The Thai Journal of Pharmaceutical Sciences

Volume 20
Issue 3 *1996*

Article 6

1-1-1996

# Study of Antiemetic Activity of Morinda citrifolia L. Fruits(การศึกษาฤทธิ์ต้านอาเจียนของผลยอ)

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Thai J. Pharm. Sci., 1996, Vol. 20, No. 3, pp 195-202

# Study of Antiemetic Activity of *Morinda citrifolia* L. Fruits

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**ABSTRACT:** *Morinda citrifolia* L. or 'Yor' is one of sixty six medicinal plants selected for the primary health care in Thailand. According to Thai traditional medicine, infusion or decoction of roasted or grilled mature, unripe fruits has been used to relieve nausea and vomiting symptom. However, pharmacological data to support this therapeutic claim are still lacking. This study was therefore conducted to determine if *M. citrifolia* fruits would possess antidopaminergic activity that would be responsible for its antiemetic action. Using antagonism of apomorphine-induced gnawing behavior model, water extract at the dose equivalent to crude drug 40 g/kg of body weight (g/kg BW) significantly reduced the duration of gnawing in rats. However, water extract at the dose equivalent to 25 g of crude drug could not prevent apomorphine-induced vomiting in dogs. In addition, the ability of the water extract to stimulate gastric emptying and GI motility or prokinetic activity, which is another action of most dopamine-antagonist antiemetics, was also determined. In mice, water extract at the doses equivalent to crude drug 10 and 20 g/kg BW increased % GI transit of charcoal in the small intestine, but 20 g/kg BW of the extract could not antagonize apomorphine-induced delay of gastric emptying. In conclusion, even though water extract could not antagonize the effects of apomorphine in dogs or mice, it decreased the duration of apomorphine-induced gnawing behavior in rats and showed prokinetic activity in mice. The results suggested that *M. citrifolia* fruits might contain a weak antidopaminergic agent responsible for its weak antiemetic effect observed in humans.

**KEY WORDS:** *Morinda citrifolia* L., antidopaminergic, antiemetic, apomorphine

## NTRODUCTION

*Morinda citrifolia* L. (Rubiaceae) or 'Yor' or 'Yor ɜaan' in Thai, are sma.l trees distributed widely throughout ıe warm region of the Pacific and tropical Asia. Accord-ıg to Thai traditional medicine, the fruit is used as car-ıinative, tonic, appetite stimulant, antiemetic, and to acce-ɔrate lochial discharge and to treat gum disease. (1, 2). Vith regard to the traditional use of the fruits as an ıtiemetic, the decoction or infusion of roasted mature unripe uits is recommended to be used as such or used as vehicle ɔr other herbs with antiemetic activity (3). Since *M. trifolia* fruits are locally used as food, this plant is regarded ɜ safe to be used in the primary health care in Thailand to ɜlieve the symptom of nausea and vomiting that is not ɜvere (3).

In a clinical study in malarial patients who experienced nausea and vomiting symptoms, it was found that *M. citrifolia* infusion taken 30 ml every 2 hours could significantly reduce the number of times that patients vomited as compared to the control group which received tea (4). The antiemetic effect of the infusion was, however, much less than that of metoclopramide.

It is known that dopamine receptors located in the chemoreceptor trigger zone (CTZ) and in the stomach play an important role in mediating nausea and vomiting symptoms and some antidopaminergic agents are now used as antiemetics and prokinetic drugs (5). This study was, therefore, conducted in order to determine whether the mechanism of antiemetic action of water extract of *M. citrifolia* is due to antidopaminergic activity. Dopamine antagonist activity of the extract was evaluated by its ability to antago-

nize the effect of apomorphine, a potent dopamine agonist, in rats, dogs and mice (6, 7).

## MATERIALS AND METHODS

### Animals

Male Wistar rats weighing 140-240 g were purchased from National Laboratory Animal Center, Mahidol University, Nakornpathom province. The animals were housed in the animal facility, National Institute of Health (NIH) building, Nonthaburi province, and allowed to acclimatize for at least one week before the day of experiment.

Female ICR mice were bread and reared in the animal facility, NIH building. The animals weighing 30-35 g were used in the experiments.

Dogs used in this study were of different breeds and ages and of both sexes. They were immunized with rabies vaccine one month prior to the day of experiment.

### Preparation of the extracts of *M. citrifolia* fruits

Mature unripe fruits of *M. citrifolia* were collected and identified by Mrs. Jaree Bansiddhi, Botany Section, Division of Medicinal Plant Research and Development, Ministry of Public Health. The voucher specimens (#Bansiddhi 93-20 and 93-22) are kept at the herbarium of the above institution. The fruits were washed, cleaned, cut into thick slices and dried by grilling over a charcoal brazier. The extraction of ground dried fruits was performed by the Phytochemistry Section, Division of Medicinal Plant Research and Development. The crude drug was extracted with water by reflux and evaporated on a water bath to obtain a 2:1 concentration.

### Chemicals

Apomorphine HCl and metoclopramide HCl were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.. Apomorphine HCl was dissolved in sterile normal saline containing 0.1% sodium metabisulfite as an antioxidant (7). Activated charcoal (Ultracarbon$^{R}$) was prepared as 10% suspension in 4% tragacanth.

### Antagonism of apomorphine-induced gnawing behavior in the rat

Rats were randomly divided into two groups and fasted 18 hours prior to the experiment but allowed to have free access to water. The animals were weighed and given *M. citrifolia* extract orally at the doses equivalent to crude drug 20, 40, 60 or 120 g/kg BW while animals in the control group received water. One hour later, the rats were injected i.v. with apomorphine at the dose of 1.25 mg/kg BW. Each individual animal was kept in a glass jar containing wood chips as bedding. The onset and duration of gnawing (chewing of wood chips) or licking itself or the cage were observed and recorded by observers who were not aware which treatment each animal received. The differences of the onsets and the durations of gnawing and licking between control and treatment groups were then determined (6). Each experiment was repeated at least twice to confirm the reproducibility of the results.

**Table 1** Dose-response relationship of apomorphine in male Wistar rats.*

| Dose (mg/kg) | % of rats having gnawing behavior | Duration of gnawing behavior (min) |
|---|---|---|
| 0.125 | 0 | – |
| 0.25 | 50 | 14.00 ± 0 |
| 0.50 | 100 | 32.25 ± 3.86 |
| 1.00 | 100 | 41.50 ± 3.87 |
| 1.25 | 100 | 49.50 ± 3.00 |

* Each value represents mean ± SD (n = 4).

### Antagonism of apomorphine-induced vomiting in the dog

Dogs were divided into control and treatment groups. The control group received 25 ml of water and the treatment group received water extract of *M. citrifolia* fruits at the dose equivalent to crude drug 25 g/ 25 ml/animal. Forty five minutes later, each animal was injected subcutaneously with apomorphine HCl at the dose of 0.1 mg/kg BW. The time when each animal received the injection and when it vomited as well as the number of times it vomited were recorded (6).

### Effect of the water extract on GI transit in the mouse

The mechanism of action of antiemetic drugs possessing antidopaminergic activity is partly due to their prokinetic activity, i.e. the ability to accelerate the emptying of gastric content and reduce GI transit time, mediated via dopamine receptors in the stomach (5). Therefore, the prokinetic activity of water extract of *M. citrifolia* fruits was determined by studying its effect on GI transit of charcoal in mice with or without the i.v. administration of apomorphine.

Eighteen hours prior to the experiment, female ICR mice were fasted but allowed to have free access to water. The animals in the treatment group received water extract of *M. citrifolia* fruits orally at the dose equivalent to crude drug 20 or 40 g/kg BW while those in the control group received water. Thirty minutes later, the mice were gavaged with 0.3 ml of 10% charcoal suspension. Some experiments in which the effect of water extract to antagonize apomorphine-induced delay of gastric emptying was assessed, after charcoal administration, the animals were also injected i.v. with apomorhine HCl at the dose of 6 mg/kg BW. Ninety minutes later, the animals were sacrificed by cervical dislocation. The distances in cm that charcoal moved from the stomach into the intestine and the total length of the small intestine were measured and the ratio of the two distances was calculated as percent GI transit of each animal. The means of percent GI transit were determined and compared between control and extract-treated groups (6, 7).

### Statistical evaluation

For each parameter measured, the difference between control group and treatment group(s) was determined by two-tailed Students' t test, or one-way analysis of variance followed by Newman Keul's range test where appropriate. The significant level was defined at p value less than 0.05.

## RESULTS

### Dose-response relationship of apomorphine and antagonistic activity of metoclopramide

In order to find the most appropriate dose of apomorphine to induce gnawing behavior in the rat, dose-response relationship of apomorphine was intially determined. The effect of apomorphine given i.v. at the doses of 0.125, 0.25, 0.5, 1.0 and 1.25 mg/kg BW on the duration of gnawing behavior in male Wistar rats was shown in Table 1. Of the doses that could induce gnawing in 100% of the animals, it was found that the most appropriate dose was 1.25 mg/kg BW because the duration of gnawing behavior was least varied.

Using this animal model, it was found that metoclopramide, a dopamine antagonist commonly used as an antiemetic, given orally at the dose of 15 and 30 mg/kg BW one hour prior to apomorphine administration, could completely inhibit gnawing behavior.

### Effect of water extract of *M. citrifolia* on apomorphine-induced gnawing behavior in the rat

It was found that oral administration of water extract of *M. citrifolia* fruits to rats at the dose equivalent to crude drug 20 g/kg BW did not affect the onset or the duration of apomorphine-induced gnawing behavior (Table 2). When the dose of the extract was increased to 40 g/kg BW, the onset of action of apomorphine was still not altered; however, the duration of gnawing was significantly reduced (17.5% reduction) as compared to that of the control groups. (Table 2).

**Table 2** Effect of water extract of *M. citrifolia* fruits on the onset and duration of action of apomorphine in the rat.#

| Dose of extract equivalent to crude drug (g/kg) | Onset of gnawing behavior (min) | Duration of gnawing behavior (min) | n |
|---|---|---|---|
| 0 | 3.0 ± 1.3 | 43.5 ± 2.4 | 8 |
| 20 | 3.1 ± 1.3 | 40.8 ± 4.4 | 9 |
| 0 | 3.7 ± 2.1 | 49.6 ± 4.9 | 9 |
| 40 | 3.5 ± 1.8 | 40.9 ± 3.9* | 9 |
| | % reduction | 17.5% | |

# Each value represents mean ± SD.
* Significantly different from control group ($p < 0.05$).

## Effect of water extract of *M. citrifolia* fruits on apomorphine-induced emesis in the dog

Water extract of *M. citrifolia* fruits was later tested for an antiemetic activity in apomorphine-induced emesis in the dog, an animal species that can vomit. Initially, dose-response of apomorphine for the induction of emesis in dogs was determined. It was found that the most appropriate dose of apomorphine for subcutaneous administration was 0.1 mg/kg BW.

The dose of water extract of *M. citrifolia* fruit that was selected for this study was equivalent to crude drug 25 g/ 25 ml/animal which was the highest possible volume of administration of this thick extract that could be given via a syringe. It was found that water extract at this dose did not have an effect on the onset or the duration of apomorphine-induced emesis or the number of times that the animal vomited (Table 3).

**Table 3** Effect of water extract of *M. citrifolia* fruits on apomorphine-induced emesis in the dog.

| Group of animal# | Onset of emesis (min) | Duration of emesis (min) | Number of times animals vomited | n |
|---|---|---|---|---|
| control | 3.2 ± 0.9 | 16.2 ± 8.5 | 8.9 ± 3.9 | 8 |
| treatment | 3.3 ± 1.3 | 15.1 ± 8.9 | 7.9 ± 3.2 | 9 |

Each value represents mean ± SD.
# Control group received 25 ml of water and treatment group received water extract of *M. citrifolia* at the dose equivalent to crude drug 25 g/animal.

### Effect of water extract of *M. citrifolia* on GI transit of charcoal in the mouse

The effect of water extract of *M. citrifolia* fruits on gastric emptying and GI motility was evaluated in mice by monitoring the movement of charcoal in the intestine with or without the i.v. administration of apomorphine. It was found that without concomitant adminstration of apomorphine, water extract at the doses equivalent to crude drug 10 and 20 g/kg BW administered 30 minutes prior to charcoal administration could significantly increase % GI transit of charcoal in the mouse intestine. In contrast, when the dose was increased to 40 g/kg BW, % GI transit of charcoal was significantly decreased (Table 4).

In order to determine if water extract of *M. citrifolia* would be able to antagonize apomorphine-induced delay of gastric emptying, the effect of apomorphine on GI transit of charcoal was initially observed in mice. It was found that the dose of 6 mg/kg BW was required to significantly reduce GI transit of charcoal (Table 5), this dose of apomorphine was then selected for the later experiments.

When the extract was administered p.o. at the doses equivalent to crude drug 20 and 40 g/kg BW thirty minutes prior to charcoal and apomorhine administration, it was found that the extract at the dose of 20 g/kg BW did not alter the effect of apomorphine on the delay of gastic emptying while the dose of 40 g/kg significantly decreased % GI transit of the charcoal as compared to the control group (Table 4).

## DISCUSSION

According to Thai traditional medicine, when *M. citrifolia* fruits are used as an antiemetic, it is recommended that the mature unripe fruits be sliced, roasted or grilled until turning brown and used in the form of decoction or infusion per se or as a vehicle for other antiemetic herbs (2). Therefore, water extract was selected to be used for

**Table 4** Effect of water extract of *M. citrifolia* fruits on GI transit charcoal in mice with or without i.v. injection of apomorphine.

| Apomorphine[1] | Dose of extract equiv. to crude drug (g/kg BW) | % GI transit[#] | n |
|---|---|---|---|
| – | 0 | 69.24 ± 13.82 | 16 |
| | 10 | 81.65 ± 18.69* | 17 |
| – | 0 | 67.10 ± 10.65 | 19 |
| | 20 | 79.65 ± 15.03* | 21 |
| | 40 | 51.14 ± 16.06* | 20 |
| + | 0 | 55.13 ± 15.09 | 30 |
| | 20 | 50.18 ± 10.73 | 30 |
| + | 0 | 62.22 ± 17.39 | 39 |
| | 40 | 26.34 ± 28.82** | 40 |

– = without apomorphine
+ = with apomorphine administration.
# Each value represents mean ± SD.
* Significantly different from control group ($p < 0.05$).
** Significantly lower than control group ($p < 0.0001$).

**Table 5** Effect of apomorphine on GI transit of charcoal in mice.[1]

| Dose of apomorphine (mg/kg BW) | % GI transit of charcoal[#] | n |
|---|---|---|
| 0 | 74.06 ± 15.04 | 7 |
| 2 | 73.61 ± 13.18 | 6 |
| 4 | 63.63 ± 8.95 | 6 |
| 6 | 55.35 ± 14.05* | 7 |

[#] Each value represents mean ± SD.
* Significantly lower than control group ($p < 0.05$).
[1] Overnight-fasted rats were first given water 10 ml/kg BW. Thirty minutes later, they were given 0.3 ml of 10% charcoal suspension p.o. and apomorphine i.v. % GI transit was determined ninety minutes therafter.

the study of antiemetic activity of *M. citrifolia* in experimental animals since active constituents similar to those in the decoction or infusion would be obtained.

Even though water extract, unlike metoclopramide, could not completely antagonize the effect of apomorphine, the extract at the dose equivalent to crude drug 40 g/kg BW showed weak antidopaminergic activity by significantly reducing the duration of apomorphine-induced gnawing behavior in rats (Table 2).

In contrast to what was observed in rats, pretreatment of the dogs with water extract at the dose equivalent to crude drug 25 g did not reduce the onset or the duration of apomorphine-induced emesis or the number of times that the animals vomited (Table 3). This dose of the extract was the highest possible dose and volume of administration that can be force-fed conveniently to the dogs. Therefore, it is not known whether higher doses of the extract would be able to mitigate the effect of apomorphine in dogs as observed in rats or not.

In the stomach, dopamine receptors mediate inhibition of gastric motility that occurs during nausea. The mechanisms of action of antiemetic drugs possessing antidopaminergic activity are not only at the level of CTZ but also party due to their prokinetic activity mediated via dopamine receptors in the stomach (5). Therefore, the prokinetic activity of water extract of *M. citrifolia* fruits was determined by studying its effect on GI transit of charcoal in mice with or without i.v. administration of apomorphine.

Without concomitant administration of apomorphine, water extract at the doses equivalent to crude drug 10 and 20 g/kg BW significantly increased % GI transit of charcoal in the mouse intestine (Table 4). During the time that we were conducting our own experiments, it was reported that only water extract of *M. citrifolia* fruits, at low concentrations, but not petroleum ether, chloroform, or n-butanol extracts, could transiently increase the force and the frequency of contraction of isolated rat fundus strips. The maximum stimulatory effect of the extract on the fundus strips was lower than that of acetylcholine but higher than that of metoclopramide (8). Their reported stimulatory effect of water extract of *M. citrifolia* fruits on the isolated rat fundus strips appeared to be consistent with the increased % GI transit in mice found in our study. Since emesis is promoted by conditions that slow gastric emptying (5), the prokinetic effect of the water extract would be beneficial for the treatment of nausea and vomiting.

The prokinetic effect of the extract at the dose equivalent to crude drug 20 g/kg BW seen in the previous experiment was not observed when mice were given apomorphine concomitantly with charcoal (Table 4). The result suggested that the active principle(s) in the water extract could not antagonize potent dopamine agonistic activity of apomorphine on the delay of gastric emptying in mice.

With or without apomorphine administration, when the dose of the extract was increased to 40 g/kg BW, % GI transit of charcoal was significantly decreased as compared to the control groups. This reduction of GI motility was

probably due to the thick texture of this dose of the extract which could markedly slow the movement of charcoal in the GI tract. Another possibility would be that there might be another substance present in the water extract that, at a high concentration, could compete with the prokinetic principle in the extract and reduce gastric motility by an unknown mechanism.

## CONCLUSIONS

The antimetic and antidopaminergic activities of *M. citrifolia* fruits was evaluated in rats, dogs and mice treated with apomorphine. It was found that water extract of *M. citrifolia* fruits could not antagonize potent dopaminergic effects of apomorphine on the induction of emesis in dogs or on the delay of gastric emptying in mice. However, the extract at the dose of 40 g/kg BW could significantly reduce the duration of apomorphine-induced gnawing behavior in rats and at the doses of 10 and 20 g/kg BW could significantly increase gastric emptying in mice. The results suggested that *M. citrifolia* fruits appear to contain some water-soluble substances possessing week antidopaminergic activity that might be responsible for its weak antiemetic action observed in humans.

## ACKNOWLEDGEMENTS

This work financially supported by the grant from the World Health Organization. We would like to thank Mr. Kamol Sawasdimongkol for his valuable advice on the project, Mrs. Jaree Bansiddhi for providing and identifying *M. citrifolia* fruits. We also would like to than Miss Siriluk Putthakote, Miss Chomyong Suasuai and Mr. Montree Klinchanhom for their technical assistance.

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# การศึกษาฤทธิ์ต้านอาเจียนของผลยอ

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**บทคัดย่อ**: ยอ (*Morinda citrifolia* L.) เป็นสมุนไพรหนึ่งในหกสิบหกชนิดที่ได้รับการคัดเลือกสำหรับใช้ในสาธารณสุขมูลฐานในประเทศไทย ตำราแพทย์แผนโบราณระบุว่า ผลยอห่ามที่นำมาคั่วหรือย่างไฟแล้วเตรียมเป็นยาซงหรือยาต้ม มีสรรพคุณในการบรรเทาอาการคลื่นไส้อาเจียน อย่างไรก็ตามยังไม่มีหลักฐานทางเภสัชวิทยาสนับสนุนคำกล่าวอ้างนี้ ดังนั้นจึงได้ทำการทดสอบเพื่อศึกษาว่าผลยอมีฤทธิ์ต้านโดปามีนหรือไม่ ซึ่งจะช่วยอธิบายกลไกการออกฤทธิ์ต้านอาเจียนของผลยอ จากการศึกษาฤทธิ์ต้านอะโปมอร์ฟีนในหนูขาวพบว่า ส่วนสกัดด้วยน้ำของผลยอในขนาดเทียบเท่าผงยา 40 กรัม/น้ำหนักตัว 1 กิโลกรัม (ก./กก.) มีฤทธิ์ลดระยะเวลาแสดงอาการกัดแทะในหนูขาวที่ได้รับอะโปมอร์ฟีนอย่างมีนัยสำคัญ อย่างไรก็ตาม ส่วนสกัดด้วยน้ำในขนาดเทียบเท่าผงยา 25 กรัม ไม่สามารถป้องกันอาการอาเจียนในสุนัขที่ได้รับอะโปมอร์ฟีนได้ นอกจากนี้ยังได้ศึกษาฤทธิ์ของส่วนสกัดด้วยน้ำของผลยอในการเร่งการบีบตัวของกระเพาะอาหารและลำไส้ซึ่งเป็นกลไกการออกฤทธิ์อีกอย่างหนึ่งของยาต้านอาเจียนกลุ่มที่ออกฤทธิ์ต้านโดปามีน พบว่าในหนูถีบจักร ส่วนสกัดด้วยน้ำในขนาดเทียบเท่าผงยา 10 และ 20 ก./กก. สามารถเร่งการเคลื่อนตัวของผงถ่านไปในลำไส้เล็กได้ แต่ส่วนสกัดขนาดเทียบเท่าผงยา 20 ก./กก. ไม่สามารถต้านฤทธิ์ของอะโปมอร์ฟีนในการลดการบีบตัวของกระเพาะอาหารได้ จะเห็นได้ว่า ถึงแม้ว่าส่วนสกัดด้วยน้ำของผลยอจะไม่สามารถต้านฤทธิ์ของอะโปมอร์ฟีนในสุนัขหรือในหนูถีบจักรได้ แต่ส่วนสกัดสามารถลดระยะเวลาแสดงอาการกัดแทะในหนูขาวที่ได้รับอะโปมอร์ฟีนและแสดงฤทธิ์กระตุ้นการบีบตัวของกระเพาะอาหารในหนูถีบจักรได้ ผลการทดลองแสดงให้เห็นว่าผลยออาจมีสารที่มีฤทธิ์ต้านโดปามีนอย่างอ่อน ๆ อยู่ จึงทำให้ผลยอสามารถแสดงฤทธิ์ต้านอาเจียนได้บ้างในคน

**กุญแจคำ**: ยอ, ฤทธิ์ต้านโดปามีน, ฤทธิ์ต้านอาเจียน, อะโปมอร์ฟีน