A Study to Identify the Effect of *Momordica Charantia* on Smooth Muscle Activity in Isolated Guinea Pig Ileum

S. Shanmugapriya*, K. Bhuvaneswari and R. Muthamizh Veena

Department of Pharmacology,
PSG Institute of Medical Sciences and Research

Abstract

**Background:** *Momordica charantia* (*M. charantia*), bitter melon is commonly used in traditional medicine for its wide variety of medicinal properties.

**Aim and objectives:** To study the effect of successive extract of *M. charantia* on smooth muscle contractility of the isolated guinea pig ileum compared to that of standard drugs and to evaluate the receptors mediating the effects of *M. charantia* on intestinal smooth muscle using appropriate antagonists.

**Materials and methods:** An adult albino male guinea pig of weight 700 g was euthanased and the ileum was dissected out for isolated tissue experiment in organ bath using tyrode as physiological salt solution, aerated with air, maintained at pH of 7.4 and temperature 37°C. Successive extract of *M. charantia* was prepared in soxhlet apparatus using petroleum ether, methanol, ethanol and water. 100 mg of this extract was diluted in 10 ml of water to get a stock solution of 10 mg/ml w/v which was used after appropriate dilutions to obtain the working standard solutions. Experimental procedures were done in accordance to the standard principles of isolated tissue experiments.

**Results:** Contractile response was noted for *M. charantia* and its dose response curve was obtained following those of standard drugs, acetylcholine and histamine. The receptor actions were studied using atropine (muscarinic antagonist) and pheneramine maleate (histamine antagonist) and adrenaline (adrenergic agonist) which are gut smooth muscle relaxants and we found that all the three drugs did not alter the response of *M. charantia* proving that none of these three receptors systems, muscarinic, histaminic or adrenergic were involved in mediating the contractile actions of *M. charantia*.

**Conclusion:** This study has evaluated the contractile effect and the underlying receptor mechanisms contributing to this effect of *M. charantia* on the intestinal smooth muscle of guinea pig using isolated tissue experiment.

*Corresponding author:*
Dr. Shanmugapriya S., Associate Professor, Department of Pharmacology, PSG Institute of Medical Sciences and Research; Email: somasundaram999@rediffmail.com
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**Introduction**

*Momordica charantia*, commonly known as bitter gourd or bitter melon is a medicinal plant belonging
to the family Cucurbitaceae and commonly cultivated in many tropical and subtropical countries. Bitter melon is known for its wide variety of medicinal properties (1). It has an array of biologically active chemicals including saponins, steroids, alkaloids, triterpenes, proteins and flavonoids due to which the fruit is shown to possess anti-bacterial, anti-fungal, anti-viral, anti-parasitic, hypoglycemic and anti-carcinogenic properties (2-5). It is especially used in the treatment of diabetes due to its effect in reducing blood glucose, enhancing insulin sensitivity and preserving the islet cell function (6). It is also used in the treatment of rheumatism, gout and diseases of liver and spleen (7).

A study done using the methanolic extract of *M. charantia* has demonstrated its antidepressant and anxiolytic properties in mice and it has been proven that the antidepressant-like effect is dependent on the agonistic activity at central serotonergic (5-HT2 receptor), noradrenergic (α1 and α2 adrenoceptors), dopaminergic (D2 receptor), and muscarinic cholinergic systems (8). The effect of *M. charantia* on the peripheral tissues like the intestinal smooth muscles expressing such receptors have not been studied. Additionally, several studies have documented a laxative effect (9, 10, 11) with the use of *M. charantia* and the mechanism underlying this effect has not been investigated. So the current study aimed to identify the change in intestinal smooth muscle contractile tone by *M. charantia* with progressively increasing doses of successive extract *M. charantia* compared to that of the spasmogens like acetylcholine and histamine in isolated tissue experiment using guinea pig ileum and to interpret the receptor mechanisms underlying such actions by using relaxants like adrenergic agonist as well as receptor antagonists of muscarinic and histamine receptors.

**Materials & Methods**

The study protocol was approved by the institutional ethics committee (proposal number: 324/2016/IAEC). The *M. charantia* was procured from the local market. It was identified by a botanist from the PSG Institutions and a voucher sample of *M. charantia* fruit is preserved in the department of Pharmacology (Pha/03/ 2014).

**Preparation of M. charantia successive extract (12):**

250 g of *M. charantia* fruit was shade dried and grounded to fine powder. 200 g of the powder was weighed and subjected to extraction in a soxhlet apparatus at room temperature using petroleum ether, methanol, ethanol and water successively. Before extraction with the next solvent, the powder was air dried to remove the adhering solvent and was evaporated to dryness. The final extract was weighed after open dish evaporation to ensure removal of the solvents and stored at 4°C in refrigerator till further use.

**Preparation of standard drugs for comparison:**

Acetylcholine (1 mg/ml in 5% w/v NaH₂PO₄), Histamine (10 mg/ml), Adrenaline (0.1 mg/ml), Atropine (1 mcg/ml), Pheniramine (1 mg/ml) and stock solution of *M. charantia* successive extract was prepared by diluting 100 mg of the extract in 10 ml of water to obtain 10 mg/ml w/v which was mixed using magnetic stirrer at 300 rpm for 15 minutes to enhance the solubility. These were used for the isolated tissue experimental procedures after appropriate dilutions to working standard solutions.

**Preparation of isolated guinea pig ileum tissue:**

An adult male albino guinea pig (*Cavia porcellus*) aged 14 months and weighing 700 g was obtained from the animal house and fasted overnight. The animal was sacrificed by overdose of pentobarbitone (100 mg/kg) injected intraperitoneally followed by cervical dislocation for confirmation of euthanasia. The ileum was identified and dissected out. The ileal tissue was divided into two segments and mounted in two separate organ baths using Tyrode as the physiological salt solution (adjusted to pH = 7.4) provided with air for aeration and temperature maintained at 37°C. One end of the each tissue segment was tied securely to the tissue holder while the other end was tied to a frontal writing lever, adjusted for suitable tension and magnification. The ink-writing kymograph drum (INCO E8 recording drum using 220V/50 Hz power) wrapped with paper was run at a speed of 0.12 mm/sec to record the
contractions. The responses were thus recorded on the paper fixed to the travelling surface of kymograph drum.

**Determination of the response of intestinal smooth muscle to Successive extract of M. charantia and receptors involved in mediating the effect:**

Following 40 min of relaxation of the mounted tissue, graded dose responses were recorded for the standards namely acetylcholine, histamine and then for the successive extract of *M. charantia* preparation. Since graded contractions of the smooth muscle were obtained for *M. charantia* successive extract, we proceeded to study the effect of antagonists/relaxants on the contraction induced by the extract on the intestinal smooth muscle.

Competitive antagonists for the receptors expressed in the smooth muscles of intestine which mediate contraction, namely atropine for muscarinic M2, 3 and pheniramine maleate for histamine H1 were utilized for receptor antagonism evaluation. Antagonism of the response to a single dose of *M. charantia* by these two antagonists was tested by adding increments of 10 times higher dose of each of the antagonists individually along with the agonist (*M. charantia* successive extract 8 mg) consequentially in 3 cycles to the isolated tissue preparation for determining antagonism by any of these doses on the smooth muscle contraction and if so, whether the antagonism signified competitive type by recording further responses keeping the dose of antagonist eliciting such reduction in response of *M. charantia* as constant and increasing the dose of *M. charantia* in geometric progression. In the event of antagonism not being demonstrable, ascertaining the nature of antagonism would not be performed and the protocol was to proceed to elucidating the role of adrenergic antagonism with a similar evaluation of the change in the contractile effect of the extract by adrenergic agonist adrenaline in order to delineate if anti-adrenergic action of successive extract on the gut smooth muscle was responsible for the stimulant effect of *M. charantia*.

**Results**

The graded dose responses were obtained for acetylcholine, histamine and the recordings ensured that the tissue was viable and sensitive. The threshold dose for acetylcholine was determined to be 0.1 µg and the maximal response was obtained for 3.2 µg (Fig. 1). Likewise for histamine, the first response was recorded for the dose of 0.2 µg and the maximal response was seen at 3.2 µg (Fig. 2). For the successive extract of *M. charantia*, the intestinal smooth muscle responses were found to be contractile and the threshold response was evident at 1mg dose. The doses were added to the inner organ bath in geometric progression as per the standard procedure. It was found that the magnitude of responses also showed progressive increase with increasing doses until the maximal response for *M. charantia* extract being recorded at 32 mg (Fig. 3).

Log dose response curves (LDRCs) were plotted (Fig. 4). The log dose response curves analyzed using Microsoft Excel 2007 revealed that the slope of the successive extract (4.740) differed significantly from those of acetylcholine (2.782) and histamine (6.389) (Fig. 5).

![Graded dose response curve for acetylcholine (Stock = 1 mg/ml) using guinea pig ileum.](image-url)
Fig. 2: Graded dose response curve for histamine (Stock = 10 mg/ml) using guinea pig ileum.

Fig. 3: Graded dose response curve for successive extract of *M. charantia* (Stock = 10 mg/ml) using guinea pig ileum.

Fig. 4: Comparison of log dose response curves of acetylcholine, histamine and *M. charantia*. 
The change in contractile responses of *M. charantia* was then tested in presence of antagonist for the cholinergic receptors namely atropine and the histamine antagonist, pheniramine maleate. The dose of *M. charantia*, 8 mg was chosen to study the effect of increasing concentrations of antagonist. The choice of this dose was essentially to avoid higher doses which on repetitive administration, may contribute to bias by reducing the response of the tissue and thus affect the sensitivity of the tissue.

Interestingly, the study of antagonists revealed that the height of response to the successive extract of *M. charantia* was neither altered by atropine at the three dose levels studied (0.01 µg, 0.1 µg, 1 µg) nor by that of histamine antagonist Pheniramine maleate (0.01 mg, 0.1 mg, 1 mg). Hence we proceeded to evaluate the effect of adrenaline, an adrenergic agonist (0.01 mg, 0.1 mg) which is an intestinal smooth muscle relaxant on the contractile response of *M. charantia* and to this end, we noticed a similar lack of change in the contraction induced by successive extract of *M. charantia* indicating that adrenoreceptors were not involved in the contractions of the smooth muscles of the ileum in response to *M. charantia* successive extract (Fig. 6).

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**Fig. 5**: Comparison of slope of log dose response curves with trendlines of acetylcholine, histamine and *M. charantia*.

**Fig. 6**: Study of antagonism of *M. charantia* response in guinea pig ileum using smooth muscle relaxants. Atropine – At; Pheniramine – P; Adrenaline – Ad; *M. charantia* - MC (4 mg)
Discussion

This isolated tissue animal experimentation study has brought out a very important effect of one of the commonly used natural products in food and traditional medicine. A contractile action of *M. charantia* on the intestinal smooth muscles of guinea pig ileum was evident and it was demonstrable that the height of responses increased with doses administered in geometric progression indicating that the magnitude of contractile responses was a function of the dose of *M. charantia* extract with $R^2=0.864$ (Fig. 5). Similar to the graded responses obtained for acetylcholine ($R^2=0.949$) and histamine ($R^2=0.923$), there was a dose dependent increase in the responses deductible up to the maximal dose, beyond which the supra-maximal dose did not show any further increase in response height; thus a complete graded dose response curve (GDRC) was obtained. The GDRC was thus demonstrative of the fact that the intestinal smooth muscle contractions induced by *M. charantia* extract were not a mere erratic phenomenon exhibited at certain high doses of the extract; on the other hand, proved to be a standard response that could be assayed on a standard tissue like the guinea pig ileum in accordance to the principles governing bioassay.

Additionally, logarithmic conversion of the dose response curve revealed a sigmoid dose response relationship akin to that of the standard spasmogens like acetylcholine and histamine. Moreover, it was evident that potency of the *M. charantia* extract was more than 1000 times lower in comparison to acetylcholine or histamine (Fig. 4). This essentially explains that it is safe for human consumption without undue spasmodic effect except at very high doses.

Literature exists for the laxative action of *M. charantia* especially at high doses and the effect illustrated in our study explicates the mechanism contributory to the increased intestinal motility with *M. charantia*. Few studies have proved that inhibition of glucose absorption in the gut is one of the mechanisms by which *M. charantia* exerts the well known antihyperglycemic effect and these studies have also shown that there is inhibition of intestinal fluid transport secondary to reduced transport of glucose and aminoacids in the rat everted gut sacs in vitro (11, 13). Such an effect could be potentially additive to increased contractility to produce a laxative effect.

Deciphering the receptors likely to be responsible for the pharmacological response of *M. charantia* elucidated in our study was done using the principles of antagonism. The change in response with addition of antagonists of the two major receptors, muscarinic and histaminergic, involved in intestinal smooth muscle contractility namely atropine, pheniramine maleate respectively were investigated apart from adrenaline, agonist for the adrenoreceptor causing gut smooth muscle relaxation. However, failure to reduce or abolish the contractile response to *M. charantia* extract was observed with all the three relaxants indicating that none of these receptors were involved in mediating the increased contractility of *M. charantia* extract (Fig. 6). The plausible explanation for this could be attributed to non-receptor mediated actions of *M. charantia*, acting directly on the ion channels like calcium or potassium ion channels. A potential increase in conductance of calcium channels or alternatively a decreased $K^+$ ion conductance could be the likely contributory mechanism for this activity. Another probable mechanism could be activation of the 5HT3 or 5HT4 receptors on the intrinsic primary afferents of the enteric nervous system, which however were not evaluated in this isolated tissue model and is therefore considered as a limitation of the study.

Moreover, literature evidence states that agonists whose log dose response curves (LDRCs) are not parallel are suggestive of acting through different receptors and hence said to have varying mechanism of action despite the same resultant effect (14). In our study, the slope of LDRC of *M. charantia* extract was significantly different from that of acetylcholine and histamine (Fig. 4). This essentially explains that it is safe for human consumption without undue spasmodic effect except at very high doses.

Thus the study has delineated the effect of increased contractility of gut smooth muscle by the successive
extract of *M. charantia* which can be utilized therapeutically for conditions affecting intestinal motility like diabetic gastroparesis. Its effectiveness in such diabetic complication, which if confirmed by clinical studies, would be dually beneficial especially in the face of paramount importance of *M. charantia* in management of diabetes patients and the huge burden of disease that this metabolic disorder contributes to, owing to the sheer high proportion of population it affects.

**Conclusion**

This is the first study done to identify the smooth muscle contractile effect of *M. charantia* in isolated intestinal smooth muscle of guinea pig ileum. This study based on the concept of reverse pharmacology has ascertained one of the salient pharmacological actions of *M. charantia* and the potential underlying mechanism, enabling future studies in further evaluating the therapeutic benefit of this effect of *M. charantia* in clinical conditions associated with reduced gut motility including diabetic gastrointestinal dysmotility disorders.

**Conflict of interest**

The authors have none to declare.

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