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Research Article

Anti-Inflammatory Activity Of Conus Toxin From Conus Kintoki

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Abstract:
Rats with swollen paws from carrageenan were used to check if *Conus kintoki* venom has anti-inflammatory effects. The goal of this study was to find out if *C. Kintoki* venom can be a helpful alternative to regular pain relievers like ibuprofen. Paw thickness, which shows how much swelling there is, was checked at different times after getting different amounts of *C. kintoki* venom (5 mg/kg and 15 mg/kg) was tested, and the results were compared to a group that got ibuprofen and another group that had inflammation caused by carrageenan. The working parts of C. Kintoki venom, which contains substances called conotoxins, is famous for its strong ability to target specific parts of the nervous system, like ion channels, receptors, and transporters. Unlike traditional NSAIDs, which often cause stomach and heart problems, this new way of working might be safer. The study highlights that conotoxins could be used as new anti-inflammatory medicines. We need to do more research to find out which conotoxins cause the anti-inflammatory effects and to understand how they work. Understanding these relationships could lead to new and better anti-inflammatory medicines that are safer to use. In short, the poison of *C. Kintoki* has good anti-inflammatory properties, which means it might be a useful alternative to regular pain relievers like NSAIDs. This work opens the door for future studies on marine poisons and how they can be used to make anti-inflammatory medicines.

Keywords: Conus, Anti-inflammatory, drugs, venom, carrageenan, NSAIDs.

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Introduction:

Long ago, people noticed five main signs to describe inflammation by just looking at it: redness, swelling, heat (only in the arms and legs), pain, and loss of function. The old Roman historian Celsus (30-38 B. C) named the first four signs, and Galen (A. D 130-200) also contributed to this named the last one while Galen gave names to the final one (Hurley 1972 *et al.*,). Not all infections cause inflammation, but we now know that inflammation is important. The immune system's reaction to damage in the body and when germs invade. It is actually much more complicated than it looks from the simple definition given above. Acute inflammation happens when there is a *Staphylococcus aureus* skin infection, like a boil. Chronic inflammation can change the walls of arteries in a condition called atherosclerosis. It can also cause swelling in the airways in asthma and chronic bronchitis, and it can lead to serious damage to joints in rheumatoid arthritis (Punchard 2004 *et al.*,). The main part of inflammation is when immune cells get activated. This causes them to

release substances that promote inflammation, like interleukins (such as IL-6) and tumor necrosis factor-alpha (TNF-α). These proteins are important for helping with different inflammatory diseases and keeping the inflammation going. Reduce swelling and ease the symptoms of long-term inflammatory diseases by stopping certain proteins called cytokines or the pathways that cause them (Punchard 2004 et al.,). In medical research, it's hard to find safer and better anti-inflammatory drugs. The treatments we have now often cause serious side effects and don't work well for a long time.

Every venomous creature can release venom, which is a complex mix of substances that affect the body (Fry 2009, *et al.*). Sea creatures have gained a lot of interest as a possible source of new anti-inflammatory chemicals because they have unique and different natural substances. To make use of their medicinal effects, new research was done on the chemical properties of snake, scorpion, and cone snail venoms to better understand how they cause harm (Mackessy 2021 *et al.*). Cone snails are unique because they belong to a group called Conus. The powerful poisons that these hunting sea snails use to stun their prey are widely known. Cone snail venom is made up of a mix of small, active substances like peptides, proteins, enzymes, and tiny molecules. These conotoxins are promising options for developing new treatments because they have various helpful effects, like pain relief, effects on nerves, and reducing inflammation. (Twede 2009 *et al.*,). One of these conopeptides, called Ziconotide (ωMVIIA from the Conus magus snail), is already being used in medicine to help treat long-lasting pain. It's a pain-relief medicine that doesn't cause addiction and is 1000 times stronger than morphine

It might take the place of morphine in the future (Behbehani 1984 *et al.*), (Livett 2006 *et al.*), (Satkunanathan 2005 *et al.*), (Umana 2013 *et al.*), (Jakubowski 2006 *et al.*). The cone snail named *Conus kintoki*, or the "Japanese ivory cone," lives only in the coastal waters of Southeast Asia and Japan. Scientists discovered that the poison from *Conus kintoki* has many conotoxins, which could be helpful in medicine. Early studies show that some substances from the *Conus kintoki* snail can help lessen swelling. These small proteins are thought to affect specific nerve signals and channels, which helps block key processes that lead to swelling (Coomans 1982 *et al.*).

The aim of this research is to examine the potential anti-inflammatory characteristicsof *Conus kintoki* venom and clarify the underlying processes responsible for these effects. This study is to evaluate the therapeutic potential of *Conus kintoki* conotoxins for the development of new treatments for inflammatory diseases by assessing their effectiveness in modifying important inflammatory pathways and mediators.

Materials and Methods:

Material/Drugs/Chemicals

The Hi-Media and Sigma Aldrich companies in India provided the chemicals, reagents, medications, equipment, and supplies used in this investigation. Analytical-grade compounds are utilized.

Venom Collection and Preparation:

Conus kintoki was collected from Paringipettai Coastal environment, Cuddalore district. Conus snails were identified through Centre for Advanced Study in Marine Biology, Annamalai University. Using a trawl net, 45 live *Conus kintoki* specimens were gathered from a depth of one to two and a half metres. The venomous gland, venom duct, bulb, and proboscis of snails were dissected following earlier research findings (Cruz 1992 et al.,) and 2 ml of 0.05% (v/v) trifluoroacetic acid (TFA; Sigma-Aldrich, Poole, UK) were used to suspend the crude venom before centrifuging (15000 g, 10 min, 4°C). After rewashing the pellet three times with 2 mL of 0.05% TFA, the remaining supernatants were combined, filtered through Millipore, Watford, UK, 0.22-µm filter membranes, and then lyophilized and kept at -20 °C.

Experimental Animals:

After receiving approval from the Animal Ethical Committee, the Saveetha Institute of Medical and Technical Sciences (SIMATS) Animal House donated 25 healthy Swiss Albino mice of both sexes (weighing 25±5 g at 6 to 8 weeks of age). The mice were housed in cages and given a week to get used to the lab environment. Testing for Acute Oral Toxicity Five female albino mice (6–8 weeks) were used to assess the acute oral toxicity of Conus venom following the recommendations set forth by the Organisation for Economic Cooperation and Development (OECD-425).

Three hours before and one hour following the venom injection, the mice fasted. A restricted dose of 30 mg/kg was administered to the animals, and neither serious toxicity nor mortality were noted.

Anti-inflammatory assay (paw edema assay):

Conus kintoki venom was tested for its anti-inflammatory properties utilising the carrageenan-induced edema method as reported by previous studies (Parmar 2010 et al.,). An hour before the experiment, 5 ml of 0.9% NaCl was used to dissolve 50 mg of carrageenan powder to prepare 1 percent of the carrageenan solution. 30 Albino rats were split into five groups at random (n = 6). A negative control group was first given saline injections and then later carrageenan injections. Conus venom at doses of 5 mg/kg and 15 mg/kg was administered to the treated groups, whereas Ibuprofen (0.5 mg/kg) was given as a reference medication to the standard drug group. For every rat, the thickness of its paws was measured at zero time. After an hour, 50 µL of 1% prepared carrageenan was subcutaneously injected into the plantar surface of each rat's right hind paw in the treatment groups. Using a skin digital calliper, paw thickness was measured 1, 2, 3, and 4 hours after carrageenan administration. The percentage of inhibition of paw edema was used to calculate the anti-inflammatory activity.

Statistical Analysis

Results are expressed as mean ± S.E.M. of triplets. One-way analysis of variance (ANOVA) was used to analyze the results with post-hoc analysis, and where appropriate, Turkey's test was employed; p values less than 0.05 were considered significant.

Results:
Anti-inflammatory Effect:

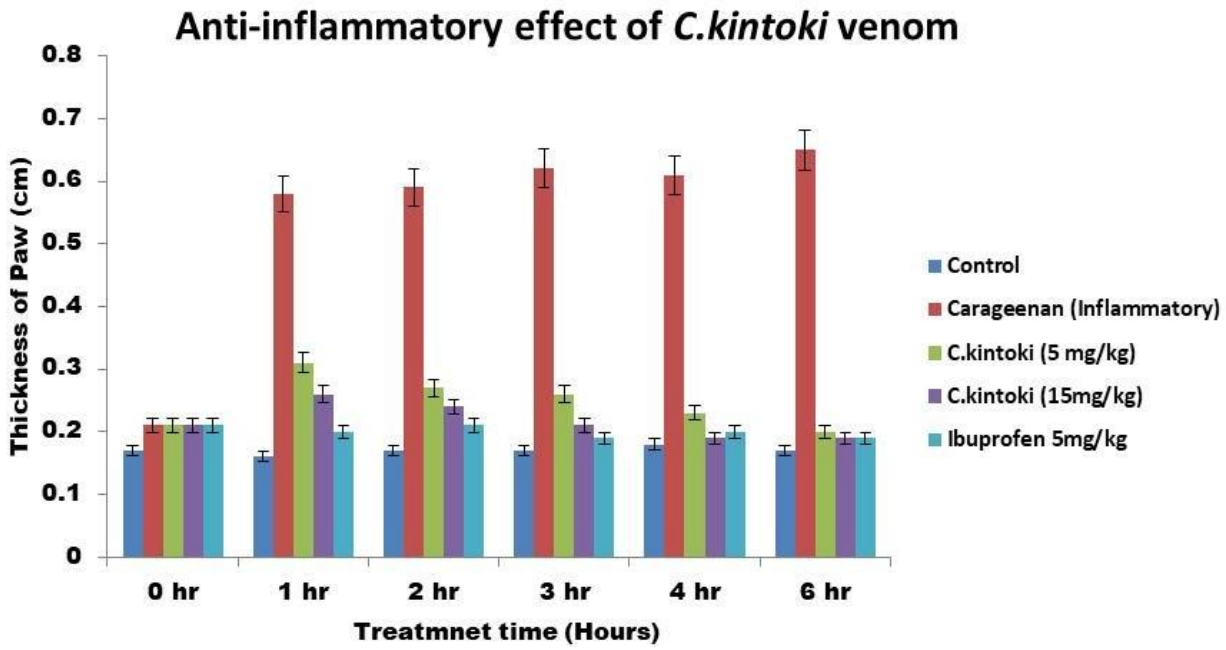

Figure 1: Graph showing the Anti-inflammatory activity of Conus venom at different time intervals

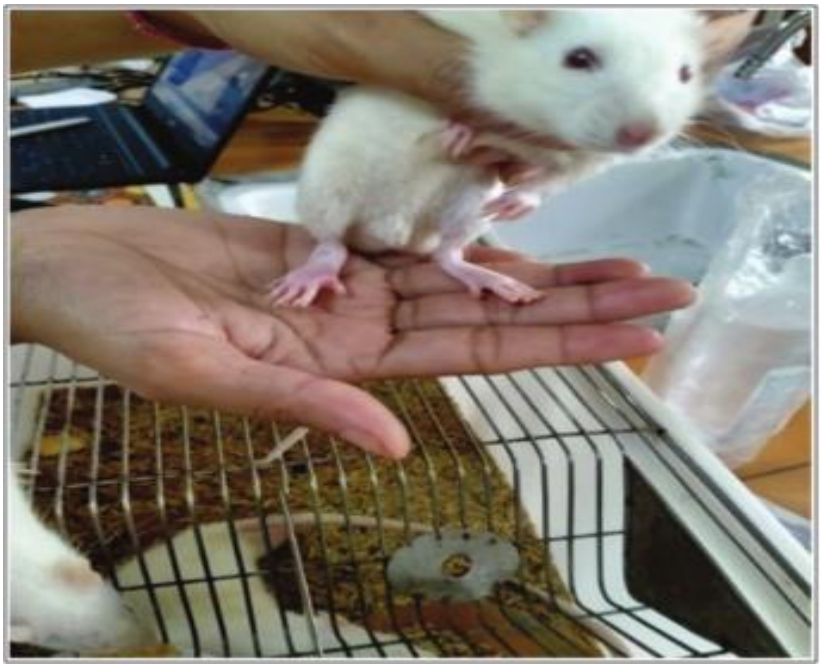

Figure 2: Albino mouse showing paw edema

Paw Edema Assay:
There are five distinct groups in the experiment:

1. Control: No inflammatory agent or treatment is administered to this group, whichacts as the baseline measurement.
2. Carrageenan (inflammatory): As a positive control for inflammation, this group receives treatment with carrageenan to cause inflammation.
3. *C. kintoki* (5 mg/kg): The venom of *C. kintoki* is administered to this group at a dose of 5 mg/kg.
4. *C. kintoki* (15 mg/kg): The venom of *C. kintoki* is administered to this group at a greater dosage of 15 mg/kg.
5. Ibuprofen (5 mg/kg): To set a standard for anti-inflammatory effects, ibuprofen is administered to this group at 5 mg/kg.

Data Analysis
Initial Observations (at 0 hour):
The paw thickness is roughly the same in all groups at the 0-hour mark, suggesting that there is no immediate inflammation or treatment effects. Before beginning any treatment or inducing inflammation, maintaining this consistency is essential for creating a trustworthy baseline.

1-hour post-treatment
The carrageenan group shows a considerable increase in paw thickness after an hour in comparison with the control

group, indicating that the induction of inflammation was successful. Paw thickness increases are less pronounced in the groups treated with ibuprofen and *C. kintoki* venom (5 mg/kg and 15 mg/kg) than in the carrageenan group. Interestingly, compared to the 5 mg/kg group, the 15 mg/kg *C. kintoki* group shows a modest reduction in inflammation, suggesting a dose-dependent anti-inflammatory action.

2 to 4 Hours Post-Treatment

The carrageenan group retains a high degree of paw thickness between two andfour hours, indicating persistent inflammation. In contrast to the carrageenan group, the groups treated with ibuprofen and *C. kintoki* venom exhibit a noticeable decreasein paw thickness. Consistent with the pattern shown at the one-hour mark, the 15 mg/kg *C. kintoki* group continues to show a more significant reduction in inflammation than the 5 mg/kg group. Paw thickness measures demonstrate that ibuprofen regularly exhibits an effective anti-inflammatory effect, equivalent to the *C. kintoki* groups.

6 hours post-treatment:

The carrageenan group continues to exhibit greater paw thickness at the 6-hour mark, albeit with a minor decrease from earlier time points. This reduction can be theresult of inflammation gradually going away on its own. Paw thickness is further reduced in the ibuprofen and C. kintoki groups; the 15 mg/kg C. kintoki group has the strongest anti-inflammatory effect. Significant anti-inflammatory effects are also shown by the 5 mg/kg C. kintoki group

Toxicity Assessment:

Using toxicity tests on mice, the safety profile of *Conus kintoki* venom was assessed. Amazingly, even at a level of 30 mg/kg body weight, the venom showed no harmful effects. The venom of *Conus kintoki* appears to be safe for medicinal usage based on its lack of toxicity, even at relatively high doses. With a low risk of toxicity or other side effects, its potential for development as a treatment for inflammatory disorders is highlighted by the lack of adverse effects at these levels.

Discussion and Conclusion:

Conus kintoki venom has demonstrated a significant anti-inflammatory impact that is similar to that of the common anti-inflammatory medication Ibuprofen. The study found that 15 mg/kg body weight was the effective concentration (EC) needed to significantly reduce paw edema in mice. The reduction in paw edema indicates that this dosage successfully reduced inflammation (see Figures 1 and 2). According to the findings, *Conus kintoki* venom is a strong anti-inflammatory agent that presents a viable substitute for traditional medical interventions.

Conotoxins are tiny, extremely powerful peptides that specifically target certain nervous system ion channels, receptors, and transporters. Their specific mode of action frequently includes blocking ion channels, which can reduce the production of chemicals that promote inflammation. Conotoxins are particularly useful for reducing inflammation at the site of injury or infection because of their capacity to modify ion channels (Buczek et al., 2005). Despite their effectiveness, traditional NSAIDs are frequently linked to negative side effects such as gastrointestinal distress, kidney impairment, and increased cardiovascular risks. Singh (2000). Conotoxins derived from *C. kintoki* may present a unique anti-inflammatory therapy strategy with a potentially distinct side effect profile. Conotoxins may help people who are intolerant to or unresponsive to traditional therapies since they function differently from NSAIDs (Ducancel et al., 2014). The promising results from this study highlight the need for further research into the specific conotoxins responsible for the observed anti-inflammatory effects. Isolating and characterizing these peptides could lead to the development of new, targeted anti-inflammatory drugs. Additionally, understanding the detailed molecular interactions between conotoxins and their targets could provide insights into novel therapeutic pathways for a variety of inflammatory conditions (Dutertre et al., 2010). In conclusion, *Conus kintoki* venom exhibits significant anti-inflammatory activity, comparable to that of ibuprofen. The dose-dependent response suggests that higher doses of *C. kintoki* venom may be more effective in reducing inflammation. This study underscores the potential of marine biotoxins as a source of new anti-inflammatory agents, offering a promising alternative to traditional NSAIDs. Further research is warranted to isolate specific conotoxins, elucidate their mechanisms of action, and evaluate their safety and efficacy in clinical settings.

Conflict of Interest: Authors declare there is no conflict of interest.

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References:

1. Cunningham, AL, and JV. Hurley. "Alpha-naphthyl-thiourea-induced pulmonary oedema in the rat: a topographical and electron-microscope study." *The Journal of pathology* 106, no. 1 (1972): 25-35.
2. Punchard, Neville A, Cliff J. Whelan, and Ian Adcock. "The journal of inflammation." *Journal of inflammation* 1 (2004): 1-4.
3. Fry, Bryan G, Kim Roelants, Donald E. Champagne, Holger Scheib, Joel DA Tyndall, Glenn F. King, Timo J. Nevalainen et al. "The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms." *Annual review of genomics and human genetics* 10, no. 1 (2009): 483-511.
4. Mackessy, Stephen P. *Handbook of venoms and toxins of reptiles*. CRC press, 2016.
5. Twede, Vernon D, George Miljanich, Baldomero M. Olivera, and Grzegorz Bulaj. "Neuroprotective and cardioprotective conopeptides: an emerging class of drug leads." *Current opinion in drug discovery & development* 12, no. 2 (2009): 231.
6. Behbehani, M. M, and A. Pert. "A mechanism for the analgesic effect of neurotensin as revealed by

behavioral and electrophysiological techniques." *Brain research* 324, no. 1 (1984): 35-42.
7. Livett, Bruce G, David W. Sandall, David Keays, John Down, Ken R. Gayler, Narmatha Satkunanathan, and Zeinab Khalil. "Therapeutic applications of conotoxins that target the neuronal nicotinic acetylcholine receptor." *Toxicon* 48, no. 7 (2006): 810-829.
8. Satkunanathan, Narmatha, Bruce Livett, Ken Gayler, David Sandall, John Down, and Zeinab Khalil. "Alpha-conotoxin Vc1. 1 alleviates neuropathic pain and accelerates functional recovery of injured neurones." *Brain research* 1059, no. 2 (2005): 149-158.
9. Umana, Iboro C, Claire A. Daniele, and Daniel S. McGehee. "Neuronal nicotinic receptors as analgesic targets: it's a winding road." *Biochemical pharmacology* 86, no. 8 (2013): 1208-1214.
10. Jakubowski, Jennifer A., Wayne P. Kelley, and Jonathan V. Sweedler. "Screening for post-translational modifications in conotoxins using liquid chromatography/mass spectrometry: an important component of conotoxin discovery." *Toxicon* 47, no. 6 (2006): 688-699.
11. Coomans, Henry, and Robert Moolenbeek. "Studies on Conidae (Mollusca, Gastropoda) 1. Conus papuensis and C. kintoki, two new species from deeper water in the western Pacific." *Bulletin Zoologisch Museum* 8, no. 15 (1982): 133-138.
12. Cruz, Lourdes J, Cecilia A. Ramilo, Gloria P. Corpuz, and Baldomero M. Olivera. "Conus peptides: phylogenetic range of biological activity." *The Biological Bulletin* 183, no. 1 (1992): 159-164.
13. Parmar, Sachin, Amit Gangwal, and Navin Sheth. "Mast cell membrane stabilization and anti-histaminic actions possible mechanism of action of anti-inflammatory action of Murraya koenigii." *J Curr Pharm Res* 2, no. 1 (2010): 21-25.
14. Buczek, O, G. Bulaj, and B. M. Olivera. "Conotoxins and the posttranslational modification of secreted gene products." *Cellular and Molecular Life Sciences CMLS* 62 (2005): 3067-3079.
15. Ducancel, Frédéric, Jordi Durban, and Marion Verdenaud. "Transcriptomics and venomics: Implications for medicinal chemistry." *Future medicinal chemistry* 6, no. 15 (2014): 1629-1643.
16. Dutertre, Sébastien, and Richard J. Lewis. "Use of venom peptides to probe ion channel structure and function." *Journal of biological chemistry* 285, no. 18 (2010): 13315-13