

In Vitro Effect of Iron Salts on Peristaltic Activity of Goat Ureter

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<http://dx.doi.org/10.18049/jcmad/235>

Abstract

Objective: To study in vitro effect of iron salts on peristaltic activity of goat ureter and to find out mechanism action of iron salts. **Material and Method:** Ureters from freshly slaughtered goats (*Capra aegagous hircus*) were collected from a local slaughter house. The effect of iron salts on peristaltic activity of goat ureter was studied by Trendelenberg's method. Parameter studied was the extent of inhibition of contractions of goat ureter. Feldberg and Lin method was used to find out mechanism action of iron salts. **Result:** All iron salts produced depressant action on peristaltic activity in goat ureter. Amongst the iron salts used in this study, ferrous sulphate (68%) was found to be the most potent antispasmodic followed by ferrous ammonium sulphate (57.5%), ferrous fumarate (55.8%), iron dextran complex (55.1%) and iron sorbitol citric acid complex (53.4%). **Conclusion:** Iron salts possess antispasmodic properties. Iron salts may follow the mechanism of inhibiting the peristaltic activity apart from their astringent action as well as alteration of intestinal flora in causing constipation. Based on Feldberg and Lin method, it was observed that iron salts neither inhibit the action of acetylcholine nor that of nicotine on smooth muscle of goat ureter suggesting its direct action on smooth muscle of goat ureter.

Key words: Goat ureter, iron salts, peristalsis

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Introduction

Iron salts are used extensively in clinical practice for management of Iron Deficiency Anemia but constipation is the most common side effect. A preliminary report on the pharmacological effect of iron salts revealed some of the probable mechanisms of constipation as, due to alteration of intestinal flora,^[1] and due to its astringent action.^[2]

The study of effects of drugs on ureteral peristalsis can help in introduction of drugs with wide range of therapeutic applications, related to ureter. Some of the species used for measuring ureteral peristalsis are: Dog ureter,^[3] Sheep ureter,^[4] Pig ureter,^[5] Human ureter,^[6] Guinea pig ureter/Rat ureter,^[7] Rabbit ureter^[8] and Chicken ureter.^[9]

In vitro experimentation using goat ureter does not require killing of the animals in the research laboratory, as the tissue can be obtained directly from the slaughter house. Domestic goat (*Capra*

aegagous hircus) is widely distributed in tropical countries and provides supply of meat, leather, and milk.^[10] The gross anatomy of the goat ureter is very much similar to that of humans; it receives an autonomic nerve supply from the pelvic nerve^[11] and blood supply from the umbilical artery.^[12] Thus easy availability of goats is an advantage for researchers, working on ureteral peristalsis in tropical countries.

Not much work has been done regarding the action of iron salts on ureteral peristaltic activity; hence exact mechanism of action is not clear. Therefore, it was of interest, to study in detail, the effect of iron salts on goat ureter.

Materials and Methods

After the ethical approval from Institutional Animal Ethics Committee, present study was carried out at Department of Pharmacology, NKP Salve Institute of Medical Sciences and Research Centre, Nagpur, India. Peristaltic

activity in goat ureter was recorded by Trendelenberg's method.^[13]

Collection of goat ureter

The ureters of freshly slaughtered goats weighing from 15 to 20 kg were immediately collected from a local slaughter house. They were then immersed in freshly prepared mammalian ringer(MR)^[14] with the composition given by Burn i.e.: sodium chloride 9.00 gm, potassium chloride 0.42 gm, anhydrous calcium chloride 0.24 gm, sodium bicarbonate 0.5 gm, dextrose 1.00 gm and distilled water 1000 ml. It was maintained at 40°C and oxygenated with 100 % oxygen and pH maintained at 7.4. Necessary precautions had been taken to avoid trauma and undue stretching of the tissue during its collection. The proximal 1/3rd of each ureter (about 4-10 cm length from the pelvic ureteric junction) was selected for experiment.

Peristaltic activity in goat ureter

Peristaltic activity in goat ureter was recorded using Trendelenberg's method with suitable modifications. The tissue was placed in an organ bath (MM 221, Inco instruments, Ambala, India) containing warm MR solution. The lower end of the ureter was mounted over the J tube

connected to a reservoir. The ureter was thoroughly washed with MR in order to expel air bubbles. The other end of the tissue was tied with thread to the lever. Before starting the experiment, optimal intraluminal pressure required to elicit peristalsis of the ureter was determined by varying the height of the MR reservoir (between 0.5-1 cm) and then maintained throughout the experiment. Raising the MR reservoir to a critical height for 2 minutes induced peristaltic activity. This rise in intraluminal pressure triggers the peristaltic reflex and the fluid inside the ureter is driven to and fro. The changes in the volume were sensed by a volume transducer attached to the reservoir. The peristaltic activity was recorded for 2 minutes following which, the MR reservoir was lowered down to stop the peristalsis. Temperature of the organ bath was maintained between 40-42°C and MR in the inner tube was constantly bubbled with 100% oxygen. Responses were only elicited when the spontaneous contraction remained stable in both amplitude and frequency.

Following Iron salts were used in this experiment:

Sr.No.	Name of Iron salt	Submaximal dose	Maximal dose
1	Ferrous sulphate (Glaxosmithkline, India)	1.5 x 10 ⁻⁶ / ml	2 x 10 ⁻⁶ / ml
2	Ferrous fumarate (Merck, India)	5 x 10 ⁻⁶ / ml	15 x 10 ⁻⁶ / ml
3	Ferrous ammonium sulphate (Zen labs, India)	5 x 10 ⁻⁶ / ml	10 x 10 ⁻⁶ / ml
4	Inferon (Iron dextran complex) (Shreya labs, India)	10 x 10 ⁻⁶ / ml	20 x 10 ⁻⁶ / ml
5	Jectofer (Iron sorbitol citric acid complex) (CFL Pharma, India)	10 x 10 ⁻⁶ / ml	20 x 10 ⁻⁶ / ml

To find out mechanism of action of iron salts by Feldberg & Lin method,^[15] Acetylcholine (Loba chemicals, Mumbai) 1 x 10⁻⁵ and Nicotine (Loba chemicals, Mumbai) 1 x 10⁻⁵ were used.

Statistical Analysis

The following null hypothesis was assumed. Firstly, it was assumed that the goat ureter will not have normal peristalsis and secondly, if normal peristalsis is present then it will not respond to drug. p < 0.05 was considered as significant, p < 0.01 was considered as very significant and p < 0.001 was considered as

highly significant. The mean height of normal contraction with standard deviation was calculated and compared with the height of contraction after drug treatment in terms of mean ±SD. The significance was calculated by using one way ANOVA using SPSS version 11.0 USA.

Results

All preparations showed spontaneous activity within 5-15 minutes following mounting in the organ bath. Mean height of the contraction

observed was 30mm/min. We also observed that tissue remained responsive for 8-10 hours with constantly maintained environmental conditions. The contraction of individual preparation demonstrated uniform pattern, with rhythmic activity, noted at times. Pre-drug activity was

observed in all preparations and only those with optimum activity were used. Ferrous sulphate was found to be the most potent iron salt in inhibiting the peristalsis while least inhibition was observed with Jectofer (Table -1 & Fig.-1).

Table 1: Effect of iron salts on peristaltic activity of goat ureter

Drug	Control (Mean Height of contraction in mm)	Partial inhibition	% change inhibition	p-value
Ferrous sulphate	29.03±4.24	9.24±1.08	68.0%	0.0002
Ferrous fumarate	28.02±4.47	12.40±2.51	55.8%	0.0000
Ferrous ammonium sulphate	29.03±3.73	12.41±2.50	57.5%	0.0000
Inferon	28.46±1.68	12.81±2.16	55.1%	0.0000
Jectofer	28.82±1.79	13.40±1.33	53.4%	0.0002

Figure -2: Effect of ferrous sulphate on peristaltic activity of goat ureter

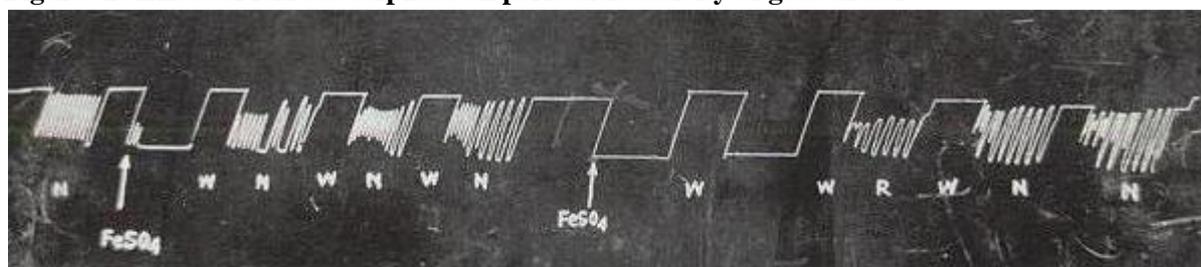


Figure- 1: Log percentage change

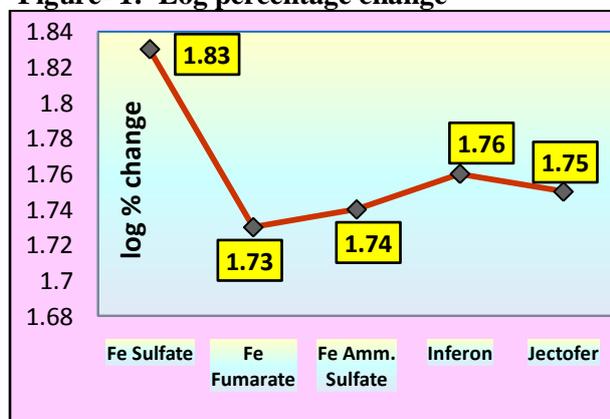
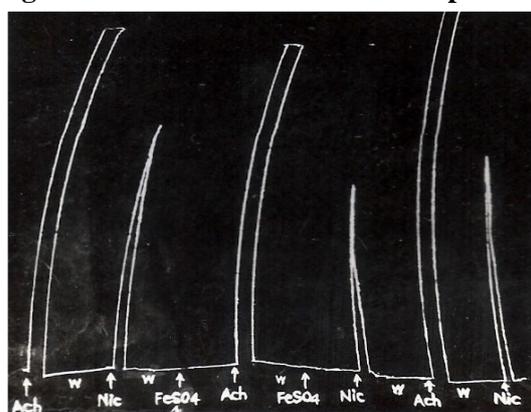


Figure- 3: Mechanism of ferrous sulphate



Effect of Ferrous Sulphate

Ferrous sulphate showed partial inhibition (68%) of spontaneous peristaltic activity at 1.5×10^{-6} /ml concentration and complete inhibition at a concentration of 2×10^{-6} /ml (Figure- 2).

Effect of Ferrous fumarate

Ferrous fumarate showed partial inhibition (55.8%) of spontaneous peristaltic activity at 5×10^{-6} /ml concentration and complete inhibition at a concentration of 15×10^{-6} /ml.

Effect of Ferrous ammonium sulphate

Ferrous ammonium sulphate showed partial inhibition (57.5%) of spontaneous peristaltic activity at 5×10^{-6} /ml concentration and complete inhibition at a concentration of 10×10^{-6} /ml.

Effect of Inferon (Iron dextran complex)

Iron dextran complex showed partial inhibition (55.1%) of spontaneous peristaltic activity at 10×10^{-6} /ml concentration and complete inhibition at a concentration of 20×10^{-6} /ml.

Effect of Jactofer

Iron sorbitol citric acid complex (Jactofer) showed partial inhibition (53.4%) of spontaneous peristaltic activity at 10×10^{-6} /ml concentration and complete inhibition at a concentration of 20×10^{-6} /ml.

Effect of iron (ferrous sulphate) in presence of acetylcholine and nicotine

The responses of acetylcholine and nicotine after administering ferrous sulphate were similar with baseline responses, indicating that ferrous sulphate does not affect the activity of acetylcholine and nicotine on peristalsis of ureter (Figure- 3).

Discussion

In our study, the activity of goat ureter was examined in terms of peristaltic activity, as described by Trendelenberg P.,1917. All the iron salts- ferrous sulphate, ferrous fumarate, ferrous ammonium sulphate, Inferon (iron dextran complex) and Jectofer (iron sorbitol citric acid complex) produced inhibitory action on peristaltic activity in goat ureter, which was dose related. Our results agree and explain the observation, that iron salts when used in anaemia, cause constipation, as a side- effect.

It is believed that iron salts cause constipation by their astringent action as well, as by alteration of intestinal flora. Our results show that, in addition to these two mechanisms, iron salts might be causing constipation by inhibiting peristaltic activity.

Feldberg and Lin., 1949 had described a method to find out the mechanism of action of a substance causing inhibition of peristaltic activity. They had shown that, if the action of acetylcholine and nicotine on longitudinal muscle of guinea pig ileum is blocked by a substance, then it acts by blocking cholinergic receptors in the smooth muscle, just like atropine. If the action of acetylcholine and nicotine is not blocked, then it acts by causing direct depression of smooth muscle, like Nitrates or papaverine. But, if the action of acetylcholine is not blocked, while that of nicotine is blocked, then it is inferred that, it acts by blocking intramural ganglion like hexamethonium.

While analyzing the mechanism of inhibitory action of iron salts on peristaltic activity, by the

method of Feldberg and Lin, 1949; it was observed that iron salts neither inhibit the action of acetylcholine, nor that of nicotine on smooth muscle of goat ureter. This shows that iron salts neither block cholinergic receptors on smooth muscle of goat ureter nor block the intramural ganglion. Thus it is inferred that they act directly on the smooth muscle of the goat ureter and inhibit it. The findings of present study were comparable with those of Sharma et al., on frog stomach and guinea pig ileum.^[16]

Ferrous sulphate caused partial inhibition of ureter peristalsis, at the dose of 1.5×10^{-6} /ml and complete inhibition at the dose of 2×10^{-6} /ml, with % change inhibition of 68%. Ferrous fumarate caused partial inhibition at 5×10^{-6} /ml and complete inhibition at 15×10^{-6} /ml, with % change inhibition of 55.8%. Ferrous ammonium sulphate caused partial inhibition at 5×10^{-6} /ml and complete inhibition at 10×10^{-6} /ml, with % change inhibition of 57.5%. Inferon caused partial inhibition at 10×10^{-6} /ml and complete inhibition at 20×10^{-6} /ml, %change inhibition being 55.1%. Jectofer caused partial inhibition at 10×10^{-6} /ml and complete inhibition at 20×10^{-6} /ml, with % change inhibition of 53.4 %. So, ferrous sulphate was found out to be the most potent iron salt in inhibiting the peristalsis, followed by ferrous ammonium sulphate, ferrous fumarate, Inferon and Jectofer.

Conclusion

The study of the effect of iron salts on peristaltic activity of goat ureter can help with the additional use of iron salts in management of various ureter related pathological conditions, such as ureteric colic, in preparation for procedures such as extra corporal lithotripsy, for avoiding retrograde propulsion of ureteral calculi, in providing relaxation of tissue preoperatively and also to ease the expulsion of ureteral calculus during flush therapy.

Source(s) of support: Nil

Conflict of Interest: None declared

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