HOKKAIDO UNIVERSITY

Title	Protective Efficacy of Clinacanthus nutans on Yellow-head Disease in Black Tiger Shrimp (Penaeus monodon)
Author(s)	Direkbusarakom, S.; Ruangpan, L.; Ezura, Y. et al.
Citation	魚病研究, 33(4), 401-404
Issue Date	1998-10-20
Doc URL	https://hdl.handle.net/2115/38352
Type	journal article
File Information	yoshimizu-134.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

魚病研究 Fish Pathology, 33 (4), 401-404, 1998. 10

Protective Efficacy of *Clinacanthus nutans* on Yellow-head Disease in Black Tiger Shrimp (*Penaeus monodon*)

S. Direkbusarakom*[1], L. Ruangpan*[1], Y. Ezura*[2] and M. Yoshimizu*[2]

*[1]*National Institute of Coastal Aquaculture, Songkhla Thailand 90000*
*[2]*Faculty of Fisheries, Hokkaido University, Hakodate 041, Japan*

(Received April 6, 1998)

The leaves of *Clinacanthus nutans* Lindua, a well-known Thai traditional medicine against viral disease in human being, were used to extract antiviral substances by ethanol using soxhlet apparatus. The extract was tested for its activity against yellow-head rhabdovirus (YRV) in black tiger shrimp (*Penaeus monodon*). Virucidal effect was investigated by observation of mortality of the shrimp injected with the extract-treated virus. The results showed that extract of *C. nutans* inhibited YRV *in vitro* with the minimum concentration of 1 μg/ml. Protective efficacy of the extract on YRV infection in shrimp was tested by oral administration of the extract mixed pellet. As the result, the protective efficacies obtained were 44.6, 57.4 and 4.2% in the shrimp groups fed the extract at 0.1, 1 and 10 g/kg pellet, respectively, indicating that the *C. nutans* extract mixed with pellet at 1 g/kg could most effectively control YRV infection in shrimp.

Key words: herb, *Clinacanthus nutans*, antiviral substance, YRV, yellow-head disease, black tiger shrimp, *Penaeus monodon*

Outbreaks of yellow-head disease of black tiger shrimp *Penaeus monodon* have been observed in Thailand since 1990. In the first period, the disease caused extensive losses to shrimp farmers in the eastern and central parts of the country, and later it has spread to the southern and caused intensive losses all over the shrimp culture areas. Affected shrimp developed light yellow coloration of the cephalothorax, hepatopancreas and gills. Cumulative mortality often reached 100% in affected populations within 3-5 days from the onset of the disease. The causative agent of the disease was identified as yellow head baculovirus (YBV) (Boonyaratpalin *et al.*, 1993). The name of the agent was changed to yellow head rhabdo- like virus (YRV) after it was found to be an RNA virus (Wongteerasupaya *et al.*, 1995). Many works have been carried out in order to control the disease however, there is no effective method to cure the disease at present.

Address for correspondence:
Satapom Direkbusarakom
National Institute of Coastal Aquaculture
Songkhla Thailand 90000
Tel. 66-74-311895 ext. 123
Fax. 66-74-442054
E-mail:nicas@t-rex.hatyai.inet.co.th

Clinacanthus nutans is a Thai medicinal plant, the fresh leaves have been used domestically for the treatment of herpes simplex skin infection, shingles and for relief of pain from insect bites (Thawaranantha *et al.*, 1992). An *in vitro* effect of *C. nutans* leaf extract on herpes simplex virus type 2 (HSV2) has been reported (Jayavasu *et al.*, 1991)

The purpose of this study is to test the efficacy of *C. nutans* against YRV in the laboratory scale as a preliminary step on application of this herbs for control of yellow head disease in cultured black tiger shrimp.

Materials and Methods

Virus

Virus stock was prepared by injecting YRV into black tiger shrimp. After 2-3 days the gills of moribund shrimp were collected and homogenized in 10 times volume of lobster haemolymph medium (LHM) (Boonyaratpalin *et al.*, 1993). After homogenization, the mixture was centrifuged at 129 × *g* for 5 min. The supernatant was then filtrated through a 0.45 μm membrane filter and stored at −80°C until use.

Preparation of herb extract

Dried *C. nutans* was used for extraction of antiviral substances by ethanol using a soxhlet apparatus. The crude extract was further prepared as complex granule with polyvinylpyrolidone (PVP) and used for antiviral study.

Antiviral test for YRV

One milliliter of the viral extract diluted to 10^6 was mixed with 1 m*l* of *C. nutans* extract at 6 different concentrations (0.1 µg ~ 10 mg/m*l*) and incubated at 25°C for 2 h. After incubation, 0.2 m*l* of the mixture was injected into each group of 20 black tiger shrimp. A positive control group (n = 20) received viral solution mixed with LHM and a negative control group (n = 20) received only LHM by injection. Antiviral activity was determined by observation of shrimp mortality in 14 days post-injection.

Toxicity

Toxicity of the herb extract was tested at concentrations of 0, 1, 10, 100, 1,000 and 5,000 µg/m*l* using 15-day-old postlarvae of black tiger shrimp. Aquaria containing 10 *l* of seawater at 29-31°C and pH 7.9-8.1 were stocked with 50 postlarvae each. Mortality was observed after 24 h and LC_{50} values were determined using probit analysis.

Protective efficacy of oral administration of herb

The experiment included 4 treatments using the herb extract mixed in feed pellet at different concentrations as follows: 0, 0.1, 1 and 10 g/kg of pellet. Groups of 15 black tiger shrimp (body weight 15-20 g) were fed these herb extract containing pellet 2 times in a day. After 7 days of feedings the shrimp were immersed in YRV suspension in seawater (1 g of gill from YRV infected shrimp/ 10 *l* of seawater) for 3 h. Clinical signs and mortality of each group were observed for 14 days after infection. Three replications of each trial were performed. The protective efficacy was calculated according to Amend (1980) using following formula:

Protective efficacy

$$= \left(1 - \frac{\%\text{ mortality in experimental group}}{\%\text{ mortality in control group}}\right) \times 100\,(\%)$$

The difference between experimental and control groups was analyzed using one way analysis of variance (ANOVA).

Results

Antiviral activity and toxicity

Table 1 shows the result on antiviral activity of the extract of *C. nutans*. It was found that the extract has antiviral activity against YRV and its minimal inhibitory concentration was 1 µg/m*l*. In contract, the LC_{50} of the extract for postlarvae (PL-15) was found as high as 2,468 ± 7.8 µg/m*l*.

Protective efficacy of oral administration of herb

Mortality of the shrimp was first observed at day 3 in the positive control group and day 4 in the group fed the extract of *C. nutans* (10 g/kg). The protective efficacy determined 14 days after infection were 44.4, 57.4 and 4.2%, respectively in the groups fed with *C. nutans* at concentrations of 0.1, 1 and 10 g/kg (Table 2). P-value analyzed by ANOVA comparison, was found at .009 which confirmed significant difference ($P < 0.05$) on pro-

Table 1. Antiviral activity of *Clinacanthus nutans* against YRV in *Penaeus monodon*

Treatment	% Mortality			Average mortality (%)
	Trial 1	Trial 2	Trial 3	
Positive control (virus)	100	100	100	100
Negative control (LHM)*1	0	0	0	0
10 mg/m*l**2	0	NT*3	NT	0
1 mg/m*l*	0	NT	NT	0
100 µg/m*l*	0	0	0	0
10 µg/m*l*	0	0	0	0
1 µg/m*l*	0	0	0	0
0.1 µg/m*l*	83.3	85	66.7	78.3

*1 LHM: Lobster haemolymph medium.

*2 virus was treated with herb exthact at given concentrations.

*3 NT: Non tested

Table 2. Protective efficacy of oral administration of *Clinacanthus nutans* extract on yellow-head disease in *Penaeus monodon*

Concentration of herb extract in pellet (g/kg of pellet)	Protective efficacy (%) (Percent of cumulative mortality)			
	Trial 1	Trial 2	Trial 3	Average
Control	– (73.3)	– (100)	– (53.3)	– (75.5)
0.1	36.4 (46.7)	46.7 (53.3)	50 (26.6)	44.4 (42.2)
1	63.7 (26.7)	46.7 (53.3)	62.5 (20)	57.6 (33.3)
10	-27.3 (93.3)	40 (60)	0 (53.3)	4.2 (68.9)

tective efficacy between the control group and the three test groups.

Discussion

The extract from the leaf of *C. nutans* has been proved to contain antiviral substances against many kinds of human pathogenic virus such as herpes simplex virus (Jayavasu *et al.*, 1992 b) and varicella-zoster (Thawaranantha *et al.*, 1992). It is also effective for fish pathogenic viruses like IHNV and OMV (Direbusarakom *et al.*, 1996). In the present study, it was shown that the herb extract inactivated YRV *in vitro*. These experimental results indicate that the extract from the leaf of *C. nutans* can inhibit both DNA and RNA viruses. On the other hand, IPNV, a non-enveloped virus, was not inactivated by *C. nutans* (Direkbusarakom *et al.*, 1996). While YRV, OMV, IHNV and herpes simplex virus are enveloped viruses. These results suggest that viral inactivation might occur by the reaction between the extract and the envelope of the virus. The known antiviral substances such as ribavirin have broad spectrum activity *in vitro* against many DNA and RNA viruses. Several mechanisms of an other antiviral substance, virazole, have been proposed such as a competitive inhibitor of IMP dehydrogenase (Streeter *et al.*, 1973). Ribavirin was also proved to interfere with 5'-capping of mRNA by inhibiting guanylyl transferase activity (Goswami *et al.*, 1979). The mechanism of *C. nutans* extract for viral inhibition is yet to be elucidated.

C. nutans has been proved to be safety for animal. Chavalittumrong *et al.* (1995) reported that the mice did not show any toxic signs after fed daily with the extract from the leave of *C. nutans* at the highest dose (1 g/ kg of body weight) for 90 days. The similar result was also observed in this study where the extract of *C. nutans* showed very low toxicity for the postlarvae of black tiger shrimp. The value of LC_{50} is approximately 2,500 times higher than the minimal effective dose.

Jayavasu *et al.* (1992 a) found that *C. nutans* extract was effective for treatment of genital herpes simplex virus infection as highly as acyclovir. It was also proved to be an effective substance for treatment of herpes zoster by Jaruvijitrattana *et al.* (1995). In the present study, the extract was slightly effective for prevention of yellow-head rhabdo-like virus infection in black tiger shrimp. This might have been due to the method of administration employed. In human being they applied directly to the infection lesion, while in our case of shrimp application was done by mixing the extract with the pellet and feeding to the animal. Since, shrimp is a slow feeding animal and has a short intestine, therefore, the absorption ability in shrimp must be lower than human being. Accordingly, small amount of extract will reach to the target.

The mortalities of black tiger shrimp in the trials 1 and 2 were higher than the trial 3 even the same concentration of viral stock was used for infection. This difference in sensitivity might be due to "immune response" of the shrimp used in the trial 3 which were naturally infected with YRV before transferred to the laboratory. The results of all trials indicate that the mixture of extract and pellet at 1 g/kg of feed is the best concentration for protection of shrimp from YRV. The concentration 10 g/kg of pellet seems to be non effective and even induced higher mortality in shrimp in the trial 1. This

ineffectiveness may be caused by the smell or taste of the extract in the pellet at the highest concentration, which consequently made the shrimp in this group refuse the pellet.

These results suggest that *C. nutans* can be used for control of yellow-head disease in shrimp. However, further studies are needed such as the method to produce encapsulated pellet with *C. nutans* to prevent the loss of the extract during feeding.

Acknowledgement

This research was partially supported by Thailand Research Fund grant no. RDG 3/01/2540.

Reference

Amend, D. F. (1981): Potency testing of fish vaccine. *In* "International syposium on fish biologics: Serodiagnostics and vaccines". Developments in Biological Standardization, 49, 447–454.

Boonyaratpalin, S., K. Supamattaya, J. Kasornchandra, S. Direkbusarakom, U. Aekpanithanpong and C. Chantanachooklin (1993): A cytoplasmic, non-occluded baculovirus, the causative agent of yellow-head disease in the tiger prawn (*Penaeus monodon*). *Fish Pathol.*, 28, 103–109.

Chavalittumrong, P., A. Attawish, P. Rugsamon and P. Chuntapet (1995): Toxicological study of *Clinacanthus nutans* (Burm. F.) Lindau. *Bull. Dept. Med. Sci.*, 37, 323–338.

Direkbusarakom, S., A. Herunsalee, M. Yoshimizu and Y. Ezura (1996): Antiviral activity of several Thai traditional herb extracts against fish pathogenic viruses. *Fish Pathol.*, 31, 209–213.

Goswami, B. B., E. Borek, O. K. Sharma, J. Fujitaki and R. A. Smith (1979): The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. *Biochem. Biophys. Res. Commun.*, 89, 830–836.

Jaruvijitrattana, S., N. Wongrattapatsorn, P. Timpatpong and M. Bunjo (1995): Double blind randomized control trial in the treatment of herpes zoster with *Clinacanthus nutans* cream. Technical report, Mahidol University, Bangkok, 8 p.

Jayavasu, C., T. Dechatiwongse, K. Balachandra, P. Chavalittumrong and S. Jongtrakulsiri (1991): The virucidal activity of herb extract to herpes simplex virus type 2: an *in vitro* study. *Virus Information Exchange Newsletter*, 8, 26.

Jayavasu, C., K. Balachandra, S. Sangkitjaporn, A. Maharungraungrat, P. Thavatsu, M. Bunjo, P. Chavalittumrong, T. Dechatiwongse and N. Sittisomwong (1992 a): Clinical trial in the treatment of genital herpes patients with *Clinacanthus nutans* extract. *Com. Dis. J.*, 18, 152–161.

Jayavasu, C., T. Dechatiwongse, K. Balachandra, P. Chavalittumrong and S. Jongtrakulsiri (1992 b): The virucidal activity of *Clinacanthus nutans* Lindau extracts against herpes simplex virus type-2: An *in vitro* study. *Bull. Dept. Med. Sci.*, 34, 153–158.

Streeter, D. G., J. T. Witkowski and G. P. Khare (1973): Mechanism of action of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamid (virazole), a new broad-spectrum antiviral agent. *Prog. Natl. Acad. Sci.*, 70, 1174–1178.

Thawaranantha, D., K. Balachandra, S. Jongtrakulsiri, P. Chavalittumrong, J. Bhumiswasdi and C. Jayavasu (1992): *In vitro* antiviral activity of *Clinacanthus nutans* on varicella-zoster virus. *Siriraj Hosp. Gaz.*, 44, 285–291.

Wongteerasupaya, C., S. Sriurairatana, J. E. Vickers, A. Akrajamorn, V. Boonsaeng, S. Panyim, A. Tassanakajon, B. Withyachumnarnkul and T. W. Flegel (1995): Yellow-head virus of *Penaeus monodon* is an RNA virus. *Dis. Aquat. Org.*, 22, 45–50.