



# INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare &amp; Pharmascope Foundation

Journal Home Page: <https://ijrps.com>

## ***In vitro* anti-motility and antispasmodic effects of *Garcinia mangostana* extracts in isolated chicken ileum preparation**

Michelle Ooi Yi Ching, Sasikala Chinnappan\*, Mogana Sundari Rajagopal

Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur, Malaysia

### Article History:

Received on: 23.10.2018  
Revised on: 15.03.2019  
Accepted on: 19.03.2019

### Keywords:

Anti-motility,  
Antispasmodic,  
Chicken ileum,  
*Garcinia mangostana*

### ABSTRACT

*Garcinia mangostana* pericarps have been traditionally used in Southeast Asia for a variety of medicinal conditions. The present study was carried out to determine the anti-motility and antispasmodic effects of methanolic and aqueous *G. mangostana* extracts (MEM and AEM) on isolated chicken ileum. Extracts were prepared from the pericarp of *G. mangostana* using maceration technique with methanol and distilled water. Isolated ileum preparations were set up for recording in Tyrode's solution at 37°C. Dose-response curves were plotted using various doses of agonist as control such as acetylcholine (ACh) and histamine. Atropine, mepyramine and extracts were used as an antagonist. The results showed that methanolic and aqueous extracts possess significant concentration-dependent inhibitory effects ( $p < 0.05$ ) on agonist-induced contractions. The half maximal effective concentration ( $EC_{50}$ ) of extracts and standard antagonists were higher than the agonist alone. Both methanolic and aqueous extract of *G. mangostana* exerts anti-motility and antispasmodic effects on smooth muscle contractions. The study provides findings that support *G. mangostana* can be the potential treatment for diarrhoea and spasm.



### \* Corresponding Author

Name: Sasikala Chinnappan  
Phone: +60186653642  
Email: hasipharm@gmail.com

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v10i2.714>

Production and Hosted by

IJRPS | <https://ijrps.com>

© 2019 | All rights reserved.

### INTRODUCTION

Herbal plants are usually preferred to treat gastrointestinal disorders because they contain multiple plant constituents which enhance the therapeutic effects, or it may have the potential to neutralize the side effects. *Garcinia mangostana* is a typical example of a remedy which is plant-based and popular as an alternative therapy. However, there is still a lack of evidence for the health benefits of these products. Therefore, the present study was undertaken to evaluate the anti-diarrhoea activity of *Garcinia mangostana* in chicken ileum model.

The results obtained could suggest a way in promoting the future use of this plant for the prevention and enhancement in the treatment of diarrhoea. Besides, mangosteen pericarp which is normally being neglected and thrown as a waste can be utilized well. Farmers, nutritionists, researchers and pharmaceutical industries will benefit from this study.

Many mangosteen health food products have been rapidly-growing worldwide in the market. Studies found that mangosteen contains secondary metabolites such as garcinone-E,  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin, methoxy-beta-mangostin, and a new geranylated biphenyl derivative. They are all highly bio-active compounds which can be largely found in mangosteen root bark, stem and latex that were extracted and it could be the causative factor for mangosteen medicinal value (Alian, 2017). Mangosteen has a long history of use in treating wounds, skin infections, and dysentery. It is also used for inflammation, cholera and diarrhoea in Ayurveda medicines. Several experimental studies have revealed that mangosteen extracts have anti-oxidant, anti-allergic, anti-inflammatory, anti-tumour, antibacterial, and antiviral activities

(Pedraza-Chaverri, 2008). Aim of this study was to investigate the anti-motility and antispasmodic effects of *Garcinia mangostana* extracts on isolated chicken ileum contractions induced by acetylcholine and histamine.

## MATERIALS AND METHODS

**Drugs and chemicals:** Tyrode's solution (composition: NaCl 136.7 mM, KCl 2.68 mM, MgCl<sub>2</sub> 1.05 mM, NaH<sub>2</sub>PO<sub>4</sub> 0.42 mM, CaCl<sub>2</sub> 1.80 mM, NaHCO<sub>3</sub> 11.90 mM, glucose 5.55 mM), methanol, *Garcinia mangostana* extracts, acetylcholine, histamine, atropine and mepyramine.

### Preparation of *Garcinia mangostana* extracts

Fresh fruits of *Garcinia mangostana* were purchased from a local market in Taman Connaught Cheras, Kuala Lumpur. Fresh, dried pericarps were collected and powdered. It was followed by successive extraction in methanol and distilled water with the maceration technique at a ratio of 1:20 (Mohamadi, 2012). The methanol extracts were filtered and concentrated on vacuuming under reduced pressure in a rotary evaporator and dried. The vacuum reduces the boiling point of the solvent and separates the solvent from the organic materials (Melanie, 2018). The water extracts were freeze-dried. Both the extracts were kept in -20°C for further use.

**Pharmacological test:** The fresh entire gastrointestinal tract of healthy chicks (*Gallus gallus domesticus*; 1-1.5 kg in 8-10 weeks) was obtained from a slaughterhouse in Cheras, Kuala Lumpur. Terminal segments of ileum about 1-1.5 cm in length was prepared and placed in 30 ml organ baths filled with Tyrode's solution. The solution was kept at 37°C and oxygenated continuously. Ileum tissues were allowed to be stabilized in the organ baths to reach steady contractions with initial tension on 1 g and stabilization time for 45-60 min. Isometric contractions of the ileum were recorded in power lab by using isometric transducer.

1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml and 16 µg/ml of acetylcholine and histamine was added to the bath, and the control cumulative dose-response curves for each were constructed. After obtaining the dose-response curves of standard agonist drugs, same doses of agonist were repeated in presence methanolic ((MEM 4 mg/ml, MEM 8 mg/ml) and aqueous extracts (AEM 2 mg/ml, AEM 4 mg/ml) of *Garcinia mangostana* extracts and 2 µg/ml atropine and 2 µg/ml mepyramine were added to the bath 10 minutes before corresponding dose-response curves were plotted.

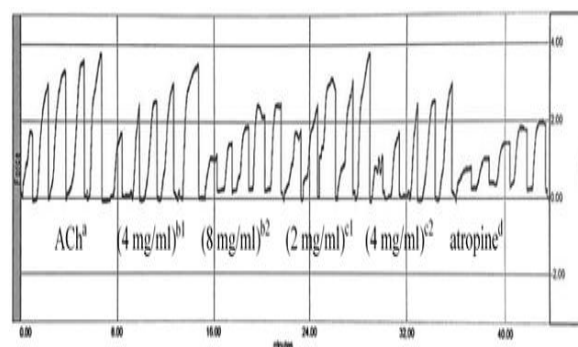
Inhibition effects of extracts and standard antagonist were compared with standard agonist in each experiment. Anticholinergic effect of extracts

(MEM 4 mg/ml, MEM 8 mg/ml, AEM 2 mg/ml, AEM 4 mg/ml), and atropine (2 µg/ml) were evaluated against a fixed minimally effective dose of acetylcholine (Ortiz-De-Urbina, 1990). The antihistamine effect of extracts (MEM 2 mg/ml, MEM 4 mg/ml, AEM 1 mg/ml, AEM 2 mg/ml), and mepyramine (2 µg/ml) were evaluated against a fixed minimally effective dose of histamine.

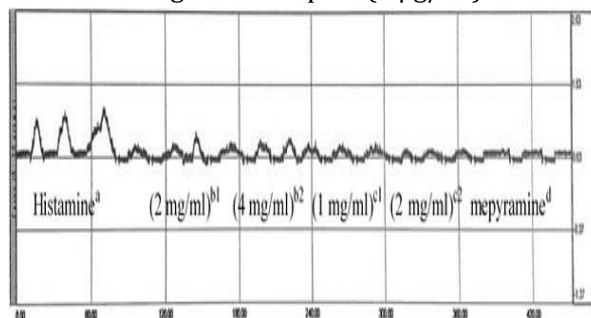
Acetylcholine and histamine dose-response curves in the absence and presence of extracts and standard antagonists were plotted using the GraphPad Prism version 7.0 software. Results were obtained and expressed as a percentage of maximum contractions. From the corresponding curves, EC<sub>50</sub>, agonist potency of acetylcholine and histamine were determined.

**Statistical Analysis:** All the values were recorded in the table as mean ± SEM of 5 experiments with significant at p<0.05. Data were analysed using the one-way ANOVA test.

## RESULTS AND DISCUSSION



**Figure 1: Dose-response tracing for acetylcholine (1-16 µg/ml) a**, respective antagonism by methanol extracts (4 mg/ml and 8 mg/ml) b1, b2 and aqueous extracts (2mg/ml and 4mg/ml) c1, c2 of *Garcinia mangostana* and standard antagonist: atropine (2 µg/ml) d



**Figure 2: Dose-response tracing for histamine (1-16 µg/ml) a**, respective antagonism by methanol extracts (2 mg/ml and 4 mg/ml) b1, b2 and aqueous extracts (1 mg/ml and 2 mg/ml) c1, c2 of *Garcinia mangostana* and standard antagonist: mepyramine (2 µg/ml) d

Acetylcholine (ACh) and histamine are the main neurotransmitters which initiate excitatory events like spasms and visceral pain in response to stimuli

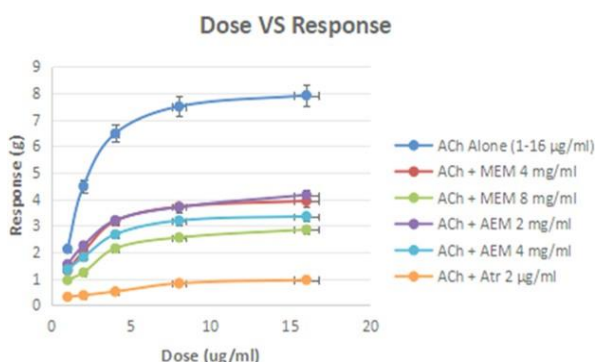
**Table 1: Pharmacodynamic values obtained for effects of *Garcinia mangostana* extracts on the acetylcholine-induced contraction of isolated chick ileum**

Parameter	ACh Alone	MEM 4 mg/ml	MEM 8 mg/ml	AEM 2 mg/ml	AEM 4 mg/ml	Atropine 2 µg/ml
Half-maximal effective concentration, EC <sub>50</sub>	1.68	1.78	2.43	1.83	1.96	3.52

**Table 2: Pharmacodynamic values obtained for effects of *Garcinia mangostana* extracts on the histamine-induced contraction of isolated chick ileum**

Parameter	His Alone	MEM 2 mg/ml	MEM 4 mg/ml	AEM 1 mg/ml	AEM 2 mg/ml	Mepyramine 2 µg/ml
Half-maximal effective concentration, EC <sub>50</sub>	0.66	0.86	0.68	0.92	0.77	1.66

(Gershon, 2007). The key finding for this study was to find out that *Garcinia mangostana* extracts have anti-motility and antispasmodic effects on the isolated preparation of chicken ileum. It could antagonize the effects of ACh and histamine-induced contractions in isolated chicken ileum tissues. *G. mangostana* extracts showed similar inhibitory effects like atropine and mepyramine. In the field of pharmacology, drug potency is a measure of drug activity and can be expressed in terms of the concentration required to produce a response of a given intensity (Neubig, 2003).

**Figure 3: Concentration response curve that shows significant ( $p < 0.05$ ) effect of *Garcinia mangostana* extracts and atropine on contractile response of acetylcholine on chicken ileum**

Acetylcholine (ACh) (1-16 µg/ml) produced a concentration-dependent contraction. *G. mangostana* produced a concentration-dependent inhibition of the spontaneous contractions of the chicken ileum (Figure 1). EC<sub>50</sub> of ACh in the presence of MEM (at 4 mg/ml, EC<sub>50</sub> = 1.78 µg/ml and at 8 mg/ml, EC<sub>50</sub> = 2.43 µg/ml) and AEM (at 2 mg/ml, EC<sub>50</sub> = 1.83 µg/ml and at 4 mg/ml, EC<sub>50</sub> = 1.96 µg/ml) was significantly ( $P < 0.05$ ) higher than EC<sub>50</sub> of ACh alone 1.68 µg/ml (Table 1). MEM at 4 mg/ml and 8 mg/ml, AEM at 2 mg/ml and 4 mg/ml showed significant ( $P < 0.05$ ) inhibitory effects on ACh-induced contractions.

Contractile responses resulted from ACh alone and in the presence of MEM and AEM were plotted respectively. Addition of the extracts to the isolated ileum reduced the height of contractions induced by ACh alone. Graph of comparison between the effects of both extracts and the standard antagonist were plotted, and a rightward shift was observed (Fig 3).

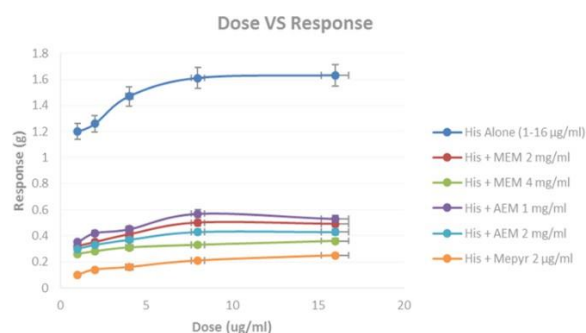
The contractile activity of ACh on ileum was significantly inhibited by *G. mangostana* extracts confirming the presence of anticholinergic components (atropine-like) in it. The antagonistic action on cholinergic receptor may explain the medicinal use of mangosteen as anti-diarrhoea and for other gastric disorder.

ACh is a major excitatory neurotransmitter from the parasympathetic nervous system and plays an important physiological role in the regulation of gut movements (Kishore, 2012). In GI smooth muscles, ileum with cholinergic nerves supply produces a contraction by activating M3 muscarinic receptors.<sup>48</sup> It may be possible that *G. mangostana* extract binds on muscarinic receptors or affects at least one of these mechanisms. Therefore, cholinergic antagonism is a conventional approach with anti-motility and antispasmodic effects for treating diarrhoea.

Histamine (1-16 µg/ml) produced a concentration-dependent contraction. *G. mangostana* produced a concentration-dependent inhibition of the spontaneous contractions of the chicken ileum (Figure 2). The EC<sub>50</sub> of histamine alone was 0.66 µg/ml, and it was increased in the presence of MEM (at 2 mg/ml, EC<sub>50</sub> = 0.86 µg/ml and at 4 mg/ml, EC<sub>50</sub> = 0.68 µg/ml) and in AEM (at 1 mg/ml, EC<sub>50</sub> = 0.92 µg/ml and at 2 mg/ml, EC<sub>50</sub> = 0.77 µg/ml). Following by the EC<sub>50</sub> of histamine obtained in the presence of mepyramine was found to be 1.66 µg/ml (Table 2). MEM at 2 mg/ml and 4 mg/ml, AEM at 1 mg/ml and 2 mg/ml showed significant ( $P < 0.05$ ) inhibitory effects on histamine-induced contractions.

Contractile responses resulted from histamine alone and in the presence of MEM and AEM were

plotted respectively. Addition of the extracts to the isolated ileum reduced the height of contractions induced by histamine alone. Graph of comparison between the effects of both extracts and the standard antagonist were plotted, and a rightward shift was observed (Fig 4). Histamine effects are predominantly due to the interaction with H1 receptors located on smooth muscle cells and moderately due to the interaction with H2 receptors present on myenteric plexus (Kishore, 2012). Antihistamines were found to block the effects of histamines secreted from mast cells and treating chronic diarrhoea condition (De Ponti, 1998).



**Figure 4: Concentration-response curve that shows significant ( $p < 0.05$ ) effect of *Garcinia mangostana* extracts and mepyramine on the contractile response of histamine on chicken ileum**

*G. mangostana* have a long history of use in gastrointestinal disorders, vomiting, and chest infection. Thus, the findings support that *G. mangostana* could be a potential treatment for the management of diarrhoea owing to its anti-motility and antispasmodic effects. Inhibitory activities occurred may due to the presence of active phytochemical contents found on the pericarp of *G. mangostana* including phenol, terpenoid, saponin, flavonoid, tannin and xanthenes. Scientific research findings have been reported on the proven benefits of xanthenes in mangosteen rind for anti-diarrhoea activity (National Germplasm Resources Laboratory, 2003). Flavonoid is one of the most numerous and widespread groups of phenolics. Inhibition of intestinal motility *in vitro* and the role as a spasmolytic have already been reviewed. Tannin, a strong antioxidant may also contribute to curing diarrhoea (Suttirak, 2014).

## CONCLUSION

EC<sub>50</sub> values obtained in the present study indicated that extracts showed anticholinergic and antihistaminic activities. *Garcinia mangostana* methanolic and aqueous extracts showed inhibitory effects in a dose-dependent manner. The dose-response curves of atropine, mepyramine and extracts were shifted towards the right when compared to ago-

nist alone. Since both extracts antagonize the contraction of chicken ileum, we concluded that *G. mangostana* showed anti-motility and antispasmodic effects and may be beneficial in different GI disorders.

## REFERENCES

- Alian D. A. et al. (2017) 'Antidiarrheal Activity of *Dissotis multiflora* (Sm) Triana (Melastomataceae) Leaf Extract in Wistar Rats and Subacute Toxicity Evaluation', Evidence-Based Complement Alternative Med.
- De Ponti, J. (1998) 'Functional gut disorders: from motility to sensitivity disorders a review of current and investigatory drugs for their management', Pharmacol. Ther., 80; 49-88.
- Gershon, M. D. and Tack, J. (2007) 'The serotonin signaling system: from basic understanding to drug development for functional GI disorders', Gastroenterology, 132;397-414.
- Kishore, D. V. and Rumana, R. (2012) 'Spasmolytic Activity of *Casuarina Equisetifolia* Bark Extract', IJPSR Vol. 3, Issue 05.
- Melanie, P. Y. and Grace, T. (2018) 'Rotary Evaporation to Remove Solvent', California Institute of Technology, JoVE Science Education Database; Cambridge: Organic Chemistry JoVE.
- Mohamadi, M., Maskook, A. M. and Mortazavi, S. A. (2012) 'Evaluation of Antioxidant Properties of Barberry Fruits Extracts using Maceration and Subcritical Water Extraction (SWE)', International Journal of Nutrition and Food Engineering, 6(9);699-703.
- National Germplasm Resources Laboratory, Beltsville, Maryland. 'Biological activities of *G. mangostana*', Phytochemical and Ethnobotanical Databases. 14 September 2003.
- Neubig, R. R. et al. (2003) International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification, XXXVIII, Pharmacol. Rev., Vol. 55;597-606.
- Ortiz-De-Urbina, A. V., Martin, M. L., Montero, M. J., Carron, R. et al. (1990) 'Antihistaminic activity of *Pulegone* on the guinea-pig ileum', Journal of Pharmacy and Pharmacology 1990, 49;295-296.
- Pedraza-Chaverri, José, Cardenas et al (2008) 'Medicinal properties of mangosteen (*Garcinia mangostana*)', Food and chemical toxicology, British: Industrial Biological Research Association, 46; 3227-39.
- Suttirak. (2014) 'In vitro antioxidant properties of mangosteen peel extract', Journal of food science and technology, 3546-3558.