Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i1.2762

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

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Objective: The primary objective of this work is to examine the antifungal properties of usnic acid, a secondary metabolite found in lichens, when combined with graphene as a carrier.

Methods: The formulation of the nano-ointment combining graphene and usnic acid was achieved using the nano-precipitation technique using sonication. The in-vitro antifungal activity was conducted in order to validate the antifungal efficacy of the nano-ointment formulation.

Result: In the context of treating fungal infections, it is more appropriate to utilize topical medication administration. Therefore, the ointment was formulated by including the nano-combination using geometric mixing. A four-day in-vitro investigation was conducted to investigate the anti-fungal properties. The study revealed that usnic acid in its regular form, as well as graphene in its nano form, had anti-fungal properties, as evidenced by the observed zone of inhibition of 3mm and 5mm, respectively.

Conclusion: In conclusion, the findings of this study demonstrate that the nano-ointment formulation of usnic acid with graphene exhibited superior anti-fungal action compared to the conventional formulation.

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

INTRODUCTION

The present study focuses on the development of an anti-fungal nano-ointment using the potential of graphene and usnic acid. The incorporation of graphene, a two-dimensional carbon-based material, is aimed at enhancing the therapeutic efficacy of the nano-ointment. The synthesis of the nano-ointment involves a nano-precipitation technique.

Fungal infections, which infiltrate epithelial tissues, are attributed to microscopic organisms. The fungal kingdom encompasses several organisms including yeast, moulds, rusts, and mushrooms. Animals are classified as heterotrophic organisms due to their reliance on the environment as their primary source of nutrients **[1]**. They do not rely on endogenous sources, such as plants utilizing photosynthesis. The majority of fungus are regarded as beneficial organisms that play a role in the process of biodegradation **[2]**. However, several fungi have been identified as potential sources of

infection when they enter the body through skin wounds or are breathed through the nasal route or lungs. In this study, we aim to investigate the effects of a particular drug on the growth of Diseases occur as a result of fungal infections, which include superficial skin infections caused by dermatophytes belonging to the genera Microsporum, Trichophyton, and Epidermophyton. Anti-fungal drugs exert their effects by selectively targeting fungal cells while sparing mammalian cells, therefore eliminating the fungal organism without inducing harmful effects on the host. Nevertheless, there is a need for synthetic antifungal medications that might provide effective responses while minimizing the occurrence of negative effects **[3].**

Nanotechnologies have garnered considerable interest in recent scientific endeavors. The advancement of nano-science is influenced by the emergence of new technologies in sample preparation and device manufacturing. Nanoparticles are utilized in the context of targeted medicine delivery systems **[4].** The bioavailability of drugs is increased by the utilization of this technique, hence enhancing their overall performance. Nanoparticles are colloidal structures with dimensions on the nanoscale, often composed of polymers that are either synthetic or semi-synthetic in origin **[5].** The nanonization method is employed to improve the dissolving rate and raise the bioavailability of chemicals that have low solubility in water. Nanoparticles are defined as drug delivery systems characterized by particle sizes ranging from 10−1000 nm. The specific size range is contingent upon the chosen manufacturing process and the materials employed. The user's text is already academic and does not need to be rewritten **[6].**

Ointments are regarded as semi-solid formulations that are applied topically on the skin. Ointments typically comprise a medicinal substance that is emulsified or blended with a base. The use of these substances is primarily intended to provide an emollient effect and protect the skin. Ointments are commonly employed as a vehicle for the topical administration of drugs or medicaments **[7].**

In contemporary times, research endeavors are focused on using herbs and specific species, such as lichens, for the purpose of treating fungal diseases. This involves the isolation and analysis of their chemical contents and secondary metabolites. The existing study provides evidence that the symbiotic relationship between algae and fungus, known as lichen, is a potential genus that exhibits a diverse range of chemical compounds with shown antibacterial characteristics. The focus of this study is to a highly active chemical compound, Usnic acid, which has been extensively investigated for its diverse range of biological activities, particularly those derived from lichen. Lichens are regarded as symbiotic associations between photosynthetic organisms and fungi. Usnic acid is widely recognized as one of the most prevalent and prolific metabolites found in lichens. Additionally, it is regarded as an antibiotic. The substance possesses the capacity to impede the proliferation of fungi and bacteria **[8].** The user's text does not contain any information to rewrite. In order to improve the bioavailability of usnic acid, a nano-combination was developed by using graphene as a carrier throughout the production process. Graphene exhibits antibacterial properties, therefore prompting the development of a nano-combination of graphene and usnic acid in order to enhance its anti-fungal action **[9].**

MATERIAL AND METHODS

THE SYNTHESIS OF USNIC ACID WITH GRAPHENE NANO-COMBINATION

The process of loading usnic acid onto the graphene involved straightforward physio-sorption. A solution of graphene at a concentration of 0.150 mg/mL was subjected to sonication with a solution of usnic acid at a concentration of 1 mg/mL, at a pH of 5, for a duration of 60 minutes. Subsequently, the mixture was stirred overnight at room temperature in the absence of light, utilizing a magnetic stirrer. Following this, the mixture was subjected to ultra-centrifugation at a speed of 15000 rpm for a duration of 30 minutes. After the ultra-centrifugation process, the liquid portion (supernatant) was removed, leaving behind a combination of usnic acid and graphene at the bottom. This solid residue was then extracted and subjected to heating at a temperature of 40°C in a

hot air oven, resulting in the formation of a powder consisting of graphene combined with usnic acid.

THE FORMULATION OF A WATER-SOLUBLE OINTMENT BASE

The water-soluble ointment bases were formulated by employing various grades of Polyethylene glycol (PEG), glycerine, surfactant, and purified water. In this study, a water soluble ointment base was made by the process of melting PEG-4000 on a hot plate/stirrer at a temperature of 70°C. Subsequently, liquid PEG-400 and glycerin were added to the melted PEG-4000. The addition of sodium lauryl sulphate was performed by incorporating it into the molten base while maintaining a consistent stirring motion. Subsequently, the base was subjected to cooling under continuous stirring until solidification occurred. According to the provided text, the user has not provided any information or content **[10].**

DEVELOPMENT OF GRAPHENE COMBINED USNIC ACID NANO-OINTMENT

Geometric dilution has been employed in the formulation of nano-ointment. The chosen water-soluble ointment base has been utilized for the formulation of a nano ointment. The concentration of the nano ointment containing a combination of graphene and usnic acid was produced at 0.5% w/w. Additionally, another formulation of the graphene-usnic acid combination was developed at the same concentration without undergoing the process of nanonization.

The focus of this study is on the development of a novel formulation involving usnic acid and graphene at the nano-scale.

RESULT

IN-VITRO PHARMACOLOGICAL ACTIVITY

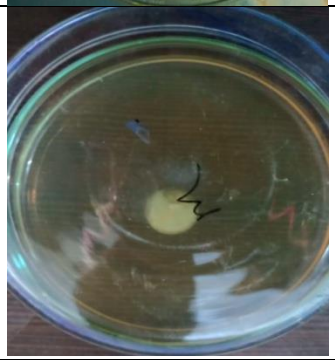
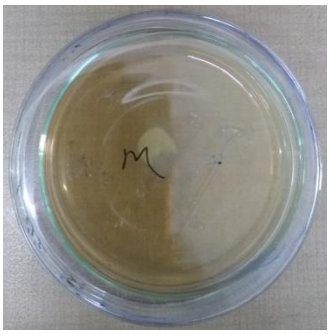
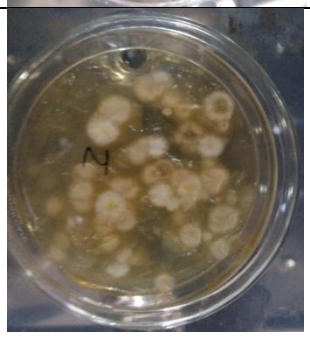
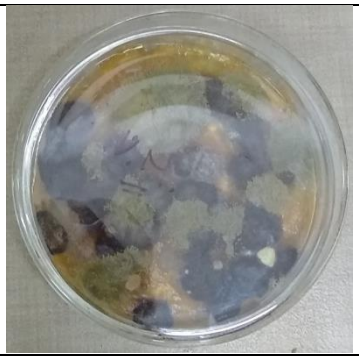
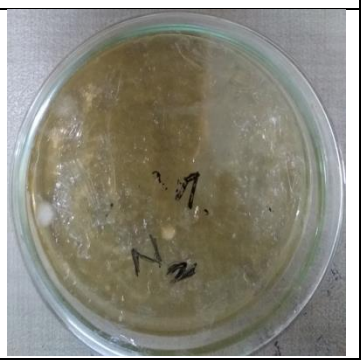
ANTI-FUNGAL ACTIVITY

The fungal colonies were developed using Candida albicans, and the anti-fungal activity was assessed using the cup and plate method.

The determination of the antifungal activity of the formulation was conducted using the cup plate technique. The Sabouraud dextrose agar was employed as a culture medium for the cultivation of Candida albicans in this experiment. The investigation started by incubating a mixture of medium and Candida albicans for a duration of two days within a BOD incubator, with the aim of cultivating fungal colonies. Subsequently, a small quantity of our conventional formulation (devoid of nano) as well as the nano-formulation were introduced onto the plates containing fungal colonies, which were then incubated for an additional duration of 2 days. Following the observation of the zone of inhibition in each group, it was determined that the UAG group exhibited a zone of inhibition measuring 3mm, whereas the UAGN group had a zone of inhibition measuring 5mm. Based on these findings, it can be inferred that the nano-formulation of usnic acid has superior inhibitory effects on fungal growth compared to the conventional formulation of usnic acid.

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

UAGN- Usnic acid and graphene nano-formulation

Group	DAY 1	DAY 2	DAY 4
Control Group			
UAG			
UAGN			
Marketed Product			

Conclusion

The findings of this study provide compelling evidence that the nano formulation of usnic acid combined with graphene has a significantly stronger antifungal effect when compared to the conventional formulation of usnic acid combined with graphene. This observation suggests that the nano formulation of usnic acid may be more effective in treating fungal infections that have entered the deeper layers of the skin, compared to the conventional form of usnic acid. Additionally, the presence of the nano form of the produced formulation suggests that it has the ability to permeate the barrier layers of the skin, such as the stratum corneum, and effectively address underlying skin infections. This study demonstrates the current and future efficacy of nano-formulations in medication formulation, highlighting the significant role that nano-technology will play in enhancing efficiency.

There is potential for conducting more study on several facets of usnic acid, as well as exploring numerous other pharmacological effects.

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