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RESEARCH ARTICLE

Anti-inflammatory Activity of Crude Extract and Zinc- Oxide Nanoparticles from the Ink of Squid (*Uroteuthis duvaucelii*)

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Abstract

Objectives: Marine natural materials can be utilized to construct a variety of medications and have produced significant leads for therapeutic development. The aim of the present study is to evaluate the anti-inflammatory activity of crude extract and zinc-oxide nanoparticles from the ink of squid. **Methods:** The anti-inflammatory activity of the synthesized zinc-oxide nanoparticles and crude extract of *Uroteuthis duvaucelii* was determined by using albumin denaturation assay and anti-proteinase activity. **Findings :** The albumin denaturation assay of the crude ink extract shows a maximum anti-inflammatory activity of 29% at a concentration of 100μl/ml and a minimum activity of 7% at a concentration of 20μl/ml. The zinc-oxide nanoparticles of *U. duvaucelii* exhibit anti-inflammatory activity of 8% at 20μl/ml, and 37% at a concentration of 100μl/ml. Anti-proteinase activity of the crude extract exhibits 25% of inhibition at a concentration of 100μl/ml and lowest activity of 2% at a concentration of 20μl/ml. Zinc-oxide nanoparticles of *Uroteuthis duvaucelii* exhibit maximum inhibition of 28% at 100μl/ml and minimum activity of 5% at a concentration of 20μl/ml. Comparing the two extracts the zinc-oxide of *U. duvaucelii* shows better albumin denaturation and anti-proteinase action than the crude ink extract. So, this can be used as a source of anti-inflammatory agents for drug development. **Novelty :** Research suggests that natural products may be a source of anti-inflammatory medications with higher activity and fewer side effects. Therefore, the synthesized nanoparticles from the ink of *Uroteuthis duvaucelii* can be used as an anti-inflammatory drug.

Keywords: Anti-inflammatory activity; Zinc-oxide nanoparticles; *Uroteuthis duvaucelii*; Anti proteinase; Crude ink

1 Introduction

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation [1,2]. Inflammation is therefore a defense mechanism that is essential to health [3]. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates the responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses [4]. Inflammation can be described as the rapid response of the body to injury and infection. The inflammatory reaction is recognized macroscopically by cardinal signs such as heat, redness, swelling, pain, and loss of function [5]. Neutrophils are known to be an abundant source of proteinase which carries in their lysosomal granules many serine proteinases. It was reported that leukocyte proteinase plays an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors. [6]

Current treatments for inflammatory diseases are primarily based on the use of steroidal and non-steroidal anti-inflammatory drugs, which are often reflective of the severity and responsiveness of the inflammation to the particular therapeutic regime [7]. Nonsteroidal anti-inflammatory drugs are of huge therapeutic benefit in the treatment of various types of inflammatory conditions. There is a global need for preventive and therapeutic measures of natural origin without side effects for inflammatory diseases that are safe, economical, and efficacious compared to the currently used drugs with various adverse effects.

Squids are one of the important seafood resources with pharmacological importance**.** In India, squid ink has traditional applications in food and pharmacological products. [8] The nanoparticles of metal oxides have specific physicochemical properties (thermal, surface, electrical, and optical), different from their bulk counterparts. Among different metal oxide nanoparticles, the most commercially utilized nanoparticles used in industries are zinc oxide, titanium dioxide, silver oxide, cerium dioxide, and copper-oxide nanoparticles [9]. In recent days copper, nickel, zinc, etc have been used for nanoparticle synthesis instead of noble metals such as gold and silver because noble metals are rare and high cost [10]. Hence the present study has been carried out to test the anti-inflammatory properties of crude ink and synthesized nanoparticle ink.

2 Methodology

2.1 Albumin denaturation assay

Albumin denaturation assay was done according to the method described by Sakat [8] *et al.*, 2010. The sample was dissolved in 1% of Bovine serum albumin fraction and incubated at 37°C for 20 minutes and then incubated at 51°C for 20 minutes in the water bath. After cooling the tubes at room temperature absorbance was measured at 660nm using a UV-VIS spectrophotometer. Tris HCL buffer solution was used as the control. The percentage of inhibition of albumin denaturation was calculated by using the following formula:

$$\% \text{ of inhibition} = \frac{Absorbance\ of\ Control\ OD - Absorbance\ of\ sample\ OD}{Absorbance\ of\ Control\ OD} X\ 100$$

2.2 Anti -proteinase action

Anti-proteinase action was done according to the method described by Sakat [8] *et al.*, 2010. The reaction mixture consists of 20 μl of trypsin and 1 ml of the 20 mM Tris HCL buffer. Trypsin and HCL buffer were mixed with the sample and were incubated at 37°C for 5 minutes. After 5 minutes 1ml of the 0.8% of (W/V) casein was added and allowed to inhibit for 20 minutes. 2 ml of 70% of perchloric acid was added to terminate the reaction. The final cloudy suspension was centrifuged and the supernatant was collected. The OD was measured at 210nm using a UV-VIS spectrophotometer. Tris HCL buffer solution was used as the control. The percentage of inhibition of anti-proteinase action was calculated by using the following formula:

$$\% \text{ of inhibition} = \frac{Absorbance\ of\ Control\ OD - Absorbance\ of\ sample\ OD}{Absorbance\ of\ Control\ OD} X\ 100$$

3 Results and Discussion

3.1 Albumin denaturation assay

Albumin denaturation assay of *U. duvaucelii* crude ink was shown in Figure 1. Albumin denaturation was measured at five different concentrations (20μl/ml, 40μl/ml, 60μl/ml, 80μl/ml, and 100μl/ml). The highest anti-inflammatory activity of 29% was observed at a concentration of 100μl/ml and the lowest activity of 7% at a concentration of 20μl/ml.

In the present study zinc-oxide nanoparticles prepared from the ink of *U. duvaucelii* exhibits good anti-inflammatory activity of 8% at 20μl/ml, 11% at 40μl/ml, 26% at 60μl/ml, 30% at 80μl/ml, 37% at 100μl/ml respectively. The highest activity was observed at a concentration of 100μl/ml and the lowest activity was at a concentration of 20μl/ml. The results showed that the extract exhibited dose-dependent albumin denaturation inhibition activity.

3.2 Anti-proteinase action

Anti-proteinase action of *U. duvaucelii* crude ink is shown in Figure 2. The anti-proteinase action of *U. duvaucelii* ink was measured at five different concentrations (20μl/ml, 40μl/ml, 60μl/ml, 80μl/ml and 100μl/ml). The highest anti-proteinase activity of 25% was noted at a concentration of 100μl/ml and lowest activity of 2% at a concentration of 20μl/ml.

The zinc-oxide nanoparticles from the ink of *U. duvaucelii* exhibit inhibition activity of 5% at 20μl/ml, 13% at 40μl/ml, 21% at 60μl/ml, 24% at 80μl/ml and 28% at 100μl/ml concentration. The highest activity was observed at a concentration of 100μl/ml and the lowest activity was noted at a concentration of 20μl/ml. The zinc-oxide nanoparticle of *U. duvaucelii* shows better inhibitory activity than the crude ink extract so zinc-oxide nanoparticle can be used as an anti-inflammatory agent.

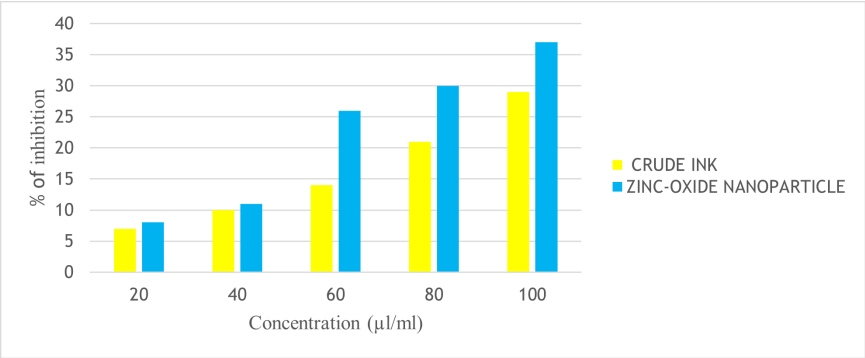

Fig 1. Albumin denaturation assay of zinc-oxide nanoparticles & crude inkextract of *U. duvaucelii*

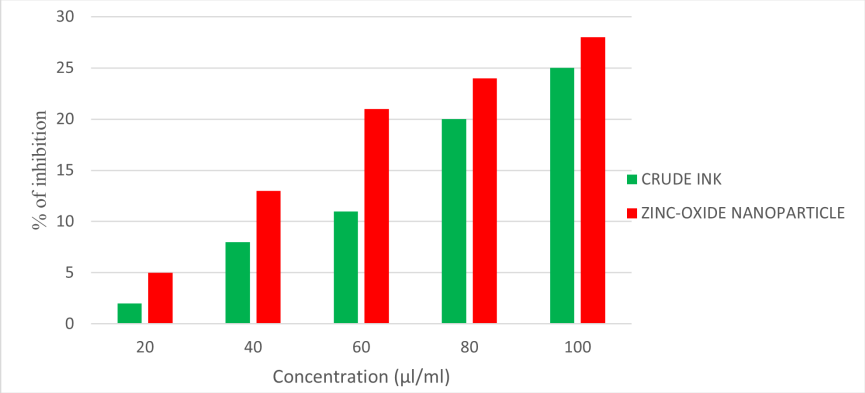

Fig 2. Anti-proteinaseaction of zinc-oxide nanoparticle & crude ink extract of *U. duvaucelii*

In the present study, the albumin denaturation assay & anti-proteinase action of *U. duvaucelii* ink were measured at five different concentrations (20μl/ml, 40μl/ml, 60μl/ml, 80μl, and 100μl/ml). The highest activity was observed at a concentration of 100μl/ml and the lowest activity was observed at a concentration of 20μl/ml. Comparing the two, zinc-oxide nanoparticles exhibit the highest anti-inflammatory activity than the crude ink. Very similar to the present study Nadarajah [11] *et al.*, 2017 studied the anti-inflammatory activity in the ethanolic ink extract of *L. vulgaris* by protein denaturation and protease inhibition activity. Inhibition of protein denaturation by the ethanolic extracts of squid ink was tested at different concentrations of 50, 100, 150, and 200μg/ml. Acetylsalicylic acid was used as a positive control. He observed the highest inhibition activity of 68.9 % at a concentration of 200μg/ml and the lowest activity of 43.2% noted at a concentration of 50μg/mL. Protease inhibition activity was measured at various concentrations in the ethanolic extracts of *L. vulgaris.* The ethanolic extracts of *L. vulgaris* exhibit

the highest anti-proteinase activity of 67.4% at a concentration of 200μg/mL and the lowest activity of 49.2% at a concentration of 50μg/mL. As the concentration of the extract increases the inhibition also increases. This study coincides with the result of the present study.

Similar to the present study, Leoline Ebenezer[12] *et al*., 2022 reported the anti-inflammatory activity of Sepia pharaonis ink using various solvents such as aqueous, DMSO (Dimethyl sulfoxide), and ethanol by egg albumin denaturation assay at five different concentrations (10,20,30,40 and 50μl). The albumin denaturation activity of 85.6% was observed in the DMSO, 79.3% in the ethanolic extract, and 78.2% in the aqueous extract at the concentration of 50μl. The egg albumin denaturation assay showed higher anti-inflammatory activity in DMSO compared to aqueous and ethanol extract. In the present study, albumin denaturation action revealed the highest anti-inflammatory activity of 29% at a concentration of 100μl/ml in the crude ink and the lowest activity of 7% at a concentration of 20μl/ml. Zinc-oxide nanoparticles in the ink showed the highest inflammatory activity of 37% at a concentration of 100μl/ml and lowest activity of 8% at a concentration of 20μl/ml.

In contrast to the present study, Kajal Chakraborty and Minju Joy[13]., 2017 tested the anti-inflammatory activity of ethyl acetate-methanol extracts of five cephalopods (*Amphioctopus marginatus, Uroteuthis duvaucelii, Sepia pharanosis, Sepiella inermis* and *Cistopus indicus*). Anti-inflammatory activity was evaluated by three methods namely, COX-1, COX-2, and 5-LOX. He reported the extracts of *Uroteuthis duvaucelii* revealed lesser inhibitory activity when compared to the other extracts of cephalopods.

Wiya[14] *et al*., 2020 studied the anti-inflammatory activity of the slime extract of an African snail (*Lissachatina fulica*) in ethanol and aqueous extract. The proteinase inhibitory activity showed the inhibition of 41.99$\pm$2.37 in aqueous extract and inhibition of 12.76%$\pm$3.61% in ethanol extract at a concentration of 1000mg/ml. Anti-proteinase activity revealed 70.81$\pm$0.40% inhibition in aqueous extract and 43.27$\pm$0.23% in ethanol extract at a concentration of 1000mg/ml. Similarly, the present study albumin denaturation activity revealed 29% at a concentration of 100μl/ml in the crude ink, and 37% was noted at a concentration of 100μl/ml in the ink zinc-oxide nanoparticles. Anti-proteinase activity of 25% was observed at 100μl/ml in the crude ink whereas 28% was noted at a concentration of 100μl/ml in zinc-oxide nanoparticles synthesized from the ink. This study corroborates the result of the present study.

Chakraborty K[15] *et al*., 2021 evaluated the anti-inflammatory properties of secondary metabolites C_{19} furano-norditerpenoid, C_{15} sesquiterpenoid, and C_{20} diterpenoid isolated from *U. duvaucelii*. The study revealed the inhibitory activities of C_{19} furano-norditerpenoid and C_{15} sesquiterpenoid against the pro-inflammatory enzyme COX-2. C_{19} furano-norditerpenoid was higher (IC_{50} value of0.74 mg/ml; $p<0.05$) than C_{20} diterpenoid analog (IC_{50} value of 0.81 mg/mL) and C_{15} sesquiterpenoid. Anti-inflammatory potencies were greater in C_{19} furano-norditerpenoid, followed by C_{15} sesquiterpenoid and C_{20} diterpenoid.

Hernández-Zazueta[16] *et al*., 2021 studied the anti-inflammatory activity of bioactive compounds from *Octopus vulgaris* ink extracts. He reported cytokines expression opposition of the control was visualized by Image-Quant. Out of 40 cytokines, 8 were differentially up-regulated (>1.5 fold). The result of the study expressed modulated cytokines recommended the octopus ink to target the immune response and cytokine production.

All these findings support that the ink of cephalopods has good anti-inflammatory activity. The extracts used in the current study have anti-inflammatory agents providing an optimal level of protection as protease inhibitors denoting that the inflammatory activity is subsided and it can be used as a natural drug for curing the inflammation.

4 Conclusion

The present study reveals the anti-inflammatory activity of crude ink and zinc-oxide nanoparticles in the ink of Indian squid (*Uroteuthis duvaucelii*). The results of the present study suggest that the zinc-oxide nanoparticles synthesized from the ink of *Uroteuthis duvaucelii* exhibited strong anti-inflammatory activity when compared to the crude extract. It can be used for therapeutic applications and in the development of new anti-inflammatory drugs.

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