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Anti-inflammatory activity of *Myristica fragrans* extract

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ABSTRACT

In simple terms, inflammation can be defined as a reaction from the body to an injury in living tissue. Anti-inflammatory drugs help in controlling and reducing this inflammation. Natural spice showing anti-inflammatory properties with no side effects, hence they can be used as an efficient anti-inflammatory drug in the near future. To determine the anti-inflammatory activity of *Myristica fragrans* (Nutmeg) using MTT Assay. The plant material was obtained as a gift sample from Life Care Phytolabs Private Limited. An extract was prepared from the sample. Cell viability assay – MTT Assay was performed, and Raw cell line 247 was used to study the anti-inflammatory potential of the extract. The results collected were put into a graph and table for discussion. A gradual decrease in the number of inflammatory cells as the concentration of the extract was increased was observed in the inflammatory cell line. The cell viability, which was 7.08% when the concentration of the extract was 1ng increased up to 30.6% when the concentration of the extract was increased up to 100ug. The MTT assay test on a raw cell line 247 showed that the *Myristica fragrans* extract exhibits some level of the anti-inflammatory property. Further research on isolating the specific component of the extract responsible for its anti-inflammatory property can be done in the future.

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INTRODUCTION

An inflammatory response is mainly triggered by mechanical injury or due to chemical toxins, sometimes invasion by microorganisms and also hypersensitivity reactions. The major events that occur during inflammation are: blood supply to the area is increased, the permeability of the capillaries is increased, and leucocytes shift from the capillaries into the surrounding interstitial space and to the site of the inflammation (Punchard *et al.*, 2004; Rankin, 2004; Pober and Cotran, 1990).

Inflammation is mostly observed as the local accumulation of leukocytes, plasma proteins and body fluids in an extravascular site of injury or infection. Normally inflammation is a part of the immune system in the host's body. It is useful for getting rid of all infectious agents that caused the inflammation from the site. However, inflammatory reactions can sometimes be harmful to the surrounding tissues and cells, especially when in an excessive manner (Medzhitov, 2010).

An ideal inflammatory response has four components: inflammatory inducers, sensors, mediators and the target tissues. The type of response and the degree of it depends on the nature of the trigger, be it bacterial, viral or parasitic and its persistence (Aderem and Ulevitch, 2000). The Bac-

terial pathogens are recognized by pattern receptors (Darveau, 2010; Baldwin, 1996), which are expressed on macrophages. Binding of these receptors induces the production of cytokines, chemokines and mediators like prostaglandins. These inflammatory mediators play a major role in establishing an inflammatory response and in the clearing of the bacteria.

After sensing the inflammatory response, the innate immunity cells trigger the production of mediators. Neutrophils, macrophages, dendritic cells and mast cells produce some proteins called cytokines that control the initiation and maintenance of inflammation and regulate its degree and duration (Hanada and Yoshimura, 2002; Rossi and Zlotnik, 2000). Chemokines are cytokines that exhibit chemo-attractant properties that cause the migration of cells to the site of the infection. Once leukocytes exit the blood vessels, they are attracted by chemotactic factors to the site of infection (Zlotnik and Yoshie, 2000; Serhan, 2009). Chemokines are produced by various cells like endothelial, epithelial and stromal cells. They are also found to be synthesized by leukocytes. Therefore inhibiting their activity can be a very effective anti-inflammatory strategy.

Excessive or large production of inflammatory mediators like prostaglandins and leukotrienes with increased sensing response to inflammatory triggers is associated with the development of acute inflammation to chronic inflammation in many cases (Higgs *et al.*, 1979).

Many anti-inflammatory drugs, e.g. aspirin-like drugs, etc. inhibit prostaglandin synthesis but are not really capable of preventing the generation of the hydroxy acid 12-l-hydroxyeicosatetraenoic acid (HETE). HETE is a chemoattractant for leukocytes. This explains why aspirin-like drugs have little effect on leukocyte migration. Thus showing, limited anti-inflammatory property (Jain *et al.*, 2017).

Myristica fragrans (Nutmeg) is an important spice derived from the genus Myristica. It is an evergreen tree, indigenous to the Banda Islands in Indonesia (Jangid *et al.*, 2014). *Myristica fragrans* has four parts - skin, fruit, seed and mace. The Skin, pulp, mace and seed have always been popularly used in traditional medicines of India, China and Thailand [15]. The major constituents of the essential oil of nutmeg are myristicin, elemicin, safrole and sabenene. Nutmeg extract may serve to be an effective anti-inflammatory drug in the future.

Thus, the cytotoxic test, that is the MTT test is done to determine how safe the extract is for therapeutic use since one of the components of the extract called Myristicin, is known to have a toxic property. Hense an inflammatory cell line 247 is used.

MATERIALS AND METHODS

The plant material for *Myristica fragrans* was obtained as a gift sample from Life Care Phytolabs Private Limited. An extract was prepared from the sample in an alcohol base after boiling for 24 hours and filtering through a filter paper. The test used to demonstrate the anti-inflammatory activity of nutmeg here was the Cell viability assay – MTT Assay, which was performed on a Raw cell line 247. This was used to study the anti-inflammatory potential of the extract. The results collected were put into a graph and table for discussion.

RESULTS AND DISCUSSION

Figure 1 shows the inflammatory cell line, raw cell line 247. In the MTT, with the drug concentration of the control, the presence of numerous inflammatory cells is observed. But at the concentration of 10ng, there seems to be a decrease in the inflammatory cells, and with 100ug, a further decrease is observed.

Table 1 Shows the table depicting the cell viability percentage associated with the concentration of the extract used. In control, the cell viability was found to be only 3.87%, at 10ng, it increased to 11.6%, and at 100ug it was as high as 30.6%. Also, having an IC50 of 163.34ug shows that it has cytotoxicity that clears away the inflammatory cells.

Table 1: Anti-inflammatory activity of Nutmeg

S.No	Concentration	Cell Viability (%)
1	SC	3.871352
2	1ng	7.087552
3	10ng	11.67362
4	100ng	15.48541
5	1ug	22.33472
6	10ug	25.31269
7	100ug	30.61346

Figure 2 In a research conducted by the Faculty of Science, Obafemi Awolowo University in Neigeria, the results showed that the myristica extract demonstrated very effective in vitro anti-inflammatory potentials with maximum percentage stability of 88 ± 0.45 %, exhibited in a biphasic mode of response which was comparable with that of Ibuprofen, which is a standard anti-inflammatory drug.

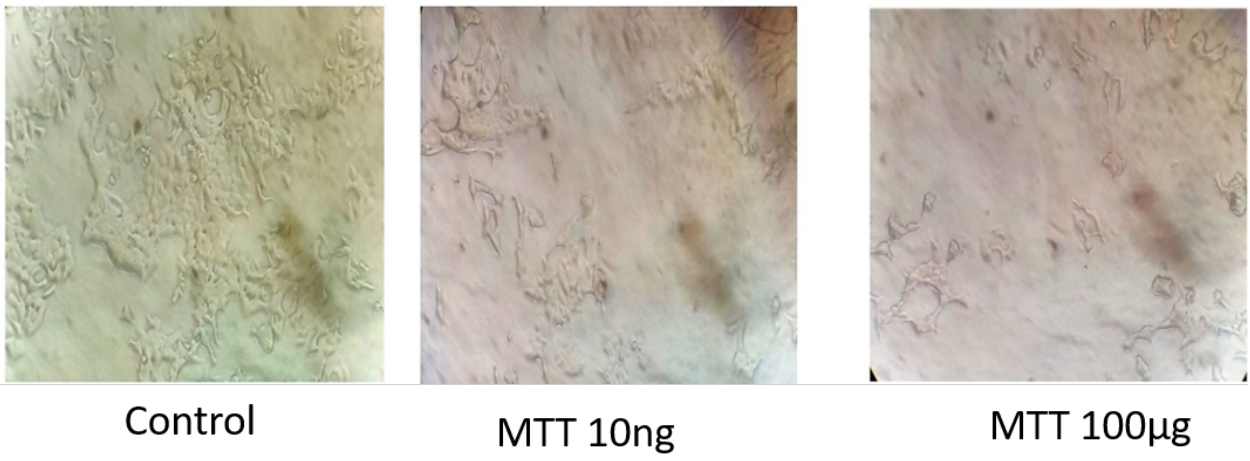

Figure 1: RAW – 247 Cell line (Inflammatory cell line)

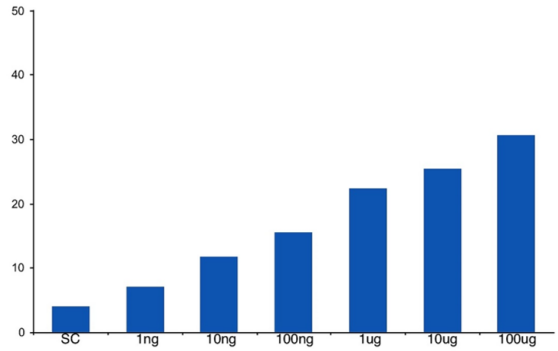

Figure 2: The details of the table in a graph form

CONCLUSIONS

Though standard synthetic drugs seem to be very efficient in their anti-inflammatory property, they are still associated with numerous side effects like abdominal pain, bloating, burning sensation, cramps, constipation, abnormal urine output, etc. However, *Myristica fragrans*, being a natural spice, has no such side effects. It also is shown to possess anti-inflammatory properties aside from antibacterial properties. Therefore, it possesses a good potential to be a source for anti-inflammatory drugs.

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