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Anti-Inflammatory and Cytotoxic Effect of Nutmeg Based Gel

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ABSTRACT
Aim: The aim of this study was to determine the anti inflammatory and cytotoxicity of Nutmeg based gel.
Introduction: Nutmeg or Myristica fragrans is commercially widely used as a culinary product in the food industry. It also is known to have inflammatory action due to the presence of anti-inflammatory compounds called mono terpenes including terpene, pinene, sabinene, etc. Since it is a naturally obtained product, it is considered better than synthetic formulations in reducing inflammation.
Materials and Methods: The gel was formulated using the plant extract of Nutmeg and the anti inflammatory action was tested via (BSA) Albumin denaturation assay. Cytotoxic analysis was made using brine shrimp nauplii. Results were tabulated in Microsoft Excel Sheets and imported to SPSS software for obtaining graphs.
Result and discussion: There was less cytotoxic action with decreasing concentration and anti inflammatory activity was assessed and percentage inhibition rate was highest in concentration of 50 microliters . Less cytotoxic activity was seen in 5-10 microliters.
Conclusion: The result shows that Nutmeg based gel has high potential in anti inflammatory activity and shows less cytotoxic activity. Hence, this nutmeg derived gel can be used for dose dependent formulations of drugs targeting inflammation in the near future.
Clinical relevance: Nutmeg gel has been used since ancient times for dental problems in children due to the plethora of medicinal uses that it contains. This study will help us to scientifically prove a basis for this explanation and further bring out its clinical use in dentistry for diseases such as periodontitis, gingivitis, inflammatory diseases of the oral cavity, etc.
Future scope:In the light of the study performed, it is safe to use 5-10µl of this formulation for drug formulations.
Keywords: Nutmeg gel , Cytotoxic, Albumin denaturation assay, anti inflammatory, novel study, innovative study

INTRODUCTION:

Nutmeg or Myristica fragrans is highly known as a culinary agent and several studies have been conducted to assess it in intro inflammatory action(1).
Nutmeg is rich in several anti-inflammatory compounds known as monoterpenes which also include other compounds called Sakinene, Terpineol and Pinene (2). These may contribute to aid in reducing inflammation in our body and are beneficial to those people with several inflammatory conditions(1,3).
In alternative medicine, the use of M. fragrans has been proven to portray aphrodisiac, memory enhancing and energizing, antidiarrheal and laxative properties, anti-inflammatory and anti-carcinogenic properties(1,3,4)
The main motive behind opting nutmeg for the present study is that, in spite of its use in various medical conditions traditionally, nutmeg has not been absolutely explored for their other benefits(5). Several potential characteristics such as its innate antioxidant and antimicrobial potential, which may be effectively shown due to the presence of a variety of active phytochemicals that includes vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans and phenolics, etc are still left to be studied about in detail(6). These compounds offer their outcomes through different processes such as free radical scavenging, metal chelation, inhibition of lipid peroxidation and quenching of nascent oxygen to play its role as antioxidants (7).

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Results from a study, established with cyclic voltammetry. Acetone extract of nutmeg mace and its subsequent TLC isolated fractions composed mainly of lignans was shown by GC–MS analysis and it further proposed the potential use of nutmeg mace and their extracts as a nutraceutical in prevention of oxidative damage to cells of living (8).
Nutmeg during the 17th century was an extremely high value commodity. Not only since it is used as an exotic spice but also for its medicinal values (9).
In recent scientific progression, components of Myristica fragrans were observed to have significant antioxidant, anti-inflammatory and chemo preventive effects(10). The anti-inflammatory activity of these proportions was looked into carrageenin-induced edema and acetic acid-induced vascular permeability in rats and was established to be very efficient(11).
A study by Kuo YH et al, F-α concentrations and hepatic DNA fragmentation in laboratory mice. Thus the concluded findings suggested that the hepatoprotective activity of myristicin could likely be related to the inhibition of TNF-α release from macrophage cells(12).
Another study showed that Myristica formulated etosome gels can control the drug's delivery and can increase its penetration transdermally(13). Hence having good bio availability. The anticariogenic compound was also isolated from the methanol extract of *M. fragrans* by a process of repeated silica gel chromatography, and its structure was recognised as macelignan by using instrumental analysis which portrayed efficient activity against several known oral microorganisms(14).
The aim of this study is to assess and determine the anti inflammatory and cytotoxic activity of Nutmeg based gel.

MATERIALS AND METHODS

Plant extract preparation

2 grams of Nutmeg powder was measured and taken . The measured amount of plant powder was then mixed with 100 ml of distilled water and then the contents was filtered (shown in figure 1).

Nutmeg gel preparation

0.1g of Methyl Paraben was mixed with 5 ml of distilled water and kept to heat for 15 to 20 minutes along with constant stirring. Then 2ml of prepared plant extract was taken and mixed with 13 ml of distilled water. 0.3 gm of Carbopol was then added to the mixture (as shown in figure 2).

Albumin Denaturation Assay

Bovine Serum Albumin, also called BSA, was used as a reagent for the assay. BSAis known to make up approximately 60% of all proteins present in animal serum and hence is very widely utilized in all cultures. 2ml of 1 % Bovine Albumin fraction was mixed in different concentrations of nutmeg gel preparation of 10ml, 20ml,30ml, 40ml and 50ml respectively and pH was adjusted to 6.8 using 1N HCL. Then the mixture was heated using a heating mantle at 55 degrees Celsius for 10 minutes in a water bath . Mixture is left to cool down and absorbance value was obtained at 660nm. Equal amount of plant extract was then replaced with DMSO for control group . Anti inflammatory activity was assessed (as shown in figure 3).

Cytotoxicity analysis

3g of sea salt was measured using an electronic weighing scale and mixed with 300ml of distilled water. A microplate was taken and 5 wells were marked 5ul, 10ul, 20ul, 30ul, 40ul and 50ul respectively along with one well marked as control . The wells were filled with the prepared salt solution and 10 brine shrimp nauplii were added to each well respectively using a transfer pipette.
Then the prepared gel was added to each well according to each concentration using the micropipette. The plate is kept undisturbed and observed after 12 hours (as shown in figure 4).

RESULTS

In vitro anti-inflammatory activity of Nutmeg based gel

A potential increase is seen in the anti-inflammatory action in the property of Nutmeg based gel in increasing concentration when compared to the standard Diclofenac (shown in graph 1). The following calculation was done on the basis of readings obtained:

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CALCULATION:

$$\text{Percentage inhibition} = \frac{AC - AS * 100}{AC}$$

where AC is the absorbance of Control and the AS is the absorbance of the sample.

Graph 1 shows Anti-inflammatory activity of nutmeg gel. Bar chart depicts correlation between concentrations of nutmeg gel and its percentage inhibition of anti-inflammatory activity respectively. X-axis represents concentrations of nutmeg gel and Y-axis represents percentage inhibition. The blue bar represents the values of the standard diclofenac used and the green bar represents the nutmeg gel values. The highest anti-inflammatory activity was observed at 50µL.

Cytotoxic activity of Nutmeg based gel

There were 60 brine shrimp nauplii in total with 10 nauplii distributed in each of the 5 wells including the control. In an interval of 12 hours the effect of the gel formulation was observed. It was observed that only 8 nauplii were alive in 5µL, 6 nauplii in 10µL, 3 nauplii in 20µL, 2 nauplii in 40µL and 1 nauplii in 80µL respectively. The control well had 10 brine shrimp nauplii alive.

The results show that there was adequate anti-inflammatory potential and reduced cytotoxicity levels with respect to decreasing concentrations.

Several studies where in the stability test of Nutmeg Gels showed reduced contents when storing at 30 degrees C till 70 degrees C and that the flux of penetration of Myristica Etosome gel had higher penetration (shown in graph 2).

Graph 2 shows Cytotoxic activity of nutmeg gel. Bar chart depicts correlation between concentrations of nutmeg gel and its cytotoxic activity on live nauplii respectively. X-axis represents concentrations of nutmeg gel and Y-axis represents the number of live nauplii. The blue bar represents the number of live nauplii on day 1 and the green bar represents the number of live nauplii on day 2. The highest cytotoxic activity was observed at 80µL.

DISCUSSION

A potential increase in the anti-inflammatory property of nutmeg gel in increasing concentration when compared to the standard diclofenac shows that this formulation can be of good therapeutic benefit in case of many inflammatory diseases.

In a study published in 2018, the essential oil and hydrolats that were obtained from Myristica fragrans seeds was utilised along with Magnesium Aluminometasilicate as an excipient in order to evaluate the potential antioxidant, antibacterial and anti-inflammatory activity (15). Their results showed that the use of magnesium aluminometasilicate as an excipient could potentially get altered and in some cases it could improve the biological activities portrayed by essential oil of nutmeg and hydrolats and prove to be beneficial (16). Moreover, researchers try to incorporate various nanotechnologies for drug delivery of Myristica fragrans oils or fruit derivatives (17).

In our study, we used the nutmeg seed in powdered form so as to derive more of its natural contents such as monoterpenes and preserve it in gel form which highly favors anti-inflammatory activity (18). The gel form of nutmeg not only makes it easier for direct application but also better drug absorption intra orally.

The results we obtained from the performed experiments showed that the positive control or standard (Diclofenac) has an equal or lesser anti-inflammatory effect compared to Nutmeg based gel. We can derive that the least anti-inflammatory was observed at 10µL and the highest was observed at 50µL, which was slightly more than the effect by the standard used.

Scientific literature has always studied various substitutes for the current line of commonly used synthetic drugs (19). In an article published by Vellapally et al., the standard used, being a synthetic drug, can be associated with many side effects such as abdominal pain, stomach bloating, burning, cramping, tarry stools, cloudy urine appearance, constipation, reduced urine output, diarrhea, dizziness, feeling of indigestion, etc (20).

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Whereas , our study chose nutmeg since it is a good source of minerals such as copper and potassium. It is also a natural compound that has very few side effects and can be used in combination with other compounds for better anti-inflammatory activity in the future.

There has been a considerable increase in the exploration of gel based therapeutics for the treatment of cancers, inflammation, allergy, infections and other oxidative stress related disorders(21).

Our results also showed the cytotoxic effect of the nutmeg gel was the least at 5µL proving as a starting point for basing future studies based on the compound. The cytotoxic activity, however, increased with increasing concentrations and was recorded the highest at 50µL.

An article published in 2020 showed that nutmeg derivatives itself possesses a strong antibacterial power category against all tested bacteria(22). Nutmeg comprises terpenes which is what is known to prevent diseases like cancer and has properties such as immunogenic modulation and anti-inflammation(23).

Due to several such inputs, our study made sure to bring light into these facts and experiment to naturally synthesize nutmeg based gel so as to be able to explain its usage in dentistry (24) Since the mucous membrane is well penetrable, our gel would be able to adopt anti-inflammation mechanisms when penetrated into the mucous membrane of the oral cavity and thereby suppress swelling (25).

Literature suggests that the medicinal properties of Myristica fragrans is due to the presence of some active principle that gives rise to a certain biologic activity that causes interference in the biologic pathways of the bacterial cells (26).

The cytotoxic activity test of Nutmeg based gel indicates the decreased cytotoxic activity exhibited in lesser concentrations. In the future, a combination of therapeutic plants can be used along with Myristica fragrans so as to get a synergistic effect. Also, this shows that dose dependent formulations of nutmeg based gel may prove to be less toxic and safe for therapeutic treatments.

CONCLUSION

Thus the study proves beneficial as Nutmeg gel is assessed to be of good anti-inflammatory potential at high concentrations such as 50µL less cytotoxic at specifically lesser concentrations such as 5µL which can be used as a base to further scientific progression. Furthermore this study may help facilitate new drug designing and targeting treatments for various inflammatory disorders with minimal side effects.

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CONFLICT OF INTEREST:

The author declares no conflict of interest.

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prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm [Internet]. Vol. 148, Computer Communications. 2019. p. 176–84. Available from: http://dx.doi.org/10.1016/j.comcom.2019.09.020

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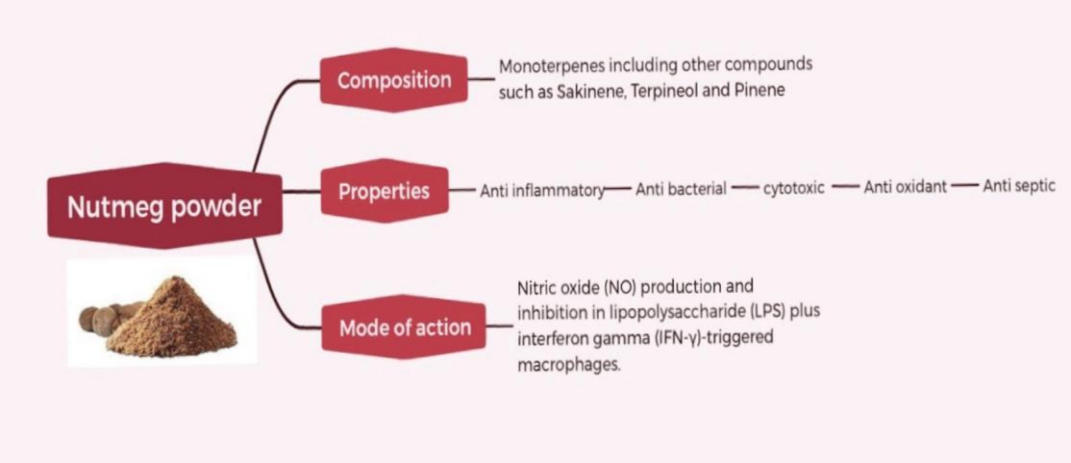
FIGURES AND TABLES



Flowchart 1. Composition, properties and mode of action of Nutmeg gel

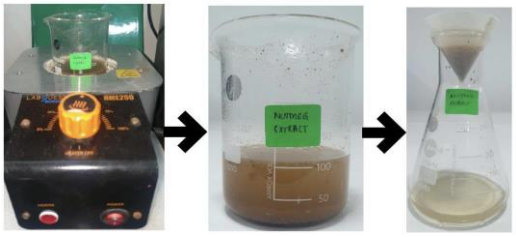


Figure 1. Plant extract preparation

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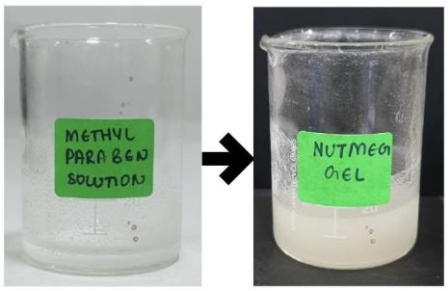


Figure 2. Nutmeg gel preparation

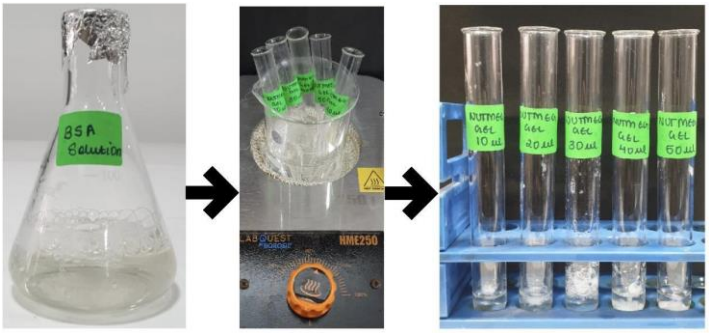


Figure 3: Albumin denaturation assay

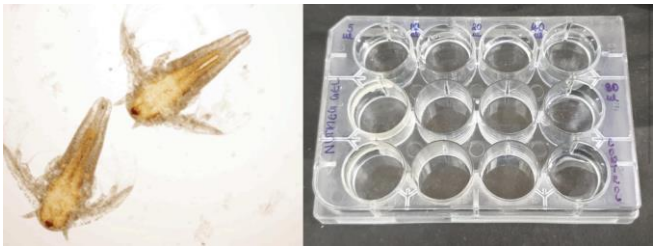
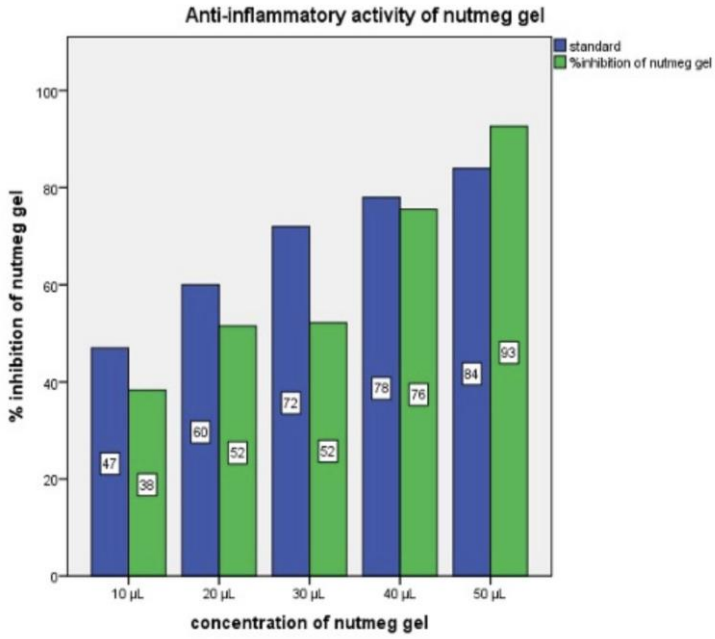


Figure 4. Cytotoxicity analysis



Graph 1. Anti-inflammatory activity of nutmeg gel.

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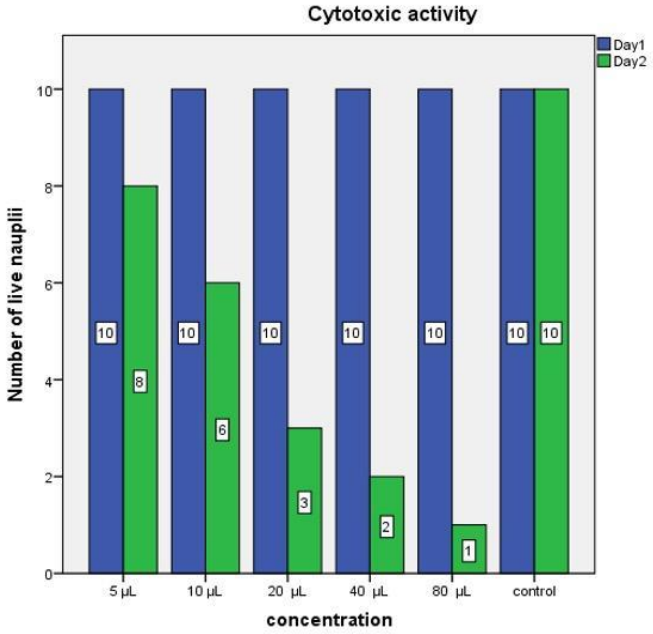

Graph 2. Cytotoxic activity of nutmeg gel.