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The Effect of High Dosage of Iron Supplementation (Fe) Against Histopathology of Gastric Mucosa in Pregnant Strain Wistar White Rats (Rattus norvegicus).

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ABSTRACT

Pregnant women are encouraged to consume iron tablets during their pregnancy or at least 90 tablets during pregnancy. Side effects of iron tablets include nausea, vomiting, diarrhea, constipation. If the tablets is consumed in excess it will cause toxicity because excess iron will cause oxidative stress which can affect the state of the stomach. Purpose: to determine the effect of high-dose iron (Fe) supplementation on the histopathology of gastric mucosa in pregnant strain Wistar white rats (Rattus norvegicus). This study used 24 pregnant rats divided into 4 groups, that is, negative controls; treatment 1 with a dose of 0.54 mg; treatment 2 with a dose of 1.08 mg; and treatment 3 with a dose of 2.16 mg. Iron (Fe) supplementation was started from the 1st day of pregnancy until the 18th day, then on the 19th day the rats were dissected and the gastric organ was taken as a preparation with Hematoxylin Eosin (HE) staining and then observed with a light microscope. This study showed the histopathological results of gastric mucosa damaged in the treatment group which was assessed using the Barthel Manja score. The One Way ANOVA test results showed a significant difference from gastric mucosa ($p = 0.000$) in the control and treatment groups of this study. High doses of iron (Fe) supplementation affect the histopathology of gastric mucosa.

1. Introduction

Anemia in pregnancy has an effect on the mother, both in pregnancy, childbirth, and in the postpartum period and thereafter. Problems that can arise due to anemia are miscarriage (abortion), premature birth, prolonged labor due to fatigue of the uterine muscles in contracting (uterine inertia), postpartum bleeding due to the absence of uterine muscle contractions (uterine atony), shock, infection both during childbirth and postpartum, and severe anemia can cause cardiac decompensation (Wiknjosastro et al., 2010).

One of the government's efforts to prevent anemia is by administering Ferrous coated tablets. Use in pregnant women is recommended every day for 3 trimesters or at least 90 tablets for 3 trimesters. Apart from blood booster tablets, to meet the needs of iron during pregnancy can be obtained from animal protein such as liver, fish and meat. Consuming blood-boosting tablets can cause side effects on the gastrointestinal tract in some people, such as discomfort in the pit of the stomach, nausea, vomiting and diarrhea, in some women experiencing constipation (Alimul, 2008).

Excess iron can cause the formation of free radicals (Perdana & Jacobus, 2015). The formation of free radicals can cause an increase in Reactive Oxygen Species (ROS) which causes oxidative stress. Excessive or increased ROS will cause lipid peroxidation which can affect the structure and function of the membrane. This affects the gastric mucosa, and initiates the formation of lesions in the gastric mucosa (Powers & Jackson, 2008).

2. Materials and Methods

In this study, researchers used experimental laboratories (true experiment) with a Post Test Only Control Group Design. The experimental group will be selected randomly or randomly, after which it will be given treatment and made observations. Furthermore, the results of observations of each group will be compared. This research was carried out at the Laboratory of Pharmacology and Anatomical Pathology, Faculty of Medicine, Brawijaya University. White female rats are the experimental animals used in the study. The white rats used had the criteria of age 8-10 weeks, weight 150-250 grams, pregnant, healthy condition which was marked by active rat movement. This study used 24 rats obtained from the Pharmacology Laboratory, Faculty of Medicine, Brawijaya University. The division of groups in this study was 4 groups, namely, the K-negative group, P-1, P-2 and P-3. Each research group contained 6 rats. The control group was not given iron supplementation, for the treatment groups 1, 2 and 3 were given iron supplementation at doses of 0.54 mg/200grBW, 1.08mg/200grBW, and 2.16mg/200grBW given once a day.

The dose of iron supplementation in this study was the independent variable, whereas the histopathology of the gastric mucosa which was given iron (Fe) supplementation was the dependent variable. Prior to the study, the rats were acclimatized for 7 days. Then the rats were mated with a ratio of male and female rats, namely, 1:1 and 1:2 each in one cage. If after mating a female rat has a vaginal plug, then the rat is declared pregnant on day 0 and put into the K-negative and treatment groups. The K-negative group will not be given iron supplementation, group P-1 will be given iron supplementation at a dose of 0.54mg/200grBW per day, P-2 will be given iron supplementation at a dose of 1.08mg/200grBW per day, and treatment 3 will be given iron supplementation at a dose of 2.16 mg/200grBB. All rats were given a standard feed of 40 grams per day and drank ad libitum.

Iron supplementation was given to rats during the 18th day of pregnancy as much as 1 ml by sonde. The rat stomach was taken and then proceeded to make preparations from the organs using Hematoxylin and Eosin (HE) staining. After that, they were observed using an Olympus BX 1 light microscope and OlyVIA Scan Dot Slide software with 400x magnification in 5 fields of view randomly. Observation of gastric preparations was assessed using a modification of the Bathel Manja mucosal epithelial integrity score (Barthel et al., 2003).

Table 1. Mucosal Epithelial Integrity Score

Score	Mucosal Epithelial Integrity
0	No pathological changes
1	Epithelial desquamation
2	Damage 1-10 epithelial cells
3	Damage > 10 epithelial cells

Data analysis technique

The SPSS 16.0 for Windows program is a program used to process data that has been obtained. Then tested for normality and homogeneity first. If the data is normal and homogeneous, then proceed with the One Way ANOVA parametric test and proceed with Post Hoc Tukey analysis.

3. Results and Discussions

The treatment of iron supplementation was given from the first day of pregnancy to the 18th day. Stomach collection of rats was carried out on the 19th day of pregnancy and then HE preparations were made. The results of observations of rat gastric epithelial cells using the mucosal epithelial integrity score were then averaged and processed using a program for analysis (McCrea & Bates, 1999).

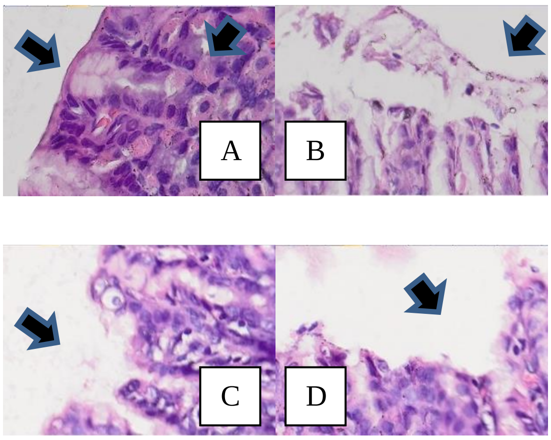
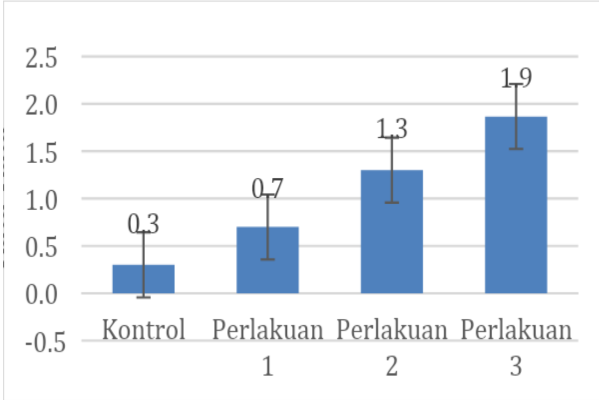


Figure 1. Histopathology of the Rat Gastric Mucosal Epithelium at 400x Magnification

(A) Control has a score of 0, i.e. no pathological/normal changes, (B) Treatment 1 has a score of 1 seen epithelial desquamation, (C) Treatment 2 has a score of 2 seen damage to 1-10 epithelial cells, (D) Treatment 3 has a score 3 shows >10 epithelial cell damage.

The following are the results of the average gastric mucosal epithelial integrity score:

Figure 2. Graph of the results of the average mucosal epithelial integrity score



The graph shows that the higher the average value, the higher the epithelial damage. In the graph it is known that the lowest damage is the control/without treatment group and the highest damage is in the treatment group 3. This shows that the increasing dose given, the damage that occurs also increases.

Normality and Homogeneity Test

The results of the normality test of the Shapiro-Wilk Test (Table 2) showed normal or significant data, namely 0.180 ($p>0.05$), which can be concluded that the mucosal epithelial integrity score data was normally distributed. While the homogeneity test used was the Levene Test (Table 3) which showed a data result of 0.827 ($p > 0.05$) which can be concluded that the mucosal epithelium integrity score data has a homogeneous data variant.

One Way ANOVA test

This test was carried out to determine whether there was a significant difference in the epithelial cells of the four groups. The difference is called significant/meaningful if $p < 0.05$. The results were 0.0000 (Table 4) so that it can be said that there were significant differences in the epithelial cells of each group

Post Hoc Tukey Test

Table 5. Post Hoc Tukey Test

Integritas Mukosa

Tukey HSD[a]

Kelompok	N	Subset for alpha = .05		
		1	2	3
Kontrol	6	.3000		
P1	6	.7000		
P2	6		1.3000	
P3	6			1.8667
Sig.		.184	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Post Hoc Tukey test results (Table 5) showed no significant differences in epithelial cells between the K-negative and P-1 groups. There were significant differences in epithelial cells seen between the K-negative and P-2 and P-3 groups.

Based on the results of the study, the highest average mucosal integrity score was treatment 3 with a dose of 2.16 mg/200 g BW. It can be concluded that the higher the dose given, the higher the score or the higher the damage to the epithelial cells. ANOVA analysis showed that there was a significant difference with the results p = 0.000 (p <0.05), the meaning of the results was that there were significant differences between groups P-1, P-2, and P-3 who were given iron supplementation at a dose of 0.54 mg /200grBB, 1.08mg/200kgBB and 2.16mg/200grBB with the control group seen from the gastric mucosal epithelium score (Sunkara et al., 2017).

Damage to gastric epithelial cells occurs due to the formation of free radicals caused by iron supplementation, then causes an increase in Reactive Oxygen Species (ROS) which will trigger oxidative stress. Free radicals themselves will trigger lipid peroxidation and tissue damage. Lipid peroxidation can cause damage to the structure and function of the membranes, one of which is the gastric mucosa, which initiates the formation of lesions in the gastric mucosa (Powers & Jackson, 2008).

The gastric mucosa naturally produces prostaglandins (PGE2) which can inhibit gastric acid secretion. Iron can inhibit the production of prostaglandins. Inhibition of prostaglandin synthesis in the stomach will increase acid secretion, and cut off the defense of the gastric mucosa (Hisakawa et al., 1998). These conditions can cause irritation to the stomach and result in damage to the mucosa, parietal cells, and endothelial cells (Shibata-Nozaki et al., 2011).

This study has results that are in accordance with other existing studies related to iron (Fe) supplementation can cause damage to the stomach. As in the case study conducted by Marginean and Melit (2017).

The histopathology of the gastric mucosa also shows inflammation and necrosis. Damage to gastric epithelial cells can be influenced by external factors that were not studied, such as the psychological state of the rats, the influence of rat feed, or Helicobacter pylori bacteria. It could also be caused by the possibility of errors in the technique of organ harvesting and preparation of preparations which is a weakness in this study.

4. Conclusion

From the research that has been done, it can be concluded as follows: Giving high doses of iron (Fe) supplementation has an effect on the histopathology of the gastric mucosa of rats. Provision of iron (Fe) supplementation at a dose of 0.54 mg/200grBB from research has had a damaging effect on gastric mucosal epithelial cells.

Based on the research that has been done and the conclusions obtained, suggestions from researchers are as follows. Further research can be carried out regarding iron (Fe) supplementation for more in-depth gastric histopathology. Further research can be carried out regarding Helicobacter pylori bacteria in the stomach given iron (Fe) supplementation. Further research can be carried out regarding iron (Fe) supplementation on variables.

Bibliography

Alimul, A. A. (2008). *Pengantar ilmu kesehatan anak untuk pendidikan kebidanan*. Penerbit Salemba.

Barthel, M., Hapfelmeier, S., Quintanilla-Martínez, L., Kremer, M., Rohde, M., Hogardt, M., Pfeffer, K., Rüssmann, H., & Hardt, W.-D. (2003). Pretreatment of mice with streptomycin provides a Salmonella enterica serovar Typhimurium colitis model that allows analysis of both pathogen and host. *Infection and Immunity*, *71*(5), 2839–2858.

Hisakawa, N., Nishiya, K., Tahara, K., Matsumori, A., & Hashimoto, K. (1998). Down regulation by iron of prostaglandin E2production by human synovial fibroblasts. *Annals of the Rheumatic Diseases*, *57*(12), 742–746.

McCrea, S., & Bates, N. (1999). Acute iron overdose: clinical features and management. *EMERGENCY Nurse*, *7*(5).

Meliţ, L. E., Mărginean, C. O., Mocanu, S., & Mărginean, M. O. (2017). A rare case of iron-pill induced gastritis in a female teenager: a case report and a review of the literature. *Medicine*, *96*(30).

Perdana, W. Y., & Jacobus, D. J. (2015). Hepcidin dan Anemia Defi siensi Besi. *Cermin Dunia Kedokteran*, *42*(12), 919–926.

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews*, *88*(4), 1243–1276.

Shibata-Nozaki, T., Ito, H., Mitomi, H., Akaogi, J., Komagata, T., Kanaji, T., Maruyama, T., Mori, T., Nomoto, S., & Ozaki, S. (2011). Endogenous prostaglandin E2 inhibits aberrant overgrowth of rheumatoid synovial tissue and the development of osteoclast activity through EP4 receptor. *Arthritis & Rheumatism*, *63*(9), 2595–2605.

Sunkara, T., Caughey, M. E., Nigar, S., Olivo, R., & Gaduputi, V. (2017). Iron pill gastritis: an under diagnosed condition with potentially serious outcomes. *Gastroenterology Research*, *10*(2), 138.

Wiknjosastro, H., Saifuddin, A. B., & Rachimhadhi, T. (2010). Ilmu bedah kebidanan. *Jakarta: PT Bina Pustaka*.