

The Effect of Ferrous Sulphate on the Gross and Histological Changes in the Body of Gastric Mucosa of Adult Albino Rats

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Abstract

Background:To observe the effects of Ferrous sulphate on the body of gastric mucosa of adult albino rats.

Methods: An experimental study was carried out on 30 adult albino Wistar rats for 12 weeks. Two groups were made; A and B. They were divided further into A1, A2, B1 and B2 for qualitative parameters and statistical purposes. Group A was kept as control and divided into 2 sub groups; A1 and A2 and was given normal food and water. The experimental group B was divided into 2 sub groups B1 and B2. Ferrous sulphate was given orally for 4 and 12 weeks respectively through gavage needle for maximum efficacy in the experimental group. Albino rat's stomach was dissected, fixed in 10% formalin and processed for light microscopy. The short and long term effects of ferrous sulphate were observed on 4th and 12th week respectively. For histological analysis H&E was used. Body of gastric mucosa of adult albino rat was considered for this study.

Results: Change in the colour of mucosa was found in 10 (100%) of cases at 12 weeks whereas only 8 (80%) cases showed changes after 4 weeks. Haemorrhage was seen scattered in 4 (40%) cases after 4 weeks whereas present in all 10 (100%) cases after 12 weeks. Ulcer was seen in 4 (40%) of cases after 12 weeks. Epithelial changes were seen in 5 (50%) cases at 4 and 8 (80%) of cases after 4 and 12 weeks respectively. Ulcerative changes were seen in 4 (40%), granulation tissue/ fibrosis was seen in 7 (70%) cases in experimental group only. There was a significant decrease in the weight of rats from 226.69 ±22.31mg to 169.43±22.39mg in the experimental group with $p < .001$. Inflammatory cell count significantly increased from (4.03±1.02) to 17.01±5.01 in the experimental group with $p < .001$.

Conclusion: Ferrous Sulphate has detrimental effects on the gastric mucosa of adult albino rats. Haemorrhages, ulcerated areas and change in colour of mucosa was observed with naked eye.

Inflammatory cell infiltrate was found to be the most common presentation.

Key Words: Ferrous sulphate, Gastric mucosa

Introduction

Iron deficiency anaemia is a common nutritional deficiency caused by either decreased use or increased loss of Iron. Iron sulphate (FeSO_4) is the most commonly administered drug to overcome iron deficiency anaemia in pregnancy and post partum period and is an important constituent of nutrition for growth of children and adults. Iron is an important metal and is considered to be an integral part of many proteins as haemoglobin and myoglobin. It also plays an important role in DNA synthesis¹⁻³ In 19th century, oral iron therapy has been the most commonly used therapy for iron deficiency anaemia⁴. Anaemia is the commonest cause of malnutrition affecting 43% of children worldwide as stated by WHO.⁵ More adverse effects were seen in patients getting simple ferrous sulphate than with extended release tablets.⁶ Iron deficiency anaemia has found to be the foremost nutritional deficiency in Pakistan. The prevalence of anaemia in Pakistan is 83%, 78%, 85% and 82.9% among pregnant, lactating women, adolescent girls and children respectively.⁷

The importance of iron is also emphasized in Surah Hadith of Holy Quran where it is stated "And we also sent down iron in which lies great dangers and it has many uses for mankind".⁸ Iron is present in ferrous (Fe^{2+}) and ferric (Fe^{3+}) states.^{4,9} Ferrous salts are 3 times more absorbed than ferric salts.⁴ FeSO_4 tablet is the most commonly used oral form of iron (325mg tablet contains 65mg iron).^{10,11,13} Other iron preparations are Ferrous Fumarate and Ferrous Gluconate.¹² Less commonly used are iron amino chelate and iron polymaltose.³ Injectable forms are Iron sucrose, Iron dextran, Sodium Ferric Gluconate and Ferrumoxytol but experienced staff is required for intravenous use as there is risk of allergic reactions.^{14,15} Oral iron tablets are easy to use, cheap, and easily

available hence no hospital staff or methods are required for their use.¹⁶

FeSO₄ is an important constituent of our nutrition yet it creates reactive oxygen species also known as free radicals.^{17,19,20} They are harmful for the body causing wide range of tissue and organ damage.^{19, 20} Injury has been observed in a few cases in gastrointestinal tract due to the usage of oral iron tablets.²¹ Oral iron mucosal injury has a prevalence of 0.7% and may be seen at only 5 days after the iron treatment has been initiated in humans.⁵

The difference between anatomy of human stomach and rat stomach is that human stomach has proper fundus, body and antrum with glandular simple columnar epithelium whereas in rat stomach, most of the stomach is covered by non glandular stratified squamous epithelium and a small lower part is composed of simple columnar epithelium. This leaves a small fundus, proper body and a very small antrum.¹⁸

Material and Methods

The experimental study was carried out in the animal house of Anatomy Department, Post Graduate Medical Institute, Lahore. Thirty albino rats 55-60 days old of either sex weighing (150-250gms) were procured from NIH, Islamabad after Ethical Committee approval. Two groups were made and each was further divided into 2 subgroups. One was control and other experimental group which was given FeSO₄ at therapeutic dosage. The duration of study was one year and the experiment was conducted for 12 weeks. They were housed separately in climate controlled environment and were kept on normal feed and water. All rats used in the study were handled with international, natural and institutional guidelines for care and use of laboratory animals as promulgated by the Canadian Council of Animal Care (1984). They were housed in cages with bar lids used to hold water bottles and feed to prevent contamination with urine or faeces. They were kept in ventilated room at ambient temperature of 28±2.0° C and humidity (60±10%) under 12 hour light /dark cycles and water ad libitum. Each rat was tagged randomly on the tail. Following acclimatization of one week, each rat was weighed at the commencement of the study and on 4th and 12th week respectively. Ferrous sulphate was obtained in the crystalline form and dose was given by dissolving ferrous sulphate in water. Dose was calculated to be 27mg/kg/day.

The dose was given through gavage needle to make sure that the drug had efficiently and completely

reached the gastric mucosa. To make a model comparable to humans as there is little compliance to multivitamins, iron sulphate was given once a day in morning with empty stomach to observe the maximal effect. After 4th and 12th week, respective rats were put in chloroform chamber and cervical dislocation was done under deep anesthesia. The surgical procedure was carried out under sterile conditions. The anesthetized animal was placed with limbs stretched on the operating board. A midline incision was given from the centre of neck extending downwards till the end with a scalpel and knife. The two flaps were divided in two parts by giving horizontal incision in the skin of both sides. Stomach was identified behind the liver and was opened along the greater curvature. It was washed with normal saline to remove food debris and secretions. The gastric mucosa was stretched out with the help of forceps. Naked eye examination was done to see change in colour, hemorrhage and ulcer. The histological analysis of the mucosa was done through light microscope. The stomach was photographed before taking sections. Sections were taken randomly from the body of the gastric mucosa and placed in cassette with labels and fixed in neutral 10% buffered formalin for 48 hours. Tissue processing was done and blocks were kept in refrigerator. They were mounted on rotary microtome and 5µm consecutive thick sections were lifted on it. Slides were labeled with a lab code and number was given accordingly.

Haemotoxylin and Eosin stain was used to see the histological changes in the mucosa. Quantitative parameters were assessed by eye piece micrometer scale which was calibrated against the stage micrometer. Quantitative variables were given as mean ± standard deviation and categorical data was shown as frequencies and percentages.

Results

On gross examination no significant change in colour of gastric mucosa in control groups A1 and B1 was observed. There was significant change in group A2 at 4 weeks ($p < .007$) and group B2 after 12 weeks ($p < .001$) was observed. The mucosa appeared dull, congested, oedematous and reddish (Table 1). In group A1 and B1, haemorrhage could not be visualized. In group A2 hemorrhagic areas were seen in 4 (40%) cases after 4 weeks ($p < .231$) and 10 (100%) cases after 12 weeks of ferrous sulphate dosage ($p < .001$). Ulcer could not be visualized in any of cases in groups A1, A2 and B1. In group B2, 4 (40%) cases showed ulceration after 12 weeks (Table 1; Figure 1). In

qualitative parameters ulcers were seen in 4 (40%) cases in group B2 only (Table 2;Figure 2).

Table 1: Comparison of gross qualitative parameters after 4 and 12 weeks on body of gastric mucosa

Qualitative Parameters	Control Gp.A1	Exp Gp.B1	P-Value	Control Gp.A2	Exp Gp.B2	p-Value	BIVs B2 p-value
Change in Color of Mucosa	0(0%)	8 (80%)	.007*	0 (0%)	10 (100%)	<.01**	.474
Presence of lesion (erosion)	0(0%)	0(0%)	NA	0(0%)	5(50%)	.101	.033*
Hemorrhage	0(0%)	4 (40%)	.231	0(0%)	10 (100%)	<.01**	.011
Ulcer	0(0%)	0(0%)	NA	0(0%)	4(40%)	.23	.087

*p<.05,**P<.01, ***P<.001, Fischer exact test used for comparison;N=number of rats: A1 (n=5); A2 (n=5);B1 (n=10); B2 (n=10)

Table 2: Comparison of histological qualitative parameters after 4 and 12 weeks on body of gastric mucosa

Qualitative Parameters	Control Gp.A1	Exp Gp.B1	P-Value	Control Gp.A2	Exp Gp.B2	P-Value	BIVs B2 p-value
Changes in epithelium	0(0%)	5(50%)	.101	0(0%)	8(80%)	.007**	.350
Presence of necrosis	0(0%)	0(0%)	NA	0(0%)	4(40%)	.231	.087
Presence of fibrosis/granulation tissue	0(0%)	0(0%)	NA	0(0%)	7(70%)	.026*	.003**

*p<.05, **p<.01, Fischer exact test used for comparison

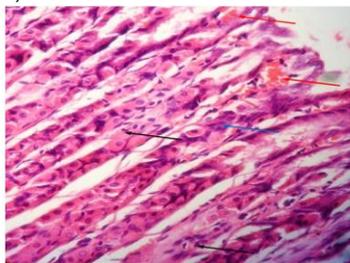


Fig 1. Body of gastric mucosa (group B1) showing congested and hemorrhagic vessels (red arrow) and neutrophils (black arrow) with mast cells (blue arrows) after 4 weeks of experiment

Granulation tissue and fibrosis was seen in 7 (70%) cases in group B (Figure 2).In quantitative parameters there was a significant increase in weight of the rats in control group; 185.39±18.02 (mg) than experimental group; 199.99±16.33 (mg) at 4 weeks (group B1) and 226.69±22.31 (mg) after 12 weeks (group B2) (table 3). In group B1, there was a slight increase in the weight of the rats from 174.61±16.33 (mg) to 183.14±22.79 (mg) after 4 weeks of ferrous sulphate usage. In group

B2, the weight of the rats significantly decreased from 177.19±18.62 (mg) to 169.43±22.39 (mg) after 12 weeks. There was significant increase in inflammatory cell count from 4.03±1.02 (µm) to 11.15±3.28 (µm) after 4 weeks of experiment (p< .001) and increased significantly to 17.01±5.01 (µm) after 12 weeks (p< .001) (Table. 4 ; Figure 3).

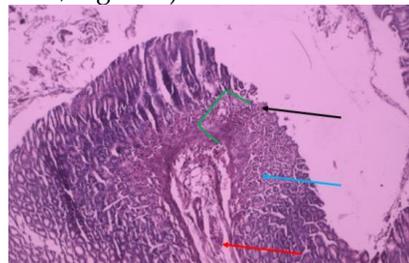


Fig 2. Body of gastric mucosa (group B2) showing erosion and ulcerative changes (black arrow), congested and hemorrhagic vessels (red arrow) and inflammatory cell infiltrate (blue arrow) after 12 weeks of ferrous sulphate ingestion.

Table 3: Comparison of groups showing significant change in weights after 4 and 12 weeks on body of gastric mucosa

Quantitative Parameter	Control	Exp Gp. B1	Exp Gp. B2	ANOVA	
	Mean± SD	Mean± SD	Mean± SD	F	P
(mg)	185.39±18.02	174.61±16.33	177.19±18.62	1.014	.376
Initial Weight	185.39±18.02	174.61±16.33	177.19±18.62	1.014	.376
Weight at 4 weeks	199.99±17.69	183.14±22.79	182.76±19.27	2.413	.109
Weight at 12 weeks	226.69±22.31	-	169.43±22.39	21.85	<.001***

***p<.001

Table 4: Comparison of groups showing significant change in inflammatory cell count after 4 and 12 weeks in body of gastric mucosa

Quantitative Parameters	Control	Exp Gp. B1	Exp Gp. B2	ANOVA	
	Mean± SD	Mean± SD	Mean± SD	F	p
Inflammatory Cell Count	4.03±1.02	11.15±3.28	17.01±5.01	33.8	<.001*

*p<.0001

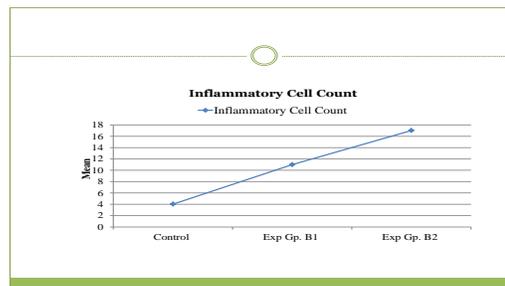


Figure 3: Escalation in inflammatory cell count in body of gastric mucosa of albino rats at 4 and 12 week.

Discussion

Most of the previous light microscopic studies on ferrous sulphate focused on intestinal mucosa.^{3,19} Little attention has been directed at changes occurring in the gastric mucosa. However, some studies showed the detrimental effect of FeSO₄ on the gastric mucosa but the information is very scarce and limited.^{1,2,22,23} Due to ethical considerations, adult albino Wistar rats were taken for experiment. The similarities in the gastric mucosa of rats are more than the differences from the human gastric mucosa so the results concluded can be considered for humans.³⁰ In the present study, the most important feature was increased inflammatory count. This finding was in accord with the one reported earlier by Toblli et al; 2008 and Kumar et al; 2013 after use of FeSO₄ in the gastrointestinal mucosa of adult albino rats that inflammatory cell count is increased in body of gastric mucosa after utilizing FeSO₄.^{3, 6} Chronic inflammation after use of iron medication was documented earlier by Hiraishi et al; 1991 augmenting the current study²². Increased inflammation was also observed by Kaye et al; 2008 similar to the current study.²³ Erosions, haemorrhages and ulcers were visualized on gross and histological study by Ji and Yardley, 2004 and Cimino-Mathews et al; 2010. These findings potentiated the current study.^{24,25} Ulcers were also observed by Marginean et al; 2006 and Hashash et al; 2013 which were similar to the present study.^{27,28} It was documented by Laine et al; 1998 and Marginean et al; 2006 that ulcers were produced in the gastric mucosa of albino rats.^{26,27} It was proved by Reagen- Shaw et al; 2007 that a few ulcerated or necrotic areas were seen along with deposits of iron in the gastric mucosa²⁹. It was observed by Toblli et al; 2008 that there was a significant change in the weight of rats receiving FeSO₄. This owed to the disturbed bowel movements caused by the drug. This study potentiated the current study where rats lost weight after 12 weeks of iron sulphate ingestion.³⁰ Formation of fibrous bands and granulation tissue was also evaluated by Zhang et al; 2008 in a study which also potentiated the current study.²⁰

Conclusion

Extensive use of ferrous sulphate used in common clinical practice can cause injury and harm to the gastric mucosa.

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