



Anti-inflammatory Activity of *Myristica fragrans* (Nutmeg) using HRBC Membrane Stabilising Method

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ABSTRACT

The aim of the study is to determine the anti-inflammatory activity of *Myristica fragrans* (Nutmeg) using HRBC membrane stabilising method. Inflammation being a common symptom for various diseases has to be treated properly. The anti-inflammatory drugs come into role here in decreasing the inflammation. Nutmeg spice is a good source of minerals like copper, potassium, calcium, manganese, iron, zinc and magnesium. Since it's a natural drug and has a lot of anti-inflammatory properties having no side effects, therefore it is better than synthetic drugs. Nutmeg being a natural drug with least side effects, in comparison with the other drugs can be used in the future to produce an efficient anti-inflammatory drug.

Keywords: *Myristica fragrans* (Nutmeg), anti-inflammatory, diclofenac, HRBC membrane stabilising

INTRODUCTION

A spice is a seed, fruit, root, bark, or other plant substance primarily used for flavoring, colouring or preserving food. They are distinguished from herbs, which are the leaves, flowers, or stems from plants used for flavoring or as a garnish. Sometimes, spices may be ground into a powder for convenience.¹ Many spices have antimicrobial properties. This may explain why spices are more commonly used in warmer climates, which have more infectious diseases, and why the use of spices is prominent in meat, which is particularly susceptible to spoiling. Spices are sometimes used in medicine, religious rituals, cosmetics or perfume production, or as a vegetable¹⁴

Myristica fragrans (Nutmeg) is one of the two spices – the other being mace – derived from several species in the genus *Myristica*. The most important commercial species is *Myristica fragrans*, an ever green tree indigenous to the Banda Islands in the Moluccas (or Spice Islands) of Indonesia⁵. *Myristica fragrans* (Nutmeg) is about 25 feet high which doesn't bloom till it is nine years old, when it fruits; it continues to do so without attention. Nutmeg has four parts - The skin, the fruit, the seed and the mace. Fruit is a pendulous, succulent pericarp, the mace arillus covering the hard endocar and a wrinkled kernel with ruminated endosperm. When the arillus is fresh it is a brilliant scarlet, when dry it is more of a horny, brittle texture, and a yellowish brown colour. The seed of nutmeg is firm, fleshy, whitish, traversed by reddish brown veins, abounding in oil. Skin, pulp, mace and seed have been widely used in traditional Ayurvedic; Chinese and Thai medicine². The major constituents in the essential oil of nutmeg contain myristicin, elemicin, safrole and sabinene which comprise 80% of the oils⁴. Used in small dosages nutmeg can reduce flatulence, aid

digestion, improve the appetite and treat diarrhea, vomiting and nausea.³

Inflammation is the most common symptom for most of the diseases. It has to be treated prior to the disease treatment since decreasing pain is the first step in a treatment procedure. This is where the anti-inflammatory drugs act and eventually decrease the cause inflammation. Inflammation is the body's attempt at self-protection to remove harmful stimuli and begin the healing process. Inflammation is a part of body's immune system. The first stage of inflammation is called irritation, which then becomes inflammation – the immediate healing process.¹¹

MATERIALS AND METHODS

Preparation of extract

The *Myristica fragrans* (Nutmeg) powder was purchased and used for the experiment. The solvent, Dichloromethane was mixed with the powder. The solution was stored for 24 hours. Then, the extract was filtered using a filter paper into a conical flask. The conical flask was kept on the hot plate and left till the volatile solvent gets evaporated and the semi-finals solid nutmeg powder was left behind for further procedures.

Isolation of RBC from blood sample for membrane stabilization assay

Blood sample was collected from a healthy human volunteer and used for the undergoing experiment. The human red blood cell (HRBC) membrane stabilization has been used as a method to study and evaluate the in vitro anti-inflammatory activity of *Myristica fragrans* (Nutmeg). The blood sample collected was stored at 4°C for 24 hours before it was used for the experiment. Then, it was centrifuges at 2500 rpm for 5 minutes and the supernatant was removed. The cell suspension was



washed with sterile saline solution (0.9% w/v NaCl) and centrifuged at 2500rpm for 5 minutes. This was repeated three times till the supernatant was clear and colour less and the packed cell volume was measured.

The cellular component was reconstituted to a 40% suspension with phosphate buffered saline (16g NaCl, 0.4g of KCl, 2.88g of Na₂HPO₄, 0.48 g of KH₂PO₄, 100 ml of H₂O) and used in the assay.

Hypotonicity Induced Human Red Blood Cell (HRBC) membrane stabilising method:

1.0 mL of test sample of different concentrations (200, 400, 600, 800, 1000 µg) in 1 ml of 0.2 M phosphate buffer and 0.5 mL of 10% HRBC suspension, 0.5 ml of 0.25 % hyposaline were incubated at 37C for 30 min and centrifuged at 3,000 rpm for 20 min and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm¹⁰. Diclofenac was used as standard and a control was prepared by distilled water instead of hypo saline to produce 100 % haemolysis without samples. The percentage of HRBC haemolysis and membrane stabilization or protection was calculated by using the following formula:

$$\% \text{ of Hemolysis} = \frac{\text{Optical density of test sample}}{\text{Optical density of control}} \times 100$$

$$\% \text{ Protection} = 1 - \left[\frac{\text{Optical density of test sample}}{\text{Optical density of control}} \times 100 \right]$$

RESULT AND DISCUSSION

The following results were obtained during the performed experiments:

Table 1: Shows the Haemolytic Activity % of the sample and positive control (Diclofenac)

Concentration (µg)	% haemolysis	
	Sample	PC
200	55.09766	17.02082
400	46.91994	10.968019
600	42.584246	10.58167
800	33.784074	5.022537
1000	24.125349	2.5541962

Hemolytic activity

The *Myristica fragrans* (Nutmeg) extract exhibited membrane stabilising effect by inhibiting hypotonicity induced lysis of erythrocyte membrane.

The lysosomal membrane is similar to the erythrocyte membrane and its stabilisation implies that the extract may as well stabilise lysosomal membranes. The importance of stabilising the lysosomal membrane is in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as protease and bactericidal enzymes which cause

cellular release. Though the exact mechanism of the membrane stabilisation by the extract is not known yet, hypotonicity induced haemolysis may rise from shrinkage of the cells due to osmotic loss of intracellular electrolytes and fluid components. The sample is compared to a positives control to check the percentage of haemolysis of HRBC cells. As seen in table 1, it started with a minimal concentration of 200 µg the sample showed a haemolysis of 55.09% while the positive control's activity on haemolysis is 17.02 %.The percentage of haemolysis is depicted in Figure 1.

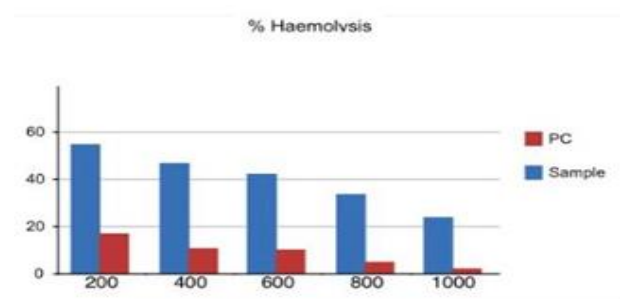


Figure 1: Shows the percentages of Hemolytic activities of the sample and positive control (Diclofenac)

As and when the concentrations of the extract increase, the haemolysis activity decreases. When the concentration was 1000 µg, the samples activity on haemolysis was 24.12% while the positive control activity was 2.55%. Thus, lower the percentage of haemolytic activity, greater the anti-inflammatory. In the verified results, both the sample and positive control showed anti-inflammatory activity, but positive control being more efficient.

Protective activity

Table 2: Shows the Protective Activity % of the sample and positive control (Diclofenac)

Concentration (µg)	% Protection	
	Sample	PC
200	44.90234	82.97918
400	53.08006	89.031981
600	57.415754	89.41833
800	66.215926	94.977463
1000	75.874651	97.445804

The sample is compared with a positive control to check the percentage of protection of HRBC cells. As seen in Table 2, when started with a minimal concentration of 200µg the sample showed a protection of 44.90% while the positive control activity on protection was 82.97%. As and when the concentrations increases, the protective ability of both the sample and the positive control were increased. When the concentration was 100µg, the samples protective activity was 75.87% while the positive control activity on protection was 97.44%. This is depicted in the form of graph in Figure 2.



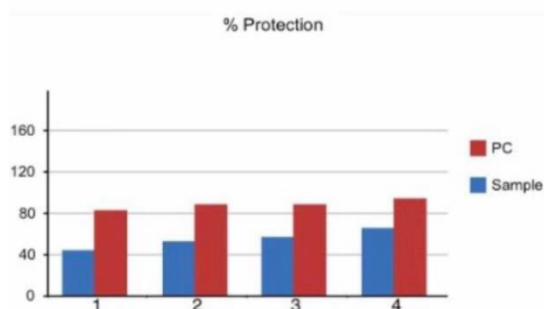


Figure 2: Shows the percentages of Protective activities of the sample and positive control (Diclofenac)

CONCLUSION

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self-cells) and to remove dead or damaged host cells⁶. In the inflammatory response there is an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines. At the same time, there is also an induction of the activity of several enzymes as well as the arachidonic acid metabolism.⁷ Inflammation involves the activation and recruitment of phagocytes (macrophages, neutrophils), NK cells, complement system and secretion of cytokines like IL-1 β , IL-6, TNF- α by activated cells which are essential for the host defence system⁸

In the above results, obtained from the performed experiments, the positive control or standard (Diclofenac) has more efficient anti-inflammatory effect compared to Nutmeg extract. But the standard being a synthetic drug, is associated with many side effects such as abdominal pain, stomach bloating, burning, cramping, belching, tarry stools, cloudy urine, constipation, decrease in urine output or decrease in urine concentrating ability, diarrhoea, dizziness, feeling of indigestion, headache, increased bleeding time, itching, skin or rash, loss of appetite, nausea and vomiting, pain in the chest below the breast bone, pale skin, severe stomach pain, swelling, troubled breathing with exertion, unusual bleeding or bruising, unusual tiredness or weakness, vomiting of blood or material that looks like coffee grounds, weight loss.¹²

Comparatively, Nutmeg spice is a good source of minerals like copper, potassium etc. being a natural compound has very less side effects and can be used in combination with other compounds for better anti-inflammatory activity in the future.

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