Research Article

ISSN 0975 – 2633
Arch Pharm Sci & Res Vol 1 No 2 189 -193 October 2009

Effect of Murraya Koenigii leaves Extracts on Gastrointestinal motility: Involving Calcium Channel Innervation in Mice

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The gastrointestinal tract is in a continuous state of contraction, relaxation and secretion. It is reported that calcium is involved in the initiation of contraction of smooth muscle. It increases the small intestinal motility through L-type calcium channels. Verapamil, a phenylalkylamine derivative, blocks the calcium channels on the surface of smooth muscle cells and relaxes the smooth muscle, thereby attenuating the intestinal motility. The present study was aim at to evaluate the influence of Murraya koenigii on small intestinal motility through involvement of calcium channel. 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 calcium channel, Verapamil (10 mg/kg p.o.) was administered 30 min prior administration of drug. The results of study indicate that MKL accelerate intestinal transit in normal mice. Verapamil inhibits the intestinal transit by 22.47% which was significant ($P < 0.05$) compared to vehicle control mice. The results indicate delaying in the GI transit time by verapamil through calcium pathway and influence of verapamil was inhibited by MKL. In presence of MKL, verapamil could able produce only 16 to 20 % inhibitions of intestinal transit of MKL, indicates that MKL partly produce acceleratory effects through calcium involvement as well as by some other pathways as verapamil could not completely prevent the acceleratory effect of MKL.

Key words: Murraya koenigii, Intestinal transit, verapamil, calcium channel.

INTRODUCTION

Gastrointestinal dysmotility impacts on the quality of life of patients 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 [1, 2, 3].

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. Many drugs affect GI transit by acting as agonists or antagonists at specific cellular receptors, such as cholinergic[4], adrenergic[5], serotonergic[6, 7], opioidergic[8, 9], calcium channels[10, 11]. Recently it is found that Murraya koenigii has positive inotropic activity[12]. The result suggest that *M. koenigii* induced positive inotropic effect possibly by increasing availability of calcium from extra cellular sites. It is also found that verapamil significantly inhibit the response by inhibiting the availability of calcium from extracellular sites[12]. It is reported that calcium is involved in the initiation of contraction of smooth muscle. It increases the small intestinal motility through L-type calcium channels[11, 13].

Thus the present study was designed to investigate the effect of Murraya koenigii leaves extracts on small intestinal motility. The main objective of the present study was to evaluate the involvement of Ca2+ in acceleration of intestinal transit time by Murraya koenigii.

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MATERIAL AND METHODS

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

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 by Verapamil
Vehicle	--	60.74 ± 2.46	--	--
EthMKL-300	--	67.68 ± 3.47	11.42[1]	--
EthMKL-500	--	76.43 ± 2.73 [a]	25.83[1]	--
PetroMKL-300	--	65.7 ± 3.13	8.16[1]	--
PetroMKL-500	--	74.92 ± 3.72 [a]	23.34[1]	--
Verapamil (1)	Vehicle	47.08 ± 3.10 [a]	--	22.49[2]
Verapamil (2)	EthMKL-300	56.67 ± 3.40 [b]	--	16.26[3]
Verapamil (3)	EthMKL-500	60.76 ±5.16	--	20.50[3]
Verapamil (4)	PetroMKL-300	54.98 ±2.71	--	16.31[3]
Verapamil (5)	PetroMKL-500	59.43 ± 6.92 [b]	--	20.67[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
[1] compared with vehicle group
[2] compared with vehicle + Vehicle group
[3] compared with Vehicle + respective dose of MKL group

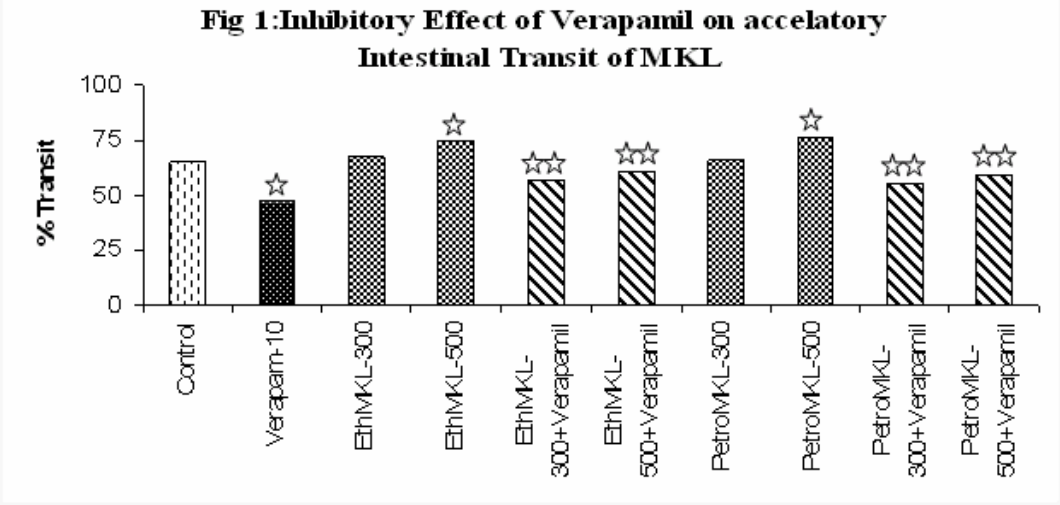
authenticated by Dr. N. M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

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 Verapamil (Torrent Laboratories Pvt. Ltd., Ahmedabad).

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



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 Verapamil group

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 Verapamil: The mice were administered with Verapamil (10mg/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, 4 and 5 were selected for evaluating acceleratory effects of MKL extracts on intestinal transit while remaining groups were selected for assessing the calcium channel in acceleration of intestinal transit.

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.

Calcium channel: Influence of MKL on delay transit by verapamil

30 minute prior treatments, group 6, 7, 8, 9 and 10 of animals were treated with verapamil (10mg/kg p.o.) for induction of delayed intestinal transit. In which group 6 served as a pure verapamil treated group for evaluating calcium channel in induction of delaying transit time, while remaining groups received ethanolic and petroleum ether extract of MKL (300 and 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[14].

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

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 Verapamil

Verapamil (10 mg/kg p.o.) produced significant ($P < 0.05$ Table 1) attenuation of intestinal transit by 22.49% when compared with vehicle treated group. In verapamil-pretreated group, administration of ethanolic and petroleum ether extracts of MKL (300 and 500mg/kg) inhibits the delay of intestinal transit produced by verapamil ($P < 0.05$ Table 1) respectively. Verapamil able to produce inhibition of intestinal transit by 16.26% and 20.50% in ethanolic extract of MKL (300 and 500mg/kg) respectively while in petroleum ether extract (300 and 500 mg/kg) by 16.31 and 20.67 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[4-11, 15].The results of the present study indicate that MKL accelerate the 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 67.68 and 65.7 for petroleum ether and ethanolic extracts respectively while at higher dose 500mg/kg the % transit were found to 76.43 and 74.92 compared to vehicle treated group (Table 1 and Figure 1). Various agents used to evaluate the pathways for acceleration or attenuation of intestinal transits e.g. verapamil used for evaluation calcium channel[11], clonidine used for adrenergic pathway[16], naloxone in opioidergic pathway[9], ondasetron in serotonergic system[7], and atropine in cholinergic mechanism[4]. In present study we used verapamil for assessing the impact of calcium channel on acceleratory intestinal transit by MKL because it used frequently as a tool for identifying mechanisms involving calcium channel pathways[11]. Calcium is involved in the initiation of contraction of smooth muscle[13]. The visceral smooth muscle has a poorly developed sarcoplasmic reticulum and the increase in intracellular calcium concentration is primarily due to Ca2+ influx from the extracellular fluid via voltagegated Ca2+ channels[17]. The L-type calcium channel is present in many cells and it is the main source of Ca2+ for contraction of smooth muscle[18]. This channel is blocked by dihydropyridines such as nifedipine, and other drugs such as verapamil and diltiazem[18]. Verapamil, a phenylalkylamine derivative, blocks the calcium channels on the surface of smooth muscle cells and relaxes the smooth muscle, thereby attenuating the intestinal motility [19, 20]. In previously it is found that Murraya koenigii has positive inotropic activity[12] possibly by increasing availability of calcium from extra cellular sites. It is also found that verapamil significantly inhibit the response of MKL by inhibiting the availability of calcium from extracellular sites[12]. In present study verapamil at the given dose (10 mg/kg) significantly *(P* < 0.05) inhibited intestinal transit, indicating the involvement of Ca2+ channels in normal physiology of small intestinal motility (Table 2). When verapamil-treated group was administered with MKL, it reverses the delay of intestinal transit induced by verapamil (Figure 1). This finding indicates that MKL possibly acts through calcium channel. The result is also supported with previous finding of inotropic effect of MKL through voltage gated calcium channel. In present study MKL increases the intestinal transit possibly by increasing the intracellular calcium concentration through calcium channel. Since, verapamil could able produce only 16 to 20 % inhibitions of intestinal transit in presence respective doses of petroleum ether and ethanolic extract of MKL (Table 1). This indicates MKL could partly produce acceleratory effect by increasing the intracellular calcium concentration through calcium channel and also by some other pathways as verapamil 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[4-11]. The results of the study indicate that MKL accelerate the intestinal transit in normal mice. In present study verapamil was used to evaluate the involvement of Ca2+ in acceleration of intestinal transit time by Murraya koenigii. The results of the study indicate that verapamil could able produce only 16 to 20 % inhibitions of intestinal transit in presence of MKL, indicates a MKL accelerate the intestinal transit by calcium as well as by some other pathways in addition as verapamil could not completely prevent the acceleratory effect of MKL. Thus there need a further studies to evaluate other pathways in acceleration of intestinal transit by MKL.

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