# The effect of Nigella sativa and Eucheuma cottonii in Collagen-induced Arthritis mice

**Article** *in* Research Journal of Pharmacy and Technology · January 2020

DOI: 10.5958/0974-360X.2020.00243.7



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ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

***RESEARCH ARTICLE***

# The effect of *Nigella sativa* and *Eucheuma cottonii* in Collagen-induced Arthritis mice

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## ABSTRACT:
Arthritis is a common autoimmune disease characterized by chronic inflammation at the synovial joint. Most treatments that are currently used have its own downside and give rise to natural plants as alternative remedy. This study investigates the effect of *Nigella sativa* and *Eucheuma cottonii* extracts in reducing inflammation and the severity in collagen-induced arthritis mice. The collagen-induced arthritis mice were fed with *Nigella sativa* and *Eucheuma cottonii* extracts for 21 days. The results were recorded from inflammation scoring and neutrophil infiltration in the knee joints. This study demonstrated that mice treated with *Nigella sativa* extract showed significant decrease ($p<0.05$) in inflammation scoring and the numbers of neutrophil infiltration as compared to other treated groups. Treatment with *Eucheuma cottonii* extract also demonstrated a significant decrease ($p<0.05$) in the inflammation scoring. The extract from *Nigella sativa* has evidently reduced the inflammation in collagen-induced arthritic mice and showed better results than *Eucheuma cottonii* treatment.

**KEYWORDS:** *Nigella sativa*, *Eucheuma cottonii*, Collagen-induced arthritis, Inflammation, Neutrophil.

## INTRODUCTION:
Arthritis is a common autoimmune disease that is characterized by chronic inflammation at the synovial joints where it is associated with progressive disability, systemic complications, early death and socioeconomics costs[1]. Rheumatoid arthritis (RA) affects about 5 in 1000 people and this disease is affecting about 1% of the human population[2]. The common physical symptoms of RA are swelling of joints areas, painful and stiff joints, fatigue, malaise, weight loss and sometimes even depression, but the main characteristic is swelling of the joints and ability to spread the disease to new joints[3,4]. The etiology of RA is multifactorial where the diseases can arise because of complex interactions between genetic, hormonal, immunological, ineffective, environmental and physiological factors.

Received on 26.07.2019 Modified on 06.08.2019
Accepted on 09.11.2019 
*Research J. Pharm. and Tech 2020; 13(3): 1319-1323.*
**DOI: 10.5958/0974-360X.2020.00243.7**

The most commonly affected joints in RA patients are the proximal interphalangeal and metacarpophalangeal joints of the hands and wrists, followed by metatarsophalangeal joints of the feet, ankles and shoulders[5].

*Eucheuma cottonii* is an edible species of pacific red seaweeds obtained from Malaysian North Borneo Sabah waters. This seaweed has variety of potential compounds like dietary fibers, vitamin C, α-tocopherol, minerals, fatty acid and protein[6]. *Eucheuma cottonni* also contain high polysaccharides, active protein with anti-inflammatory properties and high antioxidants that could help in wound healing process[7]. *Nigella sativa* also called 'black seed', Al-Habba Al-Sauda or Al-Habba Al-Barakah in Arabic is well known in the Middle East, Middle Asia and far East. It has many pharmaceutical properties such as anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities[8]. *Nigella sativa* has numerous active compounds such as 30-40% fixed oil, 0.5-1.5% essential oil and protein and pharmacologically active

components such as thymoquine, ditimoquinone and nigellin[9,10].

Collagen-induced arthritis is an experimental autoimmune disease that can be induced with type II collagen in the appropriate rodent strains and non-human primates[11]. The collagen-induced arthritis rats have similarities in clinical and pathological features with human RA[12,13,14]. This current study explores the potential of plants from different origin which is ground-based and marine-based plants as alternative remedy for anti-inflammatory disorders. It has been reported that *Nigella sativa* and *Eucheuma cottonni* extracts possessed anti-inflammatory effects in carrageenan induced paw edema and experimental ovalbumin-induced allergic asthma respectively[15,16,17]. This work describes the effects of *Nigella sativa* and *Eucheuma cottonni* extracts in collagen-induced arthritis mice.

## MATERIAL AND METHODS:

### Collection and sampling of materials:

Dried *Eucheuma cottonii* was collected from Sabah, Malaysia and the *Nigella sativa* seeds originated from Saudi Arabia was purchased from commercial market. The materials were kept in a clean and dry place before the extraction process.

### Preparation of *Nigella sativa* and *Eucheuma cottonii* extract:

The seeds of *Nigella sativa* were cleaned and washed under running water to remove foreign substances. The seeds were air dried at room temperature and grounded to powder form. In a ratio of 1:10, the *Nigella sativa* powder was then soaked in distilled water. After 24 h, the solution was continuously stirred for 1 h at room temperature before it was filtered with clean cloth and filter papers. The solution was put in a rotary evaporator (BUCHI rotavapor R-216) at 37ºC (60rpm) in order to remove excessive solvents during the extraction process. The extracts were protected from lights and stored at 4ºC.

Aqueous extraction of *Eucheuma cottonii* was prepared according to the method described previously[18] with minor modification. 150 gm of dried *Eucheuma cottonii* were washed under running water to remove dirt and unwanted particles. The plant is air dried at room temperature and grounded until it became powder-like form. The powders were weighed and soaked in distilled water at a ratio of 1:10 dilution for 24 h. After that period, the solution was continuously stirred for 1 h at room temperature. The solution was then filtered with clean cloth and filter papers and the extracts obtained were stored at 4ºC.

### Preparation of collagen type II emulsion and complete Freund's adjuvant (CFA):

The collagen type II and CFA were prepared according to the method described previously[19]. The volume of collagen type II and CFA were calculated in a ratio of 1:1. Even though 50 µL of emulsion is required per mouse, extra must be made owing to both losses during processing and the dead space in the syringes during injection.

### Induction of arthritis and treatment:

40 male BALB/c mice (20-25 gm) between 9-12 weeks old were used for this study. They were housed in controlled environment with standard diet and water according to the guidelines provided by UniKL Animal Ethics Committee (Fyp/Aec/Mestech-Unikl/2016/15). The mice were induced with collagen type II emulsion and CFA and divided into separate groups. Slowly 50 µL of collagen type II emulsion and CFA were injected subcutaneously on the hind limb of the mice. After 21 days, second injection was given with the same volume as the first injection. The first injection was given to suppress the immune system, while the second injection was given to induce arthritis and allows neutrophil infiltration in the knee joints of the animals[11, 20].

The first and second group of mice was treated with *Eucheuma cottonii* and *Nigella sativa* extracts respectively, at 200 mg/kg of body weight orally once in a day[21,22]. The third group is the positive control group and was treated with 0.5 mg/kg of betamethasone daily. The fourth group represents the negative control and no treatment was given.

### Grading of histological inflammation:

The mice were evaluated on the first day and the last day (22nd day) for arthritic incidence. Each paw was evaluated and scored individually on a scale of 0 to 4, with 4 indicating the most severe inflammation[11]. Table 1 describes the severity score based on the degree of inflammation.

**Table 1: The scale for severity scores in collagen-induced arthritis mice based on the degree of inflammatory reactions**

| Severity score | Degree of inflammation |
|---|---|
| 0 | Normal |
| 1 | Swelling of one group of the joint (wrist or ankle) |
| 2 | Two groups of swelling joints |
| 3 | Three groups of swollen joints |
| 4 | Swelling of entire paw |

### Grading of neutrophil infiltration:

The mice were sacrificed on the 22nd day and the hind limbs were taken for assessment. The bones in the hind limbs were decalcified and fixated according to the previous method[23]. The specimens were then prepared for staining and histological analysis as described previously[24]. Each of the specimens was observed under the microscope to identify neutrophil infiltrations. The

neutrophil infiltration was recorded according to the histological activity index as describes in Table 2[25].

**Table 2: The scales for inflammatory activity scores in collagen-induced arthritis mice based on histological characteristics of neutrophils infiltration**

| Inflammatory activity | Score | Histological characteristics |
|---|---|---|
| Inactive / normal | 0 | No epithelial infiltration by neutrophils |
| Mildly active | 1 | Neutrophil infiltration of <50% of sampled cross sections, no ulcers or erosions |
| Moderately active | 2 | Neutrophil infiltration of ≥50% of sampled cross sections, no ulcers or erosions |
| Severely active | 3 | Erosion or ulceration, irrespective of other features |

**Statistical analysis:**

The statistical analysis performed includes normality test with a correlation of 98%. The results recorded were presented as mean ± Standard Error Mean (S.E.M.). The data was analyzed using one-way ANOVA with post hoc corrected t-test for significant differences among the different groups. The differences among the groups were considered to be significant at $p<0.05$.

## RESULTS:

**Inflammation severity score:**

Inflammation scoring was performed on the 22nd day of the experiment before the mice were sacrificed. Each of the knee joints of the mice were assessed and graded based on the severity score in Table 1. Both groups of mice treated with *Eucheuma cottonii* and *Nigella sativa* extracts presented a substantial reduction in inflammatory reactions with severity score of 1.6 ± 0.24 than the negative control group (severity score of 3.4 ± 0.24).Fig. 1 shows the severity score in the knee joints of the mice. As a result of inflammation induced by the collagen emulsion, mice in the negative control group exhibited the most noticeable increase in inflammatory reaction. *Eucheuma cottonii* and *Nigella sativa* treated mice showed mild inflammation which is comparable to the outcomes for the positive control group (severity score of 1.2 ± 0.2).

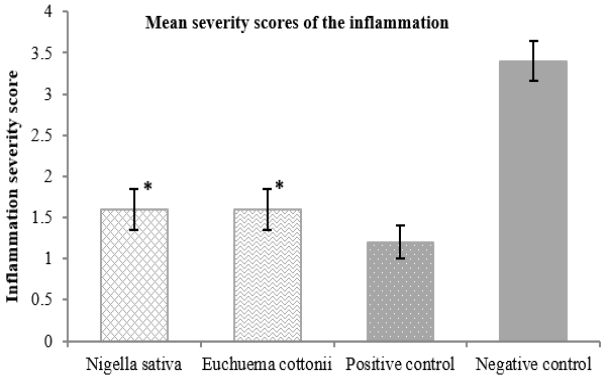


**Fig. 1: Mean severity scores of the collagen-induced arthritis mice. * p<0.05.**

**Neutrophil infiltration score:**

The presence of neutrophil bodies were calculated during histopathological analysis in order to rate its infiltration in the knee joints of the mice (Fig. 2). The infiltrations were scored based on the histological characteristics in Table 2. The results indicate that neutrophil infiltrations in mice treated with *Nigella sativa* were significantly reduced (62.2 ± 14.87) as compared to the negative control group (103.6 ± 9.44).

Fig. 3 depicts the number of neutrophils present in the histopathological specimens of the knee joints in the mice. The results demonstrates that mice treated with *Nigella sativa* extract has a lower number of neutrophil infiltration than the positive control group (78.6 ± 14.51) and significantly different as compared to the negative control group ($p<0.05$). Neutrophil infiltration in *Eucheuma cottonii* treated mice however showed no significant differences.

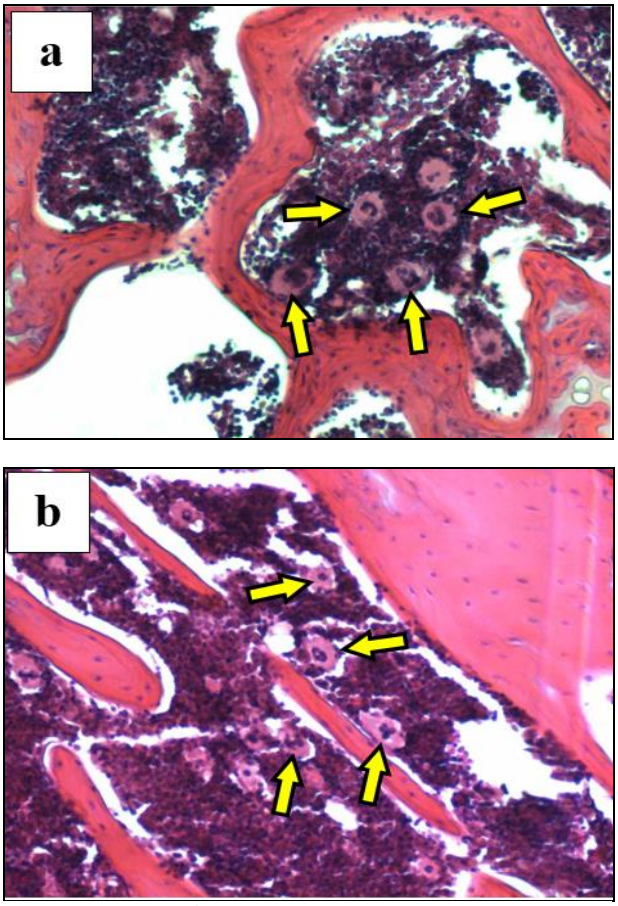


**Fig. 2: Neutrophil infiltrations (yellow arrow) in mice treated with (a) *Nigella sativa* and (b) *Eucheuma cottonii*. Mouse neutrophils have multilobed nucleus and appears in 'U-shaped' or 'Ring-shaped' form.**

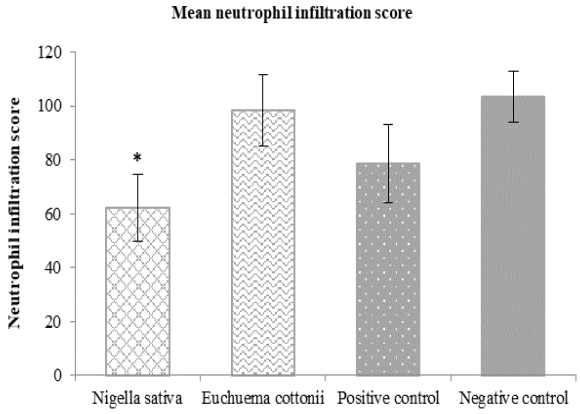


**Fig. 3: Mean neutrophil infiltration scores of the collagen-induced arthritis mice. *p<0.05.**

## DISCUSSION:

RA is a disease that can affect the health and life of a person who is suffering from the chronic joints damage[26,27]. During autoimmune incidence in RA patients, T cell lymphocytes are activated and proliferate to secrete cytokines[1,28]. Cytokines then stimulates the macrophage. Activated macrophages in the synovial joints will involve in the activation of inflammatory cells, cell contact and overexpression MHC Class II molecules. The progression and persistence of acute or chronic state of inflammation is mediated by a mediator that secreted by inflammatory cells and this can cause fluid accumulation in the synovium[29,30].

Natural plant based treatments have a wide-ranging biological activity and its molecular targets are not well defined[31, 32]. In this study, mice treated with *Nigella sativa* exhibited swelling which is confined to the tarsal/ankle joints or swelling that extended from the ankle to the tarsal area. This suggests that *Nigella sativa* extract can reduce the fluid accumulation in the synovial joints and reduce inflammatory reactions in collagen-induced arthritis model. In chronic state of inflammation, there are more neutrophils that have infiltrated the synovial joints[33,34]. The index of neutrophil infiltration in synovial joints is correlated with the number of neutrophil presents in the tissues. Low number of neutrophil counts after *Nigella sativa* treatment means the extracts could also reduce neutrophil infiltration in the synovial joints. It is believed that the presence of thymoquinone in *Nigella sativa* extracts can inhibit both, cyclooxygenase (COX) and lipoxygenase, the two main enzymes that can mediate inflammation[9, 35].

*Eucheuma cottonii* has shown to be a potential anti-inflammatory, anti-histamine and antioxidants source. Lectins, the active components found in *Eucheuma cottonii* is believed to have an anti-inflammatory properties[36]. Although mice treated with *Eucheuma cottonii* extracts showed slightly high neutrophil infiltration counts, the inflammation severity scores based on the swelling of the knee joints were evidently smaller. This effect could be due to the solvent used for the extraction process. The aqueous extract of *Eucheuma cottonii* has low active components than other solvents used for extraction[6]. In the normal condition, the volume of synovial fluid varies from joint to joint. When inflammation occurs, the vascular permeability and synovial membrane permeability will be altered and the protein content changes in diseased synovial fluid.

## CONCLUSION:

The present study indicates that *Nigella sativa* has the ability to reduce inflammation and the severity of collagen-induced arthritis in mice with comparable results to the positive control group. On the other hand, *Eucheuma cottonii* shows some potential to lessen inflammatory reactions in the animals albeit not as effective as *Nigella sativa* treatment. The anti-inflammatory properties demonstrated in this study could be attributed to the presence of active compounds such as thymoquinone in *Nigella sativa*; and terpenes and peptides in *Eucheuma cottonii*. This finding suggests that effectiveness of the extracts might be due to the intervention along inflammatory mediators by the active compounds. Thus, it can be implicated that the extracts particularly *Nigella sativa* seeds have the capabilities to reduce inflammation in the collagen-induced arthritis animal model.

## ACKNOWLEDGEMENT:

The authors gratefully acknowledged Universiti Kuala Lumpur (UniKL) and the Final Year Project research program for providing financial support.

## CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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