# ANTI-FUNGAL EFFECT OF USNIC ACID COMBINED GRAPHENE NANO-FORMULATION BY USING IN-VITRO METHOD

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## ABSTRACT

**Objective:** The present study aims to investigate the antifungal response of the lichens secondary metabolite usnic acid with the carrier graphene.

**Method:** Nano-precipitation method by sonication was adopted to formulate the graphene and usnic acid combined nano-ointment. *In-vitro* antifungal activity was performed to confirm the antifungal effect of the formulated nano-ointment.

**Results:** Topical drug administration is more suitable for the treatment of the fungal infection, so the nano-combination was incorporated into the ointment by geometric mixing. *In-vitro* anti-fungal study had been performed for 4 days. It was found that the normal form of usnic acid, graphene and the nano form both possess anti-fungal activity as the zone of inhibition was found to be 3mm and 5mm.

**Conclusion:** The present anti-fungal study revealed that the nano-form of the nano-ointment determined higher anti-fungal activity than the normal formulation of usnic acid with graphene.

**Keywords:** Anti-fungal, Graphene, Nano-precipitation, Nano-ointment, Usnic acid.

## INTRODUCTION

Microscopic organisms are the responsible ones for the fungal infections, which invades the epithelial tissues. The kingdom of fungus comprises yeast, moulds, rusts and also mushrooms. Animals considered as heterotrophic, because, environment is the nutrient obtaining source of the animals. They are not depended on endogenous sources (such as plants with the photosynthesis). Most of the fungi are considered to be good and they also involved in the process of biodegradation; it is also said that some fungi's are responsible for the infections in case, if fungi's were penetrates into the skin through the wounds, and also by the nasal passage or lungs if they are inhaled.

[**1]** Diseases happens due to fungi, also consists superficially caused skin infection by the dermatophytes in the Microsporum, Trichophyton, and Epidermophyton genera. Anti-fungal agents possess their effect by differentiating between the mammalian cells and the fungal cells to kill the fungal organism without causing hazardous effects on to the host. However the satisfactory responses of synthetic antifungal drugs are awaited without side effects.

Nanotechnologies attracted significant attention in the recent researches. New technologies both in the preparation of the sample and in fabrication of device evoke on development of nano-science. Nanoparticles are employed for the purpose of targeted drug delivery system. It enhances the performance of the drug by increasing their bioavailability. Nanoparticles are of nano-sized colloidal structures which comprises of polymers of having synthetic and semi-synthetic nature**. [2].** Nanonization process is used for the compounds which are poorly soluble in water in respect to enhance the dissolution rate and increase the bio-availability. Nanoparticles refers as drug delivery systems which has the particle size ranges between 10−1000 nm, it also depends on the preparation method and usage of materials**. [3]**

Ointment considered as the semi-solid preparations which are used for the topical application on to the skin. Basically ointments consist of a medicament which is emulsified or mixed in to the base. They are applied for the emollient effect, protection of the skin. Ointments are also used for the vehicle which is used to administer the drug or the medicament topically.

In recent times the research is going in a way to use the herbs or some special species like lichens to cure the fungal infections by isolating their chemical constituents and secondary metabolites. The current research showing that the symbiotic species between algae and fungi i.e. lichen is a promising genera that contain various chemical constituents that show their antimicrobial properties. In this context the proposed work is concerned with a potent chemical that has been explored for various biological activities isolated from lichen i.e. Usnic acid. Lichens are considered as an photosynthetic and fungal partners. Usnic acid is considered to be the most common and abundant metabolites of lichens. It is also considered as an antibiotic. It has the ability to inhibit the fungal and bacterial growth. [**4].**To enhance the bioavailability of usnic acid, nano-combination was formulated with the help of graphene used as a carrier in the preparation of nano-combination. Graphene also having antibacterial activity, so the nano-combination of graphene and usnic acid is developed to accomplished the higher anti-fungal activity.

## MATERIAL AND METHODS

### Preparation of usnic acid and graphene nano-combination

Usnic acid was loaded onto the graphene via simple physio-sorption. Graphene 0.150mg /ml was sonicated with 1mg/ml usnic acid at pH 5 for 60 minutes, then it was stirred overnight at room temperature in the dark by using magnetic stirrer instrument, then it was ultra-centrifuged at 15000 rpm for 30 minutes, after ultracentrifugation the supernatant was taken out and the combination of usnic acid and graphene was remained at the bottom, it was taken out and then it was heated at 40°c in the hot air oven, powder of graphene combined usnic acid was obtained.[**5]**

**PREPARATION OF WATER SOLUBLE OINTMENT BASE**

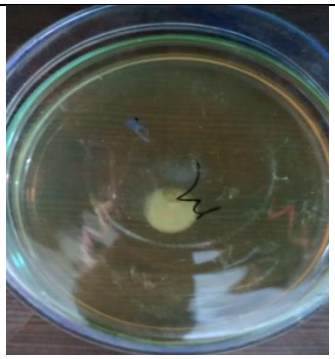
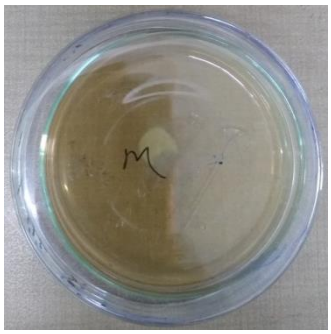
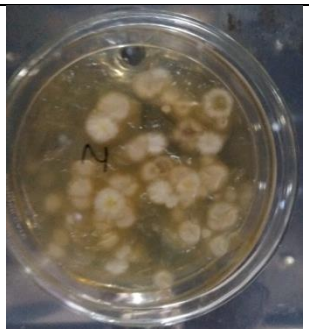
The water soluble ointment bases were prepared by using different grades of Polyethylene glycol (PEG), glycerine, and surfactant and purified water. Briefly, water soluble ointment base was prepared by melting the PEG-4000 on a hot plate/ stirrer (at 70°C) followed by addition of liquid PEG- 400 and glycerin. Sodium lauryl sulphate was mixed to the melted base with continuous stirring. Then the base was cooled with stirring until congealed. [**6]**

**FORMULATION OF NANO-OINTMENT OF GRAPHENE-USNIC ACID NANO-COMBINATION**

The process of geometric dilution has been used for the preparation of nano-ointment. The selected water soluble ointment base has been used for nano ointment. The nano ointment of graphene-usnic acid nano-combination had been formulated in a concentration of 0.5% w/w; similarly one more formulation Graphene-Usnic acid combination in the same concentration has been developed without undergoing Nanonization process.

***IN-VITRO* PHARMACOLOGICAL ACTIVITY**

**ANTI-FUNGAL ACTIVITY**

| Group | DAY 1 | DAY 2 | DAY 4 |
|---|---|---|---|
| Control Group |  |  |  |
| UAG |  |  |  |
| UAGN |  |  |  |

| | | | |
|---|---|---|---|
| Marketed Product |  |  |  |

**UAG**- Usnic acid and graphene (without nano-formulation)
**UAGN**- Usnic acid and graphene nano-formulation

*Candida albicans* was used to develop the fungal colonies and cup and plate method was applied for the anti-fungal activity.

Antifungal activity of the formulation was determined by cup plate method. In this test Sabouraud dextrose agar used as a culture media for culturing candida albicans. Study started on this by keeping the media mixed with *candida albicans* for 2 days in order to develop the fungal colonies in BOD incubator. Then little amount of our normal formulation (without nano) and nano-formulation was placed into the fungal colonies plates and kept in incubator for further 2 days. After that zone of inhibition was noted from each group, and it was found that UAG having zone of inhibition 3mm and UAGN having 5mm zone of inhibition. Due to this we can assume that nano-formulation of usnic acid inhibits the fungal growth better than the normal formulation of usnic acid.

## CONCLUSION

The present study clearly indicates that the nano form of usnic acid with graphene shows greater antifungal response as compare to the normal form of usnic acid with graphene. This provides an indication that if the fungal infection is penetrated into the deeper layers of skin the nano form of the prepared formulation would heal it better with respect to the normal form of the usnic acid. It also indicates that the nano form of the prepared formulation penetrates through the barrier layers of the skin like stratum corneum and treats the deeper infection of the skin. This study shows that how effectively the nano-formulations works in the present scenario and also into the upcoming years the nano-technology is going to play a vital role in the efficiency of the drug formulation.
Further research on various aspects of usnic acid and many more pharmacological activities are possible.

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