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## STUDY OF EFFECT OF ERYTHROMYCIN BASE ON GASTROINTESTINAL SMOOTH MUSCLES IN LABORATORY ANIMALS *IN-VITRO*

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**ABSTRACT: Background** - Drugs affecting gastrointestinal motility find place in the treatment of gastrointestinal motility disorders. **Objective:** To evaluate the effect of erythromycin base on the isolated tissue of rabbit jejunum, guinea pig ileum & rat colon in vitro and also to see whether it alters the contractile responses induced by acetylcholine. **Materials and Methods:** a) Rabbit jejunum experiment: After recording the baseline spontaneous rhythmic contractions, erythromycin was added to the organ bath and the responses were taken every 3 min for 30 min. The effect on frequency & the amplitude of spontaneous contractions were noted. (Erythromycin doses used - 1, 2, 10 & 20  $\mu$ M; in six rabbits). b) Guinea pig ileum & rat colon experiment: Initially responses were recorded with acetylcholine (1-100 $\mu$ g). After giving wash, erythromycin was added to the organ bath and response was recorded for 30 seconds; then after a contact period of 3 min with erythromycin, responses to selected dose of acetylcholine were recorded again. (Erythromycin doses used - 100, 300 500 & 1000  $\mu$ M; in six guinea pigs & rats each) **Results:** In isolated rabbit jejunum, erythromycin (1-20  $\mu$ M) produced increase in basal tone and higher concentrations (10-20  $\mu$ M) reduced both rate & height of spontaneous contractions. In isolated guinea pig ileum & rat colon, erythromycin showed no effect on basal tone. Erythromycin (100-300  $\mu$ M) reduced the magnitude of response to acetylcholine induced contractions in guinea pig ileum, but increased the magnitude of acetylcholine induced contractions in rat colon in dose dependent manner. **Conclusion:** Erythromycin has different effects on different parts of the gastrointestinal tract in different species of animals.

**INTRODUCTION:** Mammalian stomach has three layers of smooth muscles i.e. innermost circular, middle oblique and outermost longitudinal muscles. The small intestine and large intestine (colon) has only two layers of smooth muscles i.e. the inner circular and outer longitudinal muscles.

Motor function of the gastrointestinal tract is controlled by the cholinergic system (excitatory) and sympathetic system (inhibitory) and local hormones like cholecystokinin and motilin. Presence of food in the stomach, psychogenic stimuli and neurotransmitters like serotonin and dopamine can initiate and modify gastrointestinal motility.<sup>1, 2</sup> Drugs affecting gastrointestinal motility find place in the treatment of gastrointestinal motility disorders, including diarrhoea, vomiting and constipation. Erythromycin, a macrolide antimicrobial, was discovered in 1952 by McGuire

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and co-workers. It has been in clinical use for last 6 decades against gram positive bacteria, gram negative cocci and mycoplasma.<sup>3</sup> The prokinetic effect of erythromycin has been reported and confirmed in human volunteers in the fasting and fed state.<sup>4-8</sup> Acute and chronic treatment with erythromycin improves gastric emptying in idiopathic and diabetic gastroparesis<sup>9, 10</sup> as well as post vagotomy gastroparesis.<sup>11</sup> It increases the tone of lower oesophageal sphincter and relieves dyspeptic symptoms in patients with gastroesophageal reflux disease<sup>12, 13</sup> and also stimulates gall bladder emptying.<sup>14</sup>

Erythromycin has shown variable responses in animal studies. In dogs, it induces a migrating contractions originating in the stomach.<sup>15</sup> It also causes contractile response in isolated rabbit intestines.<sup>16</sup> On the other hand, it was shown to inhibit prostaglandin F-2  $\alpha$  induced uterine smooth muscle contractions in pregnant and non pregnant rats *in vitro*<sup>17</sup> and contractions of nerve muscle preparation of guinea pig intestine.<sup>18</sup> In view of this type of variable responses observed in smooth muscles of different species, it was decided to study the effect of erythromycin on acetylcholine induced contractions in guinea pig ileum and rat colon as well as on spontaneous contractions of rabbit jejunum.

## MATERIALS AND METHODS:

The study was approved by the Institutional Animal Ethics committee of T.N Medical College & B.Y.L. Nair Ch. Hospital. The rats, guinea pigs and rabbits required for the experiment were used from the departmental animal house. All experiments were conducted in accordance with guidelines of committee for purpose of control and supervision of experiments on animals (CPCSEA).

### Chemicals:

Acetylcholine (ACh) 200 mg ampoules were obtained from Sigma Chemicals. Stock solution of 1 mg/ml of acetylcholine was prepared in distilled water by adding few drops of HCl for stability. Pure powder of erythromycin base was obtained from M/S Anuh Pharma Ltd Mumbai. Stock solution of 734  $\mu\text{g/ml}$  was prepared in distilled water.

Composition of De Jalon's solution (amount per lit.): 1) Sodium chloride 9 gm, 2) Potassium chloride 0.42 gm, 3) Calcium chloride 0.06 gm, 4) Sodium bicarbonate 0.5 gm, 5) Glucose 0.5 gm, 6) Distilled water to make 1 liter.

Composition of Tyrode's solution (amount per lit.): 1) Sodium chloride 8 gm 2) Potassium chloride 0.2 gm, 3) Calcium chloride 0.2 gm, 4) Sodium bicarbonate 1 gm 5) Magnesium chloride 0.1 gm, 6) Sodium dihydrogen phosphate 0.05 gm, 7) Glucose 1 gm, 8) Distilled water to make 1 liter.

### Procedure:

#### a) *In vitro* isolated rabbit Jejunum Preparation:

Healthy rabbits of either sex weighing between 2.5 to 3.0 kg were selected for the experiment. The rabbits were kept in normal conditions and on a routine diet and sacrificed on the day of the experiment. On the day of the experiment, Tyrode's solution was prepared fresh. The rabbit was sacrificed, abdomen was quickly opened and the jejunum was identified by tracing down the stomach. The abdominal contents were kept hydrated by a wet mop. After identification, 10 cm. of jejunum was cut and kept in oxygenated Tyrode's solution. From the selected piece, 3 – 4 cm of the segment was cut and suspended in an organ bath. One end of the jejunum was attached to the oxygen tube and the other end to a frontal writing lever by means of a thread. The tissue was allowed to stabilize for 10 minutes. The physiological salt solution bathing the tissue was changed at intervals of 3 minutes. The drum was adjusted at the tip of writing lever and spontaneous contractions were recorded on the kymograph.

After two minutes of normal recording, erythromycin 1 $\mu\text{M}$  was added to the organ bath. Then responses were taken every 3 min for 30 min after the addition of erythromycin. The frequency and the amplitude of spontaneous contractions per minute were noted. The tissue was washed after each recording. Same procedure was repeated for 2, 10 and 20  $\mu\text{M}$  of erythromycin. Six such experiments were done. The time required for onset of action of erythromycin and the time required for the contractions to regain its original characteristics was noted.

**b) *In vitro* isolated guinea pig ileum preparation:**

A guinea pig weighing about 450 gm were starved for 48 hours, being allowed water ad libitum. For the purpose of the experiment it was killed. The abdomen was quickly opened and the viscera inspected. In the right lower quadrant of the abdomen, the caecum a greenish sac like structure was identified. A Peyer's patch of lymphoid tissue; using this patch as a landmark, the lower most 10 cm of ileum are removed from the abdomen and placed in a shallow dish containing warm Tyrode's solution. With the help of a 25 ml pipette, the lumen of the ileum was gently rinsed out, care being taken not to exert a hydrostatic pressure of more than 10 cm water.

The ileum was then cut into segments of about 4 cm in the fully relaxed state, and sutures were taken with needle and thread through either end of a segment. With the lower suture the ileum was fixed on to the hooked end of a hollow oxygen tube while the upper thread was tied to the frontal writing lever. The tissue was positioned in an organ bath in Tyrode's solution at 37° C aerated with oxygen or carbogen mixture.

Isotonic graded contractions were obtained with acetylcholine (ACh) using concentration ranging 1 to 100 µg/ml and were recorded on the kymograph. The lowest dose of ACh which gave an appreciable and comparable response (4-7 cm) was selected and the same dose was repeated to test for reproducibility. Then 100 µM of erythromycin was added to the organ bath and a response was recorded on kymograph for 30 seconds. It was observed if erythromycin caused contraction or relaxation as indicated by a change in baseline. Then, a contact period of 3 minutes was given to allow the drug to act on the tissue.

Afterwards, the selected dose of ACh was added to the organ bath to see if erythromycin has altered the response to ACh. The tissue was then washed twice every 5 min. The response of selected dose of ACh was again recorded for 30 sec to see if it achieved its original height. Then after a wash recording with the next higher dose of erythromycin was tested. The same procedure was repeated with 300 µM, 500 µM and 1000 µM of erythromycin. Six such experiments were done.

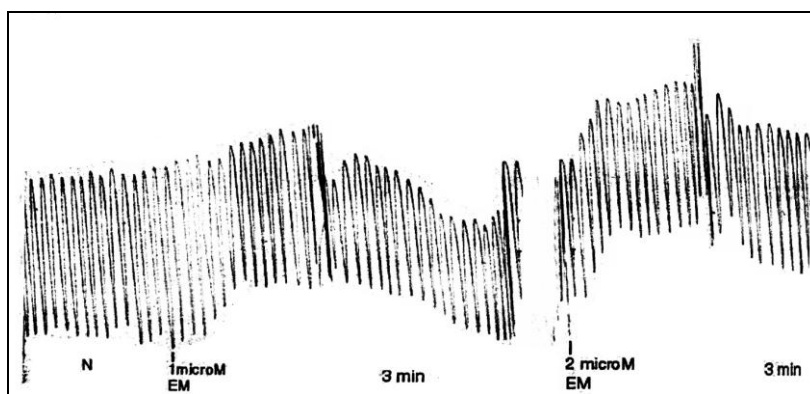
**c) *In vitro* Isolated rat colon preparation:**

Healthy rats of either sex weighing between 150-250 gm were selected for the experiment. They were kept fasting for 48 hrs and sacrificed on the day of experiment.

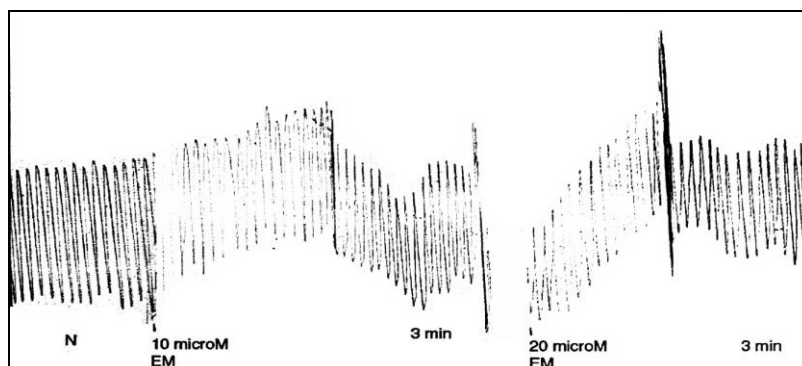
On the day of the experiment De Jalon's solution was prepared fresh. The rat was sacrificed by decapitation. The descending colon was identified by inserting polythene tube rectally. Excluding the terminal 4-5 cm, a strip of 8 cm was isolated and cut, mesocolon was separated and removed. A 3-4 cm strip of colon tissue was suspended in a Dale's organ bath (capacity 30 ml) at room temperature. One end of the colon was attached to an oxygen tube and the other to a frontal writing lever by means of a thread. The lever was loaded with tension of 1 gm and the tissue was allowed to relax for 45-50 minutes. The physiological salt solution bathing the tissue was changed at a regular interval of 5 minutes. At the end of 45 minutes the load was removed and the tissue was allowed to stabilize for a period of 5 minutes. A contact period of 30 seconds was allowed followed by a recovery period of 5 minutes for each dose of ACh. The remaining procedure was repeated exactly as in part b) i.e. for *In vitro* isolate Guinea pig ileum preparation

**RESULTS:****a) Rabbit jejunum experiments:**

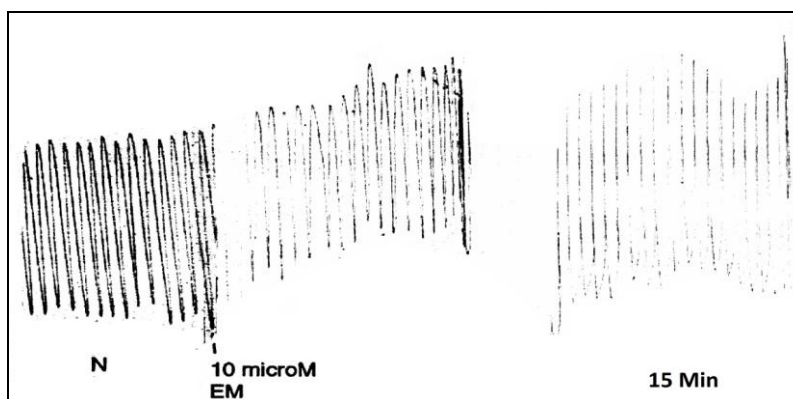
Isolated rabbit jejunum contracts rhythmically. The rate of contractions was regular and the height of contractions from the baseline was uniform and same. After addition of 1, 2, 10 and 20 µM of erythromycin, there was a change in the baseline which occurred immediately and shifted upwards, and in a curvilinear manner. Erythromycin in the doses of 1 and 2 µM did not change the rate of contraction although the height of contraction was reduced as compared to control tracing. (**Fig.1A**) When used in the doses of 10 µM and 20 µM, it reduced both the rate and height of contraction considerably. (**Fig.1B**) But after contact period of 15 minutes with 10 µM erythromycin, the tissue showed increase in height of contractions with faster recovery i.e. it relaxed faster than before. (**Fig.1C & 1D**)



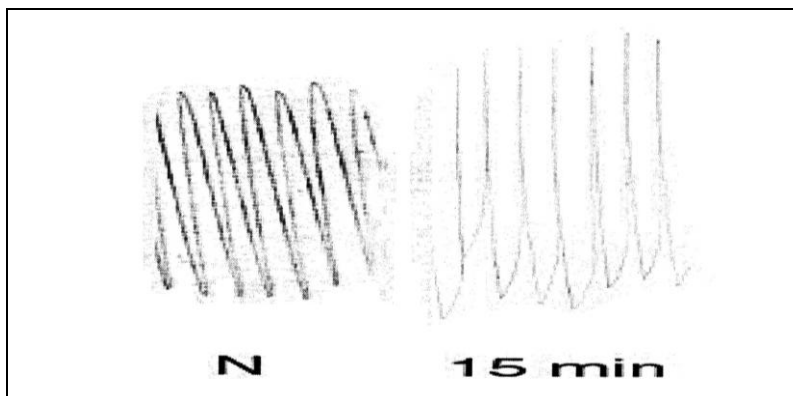
**FIG. 1A: ERYTHROMYCIN (EM) 1 & 2  $\mu$ M DID NOT CHANGE THE RATE OF CONTRACTION BUT HEIGHT OF CONTRACTION WAS REDUCED AS COMPARED TO NORMAL BASELINE TRACING (N).**



**FIG. 1B: ERYTHROMYCIN (EM) 10 & 20  $\mu$ M REDUCED BOTH THE RATE AND HEIGHT OF CONTRACTION CONSIDERABLY AS COMPARED TO NORMAL BASELINE TRACING (N).**



**FIG. 1C: ERYTHROMYCIN (EM) 10  $\mu$ M AFTER 15 MINUTES INCREASED THE HEIGHT OF CONTRACTION AND THE TISSUE APPEARS TO BE RECOVERING FASTER AS COMPARED TO NORMAL BASELINE TRACING (N).**

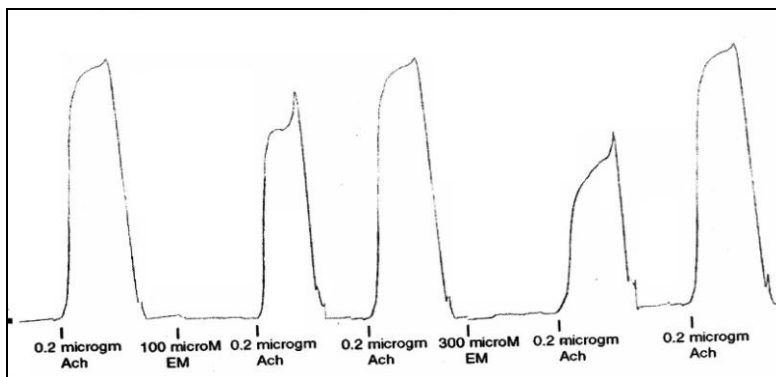


**FIG. 1D: (MAGNIFICATION OF FIG. 1C) COMPARISON OF EFFECT OF ERYTHROMYCIN (EM) 10  $\mu$ M AFTER 15 MINUTES WITH NORMAL BASELINE TRACING (N).**

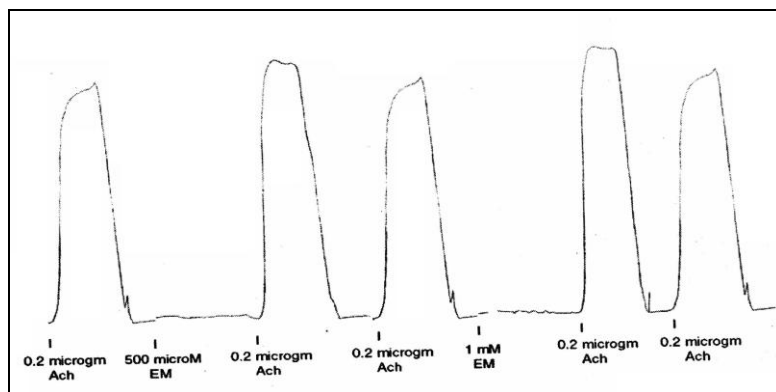
**b) Guinea pig ileum experiments:**

Erythromycin (100 - 1000  $\mu$ M) by itself neither caused contraction nor relaxation of longitudinal muscle of guinea pig ileum *in vitro*. There was no change in the baseline after the addition of erythromycin. However, ACh induced contractions of guinea pig ileum changed their character after exposure to different concentrations of

erythromycin. Lower doses erythromycin (100 & 300  $\mu$ M) reduced in the magnitude of response to ACh by approximately 25 % and 35 % respectively (**Fig. 2A**) Whereas slight potentiation of ACh induced contraction was observed in the presence of higher concentration erythromycin (500 & 1000  $\mu$ M). (**Fig. 2B**)



**FIG. 2A: ERYTHROMYCIN (EM) 100 & 300 MM REDUCED THE MAGNITUDE OF RESPONSE TO ACETYLCHOLINE (ACh) BY APPROXIMATELY 25 % AND 35 % RESPECTIVELY.**

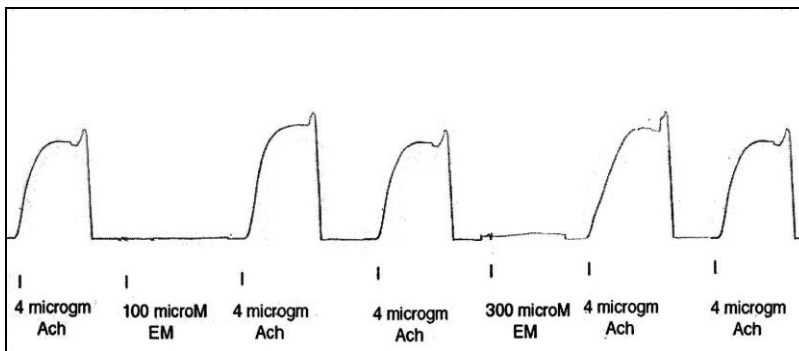


**FIG. 2B: ERYTHROMYCIN (EM) 500  $\mu$ M & 1 MM POTENTIATED THE CONTRACTION INDUCED BY ACETYLCHOLINE (ACh).**

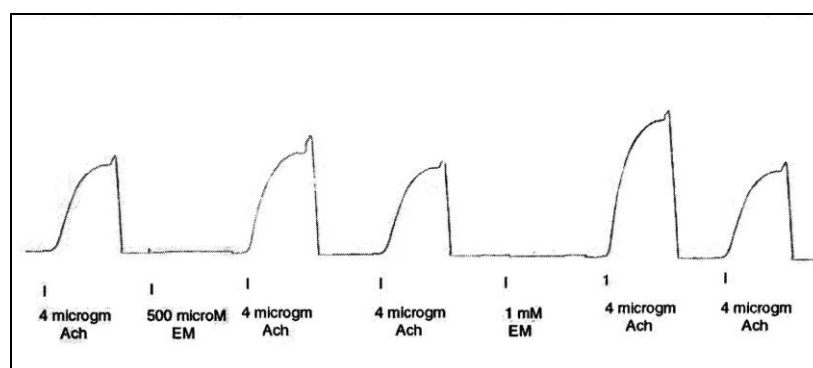
**c) Rat colon experiments:**

Erythromycin per se in concentrations of 100 to 1000  $\mu$ M did not cause either contraction or relaxation of rat colon *in vitro*. The baseline of the tracing did not change after addition of

erythromycin. However, erythromycin at all doses caused dose dependent increase in the magnitude of ACh induced contractions of rat colon. (**Fig. 3A & 3B**)



3A



3B

**FIG 3A & 3B: ERYTHROMYCIN (EM) AT ALL DOSES CAUSED DOSE DEPENDENT INCREASE IN THE MAGNITUDE OF ACETYLCHOLINE (ACH) INDUCED CONTRACTIONS.**

**DISCUSSION:** Erythromycin was discovered in 1952. Since last six decades, erythromycin has remained an important antibiotic in the treatment of infections. Despite the development of newer macrolide antibiotics (like clarithromycin, azithromycin) with extended spectrum of action and favourable pharmacokinetic properties erythromycin has an important place in therapy even today.<sup>3</sup> Erythromycin appears to produce the prokinetic effect on gastrointestinal tract by different mechanisms. Itoh Z et al (1984) reported that erythromycin at doses well below those required for antibacterial efficacy (0.03 mg/kg i.v.) induced a pattern of migrating contractions originating in the stomach in dogs which mimicked that of exogenously administered motilin, the gastrointestinal hormone. The study showed that there was increase in the blood levels of the hormone following administration of erythromycin.<sup>15</sup>

It has been shown that erythromycin interacts directly with the motilin receptor and exerts agonistic action and also causes stimulation of the cholinergic system which may be responsible for its effects on gastrointestinal motility.<sup>12, 14</sup> In animal experiments conducted both *in vitro* and *in vivo*, equivocal findings have been reported hence in the present study qualitative effect of erythromycin was studied on isolated rat colon, guinea pig ileum and rhythmically contracting rabbit jejunum.

Erythromycin caused almost an immediate effect on rhythmically contracting isolated rabbit jejunum, (Fig.1A-1D) shifting baseline of the tracing upwards and in a curvilinear manner. The

immediate effect on rabbit jejunum points to a more direct action on the muscle and rules out any indirect effect through neural mechanism. The change in the jejunum baseline indicates that erythromycin increases the tone of the longitudinal muscle. Rabbit jejunum is very sensitive to smaller doses of erythromycin as it has responded to very low doses of erythromycin i.e. 1 to 20  $\mu$ M. Unlike other smooth muscles of the gastrointestinal tract, rabbit jejunum is inherently highly electrically excitable cell and hence smaller doses of erythromycin have affected the jejunal contractions.

In isolated rat colon and guinea pig ileum, addition of erythromycin in high doses (100–1000  $\mu$ M) did not per se caused any contraction itself. But erythromycin at all concentrations altered their response to ACh. The effect of erythromycin on ACh induced contraction of isolated guinea pig ileum was different at lower and higher doses. (Fig. 2A & 2B) At lower concentrations (100 & 300  $\mu$ M), it produced significant decrease in amplitude of contraction whereas higher doses (500 & 1000  $\mu$ M) increased the amplitude of responses to ACh. One study reported that nerve mediated contraction of longitudinal muscle of guinea pig ileum was inhibited by erythromycin with  $IC_{50}$  of about 150  $\mu$ M. In the same study they also found that there was dose dependent inhibition by erythromycin of ileal contraction induced by bethanechol.<sup>18</sup> The inhibitory effects of erythromycin were also reported on pregnant and non pregnant rat uterus.<sup>17</sup>

Erythromycin (100–1000  $\mu$ M) increased the amplitude of ACh induced contraction of rat colon in a dose dependent manner. (Fig. 3A & 3B) There

are no reports in literature on effect of erythromycin on rat colon however; excitatory effect on rabbit colonic myocytes has been reported.<sup>19</sup> Despite the controversy of the cellular mechanism of prokinetic & other effects of erythromycin on various parts of the gastrointestinal tract in different species, the prokinetic effect of erythromycin is confirmed beyond doubt in humans.<sup>4-13</sup>

In conclusion, the present study shows that erythromycin base has different effects on different parts of the gastrointestinal tract in different species of animals *in vitro*. The cellular mechanism of effects of erythromycin on various parts of gastrointestinal tract is still not known with certainty. The complex nature of control of gastrointestinal motility adds to the problem of interpreting the results of experimental studies. Further work is required to resolve this difficult question. The study is first of its kind where an attempt was made to evaluate the effects of erythromycin on gastrointestinal smooth muscles of different species of commonly used laboratory animals. But since the study was carried out in *in vitro* system which is simplification of much more complex system, it would be extremely difficult to extrapolate the findings to the biology of intact organism.

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**CONFLICT OF INTEREST:** None

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