------------------|--------------|-------------------------------|--------------------------------|--------------------------------|
|                                |              |                               |                                |                                |
| Equal variances assumed         | 1.164        | .287                          | 3.828                          | 40                             | .000                           | .47143                        | .12317                         | .22250                         | .72036                         |
| Equal variances not assumed     | 3.828        | 38.354                        | .000                           | .47143                        | .12317                         | .22217                        | .72069                         |

Table 4 shows that the value of significance on Equal Variance Assumed was 0.287 (>0.05), which indicated that both sets of data in the experiment and control group were homogeneous. While the result of independent t-test obtained p-value 0.000 (<0.05), with mean difference of 0.47 gr / dl average, which indicated that there was a significant difference of mean of hemoglobin levels between the control group and the treatment group.

DISCUSSION

Findings of this study showed that there was a significant effect of rosella extract combined with Fe tablets on the increase of hemoglobin levels in pregnant women with anemia. This proves that rosella extract was effective for human, especially in pregnant women in this study, as previous study revealed that rosella extract had a significant effect in increasing the number of erythrocytes and hemoglobin level (Hb) in white rat (Rattus norvegius) (Munawaroh, 2009).

In this study the dose of Rosella flower extract given to the respondent was 115.2 mg/ kg/day/day for 10 days. Each 100 grams of dried Rosella petals contain 260-280 mg of vitamin C, including vitamin D and B2. Vitamin C content is 3 times higher that black wine, 9 times higher than citrus, 10 times higher than the star fruit, and 2.5 times higher from guava. In addition, dried rosella contains high calcium (486 mg / 100g), magnesium and omega-3, vitamin A, iron, potassium, β-carotene and essential fatty acids (Winarti, 2010).

The high vitamin C content in rosella is helping the absorption of iron. This is supported by previous research revealed that there was an increase in Hb, serum ferritin, body weight and BMI levels for those who were given vitamin C compared with the control group (Asiyah, Rahayu, & Isnaeni, 2017); while Guntur Argana, Kusharisupeni, and Diah M. Utari revealed that vitamin C is a dominant factor on hemoglobin levels. The role of vitamin C in the process of iron absorption is to help reduce iron ferri (Fe3+) to ferro (Fe2+) in the small intestine so easily absorbed. Vitamin C can increase the acidity so it can increase iron absorption by up to 30% (Guntur & Kusharisupeni, 2004).

Vitamin C is also known by other names namely "cevitamic acid", "antiscorbutic factor" and "scrvy preventive dietary essential". There are two forms of active vitamin C molecules, namely reduced form (ascorbic acid) and oxidized forms (dehydro ascorbic acid). When dehydroscobic acid is oxidized then it is converted into diethylaconic acid that is biologically inactive. Vitamin C is instrumental in the formation of hemoglobin. In addition, vitamin C can help the absorption of potassium by keeping potassium in the form of solution. The need for pregnant women increases 10 mg / day, so the need per day becomes 70-85 mg / day. Consumption of vitamin C is said to be good if consumption ≥ 100% based on Nutritional Adequacy Rate, moderate if 80-90%, less if consumption 70-80%, and deficit when <70% (Susiloningtyas, 2017). Additionally, roselle also contains protein, calcium, and other elements that are useful for the body. Amino acids contained in this plant include arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine,
aspartic acid, glutamic acid, alanine, glycine, proline and serine (Okasha, 2008).

This study also revealed that there was an increase of hemoglobin levels after given iron tablets alone. However, the mean increase of Hb levels was lower than Hb levels in the experiment group. The need for iron in a normal pregnancy is about 1000 mg. Approximately 300mg is actively in the transfer of fetus and placenta, and about 200 mg is lost along the various normal excretion pathways. The need for iron becomes considerable high during second half of pregnancy, with an average of 6 to 7 mg / day (Cunningham et al., 2014). In addition, pregnancy food with every 100 calories will produce about 8-10 mg of iron. The calculation of eating three times with 2,500 calories will produce about 20-25 mg of iron per day. During pregnancy with a calculation of 288 days, pregnant women will produce as much as 1,000 mg of iron, which still lacks. Therefore, iron intake is needed and able to increase Hb levels (Manuaba, 2010).

Each tablet of iron contains 200 mg of ferro sulfate and 0.25 mg of folic acid, given to pregnant women to overcome the problem of iron nutritional anemia. Pregnant women get 90 iron tablets during pregnancy (Kementerian Kesehatan, 2013). However, many factors influence the absorption of iron in the body, such as tea, coffee, and milk (Varney et al., 2007). This study provides the evidence that rosetta extract can help the absorption of iron in increasing Hb levels in pregnant mothers.

CONCLUSION

It is concluded that the consumption of rosetta extract combined with Fe tablet showed a higher increase of hemoglobin levels compared with the consumption of Fe tablet alone. Therefore, pregnant women who have anemia are recommended to consume Fe tablets accompanied by the consumption of Rosella extracts containing vitamin C to help the absorption and increased levels of hemoglobin. It is expected the incidence rate of anemia in Indonesia that causes complications in pregnancy can be suppressed. It is suggested for midwife to use the result of this research as an evidence practice through counseling for pregnant mother about utilization of rosetta extract that can increase hemoglobin level in pregnant woman with anemia.

Declaration of Conflicting Interest

None declared.

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Author Contribution

All authors contributed equally in this study.

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