Preparation Of Griseofulvin Nano Ointment And Its Anti Fungal Activity

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**ABSTRACT**

The study aims antifungal response of the dug griseofulvin. Nano-precipitation method by sonication was adopted to formulate the nano particles. SEM test was performed to check the shape and average size of the particles. FTIR test was performed for the chemical interaction between the drug and the carrier. Ointment was prepared by the fusion method and the viscosity test was performed by Brookfield viscometer. Spread ability test was performed by slide method. Animal activity was performed to confirm the antifungal effect of the formulated nano-ointment. Statistical analysis was done by ANOVA. SEM study shows that the particles is in the nano range and possess a spherical shape. FTIR study shows no interaction between the drug and the carrier. The result of in-vitro drug release study shows that the nano-ointment posses a higher drug release rate as compared to the drug alone. Topical drug administration is more suitable for the treatment of the fungal infection, so the nano-particles was incorporated into the ointment by geometric mixing. The viscosity and the spread ability test were performed on the different formulations of the ointment and the suitable one was selected for the topical administration. Anti-fungal study had been performed on the Wistar albino rats for 6 d. Skin culture of rats was performed for the formation of the fungal colonies. Statistical analysis by ANOVA gives p<0.001. It was found that the normal form of griseofulvin nano form both possess anti-fungal activity as 3/6 and 2/6 experimental animals are cured by normal formulation and nano-formulation. The present anti-fungal study revealed that the nano-form of the ointment possess higher anti-fungal activity than the normal formulation of griseofulvin nano-ointment.
INTRODUCTION:
Griseofulvin is an antifungal agent derived from the mold Penicillium griseofulvum that is used to treat fungal infections of the skin and nails. Griseofulvin binds to tubulin, disrupting microtubule function and inhibiting mitosis (1). The poor water solubility of drugs is a major challenge for pharmaceutical industry (2). There are reports that about 40% of the newly synthesized drug molecules are having low bioavailability arising out of their poor water solubility (3). Active pharmaceutical ingredients (APIs) having high permeability and low solubility are classified as class II APIs in biopharmaceutical classification (BCS) system (4). For the BCS Class II APIs, improved bioavailability can be achieved by increasing solubility and dissolution rate. Particle size reduction in general improves solubility. Nanosizing an formulation using water soluble polymers and surfactants also results in enhanced bioavailability. GF((2S,6 R)-7-chloro-2,4,6-trimethoxy-6-methyl-3H,4H-spiro[1-benzofuran-2,1-cyclohex[2]ene]-3,4-dione) is an antibiotic and antifungal drug (5). It is produced from the culture of some strains of the mold Penicillium Griseofulvum from which it was originally isolated (6). Griseofulvin is the first oral antifungal drug (7). Nanoparticles are good platform to increase solubility and bioavailability of hydrophobic drug (8). So nanotechnology was adopted to bring up the good dissolution and better bioavailability. Nanoparticles are referred as nanosized colloidal particle ranging in size from 10 - 1000 nm in which drug is encapsulated by polymers. These small size nanoparticles can be easily absorbed into RES and other system of body (9). Among various types of nanomaterials, polymeric nanoparticles have the advantages of relatively high biocompatibility, stability, and flexibility of conjugating ligands on their surface for targeted drug delivery (10). 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 topicaly (11).

MATERIALS AND METHOD
Materials
Griseofulvin is Antifungal drug I.e. GF was purched from Sigma Aldrich. Polyethylene glycol (PEG 400 and PEG 4000) Sodium lauryl sulphate, Glycerine were provided by college. Chemicals used in this work were of good analytical grade. All chemicals were pharmaceutical grade and used without further modification.

Preformulation studies
Determination of aqueous solubility
Saturation shake flask method was used to determine GF aqueous solubility. The excess amount of GF was dispersed in distilled water and shaken at 50 rpm and 37°C for 72h, filtered and analyzed at λmax of 299 nm using UV spectroscopy (11).

FTIR study
FTIR analysis for GF and GF nano was performed by PAAN ANALYTICAL XPERT-PRO each sample was mixed in 1:10 with potassium bromide and later compressed into pellets observed from 4000- 400cm⁻¹.

Preparation of nanoparticle
The griseofulvin nanoparticles were prepared using nano precipitation, sonication method. Griseofulvin was sonicated 10 min for 3 cycles, 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 1 hour, after ultra centrifugation the supernatant was taken out for calculating the entrapment efficiency, the griseofulvin was remained at the bottom, the purified nano particles were dried, powder of griseofulvin was obtained, then this nano-particles was taken for the characterization studies.

Evaluation of Nano-particles
Scanning electron microscopy analysis
The SEM imaging of the sample is carried out by type of electron microscope which scans it with a high energy electron beam. In this when electron gets interacted with the atoms of the sample signals is produce which contains information of the sample morphology, its composition and other properties like electrical conductivity. In the present study, the Carbon coating of the materials was done by using JEOL-JXA-8100 vacuum evacuator to make the sample conducting. Coating thickness was 20 nm. SEM images were taken by using EPA i.e. electron probe analyzer JEOL-JXA 810015.
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. Total six formulations of bases with different ratios of PEG 4000 and PEG 400 have been prepared and best one was selected on the basis of their pH, spreadability and viscosity (12).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>PEG-4000</th>
<th>PEG-400</th>
<th>Glycerine</th>
<th>S.L.S</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSB 01</td>
<td>40gm</td>
<td>60gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>WSB02</td>
<td>30gm</td>
<td>70gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>WSB03</td>
<td>20gm</td>
<td>80gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>WSB04</td>
<td>50gm</td>
<td>50gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>WSB05</td>
<td>60gm</td>
<td>40gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>WSB06</td>
<td>30gm</td>
<td>60gm</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

Formulation of nano-ointment of griseofulvin
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 Griseofulvin had been formulated in a concentration of 0.5% w/w; similarly one more formulation Griseofulvin in the same concentration has been developed without undergoing Nanonization process(13).

Physicochemical characterization

pH
The pH of the formulated products were determined by the usage of Digital pH meter (361, Systronics). The electrode which was connected to the pH meter was cleaned with distilled water and made it dry with the help of tissue paper, then immerse the electrode and temperature probe in a beaker containing ointment formulations. After that wait for few minutes then note the readings of pH of samples which were displayed on pH meter [7, 10]. Experiment was performed in triplicates and the mean values were depicted in table

Determination of viscosity
Measurement of viscosity is defined as the process of fluid resistance. Viscosity determines the process of internal friction of a fluid which is a moving state. Units of viscosity are Poise, Centipoises, Pascal-second. Viscosity test of ointment was performed by the help of Brook-field Viscometer (Model No.INSTR/04). The test was done in a triplicate manner and then the mean values of each prepared formulation were depicted in Table 2.

Spreadability
Spreadability of the semi-solid preparations, is the ability of a preparation to evenly spread on to the skin. It plays an important role in the administration of a dose of a preparation on to the skin. Spreadability is measured in respect of times in seconds. 2 sets of glass slides of standard dimension of 7.5 cm were taken. The prepared ointment was kept over on the first slide and the second slide was kept on top of the ointment, in the manner that the ointment was sand-witched in between both slides. 100g weight of ointment was kept on the upper slide in such a manner that the ointment gets compressed properly to form a thin layer. Then the previous weight of 100g was removed from the upper slide and 20g of new weight was tied to the upper slide and the slides were tilted in such a manner that only the upper slide to slips off by the applied force on to it. The time interval in which the upper slide travels the distance of 7.5 cm and separated away from the lower slide was measured. The test was performed in triplicate manner and the mean value was considered. In the spreadability study lower the time interval of separation of the slides better will be the spreadability19. Formula for the spreadability study is as follows:

\[ S = M \times L / T \]

Where, S denotes the spreadability, M denotes the weight tied on to the upper slide, L denotes the length of glass slides and T denotes the time taken by the slides to get separated.

Skin irritation study
Skin irritation study of ointment was performed on male albino wistar rates 100-120gm after receiving an approval from control and supervision of experiment on animas(SIP/IAEC/013/1019)

**In vivo study**

**Anti-fungal activity**

**Microbial strain**

*Candida albicans* (MCCB 0290) was obtained from Microbial Culture Collection Bank, Microbiology department, Prayagraj Agriculture Institute of Deemed University, Prayagraj.

**Animals**

Healthy male adult Wistar albino rats, age ranges two and three months, weights 100-150 gm was taken for the anti-fungal study. The rats were kept in polypropylene cages and also in prescribed atmosphere which comprises 12 h light and 12 hour dark at 25±2 °C and 30-55% humidity. Rat pellets were given to the rats in their daily diet. The Institutional Animal Ethical Committee, SIP, Prayagraj, India (SIP/IAEC/013/10/19) has approved the study.

**Procedure**

*Candida albicans* (MCCB 0290) was preferred to induce the mycosis in Wistar albino rats for the *in vivo* study of the formulated nanoformulation. The procedure includes, the removal of hairs from the back of the rats by using the hair removal cream and an area of 2 × 2 cm² was preferred for the application of the prepared formulations. On the next day the skin was slightly abraded with the help of sandpaper and then the inoculum of *Candida albicans* were applied on to the skin of the rats by using a cotton swab. The animals were separated into four different groups, which comprises one control group, 6 rats were taken in every group. The prepared products, i.e. nano ointment of griseofulvin and Ointment with griseofulvin, Standard marketed preparation (SURFAZ-SN 0.5% w/w) were administered topically. Daily one time application was given to the rats for the interval of six days. The control group does not received any treatment. The response of each group was compared to the control group after the period of six days. The treatment scores were given to each group as 1 (not treated), 2 (50% treated), 3 (75% treated) and 4 (100% treated). Culture study was performed to check the effect of the given treatment. Each treated site was wiped properly with 70% ethanol. The skin from each treated site was excised, minced, by the help of scissors, and then it was homogenized in 4 ml of saline by using the tissue homogenizer. Then, small portion of homogenate was streaked on the solidified Sabouraud dextrose agar medium. All plates were incubated at 25 °C for 5 d in the BOD incubator (Indosati Scientific, Ambala). The numbers of colony forming units in the agar plates were counted by using colony counter and the number of colony forming units per infected site was determined. If more than one fungal colony was found in the plates then it was termed as fungal positive

**RESULTS**

**Preformulation studies**

**Determination of solubility**

The low aqueous solubility of griseofulvin (0.05 mg/mL). Saturation shake flask technique had been recycled into complete GF wet salability.

**FTIR analysis**

FTIR spectra were recorded to assess the compatibility of the pure drug and formulated compound. FTIR spectra of drug, griseofulvin were examined. All characteristic peaks of griseofulvin were ascertained in the FTIR test of nano-particles. The results showed that there is no chemical interaction or alteration took place during formulation of nano-particles. The results showed that there is no chemical interaction or alteration took place during formulation of nano-ointment. FTIR spectra ofgriseofulvin showed characteristic peaks of C-H stretch at 2931.10 cm⁻¹, C=O stretch at 1692.03 cm⁻¹, C-O stretch 1000-1200 cm⁻¹, O-H aromatic stretch at 3091 cm⁻¹, C-H aromatic stretch at 700-900 cm⁻¹, O-H aromatic stretch at 1600-1675 cm⁻¹ were obtained. FTIR spectra of GF api showed characteristic peak at 1600 cm⁻¹ was obtained. Griseofulvin showed FTIR spectra at C-H stretch at 2929 cm⁻¹, C=O stretch at 1631 cm⁻¹, O-H aromatic stretch at 1000-1200 cm⁻¹, C=O aromatic stretch at 1600-1675 cm⁻¹ were obtained. FTIR spectra of GF api showed characteristic peak at 1600 cm⁻¹ was obtained. Griseofulvin showed FTIR spectra at C-H stretch at 2929 cm⁻¹, C=O stretch at 1631 cm⁻¹, O-H aromatic stretch at 3091 cm⁻¹. The results of the FTIR test showed that there is no chemical interaction took place during the formulation of nano particles and drug was found to be compatible.
Evaluation of drug nano particles

Scanning electron microscopy (SEM) analysis
SEM is a surface imaging method in which the incident electron beam scan across the sample surface and interact with the sample to generate the signals. The size distribution and shape of nanomaterial can be directly obtained from SEM. The average size of the prepared nano-particles was found to be in the range of 20-60 nm. The shape of the nano-particles is of spherical in nature.
Evaluation parameter of ointment formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Viscosity (cps)</th>
<th>Spreadability (gm. cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected Base</td>
<td>6.3±0.2</td>
<td>25.12±0.14</td>
<td>29.73±0.52</td>
</tr>
</tbody>
</table>

Skin irritation study
Results revealed that as ointment is a skin irritant it was produce irritation with minimal erythema after 24 hrs and definite erythema, readily visible edema was produce after 48 hrs. Compared with this both the placebo and optimized batch was not show any type of irritation up to 24 hrs after that there was little erythema found with light redness at the site of application.

(Fig. 3: SEM image of Griseofulvin nanoparticle)
(Fig. 4: A Before – skin Irritation study, B After- Skin irritation study)
**In- vivo antifungal activity**

The in- vivo efficacy of nano-ointment 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 3 depicts the efficacy of nano-ointment formulation against cutaneous candidiasis in rats as compared to that of standard preparation and normal griseofulvin formulation. It was observed that griseofulvin ointment showed moderate control over the fungal infection as three animals out of six were positive in culture test whereas griseofulvin nano ointment depicts greater efficiency in the treatment of candidiasis, as only two animal out of six exhibited a positive culture test. Fast recovery from fungal infection was found in the case of standard marketed preparation as there is only one animal exhibited trivial CFU on infected area.

(Fig. 5 A. Ointment applied on 1st day, B. Ointment applied on 2nd day, C. Ointment applied on 3rd day, D Ointment applied on 4th day, E. Ointment applied on 5th day:F Ointment applied on 6th day. Anti-fungal effect of GF nano-ointment on experimental animals)

**CONCLUSION**

The present study clearly indicates that the nano form of griseofulvin shows greater antifungal response as compare to the normal form of griseofulvin. This provides an indication that if the fungal infection is penetrated into the deeper layers of skin the nano form of the prepared formulation treats it better with respect to the normal form of the griseofulvin. 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 whereas the normal form was not penetrated through the barrier layers of skin and it remains on the top surface of the skin only. 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.

**REFERENCE**

1. R.Kumar, P.F.Siril, Preparation and characterization polyvinyl alcohol stabilized griseofulvin nanoparticles, Recent Advances in nano science and technology 2015.

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