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In Vivo Antifungal Activity of Nano-Ointment of Usnic Acid Graphene Conjugates against Candida Albicans

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Abstract
Due to its targeted delivery, enhanced skin permeability, and controlled release for the treatment of skin infections, nanoparticle-based formulations have been gaining popularity. The water soluble base was used to generate the nano-ointment of grapheme conjugate with usnic acid, which was then tested for antifungal activity using the fungus Candida albicans. The in-vivo antifungal activity is significant (p 0.001) and comparable to the activity of commercially available standard preparations. This important antifungal feature demonstrates the medication and carrier's synergistic interaction, which was previously documented for their anti-microbial property.
Key-words: Graphene, Nano-ointments, Antifungal

Introduction

Recent studies have focused a lot of emphasis on nanotechnologies. New technologies used in device fabrication and sample preparation have an impact on the advancement of nanoscience. For the goal of targeted drug delivery, nanoparticles are used. By enhancing their bioavailability, it improves the drug's performance. Nanoparticles are colloidal structures with nanoscale dimensions made of synthetic and semi-synthetic polymers. [1] In order to accelerate the rate of dissolution and increase bioavailability, compounds that are poorly soluble in water are subjected to the nanonization procedure. Drug delivery systems known as nanoparticles range in particle size from 10 to 1000 nm, depending on the technique of manufacture and the materials used. [2]

Graphene-based materials (GBMs) include graphene nanosheets, graphene oxide, and graphene with a few layers. Graphene is made up of carbon atom sheets that are only one atom thick. It is a 2D material composed of carbon atoms arranged in a honeycomb-like crystal-lattice. Graphene has anti-bacterial properties that have been discovered in numerous studies by various researchers. Graphene is active against various gram-positive and gram-negative organisms, diverse microorganisms that are responsible for the formation of biofilms, and phytopathogens.

Because of its biocidal behaviour, graphene-based materials have proven to be a potential nano-material compound for the development of anti-bacterial surfaces. Graphene possessed anti-bacterial properties that were found to be synergistic to both "physical" and "chemical" responses. When bacteria come into direct touch with graphene, a series of physical interactions occur between the graphene and the bacterial cells, resulting in physically broken cell membranes and the release of intracellular contents.

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Topical fungal infections are the most frequently diseases in society, and individuals are looking for more effective ways to cure the disease with little to no side effects, which is why better antifungal therapy is required. Graphene is a wonder biological having anti-bacterial efficacy already demonstrated, whereas usnic acid is establishing its potency as an anti-bacterial efficacy. [4] According to the literature, these compounds have considerable antibacterial properties. The graphene conjugated usnic acid nano-ointment is being developed to test the drug's pharmacological performance as a topical antifungal preparation in experimental animals. The combined/synergistic action of graphene and usnic acid as a topical antifungal preparation is described in this work.

Material and Methods Antifungal activity

For the in vivo investigation of the developed nano-formulation, Candida albicans (MCCB 0290) was preferred to produce mycosis in Wistar albino rats. The process entails removing hairs from the rats' backs using a hair removal cream, and an area of 2x2 cm^2 was chosen for the application of the developed formulations. The skin was slightly abraded with sandpaper the next day, and the inoculum of Candida albicans was administered to the rats' skin with a cotton swab. This experiment was divided into five groups of animals, with each group consisting of six rats.

The first group received no treatment, the second group received Usnic acid API dispersion treatment, the third group received Usnic acid Nanoparticles ointment (UANP), the fourth group received Usnic acid Graphene nanoconjugate ointment (UGNC), and the fifth group received Standard marketed formulation treatment. All formulation groups have been administered topically. The rats were provided with a one-time application each day for six days. After six days, the responses of each group were compared to the control group. Each group received a treatment score of 1 (not treated), 2 (50% treated), 3 (75% treated), and 4 (100% treated).

A culture study was carried out to evaluate the effectiveness of the offered treatment. Each treated area was thoroughly cleaned with 70% ethanol. The skin from each treated location was removed, chopped using scissors, and homogenised in 4 ml of saline with the tissue homogenizer. The homogenate was then streaked on the solidified Sabouraud dextrose agar medium. In the BOD incubator (Indosati Scientific, Ambala), all plates were incubated at 25°C for 5 days. Using a colony counter, the number of colony forming units in the agar plates was counted, and the number of colony forming units per infected site was calculated. If more than one fungal colony was found in the plates, it was referred to be a fungal positive. [5-6]

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Statistical analysis

The statistical analysis was performed by using GradPad Prism 5.01. The values were depicted as mean ± S.D. for all six Wistar albino rats, the data was analyzed by ANOVA by the Newman-keuls.

Results and Discussion

The in vivo efficacy of graphene conjugates usnic acid nanoointment was assessed in male albino rat model (Wistar; 100–150 g). Isolate of candida albicans was used for production of cutaneous candidiasis in albino rats. Table 1 depicts the efficacy of graphene conjugated usnic acid nano- ointment formulation against cutaneous candidiasis in rats as compared to that of standard preparation, Usnic acid Nanoparticles ointment. It was observed that Usnic acid Nanoparticles ointment (UANP) shown moderate control over the fungal infection as three animals out of six were positive in culture test whereas Usnic acid Graphene nanoconjugate ointment (UGNC) depicts greater efficiency in the treatment of candidiasis, as only two animals out of six exhibited a positive culture test. Fast recovery from fungal infection was found in the case of standard marketed preparation (Surfaz SN) as there is only one animal exhibited trivial CFU on infected area. And there's no improvement observed in case of usnic acid dispersion.

Table1: Colony forming unit of *Candida albicans* in skin of rats after treatment with different formulations

Group Number	Treatment type	No. of animals with positive culture	Total animals	Infected sites/ Mean CFU	S.D.
Group I	Control	6	6	16.10	9.01
Group II	UADN	5	6	11.13	4.22
Group III	UANP	3	6	3.78	3.20
Group IV	UGNC	2	6	1.46	1.16
Group V	Standard	1	6	1.12	0.65

UADN: Usnic acid dispersion, **UANP:** Usnic acid Nanoparticles Ointment,
UGNC: Usnic acid nanoconjugate ointment, **Standard:** Marketed Product (Surfaz SN)
The value represents the means ± S.D. for 6 rats per group. P<0.001 was considered as significant compared to control group.

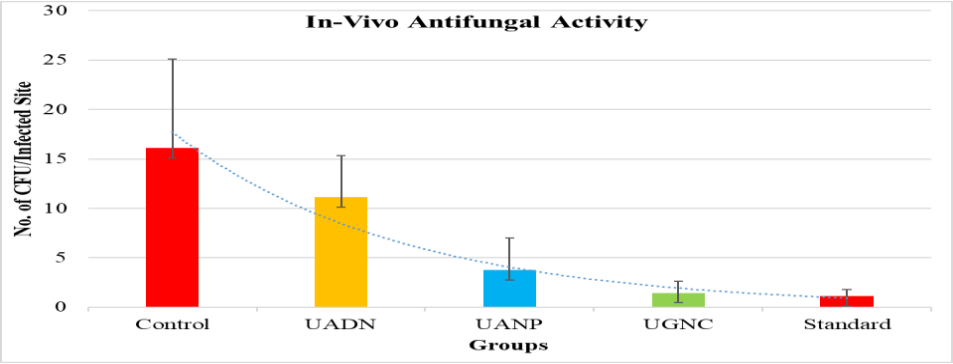

Figure 1: Effect of conjugates on no. of CFU's/infected area

Figure 1 shows the appearance of no. of CFU's spreading over entire affected area in experimental animals and scored accordingly after 6 days of treatment. UADN shown poor control over the fungal infection, as five animals out of six were positive in the culture test, whereas UANP showed moderate control over the fungal infection, as three animals out of six were positive in the culture test, whereas a synergistic effect was observed in UGNC, where out of six animals, only two were positive in the culture test. However, in the standard market formulation, only one animal out of six was positive in the culture test. The antifungal activity of the UGNC and marketed formulations is quite close despite having steroid in the marketed formulation, which could have a similar antifungal effect. This study clearly demonstrates that without having any steroid, i.e., Beclomethasone dipropionate 0.025 % in the UGNC formulation, the closer pharmacological effect proves that this formulation has more pharmacological potential than that of the marketed formulation.

Conclusion

The nanoointment of grapheme conjugate was subjected for antifungal activity by using fungal strain *Candida albicans. The* in-vivo antifungal activity shows significant ($p<0.001$) antifungal activity that corresponds similar to activity of standard marketed preparation despite having steroid in the marketed product. This study clearly demonstrates that without having any steroid, i.e., Beclomethasone dipropionate 0.025 % in the UGNC formulation, the closure pharmacological effect proves that this formulation has more pharmacological potential than that of the marketed formulation. This could be because of the graphene, as graphene first ruptures the fungal cell wall and creates a channel to penetrate drug inside the fungal cell, which further augments the eradicating process of pathogen by stopping the energy supply to the fungal cell wall through mitochondria.

References

[1] Vanderhoff J. W. and El-Aasser M.S In: Pharmaceutical dosage forms: Dispersed system, Marcel Dekker, New York ,1988;93

[2] Pandian et al., fabrication, characterization and pharmacological activity of usnic acid loaded nanoparticles, International Journal of Pharmaceutical Science and Research. 2017; 8(11): 4658- 4766.

[3] Mishra D, Hubenak JR, Mathur AB. (2013). Nanoparticle systems as tools to improve drug delivery and therapeutic efficacy. J Biomed Mater Res A. 2013; DOI:10.1002/jbm.a.34642.

[4] Pershing, L.K., Corlett, L.J., Jorgensen, C. In vivo PHARMACOKINETICS AND PHARmacodynamics of topical ketoconazole and miconazole in human stratum corneum, Antimicrob. Agent Chemother. 1994; 38:90–95

[5] Spangberg L, Engström B, Kaare L. Biologic effects of dental materials: Toxicity and antimicrobial effect of endodontic antiseptics in-vitro, Oral Surgery, Oral Medicine, Oral Pathology Dec. 1973; 36(6): 856–871

[6] Thomas R, Nair AP, Soumya KR, Mathew J, Radhakrishnan EK. Antibacterial activity and synergistic effect of biosynthesized groups with antibiotics against multidrug-resistant biofilm- forming coagulase-negative staphylococci isolated from clinical samples. Appl. Biochem. Biotechnol. 2014; 173:449–460