เชียงใหม่สัตวแพทยสาร 2560; 15(3): 199-205. DOI: 10.14456/cmvj.2017.19

เชียงใหม่สัตวแพทยสาร

Chiang Mai Veterinary Journal

ISSN; 1685-9502 (print) 2465-4604 (online)

Website; www.vet.cmu.ac.th/cmvj

Original Article

In vitro Cytotoxicity Test and Antiviral Activity of Curcuminoids from Turmeric Extract Against PRRS Virus

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Abstract Porcine Reproductive and Respiratory Syndrome (PRRS) caused by PRRS virus is an important viral infectious disease of swine that causes immense economic loss in swine industries worldwide. Presently, the defensive and controls of PRRS outbreak are mostly depend on the farm biosecurity, gilt management and successive vaccination program. However, the disease control by those strategies remained uncertain and unpredictable. Therefore, seeking an alternative antiviral compound for preventing and controlling PRRS is of great interest. In the present study, we aim to investigate the anti-PRRSv property of the curcuma, compound derived from medicinal plant in *Zingiberaceae* family. Turmeric extraction (curcuminoids) was obtained and was increase dissolved by the solid dispersion technique and found that at the concentration of 0.00005 – 1.56 µg/mL showed no cytotoxicity effect on the MARC-145 cells. Interestingly, at the concentration of 1.56 µg/mL, curcuminoids showed significantly inhibit PRRS virus replication. This result is interesting in use herb extraction against PRRS infection and should be study, in *vivo*.

Keywords: PRRSv, turmeric extract, cytotoxic effect, antiviral effect

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Article history; *received manuscript: 25 October 2017, accepted manuscript: 30 November 2017, published online: 7 December 2017*

บทความต้นฉบับ

การทดสอบความเป็นพิษต่อเซลล์และผลการต้านไวรัสของสารเคอร์คูมินอยด์จากการสกัดจากขมิ้นชันต่อเชื้อพีอาร์อาร์เอสไวรัส

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บทคัดย่อ โรคกลุ่มอาการระบบสืบพันธุ์และระบบทางเดินหายใจ (Porcine reproductive and respiratory syndrome: PRRS) เกิดจากเชื้อไวรัสพีอาร์อาร์เอส โดยเป็นโรคติดเชื้อที่สำคัญในสุกร เป็นสาเหตุความสูญเสียทางเศรษฐกิจของอุตสาหกรรมการเลี้ยงสุกรทั่วโลก ปัจจุบันการป้องกันและควบคุมการระบาดโรคพีอาร์อาร์เอสขึ้นกับระบบความปลอดภัยทางชีวภาพของฟาร์ม การจัดการสุกรสาวทดแทน และโปรแกรมวัคซีนที่ดี แต่การควบคุมโรคด้วยแผนการเหล่านี้ยังไม่แน่นอนและไม่สามารถคาดการณ์ได้ ดังนั้นการหาทางเลือกอื่นในการควบคุมป้องกันจึงเป็นสิ่งที่น่าสนใจ งานวิจัยนี้จึงศึกษาคุณสมบัติของสารสกัดของขมิ้นชัน (curcuminoids) ซึ่งเป็นพืชที่อยู่ในตระกูล *Zingiberaceae* ในการต้านไวรัสพีอาร์อาร์เอส พบว่าการสกัดขมิ้นชันและเพิ่มการละลายด้วยวิธีการเพิ่มการละลายโดยสารเพิ่มการละลาย (solid dispersion) แสดงความเป็นพิษต่อเซลล์ MARC-145 ที่ความเข้มข้น 0.00005 – 1.56 ไมโครกรัมต่อมิลลิลิตร โดยที่ความเข้มข้น 1.56 ไมโครกรัมต่อมิลลิลิตร สามารถยังยั้งการเพิ่มจำนวนของเชื้อไวรัสพีอาร์อาร์เอสได้ ผลดังกล่าวจึงเป็นที่สนใจในการใช้สารสกัดสมุนไพรในการป้องกันการติดเชื้อไวรัสพีอาร์อาร์เอสและควรมีการศึกษาในสัตว์ต่อไป

คำสำคัญ เชื้อไวรัสพีอาร์อาร์เอส สารสกัดขมิ้นชัน การทดสอบความเป็นพิษต่อเซลล์ ผลการต้านไวรัส

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Introduction

Porcine reproductive and respiratory syndrome (PRRS), which is caused by the PRRS virus (PRRSv), is a serious pandemic disease worldwide which appears to have the potential to seriously disrupt the swine industry. For example, there was a lot of pig loss from the outbreak of a new strain of PRRSv known as HP-PRRS in China (Tian *et al*., 2006). PRRSv can infect pigs of all ages, including boars, sows, weaning pigs, and finishing pigs. In its most serious forms, PRRSv can cause late-term abortion, high rates of stillbirth, increased post-weaning mortality and respiratory problems from porcine respiratory disease complex (PRDC) (Chae, 2016). At present, there is no specific treatment for PRRSv, only supportive treatment to alleviate its severity, although preventative measures, including acclimatization of gilts, vigilant biosecurity and vaccination, have been shown to help but not to provide complete protection (Labarque *et al*., 2003).

Herbs, with their many antimicrobial, antioxidant, immune stimulant, and antiviral effects, may offer promise in the fight against PRRSv. Pringproa *et al*. (2014) has shown that the crude extract of *Cynodon dactylon* can have an antiviral effect against porcine reproductive and respiratory syndrome virus. Many studies have shown that plants in the *Zingiberaceae* family, which includes Thai ginger, have antiviral properties (Oyuntsetseg et al., 2014; Sornpet et al., 2017). These include *Curcuma longa* Linn, commonly known as turmeric. Its composition are volatile oils (cineole, linalool, Alpha-terpinene, caryophyllene and germacrone) (Ingkaninan *et al.,* 2000) and curcuminoids (curcumin, demethoxycurcumin and bis-demethoxycurcumin) (Ingkaninan *et al.,* 2000) especially curcumin, which is a polyphenolic compound, has been reported to have antiviral and antioxidant activities among other properties (Zorofchian *et al*., 2014). However, Turmeric extract has the disadvantage of having low solubility in water as well as a low stability which results in low absorption and low bioavailability when administrated orally (Wang *et al*., 1997). Solid dispersion is a method which may help to circumvent that disadvantage.

The objective of this study was to evaluate the cytotoxicity and antiviral activity of turmeric extract against PRRS virus *in vitro*.

Materials and methods

Preparation of turmeric extract

This study attempted to increase the solubility in water of curcuminoids in turmeric extract which had been obtained by solid dispersion from crude turmeric extract. Turmeric extract was extracted with 95% ethanol, then the extract was evaporated to a high viscosity. The turmeric extract was analyzed by high-performance liquid chromatography (HPLC) and was compared with curcuminoids standard group. Then a crude turmeric extract of the solid dispersion was created using the solvent method as well as the solvent and melt method in which sodium lauryl sulfate (SLS) and polyvinyl

pyrrolidone K30 (PVP-K30) were used to increase solubility in water and the stability of the active ingredient.

Preparation of PRRSv

This study used PRRSv (VR2332 North American strain) as the agent and MARC-145 cells from the Microbiology Laboratory of the Faculty of Veterinary Medicine, Chiang Mai University. The cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin.

Cytotoxic test

For the cytotoxic test, the MARC-145 cells were cultured in two 96-well plates with 5,000 cells per well and incubated in an atmosphere of 5% CO_2 at 37 C° for 24 hours. Then the turmeric extract was added and diluted with media in two-fold serial dilutions starting at 100 µg/ml. (The positive control used only the media without the turmeric extract.) Incubation continued in a 5% CO_2 atmosphere at 37 C° for an additional 72 hours. The first plate was tested using the MTT assay and the second plate was stained with crystal violet. The highest concentrations of the extract which did not have an observable cytopathic effect were used in the study.

Antiviral activity test

For the antiviral activity test, the MARC-145 cells were cultured in 96-well plates with 5000 cells per well. Then they were incubated in an atmosphere of 5% CO_2 at 37 C° for 24 hours after which they were infected with PRRSv at a multiplicity of infection (MOI) of one, for 1 hour at 37 C°. After that, the virus was removed from the wells and replaced with the diluted turmeric extract at the concentration determined in the cytotoxicity test and additionally at two lower dilutions. 1% DMSO was used as the negative control. After that, the wells were incubated in an atmosphere of 5% CO_2 at 37 C° and the supernatant was collected at 24, 48 and 72 hours post inoculation (hpi) followed by immunoperoxidase monolayer assay (IPMA) to determine the viral titer.

Statistical analysis

The cytotoxicity and antiviral activity tests were analyzed by two-way ANOVA with Tukey-Kramer HSD to compare the differences of the means. $P < 0.05$ was considered statistically significant.

Results and discussion

The percentage yield of turmeric extract was 23.43%. The HPLC profile of turmeric extract compose of compose of curcumine, demethoxycurcumin, and bis-demethoxycurcumin which the drug content was 10.48 ± 0.55, 4.35 ± 0.20 and 4.50 ± 0.18%, respectively. Those chemicals were done with the standard curve in MeOH and were analyzed by high-performance liquid chromatography (Figure 1).

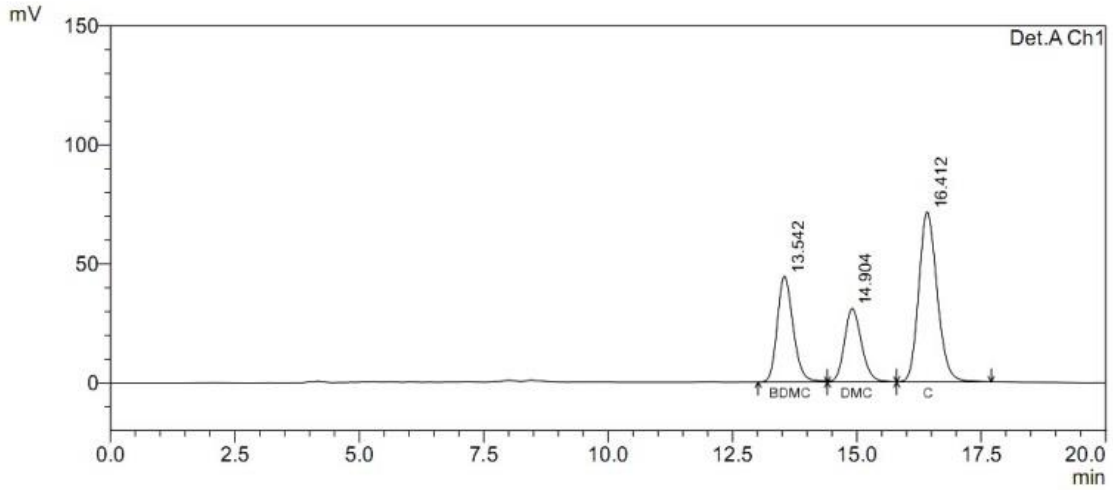

Figure 1. HPLC profile of turmeric extract was (C) curcumine, (DMC) demethoxycurcumin, and (BDMC) bis-demethoxycurcumin.

Results of the cytotoxicity test (Figure 2) showed that concentrations of turmeric extract between 0.00005-1.56 µg/mL had more than an 80% cell viability, indicating that these concentrations had no significant cytotoxic effect on MARC-145 cells. That finding was confirmed by IPMA.

Results of the crystal violet stain test compared with experimental cells with the negative control in the lower right of the figure 2. The concentrations of turmeric extract between 0.097-1.56 µg/mL had no cytotoxic effect on MARC-145 cells.

The antiviral ability test used the immunoperoxidase monolayer assay. The MARC-145 cells used in this study had been infected with the virus in each concentration. The virus titer at 24 hpi and at concentrations of 1.56 µg/mL and 0.39 µg/mL had significantly lower mean virus titers than the positive control, indicating that these concentrations of turmeric extract inhibited PRRSv replication. Comparing the virus titer at different concentrations and after different time periods showed that an extract concentration of 1.56 µg/mL had a significantly lower virus titer concentration than the others ($P < 0.05$) (Figure 3; A,B).

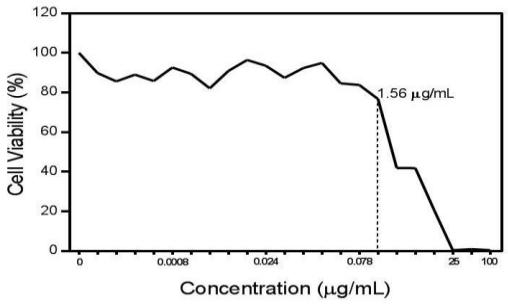

Figure 2. Cytotoxicity test results showing cell viability of MARC-145 cells at each concentration of turmeric extract

Conclusions

This study demonstrated that turmeric extract, which is composed of curcuminoids, inhibited PRRSv replication. The mechanism may be the same as that reported by Chen *et al*. (2010), i.e., interrupting virus-cell attachment and thereby inhibiting PRRSv propagation (Chae, 2016). This is the interesting report of using turmeric extract against PRRSv *in vitro*. Further study of its effect *in vivo* and its mechanism are needed.

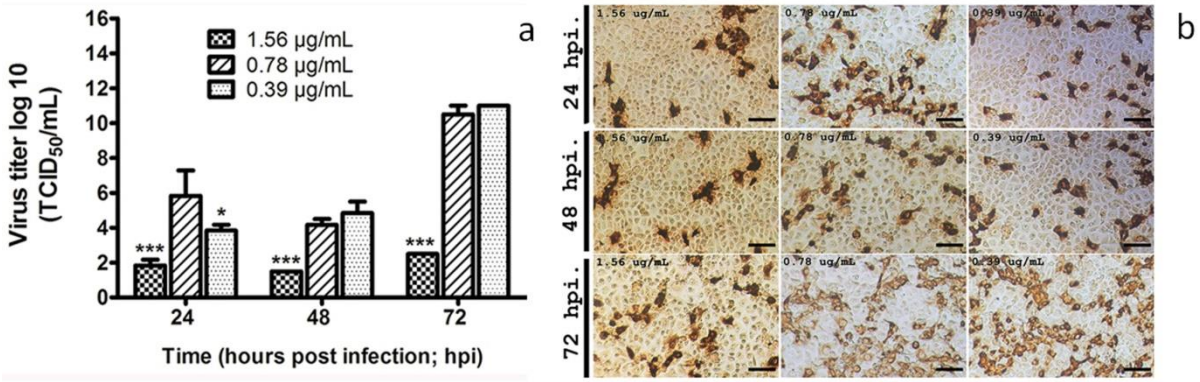

Figure 3. Comparison of virus titer of turmeric extract concentrations of 1.56 µg/mL, 0.78 µg/mL and 0.39 µg/mL at 24, 48, and 72 hpi (a). The cytopathic effect on MARC-145 cells of turmeric extract concentrations of 1.56 µg/mL, 0.78 µg/mL and 0.39 µg/mL at 24, 48, and 72 hpi (b). Symbol "*" Significantly different from control group ($P < 0.05$) ** Significantly different from control group ($P < 0.01$) *** Significantly different from control group ($P < 0.001$).

Acknowledgements

We would like to thank the Agricultural Research Development Agency (Public Organization) for financial support and the Faculty of Veterinary Medicine, Chiang Mai University, for use of its laboratory.

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