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Acceleratory Effect of Murraya Koenigii leaves Extracts in Atropine Induced Delayed Small Intestinal Transit Involved Possible Cholinergic Innervation in Mice

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ABSTRACT: Many drugs affect GI transit by acting as agonists or antagonists at specific cellular receptors, such as cholinergic, adrenergic, serotonergic, opioidergic and calcium channels. Atropine is frequently used as a tool for identifying mechanisms involving cholinergic pathway in evaluation of GI transit time. The present study was aim at to evaluate the influence to Murraya koenigii on GI transit time through involvement of cholinergic systems/mechanisms. Ethanolic and petroleum ether extract of MKL at the doses of 300 and 500 mg/kg p.o. administered 15 hrs fasted Swiss albino male mice. 4% charcoal meal was administered (10ml/kg p.o.) 1 hr after the drug treatments and 20 min after all the animals were dissected for determination of the intestinal transit. For exploration of the cholinergic mechanisms atropine (1mg/kg i.p.) was administered 30 min prior administration of drug. The results of study indicate that MKL accelerate intestinal transit in normal mice. Atropine inhibits the intestinal transit by 33.27% in normal mice. The delaying in the GI transit time by atropine involve cholinergic pathway and influence of atropine was inhibited by MKL. In presence of MKL, atropine could able produce only 20 to 22 % inhibitions of intestinal transit, indicates a possibility that MKL could partly produce acceleratory effect by some other pathways in addition to cholinergic pathway as atropine could not completely prevent the acceleratory effect of MKL.
Key words: Murraya koenigii, Intestinal transit, atropine, cholinergic system.

INTRODUCTION

GI motility and functional bowel disorders, such achalasia, gastroesophageal reflux disease, gastroparesis, functional dyspepsia, irritable bowel syndrome, colonic inertia, pelvic floor dyssynergia, and fecal incontinence, affect up to 25% of the US population. These disorders comprise about 40% of GI problems for which patients seek health care. GI motility disorders affect patients by not only causing symptoms and posing a heavy burden of illness but cause decreased quality of life with decreased work productivity. Unfortunately, these disorders are often ignored or sidelined because of a lack of understanding of mechanisms and appropriate therapy. Patients with motility disorders can be complex and difficult to treat. Understanding the GI motility dysfunction underpins the appropriate management of the patients[1-4].

Gastrointestinal dysmotility also impacts on the quality of life of patients with other disorders. For example, a significant percentage of patients with diabetes have gastrointestinal dysmotility. Gastrointestinal complications of diabetes can affect one or more parts of the gut and produce nausea, vomiting, abdominal pain, constipation and/or diarrhea. Abnormal gastric emptying, or gastroparesis, may lead to poor glucose control and complications of diabetes[5-7].

Many drugs affect GI transit by acting as agonists or antagonists at specific cellular receptors, such as

cholinergic[8], adrenergic[9], serotonergic[10, 11], opioidergic[12, 13], and calcium channels[14, 15]. Atropine is frequently used as a tool for identifying mechanisms involving cholinergic pathway in evaluation of GI transit time[8]. The present study was designed to investigate the role of Murraya koenigii on cholinergic neurotransmission in the reversal of atropine induce delayed gastrointestinal transit in mice.

MATERIAL AND METHOD

Plant: The fresh leaves of Murraya koenigii were collected in the month of November 2008 from its natural habitat at Sakoli village in Nagpur region, Maharastra, India. The plant was authenticated by Dr. N. M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Experimental animals: All the experiments were carried out in adult Swiss albino male mice. The animals were fasted for 15 hrs prior experimentation while had free access to water, and they were housed in a natural (12 hrs each) light–dark cycle. The animals were acclimatized to the laboratory conditions for at least 5 days before exposed for experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 729/02/a/ CPCSEA).

Material: Ethanolic and petroleum extracts of Murraya koenigii leaves, Activated charcoal (S.D. Fine chemical, Mumbai) and Atropine sulphate (Wockhardt Pvt. Ltd. Mumbai).

Preparation of Extracts of Murraya koenigii leaves: The collected leaves of Murraya koenigii were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using Soxhlet's extractor. The percent yield of ethanolic extract was 24.8% w/w and petroleum ether (60 Grade) extract yield 6.1% w/w. Both the extracts were concentrated by evaporation at room temperature and were used for pharmacological studies.

Administration of Extract: Suspension of ethanolic and petroleum ether extracts were prepared in 0.5% carboxymethyl cellulose using tween 20 (0.2% v/v) as a suspending agent. The extracts were administered in a dose of 300 and 500mg/kg p.o. respectively. Control groups were given only 0.5% carboxymethyl cellulose with tween 20 (0.2% v/v).

Administration of charcoal: The mice were administered charcoal meal consisting of 4 % of activated charcoal and 2% carboxy methyl cellulose orally (10ml/kg) after 1 hr. of respective treatments.

Administration of Atropine sulphate: The mice were treated with atropine sulphate (1mg/kg i.p.) 30 min prior treated with drugs.

EXPERIMENTAL DESIGN

Mice were randomly divided into 8 groups of 5 animals each. All the animals were fasted for 15 hrs. Group 1 served as a control and received vehicle only. Group 2, 3 and 4 were selected for evaluating acceleratory effects on intestinal transit while remaining groups were selected for assessing the cholinergic system in acceleration of intestinal transit by MKL.

Acceleratory effect of MKL on GI transit in normal mice: For evaluation of acceleratory effect on intestinal transit, group 2, 3, 4 and 5 received ethanolic and petroleum ether extracts of MKL (300 and 500mg/kg p.o) respectively.

Cholinergic system: Influence of MKL on delay transit by atropine

30 minute prior treatments, group 6, 7 and 8 of animals were treated with atropine sulphate (1mg/kg i.p.) for induction of delayed intestinal transit. In which group 6 served as a pure atropine treated group for evaluating cholinergic system in induction of delaying transit time, while remaining groups received ethanolic and petroleum ether extract of MKL (500mg/kg p.o) respectively.

1 hr after treatments all the groups of animals were administered 4% activated charcoal meal and 20 min later killed by cervical dislocation for determination of intestinal transit. The small intestine was removed from the pyloric sphincter to the ilioceacal junction and the distance travelled by the charcoal meal was noted and expressed as percentage of intestinal transit using following formula[16].

$$\% \text{ Transit} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of small intestine}} \times 100$$

Statistical Analysis All value are expressed as the mean ± S.D. Statistical significance was assessed by the unpaired Student's *t*test for all results.

RESULTS

Acceleratory Effect of MKL on Intestinal Transit

MKL (ethanolic and petroleum ether extract) administration at higher doses (500 mg/kg) produced a significant ($P < 0.05$ Table 1) acceleration of intestinal transit while at lower dose (300 mg/kg) unable to produce significant effects (Table 1 and Figure 1).

Cholinergic system: Inhibitory effects of Atropine

Atropine (1 mg/kg) produced significant ($P < 0.05$ Table 1) attenuation of intestinal transit by 33.27% when compared with vehicle treated group. In

atropine-pretreated group, administration of ethanolic and petroleum ether extracts of MKL (500 mg/kg) completely reverse the inhibition produced by atropine ($P < 0.001$ Table 1). Atropine able to produce inhibition of intestinal transit only by 20.18% in ethanolic extract and 21.91 % in petroleum ether extract of MKL respectively (Table 1 and Figure 1).

DISCUSSION

The gastrointestinal tract is in a continuous state of contraction, relaxation and secretion. These functions are controlled by neurohumoral systems, which in turn are regulated by various receptor systems, such as cholinergic, adrenergic, serotonergic, opioidergic and calcium channels[8-15, 17]. The results of the present study indicate that MKL accelerate the intestinal transit. MKL found to increase intestinal transit dose dependently while significant ($P < 0.05$ Table 1) effect was observed at higher dose (500mg/kg). At 300 mg/kg the % transit was found to be upto 65 to 67% for petroleum ether and ethanolic extracts while at higher dose 500mg/kg the % transit was 74 to 76% compared to vehicle treated group for both extracts (Table 1 and Figure 1). There are numerous pathways involved in gastrointestinal motility e.g. adrenergic, cholinergic, opioidergic pathway, serotonergic, calcium channel and etc[8-15]. The agents used to evaluate the pathways for acceleration or attenuation of intestinal transits are e.g. verapamil used for evaluation calcium channel[15], clonidine used for adrenergic pathway[18], naloxone in opioidergic pathway[13], ondasetron in serotonergic system[11], and atropine in cholinergic mechanism[8]. In present study we used atropine for assessing the impact of cholinergic system on acceleratory intestinal transit by MKL because it used frequently as a tool for identifying mechanisms involving cholinergic pathways[8]. It is a non-specific competitive antagonist of acetylcholine for muscarinic receptors and abolishes the effects of acetylcholine completely on the GI tract. Both in normal subjects and in patients with GI diseases, full therapeutic doses of atropine (0.5-1 mg) produce definite and prolonged inhibitory effect on the motor activity of the stomach, duodenum, jejunum and ileum[19]. Our study also confirmed inhibitory effect of atropine on intestinal transit in normal mice (Figure 1). Atropine produced significant ($P < 0.05$ Table 1) attenuation of intestinal transit by 33.27% when compared with vehicle treated group. When atropine-injected group was treated with MKL, it reverses the delay of intestinal transit induced by atropine (Figure 1). This finding indicates that MKL possibly acts through muscarinic receptors to accelerate the intestinal transit. Since, atropine could able produce only 20 to 22 % inhibitions of intestinal transit in presence of petroleum ether and ethanolic extract of MKL groups respectively (Table 1). This indicates a possibility that MKL could partly produce acceleratory effect by some other pathways in addition to cholinergic pathways as atropine could not completely prevent the acceleratory effect of MKL.

CONCLUSION

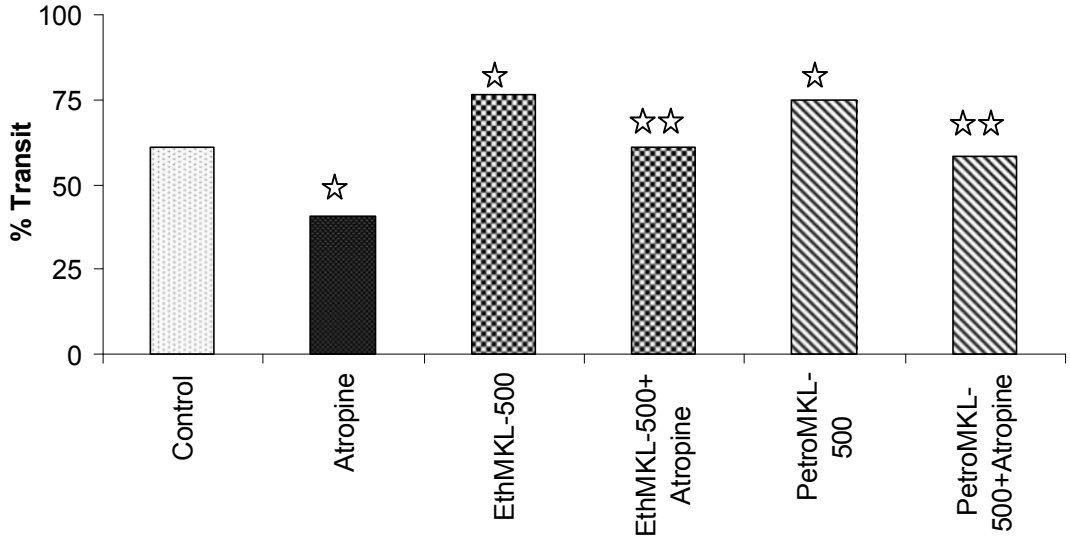
There are numerous pathways involved in acceleration or attenuation of transit time e.g. adrenergic, cholinergic, opioidergic pathway, calcium channel and etc[8-15]. The results of the study indicate that MKL accelerate the intestinal transit in normal mice. In present study atropine was used to evaluate the cholinergic effect of MKL in acceleration of intestinal transit. The results of the study indicate that atropine could able produce only 20 to 22 % inhibitions of intestinal transit in presence of MKL, indicates a possibility that MKL could partly produce acceleratory effect by some other pathways in addition to cholinergic pathways as atropine could not completely prevent the acceleratory effect of MKL. There need a further studies to evaluate other pathways in acceleration of intestinal transit by MKL.

Table 1: Influence of MKL on Intestinal Transit time

Pretreatments	Treatments of MKL (500 mg/kg)	%Intestinal Transit	% Acceleration of Intestinal Transit	% Inhibition of Intestinal Transit
Vehicle	Vehicle	60.74 ± 2.46	--	--
EthMKL-300	Vehicle	67.68 ± 3.47	11.42[1]	--
EthMKL-500	Vehicle	76.43 ± 2.73 [a]	25.83[1]	--
PetroMKL-300	Vehicle	65.7 ± 3.13	8.16[1]	--
PetroMKL-500	Vehicle	74.92 ± 3.72 [a]	23.34[1]	--
Atropine (1)	Vehicle	40.53 ± 2.44 [a]	--	33.27[2]
Atropine (2)	EthMKL	61.01 ± 2.96 [b]	--	20.18[3]
Atropine (3)	PetroMKL	58.5 ± 1.62 [b]	--	21.91[3]

Each value represents the mean ± SD (n = 5) or %.
[a] *denotes significant* ($P < 0.05$) compared with vehicle group
[b] *denotes significant* ($P < 0.05$) compared with atropine group
[2]compared with vehicle group
[3]compared with respective MKL group

Figure 1: Inhibitory Effect of Atropine on Acceleratory Intestinal Transit of MKL



Each value represents the mean ± SD (n = 5) or %.
☆*Denotes significant* ($P < 0.05$) compared with vehicle group
☆☆*Denotes significant* ($P < 0.05$) compared with atropine group

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