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ACCELARATORY EFFECT OF ETHANOLIC EXTRACT OF PIPER BETEL ON GASTROINTESTINAL TRANSIT: INVOLVING CALCIUM INNERVATIONS IN MICE

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ABSTRACT: *Piper Betel* Popularly known as 'Pan' in India belongs to family Piperaceae. Medicinally it has stimulant, antiseptic, sialogogue activity. It is reported that calcium is involved in the initiation of contraction of smooth muscle. It increases small intestinal motility through L-Type channel. Chlorpromazine blocks the calcium channel on smooth muscle and relaxes by attenuating intestinal motility. The present study was to evaluate the influence of ethanolic extract of *Piper betel (EtPB)* on small intestinal motility (SIT). EtPB (400 and 800 mg/kg p.o.) administered to 15 hrs fasted Swiss albino mice. 4% charcoal meal was administered (10 ml/kg p.o.) 1hr after the drug administration and after 20 min all animals were dissected for determination of SIT. For exploration of calcium channel in gastrointestinal motility chlorpromazine (5mg/kg p. o.) was administer 30 min prior administration of drug respectively. The results of study indicate that EtPB accelerate the intestinal transit in normal mice. A highest dose (800mg/kg) it was found to accelerate the transit upto 91.4% compared to normal mice which was found to significant at ($P < 0.05$) compared to vehicle group. Chlorpromazine inhibits GI transit by 28.85% in normal mice. In presence of EtPB it able to reverse the effect of chlorpromazine, while the significant effect was observed only at 800mg/kg EtPB. The results of EtPB are comparable to standard Metaclopramide (10mg/kg p.o.). In presence of EtPB chlorpromazine could able to produce upto 26.68 % inhibitions of SIT indicates EtPB could partly produce acceleratory effect through calcium involvement as well as by some other pathway as chlorpromazine could not completely inhibit the acceleratory effect of *Piper betel* on small intestinal transit.

Key words: *Piper betel*, Intestinal transit, Chlorpromazine, 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 cholinergic4, adrenergic[5], serotonergic[6, 7], opioidergic[8, 9], and calcium channels[10, 11].

In present study we used Piper Betel for screening of gastrointestinal motility. *Piper Betel* popularly known as 'Pan' in India belongs to family piperaceae. In India Peoples are widely using this after having meals for digestion and warm taste, medicinally it has stimulant, antiseptic, sialogogue activity[12]. It is reported that calcium is involved in the contraction of smooth

muscle. It increases small intestinal motility through L-Type channel[10, 11]. Thus the present study was designed to investigate the effect of ethanolic extract of Piper betel 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 using chlorpromazine which blocks calcium channel on smooth muscle cell and relaxes by attenuating intestinal motility.

MATERIAL AND METHOD

Preparation of Extract: The fresh leaves of Piper betel were collected from local market at village Pusad in Yavatmal district region, Maharashtra, India. The collected leaves of Piper betel were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using Maceration in a air tight closed container by using ethanol as a solvent. The extract was concentrated by evaporation at room temperature and used for testing a gastrointestinal motility.

Material: ethanolic extract of Piper betel leaves, Activated charcoal (S.D. Fine chemical, Mumbai) and Chlorpromazine (LA Pharmaceutical Pvt. Ltd., Ahmedabad), Metaclopramide (Wallace Pharmaceutical Ponda,Goa.)

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).

Administration of Extract: Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using tween 20 (0.2% v/v) as a suspending agent. The extract was administered in a dose of 400 and 800mg/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 Chlorpromazine: The mice were administered with Chlorpromazine (5mg/kg p.o.)30 min prior treated with drugs.

Administration of Metaclopramide: The mice were administered with Metaclopramide (10mg/kg p.o.) as similar with test extract.

EXPERIMENTAL DESIGN Mice were randomly divided into 7 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 were selected for evaluating acceleratory effects of Piper betel on intestinal transit while group 4, 5, 6 were selected for assessing the calcium channel in acceleration of intestinal transit by using chlorpromazine while group 7 was selected as standard Metaclopramide.

Acceleratory effect of Piper betel on GI transit in normal mice: For evaluation of acceleratory effect on intestinal transit, group 2, 3 received ethanolic extract of Piper betel (400 and 800mg/kg p.o) respectively.

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

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 Piper betel on Intestinal Transit

Ethanolic extract of Piper betel administration at higher doses (800 mg/kg) produced a significant ($P < 0.05$) acceleration of intestinal transit while at lower dose (400 mg/kg) unable to produce significant effects (Table 1 and Figure 1).

Calcium channel: Influence of Piper betel on delay transit by Chlorpromazine

30 minute prior treatments, group 4, 5, 6, 7 of animals were treated with Chlorpromazine (5 mg/kg p.o.) for induction of delayed intestinal transit. In which group 4 served as a pure Chlorpromazine treated group for evaluating calcium channel in induction of delaying transit time, while remaining groups received ethanolic extract of piper betel (400 and 800mg/kg p.o) and standard Metaclopramide (10mg/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 formula13.

Cholinergic system: Inhibitory effects of Chlorpromazine

Chlorpromazine (5 mg/kg p.o.) produced significant ($P < 0.05$ Table 1) attenuation of intestinal transit by 28.85% when compared with vehicle treated group. In Chlorpromazinel-pretreated group, administration of ethanolic extract (400 and 800mg/kg) inhibits the

delay of intestinal transit while the significant effect was observed at higher (800mg/kg p.o.) dose only ($P < 0.05$ Table 1). Chlorpromazine able to produce inhibition of intestinal transit upto 26.68 % in at lowest dose ethanolic extract of Piper betel.

DISCUSSION

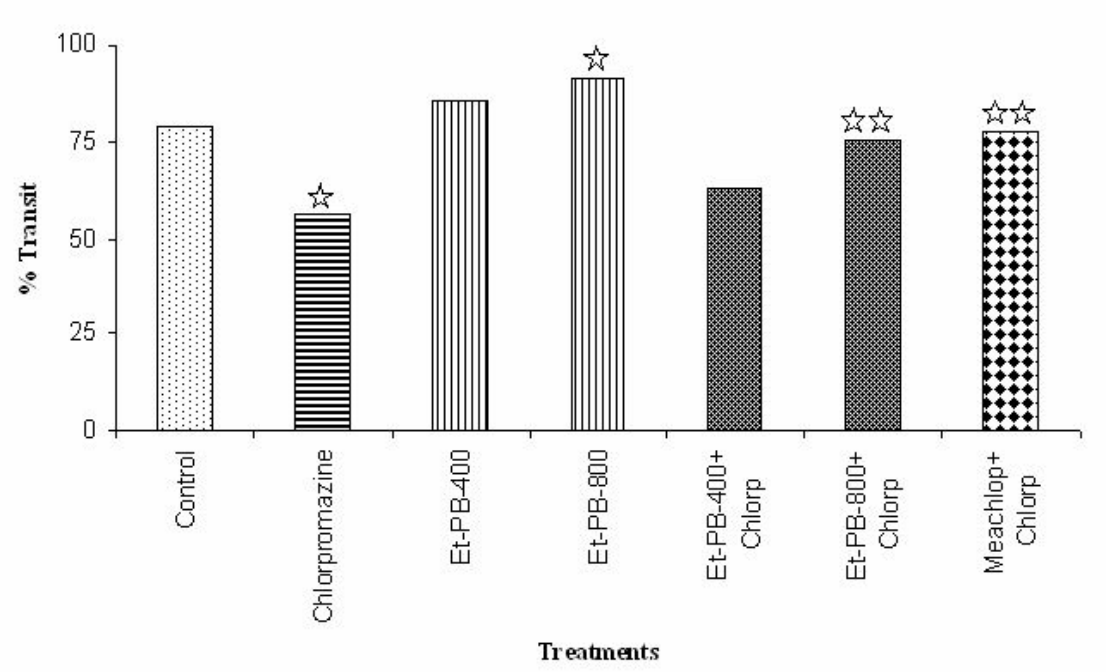
The gastrointestinal tract is in a continuous state of contraction, relaxation and secretion. These functions are controlled by neurohumoral systems, which in turn are regulated by various receptor systems, such as cholinergic, adrenergic, serotonergic, opioidergic and calcium channels[4-11, 15].The results of the present study indicate that Piper betel accelerate the intestinal transit dose dependently. I found to accelerate the transit by 85.34 and 91.4% at dose of 400 and 800mg/kg of ethanolic extract of Piper betel respectively. The significant ($P < 0.05$ Table 1) acceleration was observed at higher dose (800mg/kg) 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. Chlorpromazine used for evaluation calcium channel[10, 11], clonidine used for adrenergic pathway[14], naloxone in opioidergic pathway9, ondasetron in serotonergic system[7], and atropine in cholinergic mechanism[4]. In present study we used chlorpromazine was used for delaying in the transit time by interfering calcium channel pathways[11]. Calcium is involved in the initiation of contraction of smooth muscle[10, 11]. 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[15]. The L-type calcium channel is present in many cells and it is the main source of Ca2+ for contraction of smooth muscle[16]. The result of the study found that chlorpromazine significantly inhibit the response of Piper betel by inhibiting the availability of calcium from extracellular sites represent from the result of inhibition of intestinal transit upto 26.68% compared to vehicle control (Table 1) indicating the involvement of Ca2+ channels in normal physiology of small intestinal motility (Table 1). When Chlorpromazine treated group was administered with Piper betel, it reverses the delay of intestinal transit induced by chlorpromazine (Figure 1). This finding indicates that Piper betel possibly acts through calcium channel. In present study Piper betel increases the intestinal transit possibly by increasing the intracellular calcium concentration through calcium channel. Since, Chlorpromazine could able produce upto 26.68% inhibitions of intestinal transit in presence lowest dose of Piper betel while in presence of higher dose chlorpromazine was able to inhibit by 17.53% only which was comparable to standard Metaclopramide (Table 1). This indicates Piper betel could partly produce acceleratory effect by increasing the intracellular calcium concentration through calcium channel and also by some other pathways as Chlorpromazine could not completely prevent the acceleratory effect of Piper betel.

Table 1: Influence of Ethanolic extract of Piper betel on Intestinal Transit time

Pretreatments	Treatments	%Intestinal Transit	% Acceleration of Intestinal Transit	% Inhibition of Intestinal Transit by Verapamil
Vehicle	--	78.98 ± 4.96	--	--
Et PB-400	--	85.34 ± 6.42	8.05[1]	--
Et PB-800	--	91.4 ± 5.38 [a]	15.72[1]	--
Chlorpromazine (1)	Vehicle	56.19 ± 6.82 [a]	--	28.85[2]
Chlorpromazine (2)	Et PB-400	62.57 ± 6.30	--	26.68[3]
Chlorpromazine (3)	Et PB-800	75.37 ±6.22[b]	--	17.53[3]
Chlorpromazine (4)	Metaclopramide	77.29 ±3.78[b]	--	--

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 Chlorpromazine group
[2]compared with vehicle + Vehicle group
[3]compared with Vehicle + respective dose of Piper betel group

Fig 1: Acceleratory Effects of Piper betel on Delayed Gastrointestinal Transit



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 Chlorpromazine group

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