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Cytotoxicity and hemolytic activity of jellyfish Nemopilema nomurai (Scyphozoa: Rhizostomeae) venom

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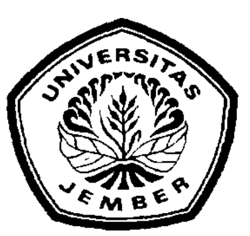

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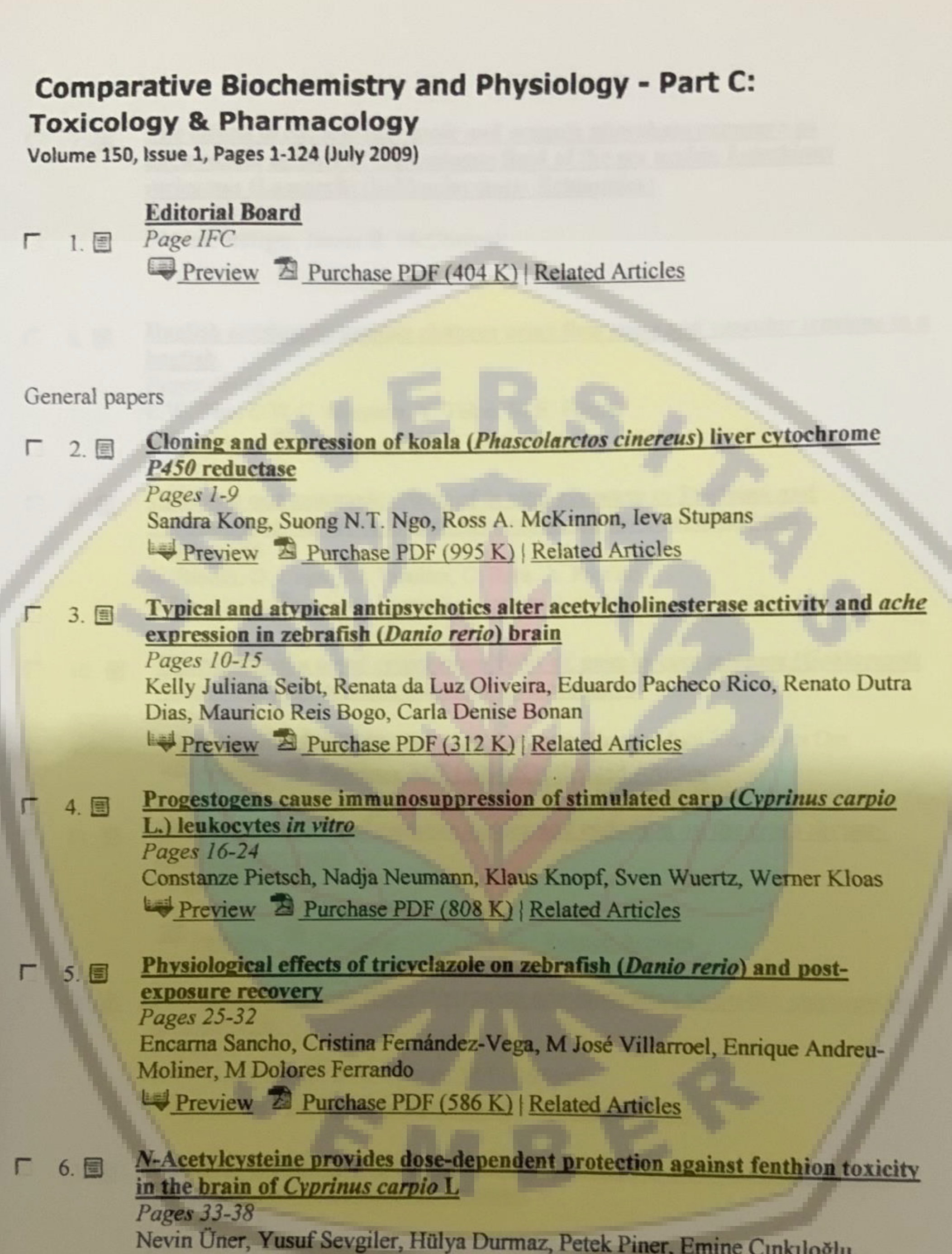

Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology

Volume 150, Issue 1, Pages 1-124 (July 2009)

1. **Editorial Board**
Page IFC
Preview | Purchase PDF (404 K) | Related Articles

General papers

2. **Cloning and expression of koala (*Phascolarctos cinereus*) liver cytochrome *P450* reductase**
Pages 1-9
Sandra Kong, Suong N.T. Ngo, Ross A. McKinnon, Ieva Stupans
Preview | Purchase PDF (995 K) | Related Articles

3. **Typical and atypical antipsychotics alter acetylcholinesterase activity and *ache* expression in zebrafish (*Danio rerio*) brain**
Pages 10-15
Kelly Juliana Seibt, Renata da Luz Oliveira, Eduardo Pacheco Rico, Renato Dutra Dias, Mauricio Reis Bogo, Carla Denise Bonan
Preview | Purchase PDF (312 K) | Related Articles

4. **Progestogens cause immunosuppression of stimulated carp (*Cyprinus carpio* L.) leukocytes *in vitro***
Pages 16-24
Constanze Pietsch, Nadja Neumann, Klaus Knopf, Sven Wuertz, Werner Kloas
Preview | Purchase PDF (808 K) | Related Articles

5. **Physiological effects of tricyclazole on zebrafish (*Danio rerio*) and post-exposure recovery**
Pages 25-32
Encarna Sancho, Cristina Fernández-Vega, M José Villarroel, Enrique Andreu-Moliner, M Dolores Ferrando
Preview | Purchase PDF (586 K) | Related Articles

6. ***N*-Acetylcysteine provides dose-dependent protection against fenthion toxicity in the brain of *Cyprinus carpio* L**
Pages 33-38
Nevin Üner, Yusuf Sevgiler, Hülya Durmaz, Petek Piner, Emine Çınkıloğlu
Preview | Purchase PDF (323 K) | Related Articles

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7. **The effects of chronic inorganic and organic phosphate exposure on bactericidal activity of the coelomic fluid of the sea urchin *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea)**
Pages 39-44
S. Anne Böttger, James B. McClintock
Preview | Purchase PDF (383 K) | Related Articles

8. **Hagfish natriuretic peptide changes urine flow rates and vascular tensions in a hagfish**
Pages 45-49
L.W. Tait, C.W.C. Simpson, Y. Takei, M.E. Forster
Preview | Purchase PDF (310 K) | Related Articles

9. **Cytotoxic and genotoxic effects of *in vitro* exposure to Triclosan and Trimethoprim on zebra mussel (*Dreissena polymorpha*) hemocytes**
Pages 50-56
A. Binelli, D. Cogni, M. Parolini, C. Riva, A. Provini
Preview | Purchase PDF (430 K) | Related Articles

10. **Expression of a novel cytochrome P450 4T gene in rare minnow (*Gobiocypris rarus*) following perfluorooctanoic acid exposure**
Pages 57-64
Yang Liu, Jianshe Wang, Yong Liu, Hongxia Zhang, Muqi Xu, Jiayin Dai
Preview | Purchase PDF (1994 K) | Related Articles

11. **Bioenergetic effects of aqueous copper and cadmium on the grass shrimp, *Palaemonetes pugio***
Pages 65-71
Teresa Manyin, Christopher L. Rowe
Preview | Purchase PDF (479 K) | Related Articles

12. **Effects of short term triiodothyronine administration to broiler chickens fed methimazole**
Pages 72-78
R.W. Rosebrough, B.A. Russell, M.P. Richards
Preview | Purchase PDF (984 K) | Related Articles

13. **Purification and characterization of a cysteine-rich secretory protein from *Philodryas patagoniensis* snake venom**
Pages 79-84
María E. Peichoto, Stephen P. Mackessy, Pamela Teibler, Flávio L. Tavares, Paula L. Burckhardt, María C. Breno, Ofelia Acosta, Marcelo L. Santoro
Preview | Purchase PDF (1710 K) | Related Articles

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14. **Cytotoxicity and hemolytic activity of jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) venom**
Pages 85-90
Changkeun Kang, Al Munawir, Mijin Cha, Eun-Tae Sohn, Hyunkyoung Lee, Jong-Shu Kim, Won Duk Yoon, Donghyun Lim, Euikyung Kim
Preview | Purchase PDF (661 K) | Related Articles

15. ***In vitro* metabolism of polychlorinated biphenyls and cytochrome P450 monooxygenase activities in dietary-exposed Greenland sledge dogs**
Pages 91-100
Jonathan Verreault, Robert J. Letcher, Christian Sonne, Rune Dietz
Preview | Purchase PDF (571 K) | Related Articles

16. **Identification of two phospholipid hydroperoxide glutathione peroxidase (*gpx4*) genes in common carp**
Pages 101-106
Edit Hermesz, Ágnes Ferencz
Preview | Purchase PDF (580 K) | Related Articles

17. **Exogenous folate ameliorates ethanol-induced brain hyperhomocysteinemia and exogenous ethanol reduces taurine levels in chick embryos**
Pages 107-112
Robert K. Barnett, Stephanie L. Booms, Tracy Gura, Mara Gushrowski, Robert R. Miller Jr.
Preview | Purchase PDF (211 K) | Related Articles

18. **Identification of a splice variant of the metal-responsive transcription factor MTF-1 in common carp**
Pages 113-117
Ágnes Ferencz, Edit Hermesz
Preview | Purchase PDF (509 K) | Related Articles

19. **Exposure to mercury reduces heat tolerance and heat hardening ability of the springtail *Folsomia candida***
Pages 118-123
Stine Slotsbo, Lars-Henrik Heckmann, Christian Damgaard, Dick Roelofs, Tjalf de Boer, Martin Holmstrup
Preview | Purchase PDF (298 K) | Related Articles

20. **26th ESCPBnew Congress, Innsbruck, Austria, September 6-10, 2009**
Page I
Preview | Purchase PDF (580 K) | Related Articles

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Cytotoxicity and hemolytic activity of jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) venom

Changkeun Kang [a], Al Munawir [a], Mijin Cha [a], Eun-Tae Sohn [a], Hyunkyoung Lee [a], Jong-Shu Kim [a], Won Duk Yoon [b], Donghyun Lim [b], Euikyung Kim [a,*]

[a] *College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, South Korea*
[b] *Headquarters for Marine Environment, National Fisheries Research & Development Institute, Shiran-ri, Gijang-eup, Gijang-gun, Busan 619-705, South Korea*

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ABSTRACT

The recent bloom of a giant jellyfish *Nemopilema nomurai* has caused a danger to sea bathers and fishery damages in the waters of China, Korea, and Japan. The present study investigated the cytotoxic and hemolytic activities of crude venom extract of *N. nomurai* using a number of *in vitro* assays. The jellyfish venom showed a much higher cytotoxic activity in H9C2 heart myoblast than in C2C12 skeletal myoblast (LC_{50} = 2 μg/mL vs. 12 μg/mL, respectively), suggesting its possible *in vivo* selective toxicity on cardiac tissue. This result is consistent with our previous finding that cardiovascular function is a target of the venom. In order to determine the stability of *N. nomurai* venom, its cytotoxicity was examined under the various temperature and pH conditions. The activity was relatively well retained at low environmental temperature (≤20 °C) and dramatically lost at high temperature (≥60 °C). In pH stability test, the venom has abruptly lost its activity at low pH environment (pH ≤ 4). Interestingly enough, however, its activity was not significantly affected even at the highest pH environment tested (pH ≤ 12) in the present study. Additionally, hemolytic activity of the venom was examined using the erythrocytes of cat, dog, human, rabbit and rat. Venom concentration-dependent hemolysis could be observed from 10 μg/mL of protein equivalents or higher with variable potencies in different species, among which dog erythrocyte was the most susceptible to the venom (EC_{50} = 151 μg/mL). SDS-PAGE analysis of *N. nomurai* venom showed the molecules of 20–40 kDa and 10–15 kDa appeared to be the major protein components of the venom.

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1. Introduction

Many marine invertebrates are included in the phylum Cnidaria, i.e. jellyfish, sea anemones and corals. One of the most distinctive aspects of cnidarian physiology is related to its biologically active components and organelles contained in specialized cells called nematocysts, which are located along the tentacles and body. These organelles are filled with toxins and discharge their content upon an appropriate stimulation. The accidental sting by venomous jellyfish can result in severe local and systemic pathologies, in some cases, can lead to death (Williamson et al., 1996). *Nemopilema nomurai* (also called 'echizen kurage') is one of the largest jellyfishes, with a maximum bell size up to 2 m and weighs up to 200 kg (Yasuda, 2004). Unusual large blooms of *N. nomurai* have occurred for the last years (Yasuda, 2004; Uye, 2008) in Yellow sea, East China Sea, and East Sea.

Biochemical and toxicological studies of jellyfish venoms have been reported by other investigators regarding their hemolytic (Torres et al., 2001), insecticidal (Yu et al., 2005a), cardiovascular (Ramasamy et al., 2005a,b), antioxidant (Yu et al., 2005b), enzymatic (Helmholz et al., 2007), and cytotoxic (Helmholz et al., 2007; Carli et al., 1996) activities. Toxic components of these jellyfish venoms are believed to be a type of proteins. On the other hand, our knowledge about *N. nomurai* jellyfish venom is extremely scarce. Based on a case report study of patients exposed to *N. nomurai* tentacles, the sting was very painful with a strong burning sensation, followed by erythematous eruption with small vesicles (Kawahara et al., 2006). Our previous study demonstrated that *N. nomurai* jellyfish venom had a cardio-depressant effect on rodent animal model, which is accompanied by a marked hypotension (Kim et al., 2006). The toxicological nature of this venom has neither been characterized nor clearly described yet. In the present study, we examined *N. nomurai* jellyfish venom in toxicological perspective using various experimental techniques, including comparative cytotoxicity test. We propose this as a novel method that can be applied for the research of natural toxins.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM), penicillin, streptomycin sulfate, trypsin, dimethyl sulphoxide (DMSO), 3-(4,5-dimethylthiazol-

* Corresponding author. Tel.: +82 55 751 5812; fax: +82 55 751 5803.
E-mail address: ekim@gnu.ac.kr (E. Kim).

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2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Alsever's solution were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All other reagents used were of the purest grade available.

2.2. Jellyfish collection and preparation

Mature specimens of *N. nomurai* jellyfish were captured from Korea Strait along the coasts of Tongyoung in September, 2007. The tentacles dissected from the jellyfish were stored in ice and transferred immediately to our laboratory for further preparation. Nematocysts were isolated from the dissected tentacles as described by Bloom et al. (1998) with a slight modification. In brief, tentacles were gently swirled with the addition of distilled water, then stood still for 1–2 h to remove debris and sea water. After decanting the supernatant, tentacles settled down at the bottom were mixed with 2× (v/v) distilled water and shaken vigorously for 3 min. The detached nematocysts were separated by filtering tentacle preparation through 4 layers of medical gauze. This was repeated for two more times with additional distilled water to harvest nematocysts from the tentacles. The filtrates were centrifuged (700 *g*) at 4 °C for 20 min and the pellets (nematocysts) were lyophilized and stored −20 °C.

2.3. Venom extraction and preparation

Venom was extracted from the freeze-dried nematocysts using the technique described by Carrette and Seymour (2004) with a minor modification. In brief, venom was extracted from 50 mg of nematocyst using glass beads (approximately 8000 beads; 0.5 mm in diameter) and 1 mL of ice-cold (4 °C) phosphate buffered saline (PBS, pH 7.4). These samples were shaken in a mini bead mill at 3000 rpm for 30-s intervals for five times with intermittent cooling on ice. The venom extracts were then transferred to a new Eppendorf tube and centrifuged (22,000 *g*) at 4 °C for 30 min. This supernatant was used as *N. nomurai* jellyfish venom for the present study. Protein concentration of the venom was determined by the method of Bradford (1976) technique (Bio-Rad, CA, USA) and the venom was used based on its protein concentration.

2.4. Cell culture and cytotoxicity assay

C2C12 (skeletal myoblast) and H9C2 (heart myoblast) cells were used for assessing the cytotoxic activity of the venom. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C with 5% CO_2. The cells were seeded in 24-well plates at a density of 10^4 cells/well and cultured for 24 h. Non-adherent cells were removed by gentle washing with fresh culture medium and *N. nomurai* jellyfish venom was treated at the indicated concentrations. After incubation (24 h), the treated cells were examined and photographed under phase contrast microscopy. Cytotoxicity was assessed by measuring mitochondrial dehydrogenase activity, using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, 100 μL of MTT solution (5 mg/mL) was added to each well and incubated for another 3 h at 37 °C. After removing the supernatant, the formazan crystal generated was dissolved by adding 150 μL/well of dimethyl sulfoxide (DMSO) and the absorbance was detected at 540 nm using a spectrophotometric microplate reader (BioTek Instruments, Inc., Winooski, USA).

2.5. Stability of N. nomurai jellyfish venom

Freshly prepared venom was incubated at the conditions of various temperature and pH, and then the retained cytotoxic activity of the venom was measured in H9C2 cells. For thermal stability study, each aliquot of the venom was incubated at the temperatures of 4, 20, 40, 60 and 80 °C for 60 min, respectively. The treated venom was added to the culture medium of H9C2 cells at the indicated concentrations for 24 h. The venom stability was then assessed by measuring its residual cytotoxic activity as described above. In order to determine the heating time-dependent venom stability, it was incubated at 40 °C for the periods of 0, 10, 30 min and 2, 6 h, respectively. The venom was then evaluated for its residual activity. We have also examined pH-dependent stability of the venom using various pH buffered solutions (Marino et al., 2004) with a little modification. Briefly, each aliquot of the venom extract was adjusted its pH for the indicated level by addition of 0.5 M of acetate (pH 2 and 4), 0.1 M of phosphate (pH 6, 7, and 8) and 0.5 M of glycine–NaOH buffers (pH 10 and 12), respectively. They were incubated for 1 h on ice, and their residual cytotoxic activity was assessed as described above.

2.6. Hemolysis assay

Hemolytic activity of the venom was tested using the erythrocytes of cat, dog, human, rabbit and rat. In brief, freshly collected blood samples were immediately mixed with anticoagulant, Alsever's solution (pH 7.4) to prevent blood coagulation. To obtain a pure suspension of erythrocytes, 1 mL of whole blood was then made up to 20 mL in phosphate buffered saline (PBS, pH 7.4), and centrifuged at 1500 *g* for 5 min at 4 °C. The supernatant and buffy coats were then removed by gentle aspiration, and the above process was repeated two more times. Erythrocytes were finally resuspended in PBS to make 1% solution for hemolysis assay. For this, various concentrations of jellyfish venom (0.01–2 mg/mL) were added to the suspension of red blood cells obtained from five different species (cat, dog, human, rabbit and rat). The venom–erythrocyte mixtures were incubated at 37 °C for 30 min in water bath and then centrifuged at 1500 *g* for 5 min at 4 °C. The supernatants were transferred to 96-well microplates and the absorbance at 545 nm was determined by using a spectrophotometric microplate reader (BioTek Instruments) to measure the extent of red blood cell lysis. Positive control (100% hemolysis) and negative control (0% hemolysis) were also determined by incubating erythrocytes with 1% Triton X-100 in PBS and PBS alone, respectively.

2.7. SDS-PAGE

Electrophoresis was carried out according to Laemmli method (Laemmli, 1970) using 16% polyacrylamide gel with 4% stacking gel. Samples were resuspended in SDS-PAGE sample buffer (62.5 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, 0.01% bromophenol blue) and incubated at 95 °C for 5 min, then stored at −20 °C until use. Jellyfish venom protein (250 μg) was electrophoresed for 90 min at 100 V constant voltage at room temperature, using Tris–glycine running buffer. The molecular size marker, 3.5–260 kDa (Novex Sharp prestained protein standards, Invitrogen, CA, USA), was run parallel with venom sample for molecular weight estimation. Protein bands were visualized by Coomassie R-250 (Sambrook and Russell, 2001).

2.8. Statistical analysis

The results are expressed as a mean ± standard deviation (S.D.). A paired Student's *t*-test was used to assess the significance of differences between two mean values. $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Cytotoxicity of N. nomurai jellyfish venom

Heart myoblast H9C2 and skeletal myoblast C2C12 cells were incubated for 24 h with various concentrations of *N. nomurai* jellyfish venom. A comparison of the relative cytotoxicity on the cells was

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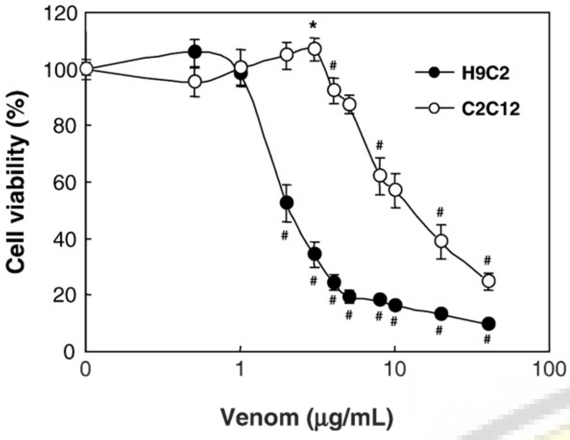

Fig. 1. Cytotoxicity of *N. nomurai* jellyfish venom on H9C2 and C2C12 cells. Exponentially growing H9C2 (cardiac myoblast cell) and C2C12 (skeletal muscle cell) were treated with various concentrations of *N. nomurai* jellyfish venom for 24 h. The cytotoxic effect was assessed by measuring mitochondrial dehydrogenase activity, using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The formazan crystal generated was dissolved by adding dimethyl sulfoxide (DMSO) and the absorbance was determined at 540 nm using a spectrophotometric microplate reader (BioTek Instruments, Inc., Winooski, USA). Cells incubated with no venom (PBS) were taken as proper controls. The results are expressed as mean ± S.D. ($n=4$), $^{*}P<0.05$, $^{\#}P<0.01$, as compared with the control value.

illustrated in Fig. 1. H9C2 and C2C12 cells showed a venom concentration-dependent cell death and their calculated LC_{50} (the concentration that kills 50% of the cells) were 2.01 and 12.25 µg/mL, respectively. As shown, 10 µg/mL of *N. nomurai* jellyfish venom was sufficient to induce an almost complete cell death in H9C2 cells. On the other hand, C2C12 cells appeared to be much less susceptible to *N. nomurai* jellyfish venom comparing with H9C2 cells. This result suggests that cardiac myoblast is much more vulnerable than skeletal myoblast to *N. nomurai* jellyfish venom.

H9C2 and C2C12 cells were also carefully observed using an inverted microscope and photographed in order to determine if *N. nomurai* jellyfish venom would induce any visible morphological changes (Fig. 2). In comparison to control, the treated cells showed a decrease in cell density as well as an increase of rounded cell type, less contact with one another and some cell detachment in both H9C2 (upper panel) and C2C12 (lower panel) cells after the 24 h incubation with 1 µg/mL and 4 µg/mL, respectively. Higher concentration of *N. nomurai* jellyfish venom induced more serious morphological alterations in both cell types, including severe cell shrinkages. The morphological changes corresponded well with the above cytotoxicity tests. These findings suggest that treatment with *N. nomurai* jellyfish venom can induce toxicity against heart muscle and skeletal muscle with a higher potency in the heart.

3.2. Stability of N. nomurai jellyfish venom

To investigate the venom stability under the various conditions of temperature and pH, we used the cytotoxicity test with H9C2 heart myoblast, which can be clinically more relevant as well as more sensitive comparing with C2C12 skeletal myoblast. Thermal stability study of *N. nomurai* jellyfish venom showed that it can be progressively affected upon the increases of its environmental temperature (Fig. 3A). After the incubations for 60 min at 4 °C or 20 °C, the venom was scarcely affected with retaining its full cytotoxic activity, whereas it almost completely lost its activity at over 60 °C (Fig. 3A). Interestingly, the thermal stability was variable at 40 °C depending on the concentration of the venom, which is much more stable at a relatively higher concentration than at lower concentrations. We have also evaluated the retained activity of venom after incubating it at 40 °C for various periods of time. As the incubation time increased, the venom drastically lost its cytotoxic activity (Fig. 3B). This indicates that *N. nomurai* jellyfish venom is susceptible to heat treatment at high temperature. However, it appears that the venom can retain a significant cytotoxicity for a relatively long period of time at ambient temperature depending on the venom concentration. We can also expect that the venom can be much more stable when it is encapsulated in nematocyst, which is often encountered from dead jellyfishes outside seashore and fishing nets contacted with jellyfish in nature.

For the assessment of pH-dependent stability, we treated *N. nomurai* jellyfish venom at various pH conditions for 60 min on ice. Then cytotoxicity of the treated venom was examined using H9C2 cells

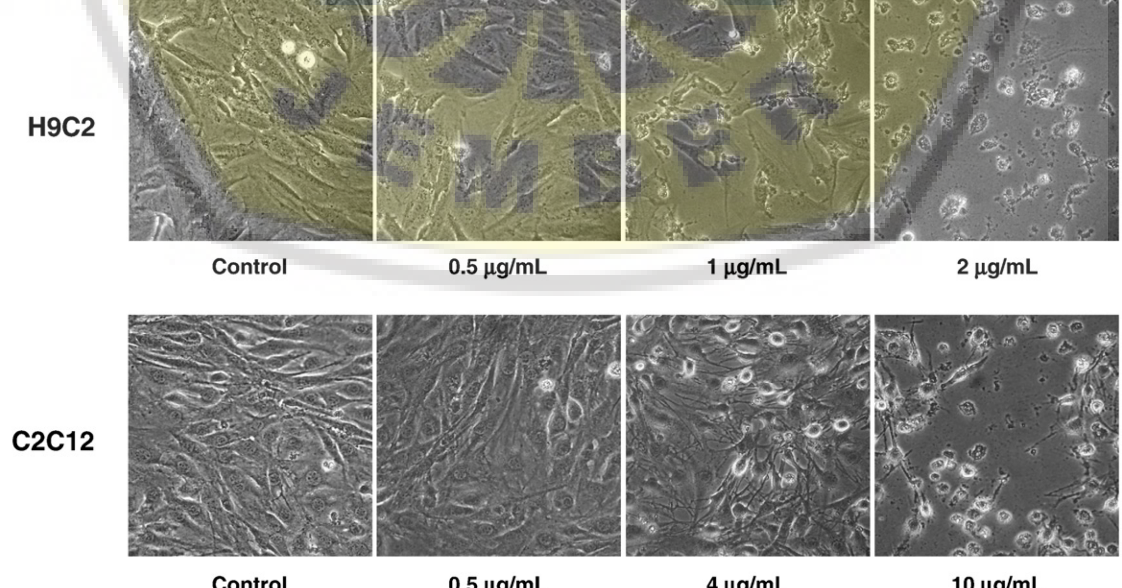

Fig. 2. Morphological changes of H9C2 (upper panel) and C2C12 cells (lower panel) treated with *N. nomurai* jellyfish venom. The cells were incubated in the absence or the presence of *N. nomurai* jellyfish venom at the indicated concentrations for 24 h. The morphological changes were examined using phase contrast microscopy.

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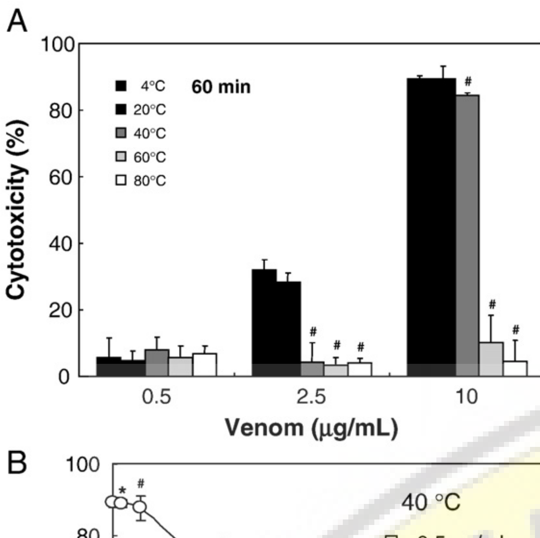

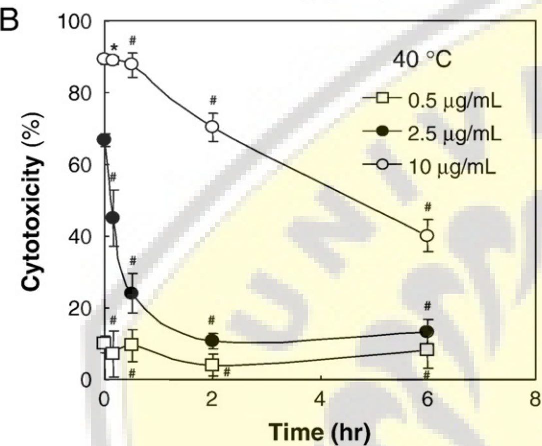

Fig. 3. Thermostability of *N. nomurai* jellyfish venom. The extracted jellyfish venom was divided into microfuge tubes in a small volume and each venom aliquot was separately treated as follows with intermittent stirring. In brief, the venom aliquots were pre-incubated at (A) different temperatures (4, 20, 40, 60 and 80 °C) for 60 min, or for (B) different periods (0, 10, 30 min, and 2, 6 h) at 40 °C, respectively. Then, the biological activity of each heat-treated venom sample was evaluated by assessing its remaining cytotoxic activity after 24 h incubation on H9C2 cells as described earlier. The venom tested immediately after extraction from nematocysts served as control. The results are expressed as mean ± S.D. ($n=4$). $^{*}P<0.05$, $^{\#}P<0.01$, as compared with the control value.

as described above. As shown in Fig. 4, the treated venom drastically lost its activity at low pH conditions (pH<6). Interestingly, however, it still preserves its full activity even after the treatment of high pH conditions (up to pH≤12) in our present study.

3.3. Hemolytic activity of N. nomurai jellyfish venom

N. nomurai jellyfish venom (10 to 2000 μg/mL) was assessed for its hemolytic activity using the blood samples of various species. The venom showed concentration-dependent hemolytic activities in all the species tested in the present study (Fig. 5). The activity could be abolished after treating the venom sample in a boiling water bath for 30 min. All the data were obtained using aliquots of the same venom extract and the diluted blood samples. The venom caused 50% hemolysis in dog, rat, cat, rabbit and human erythrocytes at concentrations of 151, 497, 685, 729 and 964 μg/mL, respectively. As shown, dog erythrocyte was the most susceptible among others.

3.4. SDS-PAGE of N. nomurai jellyfish venom

To characterize the proteinous components of *N. nomurai* jellyfish venom, we separated the venom proteins using SDS-PAGE. As shown in Fig. 6, the venom extract contains numerous proteins with various sizes of molecular weight. Most of the proteins were in the ranges between 10 and 50 kDa and there were also some minor protein bands

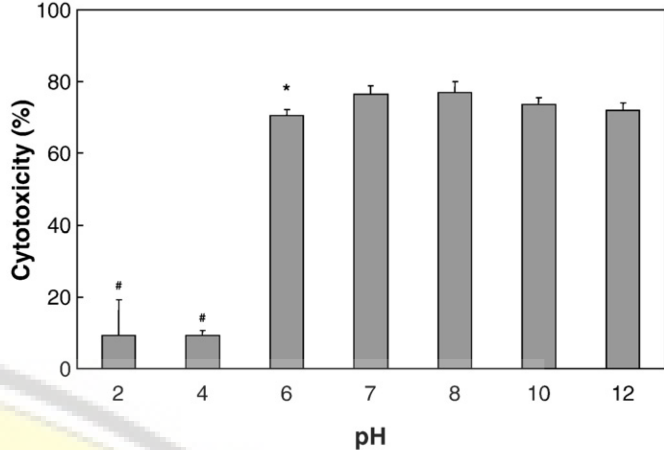

Fig. 4. pH stability of *N. nomurai* jellyfish venom. The jellyfish venom extract was divided into microfuge tubes in a small volume and they were separately adjusted to an appropriate pH by the addition of 0.5 M acetate (pH 2 and 4), 0.1 M phosphate (pH 6, 7, and 8) or 0.5 M glycine–NaOH (pH 10 and 12) buffers, respectively. These pH-adjusted venom preparations were allowed to stand for 1 h on ice with intermittent stirring. After pretreatment, the retained cytotoxic effects of the venom (10 μg/mL) were evaluated by using the cytotoxicity test as described earlier. Cells incubated with fresh venom (PBS) of no pretreatment were taken as proper controls. The results are expressed as mean ± S.D. ($n=3$). $^{*}P<0.05$, $^{\#}P<0.01$, as compared with the control value.

over 50 kDa. Especially, the molecules of 20–40 kDa and 10–15 kDa appeared to be the major protein components of the venom. Further study will be required to identify these molecular entities in the near future.

4. Discussion

The cytotoxicity test has been proposed as a reliable and reproducible assay for assessing the toxic potency of jellyfish venom with a higher sensitivity than mouse lethality test (Ordóñez et al., 1990). Although the cytotoxicity test of jellyfish venom has not been widely used, there are previous literatures, including the LC_{50} values of jellyfish venoms from *Chrysaora quinquecirrha* on CCL-13

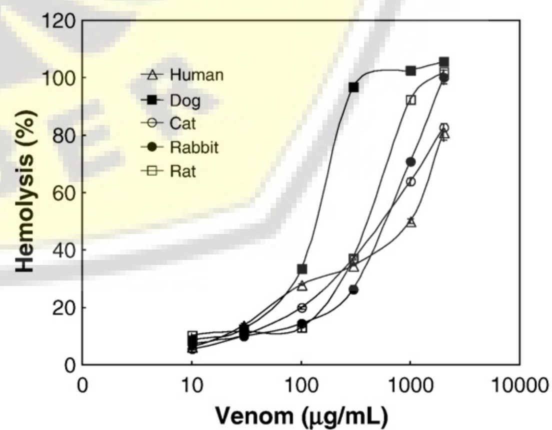

Fig. 5. Hemolytic activity of *N. nomurai* jellyfish venom. Freshly prepared erythrocytes (from cat, dog, human, rabbit and rat) were resuspended in PBS to make 1% solution and incubated with various concentrations of jellyfish venom extract for 30 min at 37 °C water bath. The mixtures were centrifuged at 1500× *g* for 5 min at 4 °C, and the supernatants were transferred to 96-well microplates and the absorbance at 545 nm was measured using a spectrophotometric microplate reader to quantify the extent of red blood cell lysis. Positive control (100% hemolysis) and negative control (0% hemolysis) were determined by treating erythrocytes with 1% Triton X-100 (in PBS) or PBS alone, respectively. The results are expressed as mean ± S.D. ($n=3$).

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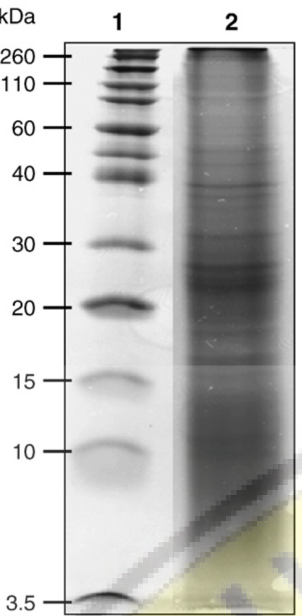

Fig. 6. SDS-PAGE separation of *N. nomurai* jellyfish venom proteins. Electrophoresis was carried out according to Laemmli (21) using 16% polyacrylamide gel. Jellyfish venom protein (250 μg) was electrophoresed for 90 min at 100 V constant current in room temperature, using Tris–glycine running buffer. The molecular weight size marker (lane 1), in the range of 3.5–260 kDa, was run parallel with venom sample (lane 2) for molecular weight estimation. Protein bands were visualized by staining gels with Coomassie R-250 dye.

hepatocyte (LC_{50}<1 μg/mL) (Cao et al., 1998), from *Cyanea capillata* on HepG2 hepatoma cells for 48 h (LC_{50} = 20.3 μg/mL) (Helmholz et al., 2007), and from nematocyst free-tissue of *Rhizostoma pulmo* on V79 lung fibroblast for 3 h (LC_{50} = 37.6 μg/mL) (Allavena et al., 1998). The present study is the first report demonstrating the toxicological aspects of *N. nomurai* jellyfish venom using mammalian cell culture model. Exposure to the venom resulted in concentration-dependent cell deaths in H9C2 and C2C12 with a much higher potency in H9C2 than C2C12. It has been previously demonstrated that H9C2 and C2C12 cells exhibit similar sensitivities (cytotoxicities) against a number of agents (Cecchi et al., 2005; Smith et al., 2007). The analysis of the data shows that *N. nomurai* jellyfish venom has a moderate toxicity in comparison with other cnidarian venoms. Our results evidently show that *N. nomurai* nematocysts contain at least one or more toxic components that have a strong cytotoxic activity against heart muscle. This result is also consistent with our earlier findings (Kim et al., 2006) that described the cardio-depressant effect of the venom in rat animal model.

The activity of *N. nomurai* jellyfish venom was accompanied by poor stability that was readily inactivated by either moderate pre-heating or lowering environmental pH. The activity was relatively well preserved at low temperatures (≤20 °C), but sharply reduced at high temperatures (≥60 °C) regardless of the venom concentrations (Fig. 3A). Interestingly, at 40 °C, which is near our body temperature, the activity was largely dependent on venom concentration. These results in accordance with other previous studies show that jellyfish venoms are proteins with biological activities labile to temperatures above 40 °C (Carrette et al., 2002; Loten et al., 2006). Other investigators have suggested that cnidarian venom stability against environmental pH is ranging from pH 4 to 10 (Yu et al., 2007), where optimal pH should be chosen to make cnidarian venom stable at pH 7.5. Interestingly, the cytotoxicity of *N. nomurai* jellyfish venom was not abolished even at the highest pH environment tested (pH ≤ 12) in the present study. On the other hand, it was almost entirely lost at relatively low pH environment (pH ≤ 4). The reasons that pH influenced venom activity may be explained as follows: (i) Acid or alkali could destroy the activity of venom by inducing conformational changes of protein structures. (ii) When pH did not cause protein denaturation, it could still affect the dissociated state of substrates, intermediate complexes, and some groups of protease active sites. (iii) pH could influence the decomposition of functional groups keeping the spatial structure of protease molecule stable, resulting in the conformational change of protease active sites with the loss of its activity (Li et al., 2005). Our results suggest that *N. nomurai* jellyfish venom is most likely to have a proteinic character, as shown by thermal instability and pH dependence of its activity (Figs. 3 and 4).

Hemolytic activity has been described on a variety of cnidarian venoms against erythrocytes of many different species. Rottini et al. (1995) demonstrated that *Carybdea marsupialis* jellyfish venom exhibits unpredictable hemolytic activities in different species, such as sheep, human, and rabbit. They showed the sheep RBCs, but not human or rabbit RBCs, were susceptible to lysis by the toxin. On the contrary, *Cassiopea xamachana* jellyfish venom showed a higher hemolytic activity in human RBCs than sheep RBCs (Torres et al., 2001). These results suggest that jellyfish venom can have a unique hemolytic activity profile which can be highly variable among different species. In the present study, we described the hemolytic activity of *N. nomurai* jellyfish venom and compared its potency in cat, dog, human, rabbit and rat erythrocytes. The results show that dog erythrocyte was the most sensitive (EC_{50} = 151 μg/mL) among the species tested. Cytolytic toxins are generally known to operate by either of two mechanisms. One is an enzymatic mechanism, in which cytolytic components of marine invertebrates bind preferentially to membrane glycolipids or glycoprotein (Burnett and Calton, 1987). The other is a stoichiometric mechanism, which contains the binding and insertion of toxin molecules into plasma membrane followed by oligomerization to form transmembrane pores, and resulting in colloid osmotic lysis (Bhakdi and Tranum-Jensen, 1988). It is not clearly understood now how *N. nomurai* jellyfish venom elicits various hemolytic potencies in different species as well as its mechanism of action, which may need further investigation in the future.

Protein components of *N. nomurai* jellyfish venom were separated by using SDS-PAGE, and the two major ranges with molecular weights of approximately 20–40 kDa and 10–15 kDa, respectively, were identified. We have now no evidence, however, whether some of these proteins contribute to the cytolytic activity of this cnidarian venom, which was observed in the present study. Recently, cnidarian venoms of sea anemones have been classified into four polypeptide groups based on their primary structure and functional properties as below (Anderluh and Maček, 2002). Group 1 (5–8 kDa) is constituted by peptides with antihistamine activity that is not inhibited by sphingomyelin. Group 2 (~20 kDa) includes basic proteins inhibited by sphingomyelin. Group 3 (~30–40 kDa) is formed by cytolysins with or without phospholipases. Group 4 (80 kDa) includes a single cytolysin whose activity is inhibited by cholesterol or phospholipids. In case of sea anemone, the 20 kDa pore-forming cytolysins are most studied for their powerful membranolytic and cytostatic activities. They can also form rectified cation-selective pores in lipid membranes. As described above, *N. nomurai* venom contains numerous proteins with various molecular masses between 10 and 50 kDa. This suggests that one or more proteins of *N. nomurai* venom can be related to sphingomyelin binding protein or phospholipases. Further analysis of these proteins is required to determine if they are, indeed, a phospholipase A_2 and/or inhibited by sphingomyelin.

In conclusion, our results showed that *N. nomurai* jellyfish venom has strong cytotoxic and hemolytic activities, which might be attributed to the proteinous factor of the venom. Further biochemical investigations are in progress to characterize the different proteinic components of *N. nomurai* nematocyst extract from a toxicological barometer and to clarify their mechanism of action.

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