

Nutmeg Oleoresin Assisted Copper Sulphide Nanoparticles and Its Anti-Inflammatory Activity

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ABSTRACT

Introduction: Nutmeg is the seed or ground species of several species of the genus *Myristica*. *Myristica fragrans* is a dark leaved evergreen tree Cultivated for two species derived from its fruit. Nutmeg oleoresin is naturally obtained by the pleasant, warm taste. It is semi solid, waxy resin with colours ranging from white to light orange. Nutmeg oleoresin is primarily used in flavouring applications and it is also used in baked products, meat, poultry, seafood and seasonings. Nutmeg is rich in anti-inflammatory compounds called monoterpenes, including Sabinene, terpineol and pinene. This may help to reduce inflammation in our body and benefit those with inflammatory conditions.

Aim : The aim of the present study was to evaluate the anti - inflammatory activity of nutmeg oleoresin assisted copper sulphide nanoparticles.

Materials and methods: The nutmeg oleoresin mediated copper sulphide nanoparticles were synthesized and were confirmed by UV spectroscopy. The anti-inflammatory property of the prepared nutmeg oleoresin mediated copper sulphide nanoparticles was assessed using albumin denaturation inhibitory assay technique.

Results: The present study showed an inhibition of 30% in 10µL and 90% in 50µL of NMO CuNPs. The nutmeg oleoresin mediated copper sulphide nanoparticles showed good anti-inflammatory activity with increasing concentration of the nanoparticle.

Conclusion: In the present study the nutmeg oleoresin mediated copper sulphide nanoparticles have shown good anti-inflammatory activity. In the future this can be developed into a very successful drug for anti-inflammatory action after evaluating the anti inflammatory activity in animal models.

KEYWORDS:

Nutmeg; inflammation; nanoparticles; UV Spectroscopy; oleoresin

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INTRODUCTION

Nanotechnology is a branch of technology that deals with various dimensions and tolerances with less than 100 nanometres, especially with individual atoms and molecules (1) Nanotechnology has become an emerging field in medical science, because it offers the possibility of examining biological processes in ways that were not previously possible. The medical field of nanotechnology mainly deals with diagnostic and screening purposes, it also helps in developing

artificial cellular proteins such as receptors, DNA and protein sequencing using nanopores and nano sprays and it is also used in gene therapy and tissue engineering applications (2). Nanotechnology provides a range of tools which are capable of invigilating individual cells at the level of individual molecules. It enables researchers to investigate and monitor cellular and molecular function and to alter systems that are deregulated in disease. It is possible that nanomachines with the ability to circulate through the bloodstream, kill microbes, supply oxygen to hypoxic organs, or undo tissue damage could

one day be used to deliver the human body with medicines or even foods (3). Nanotechnology also plays a very important role in transforming cancer diagnostics and treatment, nanotechnology is capable of manipulating interactions which occur across microscopic and molecular length scales in the microbiome and the tumour microenvironment (4). The main aim of using nanoparticles in drug deliverance is because it has high efficacy in delivering drugs to the target site with less damage to the tissues(5).

Nutmeg (*Myristica fragrans* Houtt.) is one of the most popular spices, which belongs to the family Myristicaceae(6) . It is a tropical tree and commonly available in Malaysia, India, Indonesia and SouthEast Asia (7) . Nutmeg's flavor varies depending on its origin. Its flavor can vary from a sweet to a heavier taste (8). Oleoresins have the potential to withstand high temperature and undergo processes to a greater extent than essential oils (9). Oleoresin is a naturally occurring mixture of essential oil and a resin extracted from various spices with organic solvents. The type of solvent affects the quality and quantity of oleoresin obtained (10). Nutmeg oleoresins are often used in flavoring soft drinks, canned foods, and cosmetics (11). Nutmeg oleoresin is also used in the preparation of meat and vegetable dishes (12). According to various researches that have been done on nutmeg oleoresin has antioxidant, antibacterial, antidiabetic and anti-inflammatory activities . Previously, studies conducted includes other oleoresin , oils and plant extract directly or after preparing nanoparticles and concluded various pharmacological activity.(13)- (14)

Chronic anti-inflammatory diseases including gingivitis and rheumatoid arthritis are still one of the main health problems affecting a major population. At present, although synthetic drugs are dominating the market, their toxicity level is generally ruled out. Their prolonged use may cause severe adverse effects such as gastrointestinal bleeding and peptic ulcers (15). Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Our team has extensive knowledge and research experience that has translated into high quality publications which was a real motivation to take up the present study (16-20) (21). So the aim was to synthesise nutmeg oleoresin mediated copper sulphide nanoparticles and evaluate its anti-inflammatory activity.

MATERIALS AND METHODS

Study Setting

The present study was conducted in the Nanomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals after getting ethical approval. The ethical approval

number is IHEC/SDC/UG-1930/21/132.

Preparation of copper sulphide nanoparticles

0.5g of nutmeg oleoresins in 100ml distilled water was heated for 5-10min at 50 degree celsius using a heating mantle. To that 20millimolar of copper sulphate was measured and added to the previously prepared nutmeg oleoresin extract. After 2-3 days the formation of the nanoparticle was confirmed using UV- Visible spectroscopy and was then centrifuged and stored in airtight containers for further use.

Study of anti inflammatory activity

10µL, 20µL, 30µL, 40µL, 50µL of nutmeg oleoresin mediated copper sulphide nanoparticle was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

In order to calculate the percentage of zone of inhibition we use a formula that is,

$$\% \text{ inhibition} = \frac{\text{Control O.D} - \text{Sample O. D}}{\text{Control O. D}} \times 100$$

RESULTS

The synthesis of nanoparticles is a boon to modern nanotechnology. Even though there are advanced drugs available in the market, they have some side effects if they are consumed on a regular basis, because they are synthetic. So , nowadays researchers are focusing on drugs which can be obtained from natural products and hence, these natural products are under experimental process for the synthesis of nanoparticles. The reaction mixture showed a gradual increase in the colour development from pale yellow to mild blue colour and exhibited a strong absorbance at 280 nm in UV - Visible spectroscopy (Figure 1 and Figure 2). This indicated the formation of copper nanoparticles. The reduction in the copper ions present in the aqueous solution of copper complex in the nutmeg extract demonstrated the change in the colour.

When the anti-inflammatory property of nanoparticles was assessed (Figure 3 and Figure 4), it was found that the percentage of inhibition kept increasing with the increase in concentration of the nutmeg oleoresin mediated copper nanoparticles. This proved the effect of nutmeg against inflammation. In the production of nanoparticles, pH also plays a major role in it. pH affects both productivity and stability of a nanoparticle(22).

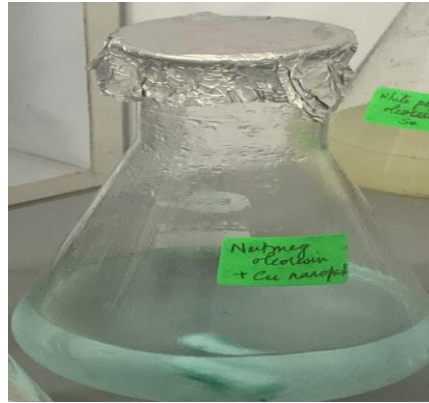


Fig.1: Nutmeg oleoresin mediated copper sulphide nanoparticles

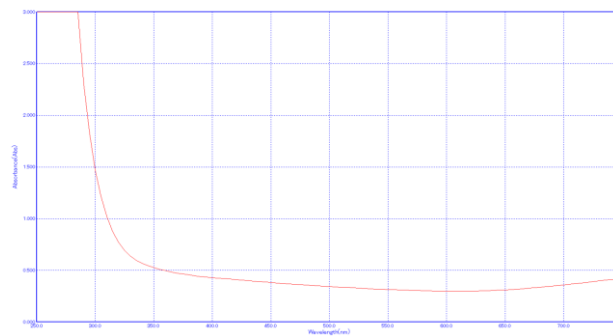


Fig.2: Graph showing the absorbance of nutmeg mediated copper sulphide in UV - visible Spectroscopy . X -axis represents wavelength in nm and the Y- axis represents absorbance.

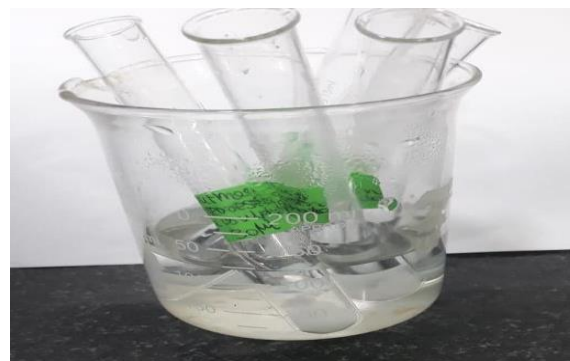


Fig.3: Image for anti inflammatory activity procedure.

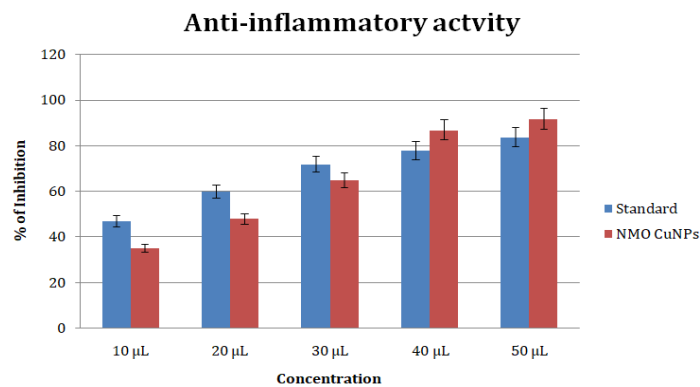


Fig.4: This figure shows the comparison of concentration of nutmeg oleoresin mediated with copper nanoparticles and percentage of inhibition. The standard value used here is Diclofenac Sodium.X-axis shows the concentration and Y-axis shows the Percentage inhibition.(n= 3 , mean +SD).Blue color represents standard diclofenac and red color represents Nutmeg oleoresin mediated copper nanoparticles.

DISCUSSION

The present study showed an inhibition of 30% in 10µL and

90% in 50µL of NMO CuNPs.The nutmeg oleoresin mediated copper sulphide nanoparticles showed good anti-inflammatory activity with increasing concentration of the nanoparticle.

In a study done by Priyanka Singh et al 2017 stated that *P. serrulata* fresh fruit extract is a green resource for the eco-friendly synthesis of P-AgNPs and P-AuNPs, which can further be utilized as a novel therapeutic agent for prevention and cure of inflammation due to their biocompatible nature(23) In a study done by Happy Agarwal et al 2019 stated that ZnONPs have good anti-inflammatory activity, stable nature and selective targeting property.(24) In a study done by Philippe Belle Ebanda Kedi et al, 2018 stated that the percentage of inhibition was 99% and 60% in vitro and in vivo respectively for silver nanoparticle-mediated *Selaginella myosurus* aqueous extract(25). Our college has conducted various studies and published data (26)-(27).

Limitations

Anti-inflammatory activity could have been done with different types of nanoparticles such as silver, gold or copper oxide which would have given different results when they are compared to each other that would have helped to enhance the result of the present study.

CONCLUSION

Our study concluded that the nutmeg oleoresin mediated copper sulphide nanoparticle showed an excellent anti-inflammatory activity at a higher dose of concentration (50µL) when compared with the standard drug, diclofenac sodium. In the future this can be developed into a very successful drug for anti-inflammatory action(28)-(29). This drug may be used for treating periodontitis and gingivitis after proper in vivo studies in future(30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44).

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CONFLICT OF INTEREST

Nil

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