ABSTRACT:
Pain from a spastic viscus occurs in the form of cramps, with the pain increasing to a high degree of severity and then subsiding. This process continues intermittently, once every few minutes. The intermittent cycles result from periods of contraction of smooth muscle. For instance, each time a peristaltic wave travels along an overly excitable spastic gut, a cramp occurs. Anti-cholinergic agents like atropine are administered for relief of the abdominal colic but it often produces side effects like palpitation, dry mouth, urinary retention, mydriasis. Drug like dicyclomine are preferred in the treatment of intestinal spasm, which is not producing severe type of side effect due to blockade of muscarinic receptors. Dicyclomine is a competitive antagonist at muscarinic cholinergeric neurons. Rats were divided into two groups (N=6), fasted for 24-hours before the experiment but water was supplied ad libitum, were sacrificed by cervical dislocation. The abdomen of the rat was opened; the part of colon was dissected out which was fixed to stand and the tension adjusted such that it gives maximum contractions. All the drug containing solutions were freshly prepared before the experiments Dicyclomine, Atropine and Acetylcholine respectively. Muscarinic receptor blocker Atropine was added to the biophase in addition to selected sub maximal dose and the contraction of muscle till the 70-80% of inhibition was produced and the difference from selected dose contractions was recorded. Same procedure repeated with Dicyclomine. The dose of dicyclomine that caused half-maximal acetylcholine-induced isolated rat colonic smooth muscle contraction was $0.30 \pm 0.17 \mu g$, while that of atropine it was found to be $31.48 \pm 22.73 \mu g$. There is highly significant difference ($P < 0.01$). Atropine was 103.87 times more potent as an antispasmodic drug than dicyclomine.

Keywords: Spasmodics, Dicyclomine, Atropine, Rat colon

INTRODUCTION
Spasm of a portion of the gut, the gallbladder, a bile duct, a ureter, or any other hollow viscus can cause pain, possibly by mechanical stimulation of the pain nerve endings or the spasm might cause diminished blood flow to the muscle, combined with the muscle’s increased metabolic need for nutrients, thus causing severe pain [1]. Often pain from a spastic viscus occurs in the form of cramps, with the pain increasing to a high degree of severity and then subsiding. This process continues intermittently, once every few minutes. The intermittent cycles result from periods of contraction of smooth muscle [2]. For instance, each time a peristaltic wave travels along an overly excitable spastic gut, a cramp occurs. The cramping type of pain frequently occurs in appendicitis, gastroenteritis, constipation, menstruation, parturition, gallbladder disease, or ureteral obstruction. Muscle spasm or cramp is an involuntary contraction of a muscle. Muscle spasms occur suddenly, usually resolve quickly, and are often painful. A muscle spasm is different than a muscle twitch. A muscle twitch or fasciculation is uncontrolled fine movement of a small segment of a larger muscle that can be seen under the skin [3].
Anti-cholinergic agents like atropine is administered for relief of the abdominal colic but it often produces side
effects like palpitation, dry mouth, urinary retention, mydriasis and others by blocking the muscarinic type of
acetylcholine receptors in other organs or system. So, drug like dicyclomine are preferred in the treatment of
intestinal spasm, which is not producing severe type of side effect due to blockade of muscarinic receptors.
Dicyclomine is a competitive antagonist at muscarinic cholinergic neurons. In addition there is evidence of a direct
antispasmodic effect on smooth muscles of both guinea-pig ileum and human bladder. Though dicyclomine is used
frequently clinically in irritable bowel syndrome, urinary incontinence, infantile colic, there is hardly any
comparison of its anti-cholinergic potency with that of atropine [4].
The present study is taken up as an attempt to compare the anti-muscarinic effect of atropine and dicyclomine on
isolated rat colon smooth muscle.

MATERIAL METHODS:
Healthy Wister rats of either sex weighing between 150-200 gm were divided into 2groups, in each group six
animals. They were maintained on synthetic pellet feed supplied by Pranav Agro Industries Ltd. Sangli,
Maharashtra and water ad libitum in the Central Animal House of the Institution. Animals are housed in a
temperature 35 ± 2°C and 12/12 hour light- dark cycle (6.00 AM – 6.00 PM) environment, in cages (6 per cage)
with mesh bottom to prevent coprophagy. The study was performed in the Experimental Laboratory in the
Department of Pharmacology after obtaining approval from the Institutional Animal Ethics Committee. It was an
in vitro study.
The rats were fasted for 24-hours before the experiment but water was supplied ad libitum, were sacrificed by
cervical dislocation. The abdomen of the rat was opened with a vertical incision by a scalpel. The right flexure of
colon, the part where the ascending colon turns to become the transverse colon was identified. The part which lies
in the sub-hepatic region of the abdomen. That part of colon was dissected out and was placed in a petri dish
containing modified Ringer’s solution. Threads were attached to the top and bottom of each piece by purse string
suture. The lower end was tied to aeration tube with help of a fine thread. The upper end was tied to the free end of
the frontal writing lever. Which was fixed to a stand and the tension adjusted such that it gives maximum
contractions [5]. The tissue was mounted in the organ bath containing modified ringer’s solution(pH 7.4)
maintained of 25°C and bubbled with air allowed to equilibrate for 45min under 500mg tension. The capacity of
organ bath was 15ml fluid was taken till it just immersed the tissue. Proper tension and magnification was adjusted
by altering the height of levers. Record the concentration dependent responses due to Ach using frontal writing
lever contact time of 60 sec [6].
All the drug containing solutions were freshly prepared before the experiments Dicyclomine, Atropine
(0.01,0.1,1,10,100µg/ml and 1mg/dl) respectively Acetyl choline (1, 10,100µg/ml and 1mg/dl). Acetylcholine
solution in various strength was prepared starting from 0.1% to 0.0001 %. [5] Dose was added by starting from the
highest dilution (10⁶ solution) and increased in geometric progression (log dose). The dose was increased till there
was no further increase or a decrease in the effect with the highest dose. Among these the dose producing sub
maximal contractions was selected. The Muscarinic blocker Dicyclomine was added to the biophase in addition
to selected dose (0.8µg or 1.6µg) and the contraction of muscle till the 70-80% of inhibition was produced and
the difference from sub maximal contractions (inhibition in height of contraction) was recorded. The procedure
was repeated by Muscarinic Atropine in the dose of 0.1µg, 0.2µg, 0.4µg and 0.8µg respectively. The effect
Muscarinic receptor blocker Atropine was added to the biophase in addition to selected sub maximal dose and
the contraction of muscle till the 70-80% of inhibition was produced and the difference from selected dose
contractions (inhibition in height of contraction) was recorded. The procedure was repeated by adding Muscarinic
blocker Dicyclomine in the dose of 0.1 µg, 0.2 µg, 0.4 µg, 0.8µg, 1.6 µg, 3.2µg, 6.4 µg 12.8 µg, respectively.(5)
Individual findings were recorded on smoked drum cylinder, fixed with resin (colophony) and the recordings are
measured. In this study, dose response curve of acetylcholine was observed on isolated rat colon muscles and the
sub-maximal dose of contraction was determined from it. Then the effect of graded doses of the anticholinergic
drug, either atropine or the test drug dicyclomine on acetylcholine (submaximal dose)-induced contraction on rat
colon muscles were observed. The experiment was repeated on six isolated preparation in each group.
RESULTS

Table 1: Comparison of Log ID$_{50}$, ID$_{50}$ (PA$_2$) value of Dicyclomine and Atropine

<table>
<thead>
<tr>
<th>Log ID$_{50}$</th>
<th>ID$_{50}$ Value</th>
<th>Dicyclomine</th>
<th>Atropine</th>
<th>Dicyclomine</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.65</td>
<td>1.20</td>
<td>0.22</td>
<td>15.85</td>
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<tr>
<td>-0.75</td>
<td>1.00</td>
<td>0.18</td>
<td>10.00</td>
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<td>-0.35</td>
<td>1.60</td>
<td>0.45</td>
<td>39.81</td>
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<td>-0.55</td>
<td>1.00</td>
<td>0.28</td>
<td>10.00</td>
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<tr>
<td>-0.25</td>
<td>1.70</td>
<td>0.56</td>
<td>50.12</td>
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<td>-0.90</td>
<td>1.80</td>
<td>0.13</td>
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<tr>
<td>Standard Deviation</td>
<td>0.17</td>
<td>22.73</td>
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<td>Standard Error</td>
<td>0.14</td>
<td>2583.47</td>
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<tr>
<td>Sum of Square Deviation</td>
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<td>9.28</td>
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<tr>
<td>95% Confidential Limit (U)</td>
<td>0.48</td>
<td>55.33</td>
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<tr>
<td>95% Confidential Limit (L)</td>
<td>0.13</td>
<td>7.62</td>
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<td>Student’s Unpaired ‘t’</td>
<td>3.36</td>
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<tr>
<td>P value</td>
<td>&lt; 0.01</td>
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<tr>
<td>Ratio</td>
<td>103.87</td>
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</table>

DISCUSSION

Smooth muscle cramps are very commonly seen clinically. It may be in the form of intestinal colic experienced during diarrhoea, dysentery or irritable bowel syndrome, uterine cramps during dysmenorrhea, ureteric colic, urinary bladder and gall bladder pain due to spasm of smooth muscle lining the wall [7]. The cramping pain is often treated symptomatically by anticholinergic drugs. Atropine the naturally occurring antimuscarinic agent is used form time immemorial for relieve of the pain due to smooth muscle spasm in hollow viscous [8]. Though atropine is a very efficient antispasmodic drug, it is associated with so many dose-limiting side effects. Especially it produces side effects related to CNS like ataxia, excitement, psychotic behavior, delirium and hallucination as it crosses the blood-brain barrier very easily. [9] It is a non-specific drug and producing side effects due to its antimuscarinic action on other organs. For example, dry mouth and difficulty in swallowing or taking due to drying of pharyngeal secretions, photophobia and blurring of near vision due to papillary dilation and cycloplegia, palpitation due to increase in heart rate etc. It is contraindicated in individuals with a narrow iridocorneal angle as it may precipitate acute congestive glaucoma and should be given cautiously to elderly males with prostatic hypertrophy – urinary retention [10].

So to obviate the disadvantages of atropine many semisynthetic and synthetic atropine substitutes are developed. Among the atropine substitutes that are used frequently as antispasmodic, the drug dicyclomine is clinically important. It is a tertiary amine, so cannot cross blood brain barrier, and having direct smooth muscle relaxing effect besides anticholinergic action. Also the doses that antispasmodic action is seen, there is hardly any atropinic action is seen. But there is hardly any literature is found to compare the antispasmodic potency of dicyclomine with that of atropine, their antispasmodic potency was compared with that of atropine on isolated rat colon smooth muscle.

CONCLUSION

In this study, the PA$_2$ or ID$_{50}$ value of dicyclomine or the dose of dicyclomine that caused half-maximal acetylcholine-induced isolated rat colonic smooth muscle contraction was 0.30 ± 0.17 µg (95% confidential limit 0.48 – 0.07 µg) while that of atropine it was found to be 31.48 ± 22.73 µg (95% confidential limit 55.33 – 7.62 µg). There is highly significant difference (P < 0.01) between the mean ID$_{50}$ values of dicyclomine and atropine as found by Student’s Unpaired ‘t’ Test. In this study, the potency ratio of atropine to dicyclomine was found to be 103.87 i.e. atropine was 103.87 times more potent as an antispasmdic drug than dicyclomine.
REFERENCES


