Original Article

Analgesic Properties of *Nigella Sativa* and *Eucheuma Cottonii* Extracts

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Abstract

Background: This study investigates the analgesic properties of the aqueous extracts of *Nigella sativa* and *Eucheuma cottonii* in mice. The analgesic properties of both extracts were evaluated in an experimental model of acetic acid-induced writhing test. **Materials and Methods:** The mice were divided into four different groups and received the test extracts and the standard drug (aspirin) for 14 days via force-feeding. On day 15, the mice were injected with 5% acetic acid, and the number of abdominal constriction and elongation of hind limb (writhes) were counted for 20 min. **Results:** The numbers of writhes were counted starting after 5 min of the acetic acid injection. The *N. sativa* extracts significantly reduced the number of writhes as compared to the control group. Both of the extracts revealed a comparable result as referred to the aspirin effects in the mice. **Conclusions:** These findings indicate that *N. sativa* and *E. cottonii* may possess protective active constituent that is effective in reducing the sensation of pain in mice.

Keywords: Acetic acid, analgesic, *Eucheuma cottonii*, *Nigella sativa,* writhing test

Introduction

Sensation of pain is vital for survival because it acts as an indicator to tissue-damaging condition in the body. Sensation of pain is initiated in peripheral pain receptors known as nociceptors that are only excited when there is noxious stimulus.[1] Nociceptors are free nerve ending that innervate every tissue of the body except the brain and it can be activated by intense thermal, mechanical, or chemical stimuli. Tissue irritation or injury liberates chemicals such as prostaglandins (PGs), kinins, and potassium ion that stimulate nociceptors.[2] Analgesic drugs are medication that can reduce pain and have several mechanisms of action to provide relief. Drugs such as aspirin and ibuprofen block the formation of PGs; thus, nociceptors will not be stimulated.[3] Local anesthetics such as novocaine block the conduction of nerve impulse along the axons of first-order pain neurons, giving short-term pain relief, while morphine and other opiate drugs act by changing the quality of pain perception in the brain. Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most prescribed medications and have proved to be useful in the management of chronic pain disorders, but its prolonged usage could lead to renal and gastrointestinal side effects.[4] The use of NSAIDs among patients with first-time myocardial infarction was associated with persistent increased coronary risks.[5] There are also the tendency of increased new acute myocardial infraction risk with current use of some NSAIDs, especially parenteral NSAIDs.

Natural products have been considered an alternative remedy to synthetic chemicals in many clinical conditions. These research works explore the analgesic properties of *Eucheuma cottonii* or red seaweed and *Nigella sativa* plant. Marine organisms including seaweeds contain biological compounds that have medicinal properties such as analgesic and anti-inflammatory.[6] The discovery of biological activities of seaweed metabolites has increased in recent years, and some of the compounds have been reported to exhibit antibacterial, cytotoxic, and anticoagulant activities and stimulate cell migration.[7] *E. cottonii* is edible species of red seaweed that is rich in nutrients in the form of protein, soluble fiber, macrominerals, trace elements, and iodine. *E. cottonii* also

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has high polyunsaturated fatty acid content and low saturated fatty acid.[8] The antioxidant properties of seaweed compounds can significantly prevent tissue damage and stimulate wound healing process.[9] *N. sativa* is a flowering plant with seeds which is used as spice called black cumin. These potentially active seeds are small, black and possess aromatic odor and taste. It has been previously reported that *N. sativa* showed anti-inflammatory and antibacterial activities[10] and antiulcer property[11] and possess protective effect against liver injury caused by restrictive blood supply. In addition, *N. sativa* is also effective in eradicating *Helicobacter pylori* infection in nonulcer dyspeptic patients and the seed of *N. sativa* was found to contain many active compounds such as thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, and alpha-hederin.[12] The needs for new efficacious analgesic medication with fewer side effects from natural sources have gained a lot of interests in recent years. This study investigates the effectiveness of *E. cottonii* and *N. sativa* extracts to reduce pain in an experimental animal model.

Materials and Methods

Eucheuma cottonii plant extraction

E. cottonii extracts were prepared using modified method as described previously.[6] 150 g of dried *E. cottonii* or red seaweed was washed with distilled water to remove salt and foreign substances. The seaweeds were left to dry in a clean environment at room temperature for 7 days. After 7 days, the seaweeds were grounded until it became a powder-like form. The seaweed powders were weighed and soaked in distilled water at a ratio of 1:20 dilution for 48 h. After that period, the seaweed solution was placed in a water bath at 50°C for 6 h. The solution was then filtered with clean cloth and filter papers, and the extracts obtained were stored at 4°C.

Nigella sativa seeds extraction

N. sativa seeds were cleaned with distilled water to remove any foreign substances. The seeds were properly dried and grounded to powder form. The *N. sativa* powder was then soaked with distilled water at 1:10 ratio. After soaking the powder in distilled water for 48 h, the solution was placed in a water bath at 50°C for 6 h before it was filtered with clean cloth and filter papers. The solution was put in a rotary evaporator (BUCHI rotavapor R-216) at 37°C (60 rpm) to remove excessive solvents during the extraction process. This yielded a blackish-brown concentrated *N. sativa* seed extracts. The extracts were protected from lights and stored at 4°C.

Experimental animal model

Forty male BALB/c mice of 9 weeks old were kept in the animal house according to the Animal Ethic rules and regulation at Universiti Kuala Lumpur, Institute of Medical Science Technology (FYP/AEC/MESTECH-UNIKL/2016/16). The animals were housed in a clean environment and were fed with normal diet and water *ad libitum*. The mice were divided into 4 groups, and each cage was labeled according to the designated group.

Preparation of test substance and reagents

5% acetic acid was prepared from 99.8% glacial acetic acid (HmbG) to induce pain in the mice. Aspirin (Bayer) containing 0.5 g acetylsalicylic acid per tablet was diluted and used as the positive control drug.

Acetic acid-induced writhing test

The mice were divided into four different groups. Mice in the positive control group were given 25 mg/kg of diluted aspirin through intraperitoneal injection. Moreover, both groups of mice treated with either *N. sativa* or *E. cottonii* received 0.5 ml of the extracts, respectively. The mice in the negative control group were not given any treatment. Aspirin and the extracts were administered to the mice daily for 14 days via force-feeding. On the 15th day, acetic acid-induced writhing test was conducted on each group. The mice were injected with 5% acetic acid (0.1 ml) intraperitoneally, and the number of writhes exhibited was recorded for 20 min. This acetic acid-induced writhing test was a modification of the method described previously.[13]

Statistical analysis

The results on the number of writhes recorded were presented as mean + standard error mean (SEM). The data were analyzed using one-way ANOVA and Student's *t*-test for significant differences among the different groups. $P < 0.05$ was considered statistically significant difference.

Results

The number of writhes displayed by each mouse was counted accordingly for 20 min. Mice treated with *N. sativa* extract demonstrated a low count (21.33 + 3.04) of abdominal constrictions and hindlimb elongation during the period. This result is comparable to the number of writhes (20.57 + 3.25) recorded by the mice treated with aspirin. The average count of writhes in mice treated with *E. cottonii* extract also showed a potentially good result (24.29 + 2.65) as compared to the positive control group.

Figure 1 shows the average counts of writhes exhibited by mice in each group. The number of writhes was carefully recorded within the designated time period. The average counts of writhes in the negative control group were the highest (35.17 + 5.86) among all tests group, whereas mice treated with aspirin (positive control group) have the lowest count of writhes. However, the treatment of *N. sativa* for 14 days has significantly reduced the writhing counts in the mice as compared to the positive control group.

Discussion

In this study, acetic acid was administered to assess the prophylactic analgesic properties of *E. cottonii* and *N. sativa* extracts in an animal model. The extracts were given as a supplementary food in addition to the mice normal diet. Pain induced by acetic acid occurs due to the liberation of endogenous substances such as serotonin, histamine, PGs, bradykinins,

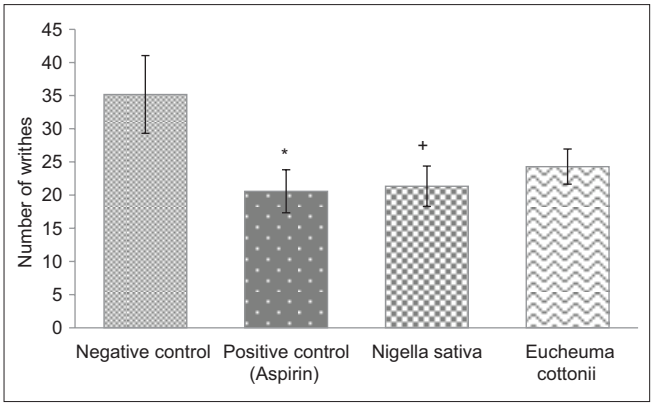

Figure 1: The average counts of writhes recorded in acetic acid-induced mice

substance P, and other pain mediators that stimulate the nerve endings.[14] This method of pain induction will increase the levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluids together with lipoxygenase products. Arachidonic acid was released from the tissue phospholipids via cyclooxygenase (COX) and produced PG. PG and lipoxygenase caused the capillaries permeability to increase and lead to inflammation and pain. Any substance or compound that can reduce the number of writhes in an animal model will possibly exhibit the analgesic effect by inhibiting PG synthesis.[15]

Dichotomaria obtusata is another type of marine red seaweed from the same species with *E. cottonii*. It was found that the aqueous extract of *D. obtusata* can inhibit ear edema in mice and significantly reduce abdominal writhes in the animal.[6] The study reported that the aqueous extract contains compounds such as terpenes, peptides, and sulfated polysaccharides that were known to produce analgesic effect by inhibiting the release of endogenous mediators in response to acetic acid injection. Most red seaweeds were reported to contain polysaccharides that possess biological activity of potential medicinal value. Sulfated polysaccharides are found abundant in the extracellular matrix and involved in ionic, mechanical, and osmotic functions in marine algae.[16,17] They are found to modulate various biomedical activities such as anticoagulant, antinociceptive, antioxidant, and anti-inflammatory.

A clinical evaluation on volatile oil of *N. sativa* found that there was a significant inhibition of inflammation and pain when rats were treated with aqueous extract of the *N. sativa* seeds.[18] Analgesic effects shown by *N. sativa* may be due to the inhibition of lipoxygenase or COX by the active compounds present in the extract. Thymoquinone is one of the main biological compounds in *N. sativa* extracts and was reported to inhibit the production of thromboxane A_2 and leukotriene B_4.[19] The presence of thymoquinone is believed to reduce the pain sensation in the mice when acetic acid was injected by interfering with the PG synthesis. It was described that thymoquinone and its structural analogs, para-benzoquinones, can reduce pain in mice.[20]

Conclusions

The findings indicate that *E. cottonii* and *N. sativa* extracts can reduce pain in the animals when compared to the positive control group. The analgesic effect of the extracts could be contributed by the presence of several active compounds such as thymoquinone in *N. sativa* and sulfated polysaccharides, terpenes, and peptides in *E. cottonii*. Based on previous reports, the active compounds from both extracts have a very broad biological activity. The outcomes from this study suggest that the effectiveness of the extracts might be due to the inhibition of pain mediators by the active compounds. Therefore, it can be implicated that *E. cottonii* and *N. sativa* seed extracts have the capabilities to reduce pain and may have an analogous mechanism of action as acetylsalicylic acid.

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Conflicts of interest

There are no conflicts of interest.

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