

Synthesis of Ferrous Fumarate from Indonesian Iron Sand and *In Vivo* Body Weight Gain Test in Rats

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ABSTRACT

Iron deficiency anemia (IDA) is a health problem in Indonesia. Prevention and treatment of IDA is carried out by giving fortified foods and oral iron therapy. Both can use ferrous fumarate which is made with reacting bivalent iron and disodium fumarate. Bivalent iron can be obtained from iron sand in Indonesia. This study aims to synthesize ferrous fumarate from Indonesian iron sand, which is from Malang, Sukabumi, and Cianjur area, and to determine its absorption through in vivo body weight gain test in male Wistar white rats (*Rattus norvegicus*). First, ferrous fumarate was synthesized through reaction of ferrous sulfate, which was made from Indonesian iron sand with the highest iron content, which was from Sukabumi area, and disodium fumarate. Second, *in vivo* body weight gain test was conducted to 3 rat groups (negative control, ferrous sulfate group, and ferrous fumarate group, respectively) and monitored for two weeks. The results showed that ferrous fumarate was successfully obtained as brownish red-orange fine powder with yield of 62.17 ± 1.66 %. In addition, the *in vivo* body weight test suggested that the rats from ferrous fumarate group showed similar weight gain (35.1%) compared to those from the ferrous sulfate group (30.6%), indicating a possibility of iron absorption from ferrous fumarate.

Keywords: anemia; iron sand; ferrous fumarate; atomic absorption spectrophotometry; iron absorption

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INTRODUCTION

Anemia is a global health problem, including in Indonesia (Suryani et al., 2015 and Rah et al., 2021). Anemia is mostly caused by iron deficiency (Suchdev et al., 2016; World Health Organization, 2015). According to an Indonesian report, *Riset Kesehatan Dasar* (Basic Health Research), conducted by *Badan Penelitian dan Pengembangan Kesehatan* (Health Research and Development Agency) (2018), there had been an increase in the proportion of anemia in pregnant women from 37.1% (2013) to 48.9% (2018). Adolescent girls who used iron tablet supplements were 76.2%, while those who did not use the supplements were only 23.8%. This is contradictive, considering that Indonesia has abundant natural resources, so it is expected that Indonesia can meet the needs of iron micronutrients.

The management of iron deficiency anemia is the administration of reduced iron ion or Fe^{2+} (ferrous) because Fe^{2+} is absorbed better than Fe^{3+} (ferric) (Dipiro et al., 2017). Iron is an essential mineral which is important in the formation of haemoglobin and myoglobin, which are protein component of red blood cells and muscles (Seriki et al., 2017 and Taşğın, 2017). Therapy is generally given orally because it

is cheaper, safer, and has the same effectiveness as parenteral administration, except in a certain condition that parenteral administration is recommended (Fitriyani and Saputri, 2018 and Özdemir, 2015). The adherence to consumption of Fe tablets have an effect on the incidence of anemia in pregnant women (Ernawati and Sety 2023; Salma, 2021; and Nurmasari and Sumarmi, 2019). Oral iron preparations are generally in the form of ferrous iron salts, such as ferrous sulfate, fumarate, and gluconate, all of which are absorbed equally well (Dipiro et al., 2017). Each ferrous iron salt contains a different percentage of the element iron, namely 20% for ferrous sulfate heptahydrate, 12% for ferrous gluconate, and the highest content is in ferrous fumarate which is as much as 33% (Dipiro et al., 2017). Ferrous fumarate has less toxic potential because ferrous fumarate causes fewer side effects due to its low solubility and slower dissolution after oral administration than ferrous sulphate (Crichton et al., 2008). In addition, ferrous fumarate is considered as the cost-effective preference to treat iron deficiency anemia (Panicker et al., 2016).

In addition to medication, the use of iron fortification in food is another method that can be utilized throughout the world in dealing with anemia due to iron deficiency (Uauy et al., 2002 and Wan et al., 2019). Priambudi et al (2017) suggested that the seasoning of instant noodles is

a good candidate for iron fortification. Food fortification is the intentional addition of one or more micronutrients to certain foods with the intention of increasing the intake of micronutrients (Shamah et al., 2008). Ferrous fumarate is one of the most bioavailable iron components in the fortified form (Shamah et al., 2008).

Ferrous fumarate can be used as an oral iron deficiency anemia medication and iron fortification in Indonesia. This research will utilize Indonesia's natural resources to produce ferrous fumarate raw materials as an effort to increase domestic production of medicinal raw materials. The sources that will be used to make ferrous fumarate raw materials in this study are fumaric acid and bivalent iron (ferrous) derived from iron sand in Indonesia.

Iron sand is a deposit of sand containing iron particles (magnetite) and mostly can be found along the coast (Hilman et al., 2014). In general, the composition of iron sand is iron oxide (Fe_2O_3 and Fe_3O_4), silicon oxide (SiO_2), and other compounds with lower levels (Suroso, 2017). In Indonesia, iron sand is generally mined and used as a raw material, sold directly without processing, or only used for the cement and steel industries (Hilman et al., 2014). Therefore, iron sand has the potential to be processed into useful products in the health sector for the treatment of iron deficiency anemia.

This study aims to synthesize ferrous fumarate using Indonesian iron sand sources and to conduct *in vivo* body weight gain test in male Wistar white rats (*Rattus norvegicus*) to assess the absorption ability of ferrous fumarate which has been synthesized.

METHODS

Instruments and Materials

Various instruments and tools used in this study were the Shimadzu AA-6300 atomic absorption spectrophotometer equipped with a Hollow Cathode Lamp Fe (Japan); Shimadzu FTIR Infrared Spectrophotometer – 8400S (Japan); Memmert ovens (Germany); Shimadzu AP225WD analytical balance (Japan); OHAUS balance (United States); Branson ultrasonic homogenizer (United States); Hettich centrifuge (Germany); Socorex Acura 825 micropipette (100 – 1000 μL) (Switzerland); beaker glasses; Erlenmeyer; stir sticks; vaporizer cup; measuring cup; volumetric flask; crustang; filter paper; air gas, acetylene and nitrogen gas; tara bottle; centrifuge tubes; and rat pen.

Various materials used in this study were iron sand from 3 different regions, namely Malang, Sukabumi, and Cianjur, Indonesia; fumaric acid (PT Justus Sakti Raya, Indonesia and Merck, Germany); ferrous fumarate; 1 N Sodium hydroxide (Merck, Germany); aquadestilata;

hydrochloric acid 37% (Merck, Germany); nitric acid 68% (Merck, Germany); potassium bromide P (Merck, Germany); iron standard (Merck, Germany); phosphate buffer; hydrochloric acid 0.1 N; male Wistar white rats (*Rattus norvegicus*); sawdust; rat feed Rat Bio (PT Citra Ina Feedmil, Indonesia); drinking water; ferrous fumarate samples; and FeSO_4 standard.

Synthesis of $\text{FeCl}_2\text{-FeCl}_3$ from Iron Sand to Determine the Highest Iron Content

Iron sand from 3 regions, namely Malang, Sukabumi, and Cianjur, each was weighed approximately 55 grams. Iron sand was put into a beaker glass and washed with distilled water 3 times. Iron sand that had been washed was put into an evaporating cup that had a fixed weight. Iron sand was heated in an oven at 120°C for 30 minutes. The iron sand was left to room temperature and then weighed with an analytical balance. The process was repeated until a stable weight achieved.

After a stable weight achieved, the iron sand was weighed as much as 10 grams, put into a beaker glass, then dissolved in 5 mL of 37% HCl and 15 mL of distilled water. The mixture was stirred with heating for 15 minutes. The formed $\text{FeCl}_2\text{-FeCl}_3$ solution was separated into the Erlenmeyer flask. The iron sand residue was dissolved with 2 mL of 37% HCl and 15 mL of distilled water, then stirred with heating again for 15 minutes. The formed $\text{FeCl}_2\text{-FeCl}_3$ solution was separated back into the same Erlenmeyer flask. The iron sand residue was rinsed with 5 mL of distilled water twice. The solution of $\text{FeCl}_2\text{-FeCl}_3$ was put to 50 mL volumetric flask, added with distilled water up to 50 mL, then homogenized. Determination of iron content from three $\text{FeCl}_2\text{-FeCl}_3$ solutions from three different regions was carried out using an atomic absorption spectrophotometer. The solution containing the highest amount of iron was chosen to make FeSO_4 and, in turn, ferrous fumarate.

Synthesis of FeSO_4 from Iron Sand with the Highest Iron Content

Iron sand from the region with the highest iron content was selected based on the results of the assay of $\text{FeCl}_2\text{-FeCl}_3$ above. FeSO_4 was prepared with dissolving 5 grams of iron sand, that had been washed and dried, in 50 mL of 6 M H_2SO_4 . The dissolving process was carried out for 5 – 6 hours at a temperature of $112\text{--}115^\circ\text{C}$. After heating, the solution was cooled down to about 15°C . The solution was put in the refrigerator to produce a greenish-white FeSO_4 precipitate. The precipitate was separated with filtration and decantation, then dried at room temperature on filter paper. The process was adapted from Kapor et al., 2012. Determination of FeSO_4 levels was carried out using an atomic absorption spectrophotometer (AAS).

Synthesis of Ferrous Fumarate Complex from FeSO₄
Fumaric acid was dissolved in 1 N NaOH in an amount according to stoichiometric calculations at a molar ratio of 1:2. Fumaric acid was stirred until completely dissolved in the NaOH solution to produce disodium fumarate. The ferrous fumarate complex was prepared with mixing FeSO₄ into a solution of disodium fumarate in an amount according to stoichiometric calculations at a molar ratio of 1:1. The mixing process was put under N₂ gas so that the reaction was free of O₂ gas. The mixture was then heated while stirring at 90°C for 30 minutes. The mixture was allowed to stand until a brownish red precipitate was obtained.

The filtrate was separated from the precipitate with decantation. The precipitate was then rinsed with water and separated with centrifugation. Impurities from other salts that might be formed were removed with washing the residue of the precipitate with distilled water, centrifuging the precipitate again, and then taking the precipitate. The washing and centrifugation stages were carried out three times. The precipitate, that had been washed, was then dried at 105°C for 12 hours until a stable weight achieved. The process was adapted from Kapor et al., 2012. Determination of ferrous fumarate levels was carried out using an atomic absorption spectrophotometer.

Identification of Ferrous Fumarate and Loss on Drying

Identification test was carried out based on the Indonesian Pharmacopoeia VI (*Farmakope Indonesia Edisi VI*, published by *Direktorat Jenderal Kefarmasian dan Alat Kesehatan, Kementerian Kesehatan Republik Indonesia*, 2020). After going through the test reaction procedure on ferrous fumarate from the monography, there were dry precipitate and filtrate. The dry precipitate was used for the fumaric acid identification test with an infrared spectrophotometer. The sample spectra was compared to the reference fumaric acid spectra. The filtrate was used to identify iron based on general identification in the Indonesian Pharmacopoeia VI. The identification of iron used was identification using 1 N NaOH. The addition of 1 N NaOH to the filtrate caused the formation of a green precipitate which became brown or red-brown when shaken. In addition, for the loss on drying test, 1000 – 2000 mg of ferrous fumarate was dried at 105°C for 16 hours. The loss on drying requirement for ferrous fumarate was not more than 1.5%.

Assay of Ferrous Fumarate with Atomic Absorption Spectrophotometer

Iron standard solutions were prepared with concentrations of 5; 10; 15; 20; 30, and 50 ppm, respectively. The assay was carried out using an atomic absorption spectrophotometer. The relationship between

the concentration of the solution as the x-axis and the absorption (A) obtained as the y-axis was plotted so that a linear regression equation $y = a + bx$ and a coefficient correlation r were obtained. Ferrous fumarate sample of 50 mg was dissolved with 10 mL HNO₃ at 100°C, then put into a 50 mL volumetric flask. Aquadest was added up to the 50 mL limit. The ferrous fumarate sample solution was then diluted to a concentration of 80 ppm, containing about 26.3 ppm iron, which was proportional to the atomic weight of iron (55.8 g/mol) compared to the molecular weight of ferrous fumarate (169.9 g/mol). Then, the determination of the iron content of sample was carried out using the same atomic absorption spectrophotometer instrument.

In Vivo Body Weight Gain Test in Rats

Acclimatization of rats was carried out for 1 week. Rats were divided into 3 groups. The first group was given rat food only as the negative control group. The second group was given rat food containing 300 mg FeSO₄/kg of food as the comparator group. The third group was given rat food containing 300 mg ferrous fumarate/kg of food as the test group. The number of rats for each treatment group was 7. Rat food was given every day as much as 15 grams/rat in the first week. The amount of food per day was then increased to 20 grams/rat in the second week. The weighing data of rats measured was the initial weighing data (after acclimatization) and subsequent weighing every week for 2 weeks. The increase in weight was compared between the negative control group, the comparator group (FeSO₄), and the test group (ferrous fumarate). Quantitative data on the body weight increase of the rats were analyzed statistically using the one-way ANOVA test method with the post-hoc test with the smallest significant difference or the Kruskal-Wallis test which was determined by the normality test. Data were analyzed with SPSS statistical software. All data is shown as the mean \pm standard deviation (SD). This test was carried out based on the research by Lawless et al (1994) and Kilic et al (2014).

RESULTS AND DISCUSSION

Synthesis of FeCl₂-FeCl₃ from Iron Sand to Determine the Highest Iron Content

Bivalent iron FeCl₂ was selected because HCl, as a material for the synthesis of FeCl₂, is more reactive than H₂SO₄, as a material for the synthesis of FeSO₄, so that in theory it can extract more iron than H₂SO₄ (Aliwarga et al., 2019). In determining the iron content in the FeCl₂-FeCl₃ solution, 1 mL of the solution was taken and dissolved in distilled water in a 100 mL volumetric flask. The solution was then used for the determination of iron content. Table 1 below shows the results of the determination of the FeCl₂ – FeCl₃ content of iron sand from 3 regions, namely Malang, Sukabumi and Cianjur.

Table 1. Content of FeCl₂-FeCl₃ synthesized and extracted from Indonesian iron sand

Region	Extracted iron content from iron sand (mg/g)	Percentage (%)
Malang	15.56	1.56
Sukabumi	31.81	3.18
Cianjur	8.85	0.89

In iron sand, although the highest content is iron (45 – 48%, and can be up to 79.12%), there are still other substances in smaller amounts, such as 0.66% magnesium, 1.62% aluminum, and titanium 1.32% (Rochani et al., 2008; Ansori et al., 2019; and Gunanto et al., 2018). In addition, the heating time of HCl on iron sand was also thought to be the cause of the low extracted iron content. The short heating time was intended to prevent more FeCl₂ from turning into FeCl₃ due to oxidation during heating.

However, this might cause an incomplete reaction between iron sand and HCl. So, these could be the factor that caused of the relatively low content of the extracted iron from the iron sand. Nevertheless, it can be seen from the assay results that iron sand from the Sukabumi area has the highest iron content.

Synthesis of FeSO₄ from Iron Sand with the Highest Iron Content

Iron sand from Sukabumi was selected for FeSO₄ production because it had the highest iron content. A greenish-white FeSO₄ precipitate was successfully obtained from the reaction of iron sand with H₂SO₄. The FeSO₄ precipitate was then weighed as much as 50 mg and diluted in 50 mL of distilled water. The solution was then measured using AAS to determine the extracted iron content from the Sukabumi iron sand. Table 2 shows the content of FeSO₄ synthesized and extracted from the

Table 2. Content of FeSO₄ synthesized and extracted from Sukabumi iron sand

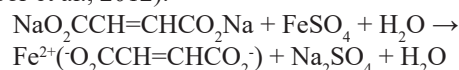
Batch	Extracted iron content from iron sand (mg/g)	Percentage (%)
1	151.54	15.15
2	139.58	13.96
Average	145.56	14.56

iron sand of Sukabumi.

These results show different levels of Fe²⁺ iron compared to a study by Aliwarga et al (2019) who obtained an iron content of 76%. This is because in this study, the determination of iron content was carried out in solutions without forming FeSO₄ precipitate. This suggests that in the process of forming FeSO₄ precipitate, there is

Fe²⁺ still left in the solution, together with Fe³⁺, which does not form precipitates. The small amount of FeSO₄ precipitate formed could be due to a lack of water added in the process of FeSO₄ precipitate formation. It needs 7 water molecules to form a precipitate of FeSO₄ at a cooling temperature of around 15°C (Vidianti, 2014). The small amount of extracted iron could also be due to impurities that interfere with the formation of FeSO₄ precipitate, such as TiSO₄. In addition, different results may also occur due to differences in the conditions of the synthesis process, especially during the heating process of iron extraction. The characteristics of iron sand, composition, and impurities in iron sand could be factors that cause the differences in the results of iron extraction from iron sand with H₂SO₄ (Aliwarga et al., 2019).

Synthesis of Ferrous Fumarate Complex from FeSO₄
Ferrous fumarate was synthesized through reacting bivalent iron FeSO₄ with disodium fumarate solution. The chemical reaction between disodium fumarate and FeSO₄ to produce ferrous fumarate is shown below (Kapor et al., 2012).



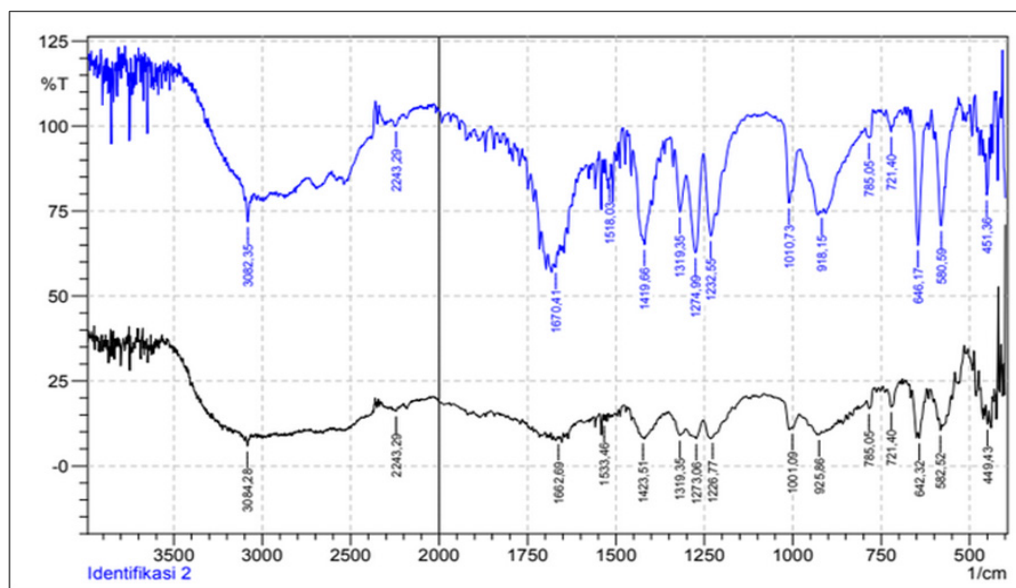
The bivalent iron FeSO₄ was selected because the substance was usually used in the manufacture of ferrous fumarate, as in the study by Kapor et al (2012). The ferrous fumarate precipitate, from the reaction of FeSO₄ with disodium fumarate, was then dried and weighed to calculate the yield. Table 3 shows the yield of ferrous fumarate. The synthesized ferrous fumarate is shown in Figure 1.

**Figure 1. The synthesized ferrous fumarate obtained from the reaction between FeSO₄ and disodium fumarate**

The yield obtained did not match the theoretical calculations based on stoichiometric calculations and literature, as in research by Kapor et al (2012). This could be caused by problems during the synthesis

Table 3. Yield of ferrous fumarate synthesized from FeSO₄ and disodium fumarate

Results	Yield (%)			
	Batch 1	Batch 2	Batch 3	Average
Theoretical yield	98.83	98.83	98.83	98.83 ± 0.00
Obtained yield	61.09	64.08	61.34	62.17 ± 1.66
Characteristic	Brownish red-orange fine powder	Brownish red-orange fine powder	Brownish red-orange fine powder	-

**Figure 2. Comparison of the infrared spectra of the sample fumaric acid (blue), from ferrous fumarate sample, with that of the reference fumaric acid (black)**

process, such as unstable heating temperature, less airtight atmosphere, even though it has been supplied with nitrogen, and less homogenized mixing. This might make the reaction process incomplete so the obtained results were less than expected. In addition, there could be unknown impurities from the extraction of FeSO₄ which could affect the reaction. One of the predicted impurities was excess acid content (H₂SO₄). Excess acid can be known by measuring the pH of dissolved FeSO₄ based on the Indonesian Pharmacopoeia VI (2020). H₂SO₄ which is acidic could dissolve some of the ferrous fumarate that had been formed or hinder the process of forming the ferrous fumarate by binding to NaOH which played a role in the reaction between fumaric acid and FeSO₄. Even in small amounts, the presence of H₂SO₄ may produce other contaminants, namely Fe₂(SO₄)₃ due to oxidation of FeSO₄ during storage (Herrly et al., 1922). However, FeSO₄ precipitate undergoes slow oxidation, even though it has been exposed to air, so that the amount of Fe₂(SO₄)₃ could be very small.

Identification of Ferrous Fumarate and Loss on Drying

Identification test of ferrous fumarate, synthesized from FeSO₄ and disodium fumarate, was carried out based on the Indonesian Pharmacopoeia VI. The precipitate, obtained from the monograph procedure, was used for the fumaric acid identification test, while the filtrate was used for the iron identification test.

The precipitate, which should be fumaric acid, was then identified using an infrared spectrophotometer. Figure 2 below is a comparison of the infrared spectra of the sample fumaric acid with that of the reference fumaric acid. The sample fumaric acid shows maximum only at the same frequency or wave number as the reference fumaric acid, so that the identification of fumaric acid precipitates meet the requirements according to the Indonesian Pharmacopoeia VI.

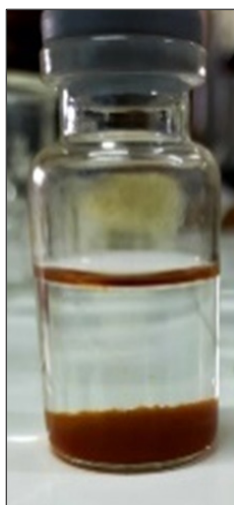


Figure 3. The results of the iron identification test from ferrous fumarate sample

In the iron identification test, identification was carried out to determine the presence of iron. The result obtained was a red-brown precipitate, indicating the presence of iron in the solution, as shown in Figure 3. Based on the results, the identification of iron meets the requirements of the Indonesian Pharmacopoeia VI.

Table 4. The results of loss on drying test of ferrous fumarate sample

No	Sample	Ferrous fumarat
1	Before drying	1.0935 g
2	After drying	1.0883 g
	Difference	0.0052 g
	Loss on drying	0.48%

The loss on drying test was carried out on ferro fumarate, which had been identified according to the Indonesian Pharmacopoeia VI (2020). Loss on drying is the difference in the weight of the substance before and after drying, divided by the weight of the substance before drying, multiplied by one hundred percent. The requirement of the loss on drying shrinkage for ferrous fumarate based on the Indonesian Pharmacopoeia VI (2020) is no more than 1.5%. The ferrous fumarate sample meets the loss on drying requirement, as can be seen in Table 4.

Assay of Ferrous Fumarate with Atomic Absorption Spectrophotometer

The calibration curve equation obtained from iron standard solution is $y = 0.0159x + 0.1480$ with $r = 0.9956$. The sample solution had a concentration of 80 ppm ferrous fumarate, containing about 26.3 ppm iron. The iron content obtained from the samples is shown in Table 5. The average iron content obtained was 13.01 ppm from 80 ppm ferrous fumarate, or 16.26%. According to Dipiro et al (2017), the percentage of iron content in pure ferrous fumarate is 32.87%, which roughly corresponds to the ratio of the atomic weight of iron (55.8 g/mol) to the molecular weight of ferrous fumarate (169.9 g/mol). Therefore, the purity of the synthesized iron fumarate samples is about 49.47%.

One of the factors causing the low purity of ferrous fumarate samples could be the presence of foreign substances that interfere with the reaction process between FeSO_4 and disodium fumarate, such as sulfuric acid (H_2SO_4). Sulfuric acid could dissolve the ferrous fumarate precipitate back to the solution. Sulfuric acid might also inhibit the process of ferrous fumarate

Table 5. Iron content of ferrous fumarate sample

No	Absorbance	Iron Concentration (ppm)	Average (ppm)	Ferrous Fumarate Concentration (ppm)	Iron Content (%)	Average (%)
1	0.3551	13.07			16.34	
2	0.3541	13.00	13.01 ± 0.05	80.00	16.25	16.26 ± 0.07
3	0.3535	12.97			16.21	

Table 6. Weight gain of control, FeSO_4 , and ferrous fumarate group

Group	Average Weight (g)			Average Weight Gain (g)		
	Initial	Week 1	Week 2	Week 1	Week 2	Cumulative
Control	124.5 ± 11.0	142.3 ± 9.0	159.9 ± 16.0	17.8 ± 5.3	17.6 ± 9.7	$35,4 \pm 10.1$
FeSO_4	125.2 ± 14.3	139.7 ± 12.4	163.5 ± 10.2	$14,5 \pm 5.0$	$23,8 \pm 7.0$	$38,3 \pm 8.5$
Ferrous Fumarate	117.8 ± 8.3	137.7 ± 6.7	159.2 ± 10.9	$19,9 \pm 2.6$	$21,5 \pm 6.7$	$41,4 \pm 6.3$

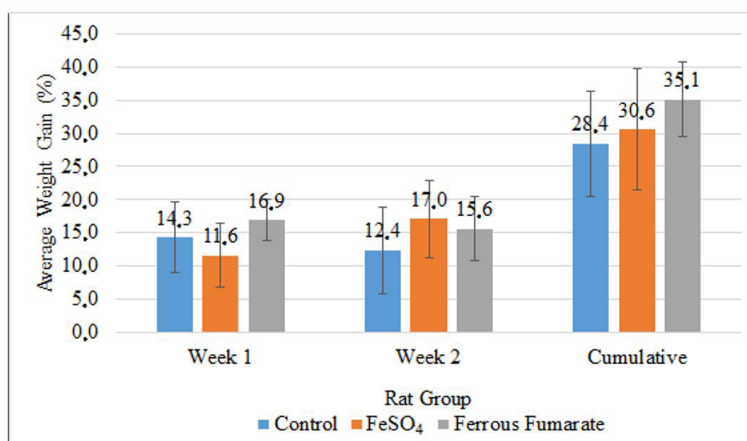


Figure 4. Percentage of average weight gain of rats

formation by binding to NaOH, which had a role in the reaction between fumaric acid and FeSO₄. In addition, the unreacted fumaric acid, during the synthesis process of ferrous fumarate, may be mixed into the sample precipitate, diluting the ferrous fumarate content in the precipitate.

In Vivo Body Weight Gain Test in Rats

Table 6 and Figure 4 show the increase of rat body weight, in absolute number (gram) and percentage (%), respectively, in the first week, the second week, and the cumulative weight (total) for two weeks. Although the three groups did not show significant differences ($p > 0.05$) in weight gain statistically, it appears that ferrous fumarate group has the highest effect on weight gain. The increases in total body weight in the ferrous fumarate and ferrous sulfate groups are also higher than the control group. This suggests that the absorption of iron from the two active substances leads to the effect of increasing body weight, albeit insignificant. This might be due to the observation was only for 2 weeks in this study. There is a possibility that with enough iron level in blood, the blood function to distribute oxygen and nutrition to body cells would be optimal, so the body growth, including weight gain, would also be optimal. However, more studies need to be done to elaborate the correlation (or not) between iron absorption (i.e. the increase of iron level in blood) and body weight gain. As an information, the normal iron content in blood serum is 80-180 mcg/dL (14-32 $\mu\text{mol/L}$) in men and 60-160 mcg/dL (11-29 $\mu\text{mol/L}$) in women (Devkota, 2019). It is also recommended that the observation of body weight gain is conducted in longer time frame (for more than 1 month) to assure the significance of the result difference between groups. According to the National Health Service (United Kingdom) (2019), generally it takes up to 4 weeks for ferrous sulfate and ferrous fumarate to have full effect as anemia treatment.

The results of this study are in accordance with the results of a study by Harrington et al (2011) about the absorption of iron fortified foods in children. The absorption of ferrous fumarate and ferrous sulfate was equally good because the difference was not significant ($p > 0.05$) between the two substances. In that study, absorption of iron ferrous fumarate and ferrous sulfate respectively in the mother group was 17.5% and 20.5%; babies 7% and 7.2%; and children 6.3% and 5.9%. According to the study of Moreno-Fernandez et al (2019), there was a body weight gain effect due to iron therapy in a group of anemic rats with various action mechanisms of the body's homeostasis. Research by Lawless et al (1994) and Kilic et al (2014) also showed an increase in body weight due to iron supplements in children and adults with and without anemia. The increase in body weight could be said to be proportional to the absorption of iron fortification.

CONCLUSION

Ferrous fumarate was successfully synthesized through the reaction of disodium fumarate and bivalent iron FeSO₄, which, in turn, was extracted from Indonesian iron sand with H₂SO₄. The synthesized ferrous fumarate met the identification and loss on drying requirements based on the Indonesian Pharmacopoeia VI. The *in vivo* weight gain test in rats showed that ferrous fumarate had similar effect on the total weight gain of rats compared to ferrous sulphate.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest to declare.

REFERENCES

- Aliwarga, L., Reynard, R., dan Victoria, A. V. (2019). Pengendapan titanium pada larutan pasir besi dalam asam sulfat. *Jurnal Teknologi Mineral dan Batubara*, 15(2), Mei 2019: 109 – 118. <https://doi.org/10.30556/jtmb.Vol15.No2.2019.989>
- Ansori, C., Wardhani, F. A., Puswanto, E. (2019). Karakteristik unsur jejak dalam diskriminasi magmatisme batuan beku tinggian karangbolong kebumen. *Prosiding Seminar Nasional Hasil Penelitian Sains, Teknik dan Aplikasi Industri* (SINTA 2018), Universitas Lampung. No. Arsip: LIPI-20190107. Retrieved from <http://lipi.go.id/publikasi/karakteristik-unsur-jejak-dalam-diskriminasi-magmatisme-batuan-beku-tinggian-karangbolong-kebumen/27287>.
- Badan Penelitian dan Pengembangan Kesehatan. (2018). Hasil utama Riset Kesehatan Dasar 2018. Kementerian Kesehatan Republik Indonesia. Accessed from: <https://www.kemkes.go.id/resources/download/info-terkini/hasil-risikesdas-2018.pdf>
- Devkota, B.P. (2019). Iron. *Medscape*. Retrieved from <https://emedicine.medscape.com/article/2085704-overview#:~:text=The%20serum%20iron%20reference%20ranges,%3A%20100%2D250%20mcg%2FdL>.
- Dipiro, J. T., Talbert, G. C., Yee, G. R., Matzke, B. G., dan Wells, L. M. P. (2017). *Pharmacotherapy: A pathophysiology approach 10th edition*. In McGraw Hill Medical. The McGraw-Hill Companies: United State of America.
- Direktorat Jenderal Kefarmasian dan Alat Kesehatan. (2020). Farmakope Indonesia Edisi VI. Jakarta: Kementerian Kesehatan RI.
- Ems, T., Lucia, K.St., dan Huecker, M.R. (2021) Biochemistry, Iron Absorption. [Updated 2021 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
- Ernawati and Sety L. O. M. (2023). Pengaruh pengetahuan gizi ibu dan kepatuhan konsumsi tablet Fe terhadap kejadian anemia pada ibu hamil. *Jurnal Ilmial Obsgin: Jurnal Ilmiah Ilmu Kebidanan & Kandungan*, 15(1): 46-52.
- Fitriany, J., and Saputri, A. I. (2018). Anemia defisiensi besi. *Averrous Jurnal Kedokteran dan Kesehatan Malikussaleh*, 4(2). <https://ojs.unimal.ac.id/averrous/article/view/1033/552>
- Gunanto, Y E., Izaak, M P., Jobiliong, E., Cahyadi, L., Adi, W. A. (2018). High purity Fe₃O₄ from local iron sand extraction. The International Conference on Theoretical and Applied Physics Conf. Series 1011 (2018) 012005. <https://doi.org/10.1088/1742-6596/1011/1/012005>
- Harmita. (2014). *Analisis fisikokimia: Potensiometri dan spektroskopi volume 1*. Jakarta: Penerbit Buku Kedokteran EGC.
- Harrington, M, Hotz, C., Zeder, C., Polvo, G., Villalpando, S., Zimmermann, M., Walczyk, T., Rivera, J., dan Hurrell, R. (2011). A comparison of the bioavailability of ferrous fumarate and ferrous sulfate in non-anemic Mexican women and children consuming a sweetened maize and milk drink. *European Journal of Clinical Nutrition*, 65, 20-5. <https://doi.org/10.1038/ejcn.2010.185>
- Herrly, C. J. (1922). Method of Oxidizing Ferrous Sulphate. United State Patent Office. Diambil dari: <https://patents.google.com/patent/US1430551A/en>
- Hilman, P. M., Suprpto, S. J., Sunuhadi, D. N, Tampubolon, A., Wahyuningsih, R., Widhyatna, D., Pardiarto, B., Gunradi, R., Franklin, F., Yudawinata, K., Sutisna, D. T., Dinarsih, D., Sukaesih, S., Yuningsih, E. T., Candra, C., Oktaviani, P., Rahmawati, R., Ulfa, R. M., Sukmayana, I., dan Ostman, I. (2014). Pasir besi di Indonesia geologi, eksplorasi dan pemanfaatannya. Badan geologi pusat sumber daya geologi. Bandung: Kementerian Energi dan Sumber Daya Mineral. Accessed from: <http://ftgeologi.unpad.ac.id/wp-content/uploads/2018/05/Pasir-Besi-di-Indonesia-Geologi-Eksplorasi-dan-Pemanfaatannya.pdf>
- Kapor, A. J., Nikolić, L. B., Nikolić, V. D., Stanković, M. Z., Cakić, M. D., Ilić, D. P., Mladenović-Ranisavljević, I. I., dan Ristić, I. S. (2012). The synthesis and characterization of iron (ii) fumarate and its inclusion complexes with cyclodextrins. *Advanced technologies* 1(1) (2012), 07-15. Accessed from: <http://www.tf.ni.ac.rs/casopis-arhiva/sveska1/c1.pdf>
- Kilic, H. C., Onec, B., Onec, K., Caliskan, E., dan Ankarali, H. (2014). An investigation about weight gain with treatment of iron deficiency anemia: changes of ghrelin and hepcidin levels with treatment. Madrid 22nd Congress European Hematology Association.

- Lawless, J. W., Latham, M. C., Stephenson, L. S., Kinoti, S. N., dan Pertet, A. M. (1994). Iron supplementation improves appetite and growth in anemic Kenyan primary school children. *The Journal of Nutrition*, 124(5), 645–654. <https://doi.org/10.1093/jn/124.5.645>
- MacKenzie, E. L., Iwasaki, K., dan Tsuji, Y. (2008). Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxidants & redox signaling*, 10(6), 997–1030. <https://doi.org/10.1089/ars.2007.1893>
- Moreno – Fernandez, J., Diaz – Castro, J., Alferez, M. J. M., dan Lopez – Aliaga, I. (2019). Iron deficiency and neuroendocrine regulators of basal metabolism, body composition, and energy expenditure in rats. *Nutrients*, 11, 631. <https://doi.org/10.3390/nu11030631>
- National Health Service (United Kingdom). (2019). Ferrous fumarate. Retrieved from: <https://www.nhs.uk/medicines/ferrous-fumarate/>
- National Health Service (United Kingdom). (2019). Ferrous sulfate. Retrieved from: <https://www.nhs.uk/medicines/ferrous-sulfate/>
- Nurmasari, V., & Sumarmi, S. (2019). Hubungan keteraturan kunjungan antenatal care dan kepatuhan konsumsi tablet Fe dengan kejadian anemia pada ibu hamil trimester III di Kecamatan Maron Probolinggo. *Amerta Nutrition*, 3(1), 46–51.
- Özdemir, N. (2015). Iron deficiency anemia from diagnosis to treatment in children. *Türk. Pediatri Arşivi*, 50(1), 11–9. DOI:10.5152/tpa.2015.2337
- Panicker, Naveen Kumar, A, Hridya, dan Prabhu, Raghuvier. (2016). Comparison of efficacy and safety profile of oral iron formulations in patients with iron deficiency anemia. *International Journal of Pharmaceutical Sciences Review and Research*, 41(2), November - December 2016; Article No. 46. Hal, 248-252.
- Priambudi, A. P., Kusnandar, F., dan Palupi, N. S. (2017). Stabilitas dan bioavailabilitas in vitro zat besi sebagai fortifikan dalam bumbu mi instan. *Jurnal Ilmu Pangan dan Hasil Pertanian* 1(1). Retrieved from <http://journal.upgris.ac.id/index.php/jiphp>
- Przybyszewska, J., & Zekanowska, E. (2014). The role of hepcidin, ferroportin, HCP1, and DMT1 protein in iron absorption in the human digestive tract. *Przegląd Gastroenterologiczny*, 9(4), 208–213. <https://doi.org/10.5114/pg.2014.45102>
- Rah, J. H., Melse-Boonstra, A., Agustina, R., van Zutphen, K. G., & Kraemer, K. (2021). The triple burden of malnutrition among adolescents in Indonesia. In *Food and Nutrition Bulletin* (Vol. 42, Issue 1_suppl, pp. S4–S8). SAGE Publications Sage CA: Los Angeles, CA.
- Rochani, S., Pramusanto, P., Sariman, S., Anugrah, R.I. (2008). The current status of iron minerals in Indonesia. *Indonesia Mining Journal* 11(11): 1- 17. DOI: <https://doi.org/10.30556/imj.Vol11.No2.2008.590>. Accessed from: <https://jurnal.tekmira.esdm.go.id/index.php/imj/article/view/590>
- Salma, A. (2021). Riwayat anemia pada kehamilan sebagai prediktor kejadian stunting pada anak: Literatur Review. *Jurnal Ilmiah Obsgin: Jurnal Ilmiah Ilmu Kebidanan & Kandungan*, 13(4), 29–38.
- Seriki, SA., Adebayo, OF., Odetola, AO. (2017). Iron: from dietary sources to utilization in the body. *Global Journal of Nanomedicine*, 3(3):555615. <https://doi.org/10.19080/GJN.2017.03.555615>
- Suchdev, P. S., Namaste S. M., Aaron, G. J., Raiten, D. J., Brown, K. H., Flores – Ayala, R dan BRINDA working group. (2016). Overview of the biomarkers reflecting inflammation and nutritional determinants of anemia (BRINDA) project. *Advances in Nutrition*, 7(2), 349–356. <https://doi.org/10.3945/an.115.010215>
- Suroso, Indreswari. (2017). Analisis secara fisis dan mekanis pasir besi dari pantai selatan kulonprogo berguna bagi material pesawat terbang. *Jurnal Teknik STTKD*, Vol.4, No. 1, Juli 2017. Retrieved from <https://core.ac.uk/download/pdf/249342013.pdf>
- Suryani, D., Hafiani, R., dan Junita, R. (2015). Analisis pola makan dan anemia gizi besi pada remaja putri Kota Bengkulu. *Jurnal Kesehatan Masyarakat Andalas*, 10(1), 11–18
- Taşğın, E. (2017). Macronutrients and micronutrients in nutrition. *International Journal of Innovative Research and Reviews*, 1(1), 10-15. Retrieved from <http://www.injirr.com/article/view/8>
- Uauy, R., Hertrampf, E., Reddy, M. (2002). Iron Fortification of Foods: Overcoming Technical and Practical Barriers. *The Journal of Nutrition*, 132 (4), April 2002, Pages 849S–852S. <https://doi.org/10.1093/jn/132.4.849S>
- Vidianti, I.A. (2014). Extraction of titanium dioxide from lumajang beach sand by H3PO4 leaching and NaOH as decomposition agent. Surabaya: Chemistry Department Faculty of Mathematics and Natural Sciences Sepuluh

Nopember Institute of Technology. Accessed from: https://repository.its.ac.id/63184/1/1410100075-Undergraduate_Theses.pdf

Wan, D., Wu, Q., Ni, H., Liu, G., Ruan, Z. and Yin, Y. (2019). Treatments for iron deficiency (id): prospective organic iron fortification. *Current Pharmaceutical Design*, 25. 10.2174/1381612825666190319111437.

World Health Organization. (2015). The global prevalence of anemia in 2011 Geneva: World Health Organization. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/177094/9789241564960_eng.pdf?sequence=1