

## Intracellular Signaling Mechanisms Pharmacological Action of *Jasminum amplexicaule* Buch.-Ham. (Oleaceae) on Gastrointestinal Secretion

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### Abstract

*Jasminum amplexicaule* Buch.-Ham. (Oleaceae) has been commonly used in the traditional medicine in dysentery, diarrhoea and bellyache in China. In the present work, the methanol extract of *Jasminum amplexicaule* (JME) was examined for pharmacology on human colonic epithelial cell line T<sub>84</sub> by the short-circuit current technique. The results showed that pretreatment of T<sub>84</sub> cells with JME produced a concentration-dependent (0-1000 µg/mL. EC<sub>50</sub> = 0.055 mg/mL) inhibition effect on adrenalin (Adr.)-induced Cl<sup>-</sup> secretion. The maximal response was observed at 200 µg/mL. It has been demonstrated that JME has a direct effect on the enterocyte. Our results also demonstrated that the JME exerted inhibitory effect on gastrointestinal Cl<sup>-</sup> secretion that effected by acting on basolateral β-adrenoreceptors. These results suggested that the Chinese traditional medicine of JME can be used for the treatment of acute diarrhea and bellyache.

**Keywords:** *Jasminum amplexicaule*; T<sub>84</sub> cell; Secretion; Anti-diarrhoea.

### Introduction

*Jasminum amplexicaule* Buch.-Ham. (Oleaceae) is a kind of traditional medicine mentioned above, which is a genus of about 600 species of shrubs and small trees in the family Oleaceae. It is a multipurpose plant with great potential for ethno medicinal application in some parts of China and India. The powder of its twigs and leaves has been used as a hydragogue and a febrifuge and also has been used as a kind of traditional medicine for the treatment of dysentery, diarrhoea and bellyache in China (1). Its leaves are used to take care of the quadriplegia-gall and also mixed with other ingredients to cure dysentery and bellyache

(1). A phytochemical study reported that four secoiridoids glycosides were isolated from the dried twigs and leaves parts of *J. amplexicaule* (2, 3). A report studied its anti-diarrhoea and analgesic activities (1). The anti-diarrhoea activity can be ascribed to inhibition of intestinal motility and/or the anti-secretory properties (4) on gastrointestinal (syn GI) tract.

It is well known that, as the first defense line, the GI epithelium of the host plays an important role in protecting enteric epithelia from invasion of most pathogens. Intestinal epithelial barrier function regulates epithelial ions and nutrient transport as well as host defense mechanisms. Epithelial membrane pumps, ion channels and tight junctions control epithelial transcellular and paracellular fluxes (5, 6). Epithelial Cl<sup>-</sup> channels play an important role in regulation and maintenance of normal GI physiological

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functions. Abnormal regulation of Cl<sup>-</sup> channels may result in diarrhea (7, 8) or constipation (9).

Because Cl<sup>-</sup> provides an essential driving force for lubrication of intestinal contents during regular bowel movements or artificial irritants in host defense responses (10), Cl<sup>-</sup> channels are very likely to be the targets for pharmacological intervention. Our present study focuses on examining whether ME exerted any effects on inhibition Cl<sup>-</sup> secretion and investigating which mechanism was involved in.

## Experimental

### *Plant material and extraction*

The twigs and leaves of *Jasminum amplexicaule* were collected from Botanical Garden of Department of Medicinal Plants, Southern Medical University (the first military medical university) in Guangzhou in September 2008 Guangdong, and identified by Prof. Qianhai Chen (Guizhou Academy of Sciences, P. R. China). Voucher specimens and samples were stored at the Institute of Biology, Guizhou Academy of Sciences. The methanol extract (JME) was filtered and evaporated to dryness using vacuum rotary evaporator (yield 14.2%). At the time of using, drugs tested were freshly prepared in the distilled water.

### *Cell culture*

Human colonic T<sub>84</sub> cells (American Type Culture Collection) were maintained in Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, 100 IU/ml penicillin, and 100 µg/mL streptomycin. For simultaneous measurements of I<sub>SC</sub>, the cells (1.5×10<sup>6</sup>/mL) were seeded onto a filter (Millipore, U.S.A) with a silicone rubber ring on top of it to confine the cells (0.45cm<sup>2</sup>). Cells reached confluency after 8 to 10 days, with a resistance greater than 300 Ω/cm<sup>2</sup>.

### *Short-circuit current measurement*

The measurement of I<sub>SC</sub> for human colonic T<sub>84</sub> has been described (11). The monolayers grown on permeable supports were clamped vertically between two halves of the Ussing chamber and both sides bathed in normal Krebs-Henseleit

solution (K-H solution, pH 7.4) while the I<sub>SC</sub> was measured. The K-H solution maintained at 37 °C by a water jacket enclosing the reservoir. The solution was bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, so that the pH of solution was maintained at 7.4. Drugs could be added directly to the apical or basolateral side of the epithelium. Electrodes for measuring transepithelial potential difference (PD) and passing current were connected to the chamber. The transepithelial PD were then clamped at 0 mV, and the short circuit current was recorded with VCC MC6 voltage-current clamp amplifier (Physiologic instrument, San Diego, CA) and displayed using a signal collection and analysis system (BL-420E, Chengdu Technology & Market Co. Ltd, China). The change in I<sub>SC</sub> was defined as the maximal rise in I<sub>SC</sub> following agonist stimulation and it was normalized to current change per unit area of the epithelial monolayer (µA/cm<sup>2</sup>). Transepithelial resistance (R<sub>t</sub>) was calculated by measuring the current response to a 1mV pulse. The I<sub>SC</sub> value is expressed as positive when the current flow from mucosal to serosal.

### *Solutions*

The bicarbonate-buffered Krebs-Henseleit solution contained (mM) NaCl, 117; NaHCO<sub>3</sub>, 25; KCl, 4.7; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.5; and D-glucose, 11, pH 7.4, when bubbled with 5% CO<sub>2</sub>, 95% O<sub>2</sub>. The bath temperature was maintained at 37 °C using a heated water jacket. Adrenalin, nystatin, Carbachol and propranolol were obtained from Sigma. Stock solutions of all the chemicals were dissolved in DMSO. Final DMSO concentration never exceeded 0.1% (v/v).

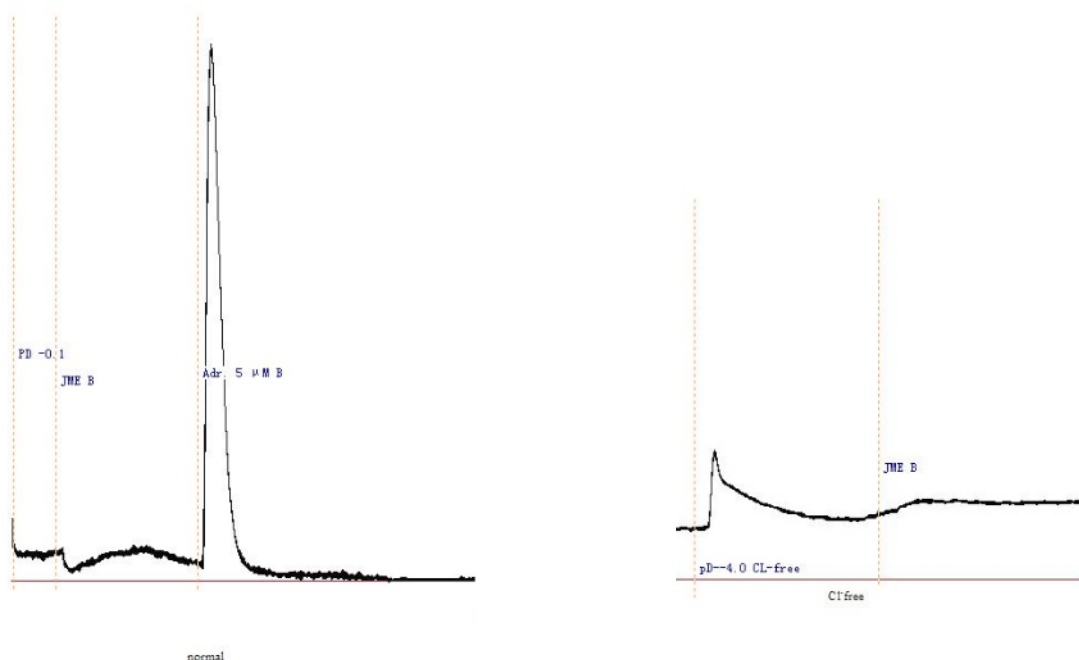
### *Statistics*

The data were expressed as X ± S.D. Concentration-response relationship was calculated with Hill equation. Statistical significances were analyzed by Student's t-test. p-value < 0.05 was considered significantly.

## Results

### *JME-induced anion secretion*

JME elicited a significant secretory response when added to the basolateral side of T<sub>84</sub> cells at a concentration-dependent manner, as shown



**Figure 1.** Representative Isc recording with arrows indicating the response to JME (0.05 mg/mL) added in normal and Cl<sup>-</sup> free K-H solutions.

in Figure 1. In order to study the ion species involved in mediating JME induced *I<sub>sc</sub>*, a series of ion substitution experiments were conducted, Cl<sup>-</sup> was removed from the bathing solution. The JME-induced *I<sub>sc</sub>* response was completely abolished by removing Cl<sup>-</sup> from bath solution (Figure 1), indicating that the JME-induced *I<sub>sc</sub>* was almost induced by Cl<sup>-</sup>.

*Short-circuit current measurement effect of JME on secretagogue-induced Cl<sup>-</sup> secretion*

JME concentration of 2.0 mg/mL was selected to study its effect on secretagogue induced Cl<sup>-</sup> secretion, since at this concentration application of JME can not induce ion transport on either side of the monolayers. In Figure 2, stimulation with Carbachol (100 μM) on basolateral side resulted in an *I<sub>sc</sub>* increase; application of JME (0.2 mg/mL) cannot further induced the *I<sub>sc</sub>* increase on either side of the monolayer (Figure 2).

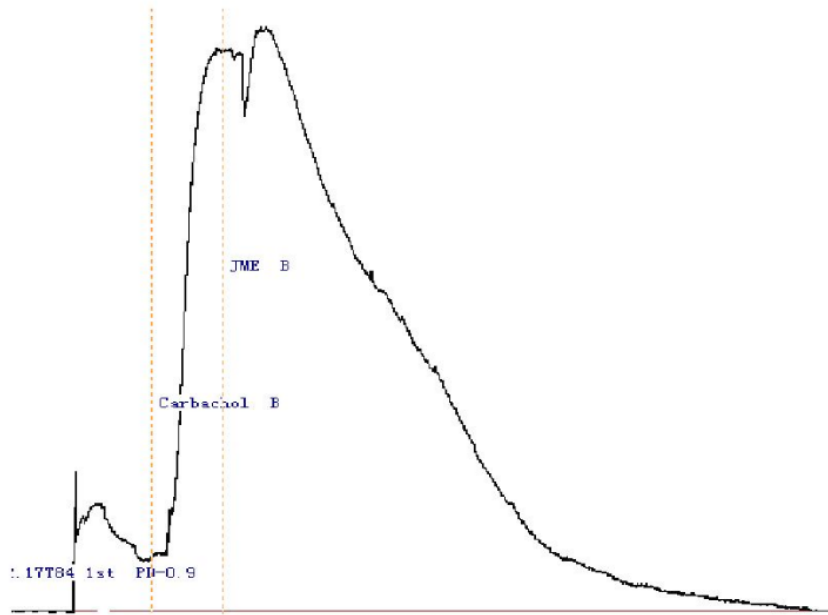
In Figure 3, the basolateral added adrenalin (5 μM, Adr.) produced a significant *I<sub>sc</sub>* increase, which lasted for at least 30 min. In order to study the ion species involved in mediating the Adr.-induced *I<sub>sc</sub>*, Cl<sup>-</sup> was removed from the bathing solution. The response was completely abolished by Cl<sup>-</sup> removal, indicating a Cl<sup>-</sup> dependence of

the Adr.-induced current. The JME (0.2 mg/mL) had also no significant effect on Adr.-induced Cl<sup>-</sup> secretion on either side of the monolayer. Pretreatment of T<sub>84</sub> cells for 20 min with JME (0.2 mg/mL, either side) had no significant effect on carbachol-induced Cl<sup>-</sup> secretion. Pretreatment of T<sub>84</sub> cells for 20 min with JME (0.2 mg/mL, apical) had also no significant effect on Adr.-induced Cl<sup>-</sup> secretion. In contrast, when T<sub>84</sub> cells were pretreated with JME (0.2 mg/mL, basolateral) for 20 min, the second area secretory response to Adr. was inhibited significantly.

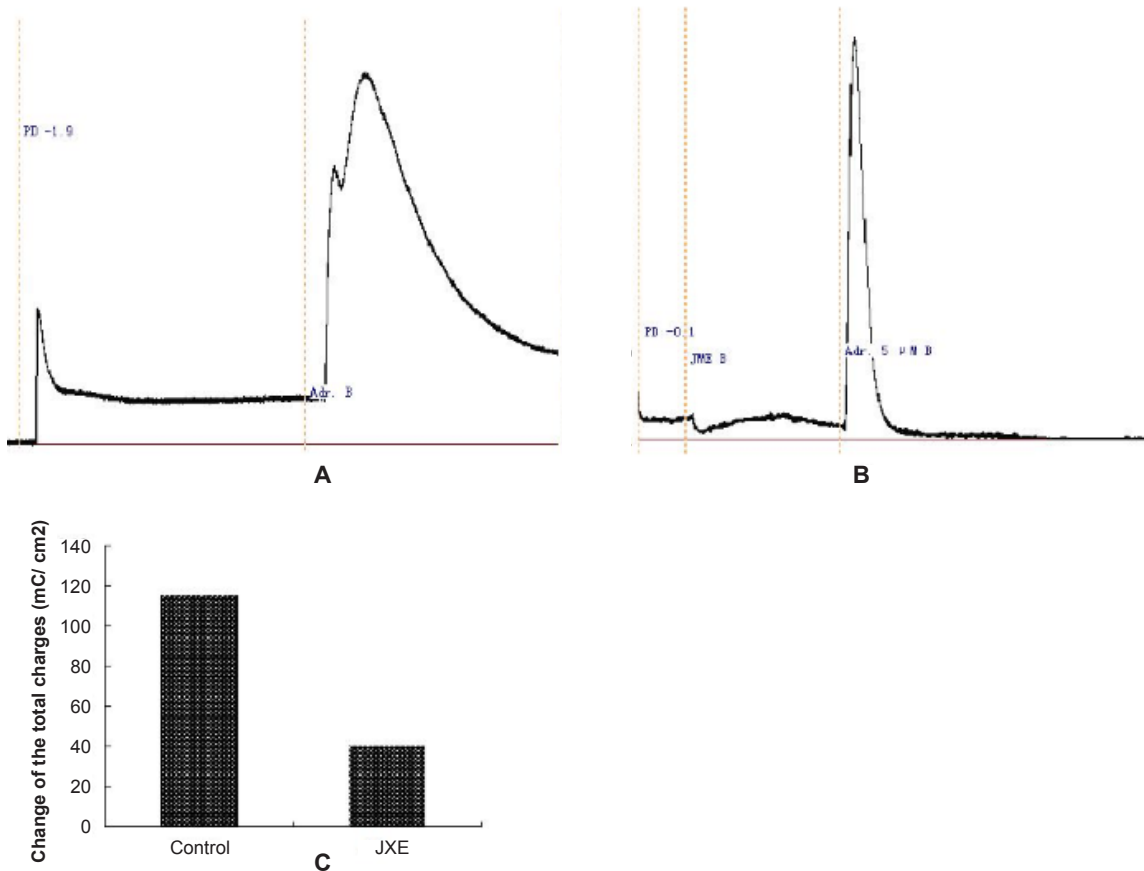
The effect of JME extract was concentration-dependent (Figure 4) with EC<sub>50</sub> of about 0.055 mg/mL. Different concentrations of JME are added to basolateral sides (Figure 5).

**Discussion**

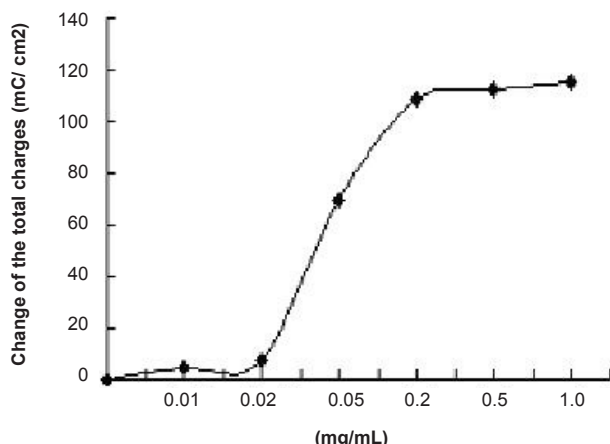
The present study is the first to investigate the pharmacological action of JME on intracellular signaling mechanisms. JME exerted a significant secretory effect at the higher concentration assayed. According to JME's traditional using, the mechanism of JME on secretagogue-induced Cl<sup>-</sup> secretion was selected for the subsequent experiments.



**Figure 2.** Representative Isc recordings of the  $T_{84}$  cell line in normal K-H solution. Stimulation with Carbachol (100  $\mu$ M) on basolateral side resulted in an increase in Isc, which was not inhibited by either side application of JME (0.2 mg/mL) (n=3). The time of the drug administration is indicated with arrow.



**Figure 3.** Representative Isc recordings of the  $T_{84}$  cell line in normal K-H solution. (n=3).  
 A: Representative Isc recordings in response to adrenalin of the  $T_{84}$  cell line in normal K-H solution.  
 B:  $T_{84}$  cells have pretreated with JME (0.2 mg/mL, basolateral) for 20 min before adding adrenalin.  
 C: Comparison between A and B in total charges.



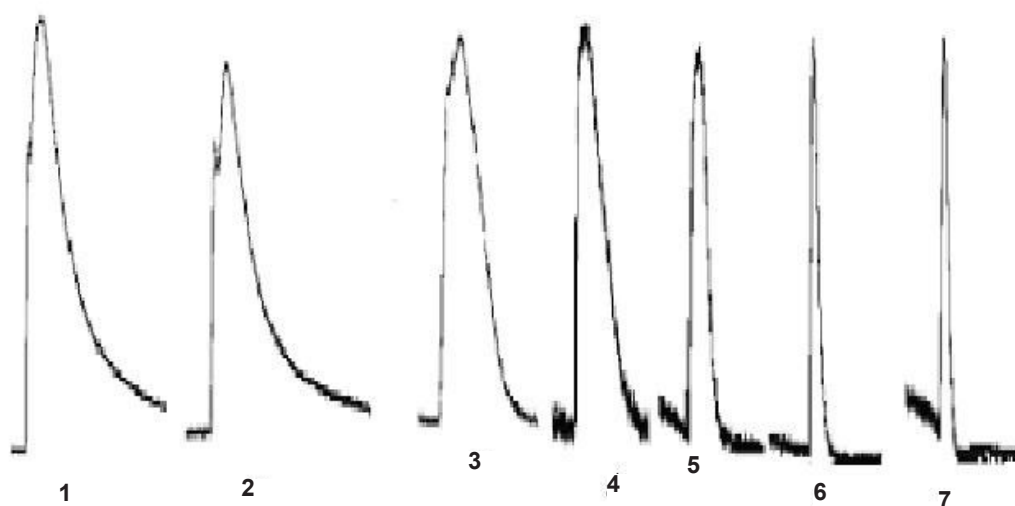
**Figure 4.** Concentration- response curve for JME-inhibited Isc was in  $T_{84}$  cell line. Values are mean  $\pm$  S.D. of maximal Isc decrease (n=3).

It has been reported that adrenalin induced  $Cl^-$  secretion in some epithelial cells through action of basolateral adrenoreceptors, such as epididymal epithelium and colonic cells (12). In normal Krebs solution, Adr. elicited a biphasic Isc response consisting of an initial spike followed by a delayed, but more sustained response. Pretreatment of  $T_{84}$  cells for 20 min with JME (0.2 mg/mL, basolateral) can abolish the second response. Using the nonselective  $\beta$ -adrenoreceptors antagonist propranolol can also abolish the components. It is suggested that JME exert a direct inhibitory effect on basolateral  $\beta$ -adrenoreceptors so that the opening

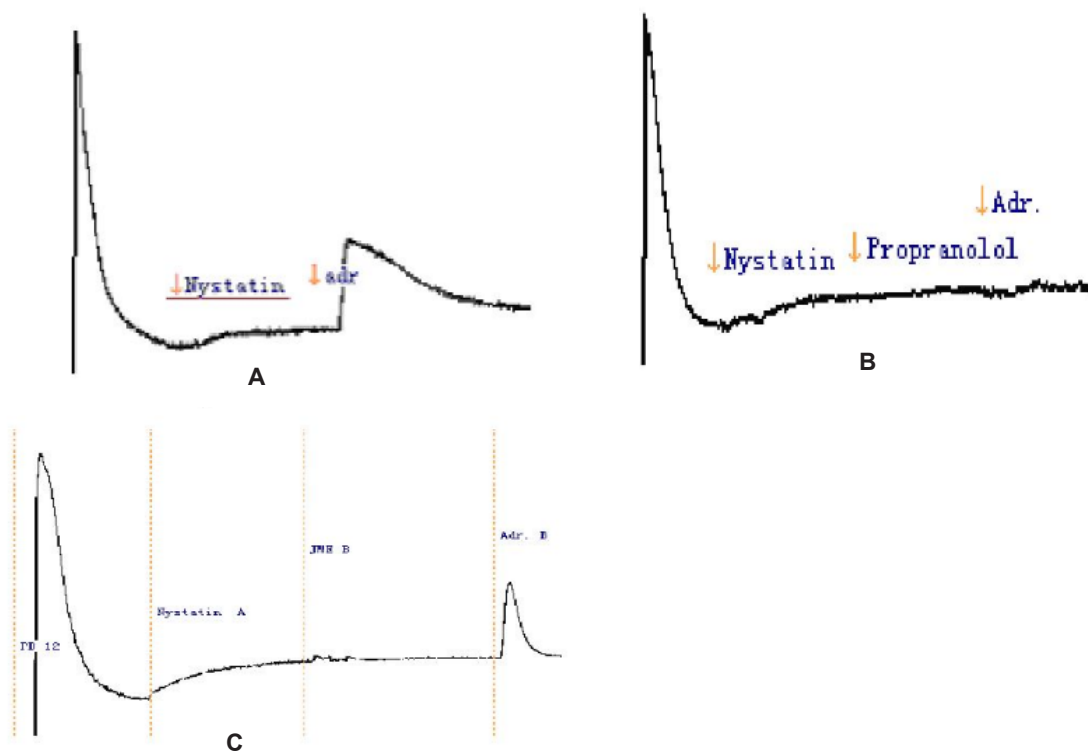
of the apical anion channels would be somehow compromised. The inhibitory effect of JME extract was concentration-dependent.

It is interesting to find that, JME was ineffective in blocking the Isc responses to carbachol. It has been reported that in  $T_{84}$  cells, carbachol can stimulate a  $Ca^{2+}$  dependent  $Cl^-$  secretion through activation of basolateral M-Ach receptor (13). The present results suggested that ME was ineffective in blocking basolateral M-Ach receptor in  $T_{84}$  cells.

We investigated the putative inhibition of secretion through blockade of basolateral  $K^+$  channels by using nystatin-permeabilised



**Figure 5.** Representative Isc recordings in response to JME of the  $T_{84}$  cell line is in normal K-H solution (n=3). Inhibition with JME 0  $\mu$ g/mL (1), 10  $\mu$ g/mL (2), 20  $\mu$ g/mL (3), 50  $\mu$ g/mL (4), 200  $\mu$ g/mL (5), 500  $\mu$ g/mL (6), 1000  $\mu$ g/mL (7) on basolateral side resulted in an increase in Isc, respectively. Values are mean  $\pm$  S.D. of maximal Isc decrease.



**Figure 6.** Representative  $I_{sc}$  recordings in response to JME of the  $T_{84}$  cell line in a low  $Cl^-$  solution ( $n=3$ ).

A:  $I_k$  recorded, firstly added with nystatin, secondly added with Adr. to the apical side;

B:  $I_k$  recorded, firstly added with nystatin, secondly added with propranolol, thirdly added with Adr. to the apical side;

C:  $I_k$  recorded, firstly added with nystatin, secondly added with JME, thirdly added with Adr. to the apical side.

human colonic mucosae in order to enucleate the effect of  $I_k$  to JME. Under these conditions of the low  $Cl^-$  solutions, mucosa to serosa  $K^+$  gradient, apical permeabilisation with nystatin ( $180 \mu g/\mu L$ ), a relationship between Adr. ( $5 \mu M$ ) and  $K^+$  gradient was observed (Figure 6a). The result showed that Adr. can't stimulate  $K^+$  secretion. Under the same condition we added the propranolol (the inhibitor of Adr.,  $10 \mu M$ ) on the basolateral of the monolayer, then, the Adr. ( $5 \mu M$ ) was added (Figure 6b).  $I_{sc}$  was completely inhibited. So, in the  $T_{84}$  cell line, Adr. induces secretion through activation of basolateral  $\beta$ -adrenoreceptors. Under the same condition when pretreatment of  $T_{84}$  cells for 20 min with JME ( $0.2 \text{ mg/mL}$ , basolateral), the second secretory response of Adr.-induced was significantly inhibited (Figure 6c).

Taken together, the present results have demonstrated that JME exerted an inhibitory effect on gastrointestinal  $Cl^-$  secretion that is effected by acting on basolateral

$\beta$ -adrenoreceptors. The ability of JME to inhibit  $Cl^-$  secretion in the GI tract can contribute to its beneficial effects, such as reducing smooth muscle movement and decreasing the frequency of defecations.

At the moment, it is uncertain whether the inhibitory effect of JME on  $Cl^-$  secretion is due to the collective effect of all the constituent herbal components or some active ingredients contained in the JME. Nevertheless, the current study has established a model for the quantitative measurement of JME effect on the GI tract and for further screening of its possible active ingredients. The study of intracellular signaling mechanisms validated the JME pharmacological action.

The mechanism of action of the JME is still not fully elucidated, but it involves a direct effect on the enterocyte. The effects are not specific for a particular signal transduction mechanism, and may be involved in the anti-secretory effect. The intracellular signaling mechanisms of JME



much more validated the JME pharmacological action.

### Acknowledgements

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