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Design, characterization and microbiological evaluation of microemulsion based gel of griseofulvin for topical delivery system

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ABSTRACT

This study was performed to develop microemulsion based gels (MEBG) of griseofulvin (GF). The impact of concentration of Carbopol 934, the concentration of oil phase; oleic acid and also the penetration enhancer; dimethylsulfoxide on the viscosity of the drug was studied. The MEBG formulations were evaluated for the physical appearance, drug content, droplet size, transmission electron microscopy and histopathological examination. Moreover the in vitro release of GF from different formulations and also the microbiological assay of the selected formulation were carried out. The prepared MEBG showed acceptable physical properties and exhibited non-Newtonian pseudoplastic shear thinning behavior. The viscosity value was increased by increasing Carbopol concentrations while oleic acid concentrations had non significant effect on the viscosity. MEBG formulations containing DMSO exhibited lower viscosity than that free from DMSO. Release rate of GF from different MEBG without DMSO was affected non significantly by the Carbopol concentrations while the rate of the release from formulations containing 0.5 % Carbopol was higher than that containing 1 % Carbopol after adding DMSO. The release of GF from the MEBG increased with the addition of DMSO in the formulations. The selected formulation showed higher antifungal properties against all the selected strains.

Keywords: Gels, microemulsion, dermal drug delivery, griseofulvin, gelling agents, hydrophobic drugs.

1. INTRODUCTION

Topical drug delivery systems have been utilized for the treatment of several local skin diseases by liberating the drug directly to the position of action. The topical drug delivery systems present the prospective advantages as avoidance of first effect, suitable, comfortable to apply, providing utilization of drugs with short half-life, and enhance patient compliance [1].

Microemulsions (ME) as carrier systems in topical drug delivery have encouraged markedly in the current years. They are optically transparent micrometric sized microemulsions with particle sizes between 100 and 500 nm, composed of the oil, surfactant, co-surfactant and water [2, 3]. However, the application of the microemulsions to the skin is undesirable due to low viscosity and to increase their viscosity; gelling agents can be used [1]. Inspite of many benefits of gels a main restriction is their incapability to deliver hydrophobic drugs. When gels and microemulsion are used in combined system the dosage forms are refereed as microemulsion based gels. Microemulsion based gels are suitable for hydrophobic drugs that cannot be easily included into gels. Among the other advantages of microemulsion based gels are enhanced stability and improved loading capacity of drugs. They have been recently applied as vehicles to deliver numerous drugs to the skin [4, 5, 6, 7].

Griseofulvin is a hydrophobic antifungal drug and it is used to treat different dermatomycoses and other cutaneous infections (8). The conventional oral route of administration of griseofulvin is combined with variable bioavailability, abundant systemic side effects and long period of treatment [9].

The objective of this work was to develop microemulsion based gel formulations of griseofulvin using Carbopol 934 as gelling agent. Oleic acid in different concentrations was selected as the oil phase of microemulsion. The formulations were evaluated for their physiochemical properties and rheological behavior. The influence of the concentration of Carbopol and oleic acid, and also DMSO as penetration enhancer on the in vitro release of the drug was investigated. Moreover, the antifungal efficiency of the selected formulation was also examined.

2. EXPERIMENTAL SECTION

2.1. Materials. Ultramicronized griseofulvin (GF) was a gift from (Nile Pharm. Industrial Company, Cairo, Egypt). Carbopol 934 was obtained as gifts from (Al-Amriya Pharm. Industry Company, Alexandria, Egypt). Oleic acid obtained from (Lobachemi Company, India). Tween 20 and span 20 were purchased from (Avonchem Ltd., UK). Methyl and propyl parabens were kindly supplied by (Mallinckrodt Specialty Chemicals Co., Paris, France). Propylene glycol, triethanolamine were obtained from (El Nasr Co., for Chemicals and Pharmaceuticals, Cairo, Egypt).

2.2. Preparation of griseofulvin microemulsion-based gel (MEBG). Microemulsion-based gel of griseofulvin (GF) was prepared by the method reported by Magdy Mohamed, 2004 [10]. The gel was prepared by dispersing Carbopol 934 in purified water with stirring and the pH was adjusted to 5.5 using triethanolamine (TEA). The microemulsion was prepared by

dissolving span 20 in light liquid paraffin. Griseofulvin was dissolved in the oil phase of the microemulsion. For formulae F4, F5, F9, F10; GF was dissolved in DMSO and then added to the fatty phase of the microemulsion. The aqueous phase was prepared by dissolving tween 20 in purified water. Methyl and propyl parabens were dissolved in propylene glycol and then added to the aqueous phase. Both the oily and aqueous phases were separately heated to 70 °C to 80°C; then the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature. The obtained microemulsion was mixed with the prepared gel with slight stirring to produce microemulsion-based gel. The composition of GF MEBG formulations is shown in Table 1.

2.3. Physical examination. The formulated GF MEBG preparations were checked visually for their appearance (color, homogeneity, consistency and spreadability). The pH of 1 % aqueous solution of the formulated MEBG was determined using a digital pH meter (pH meMttler-Toledo GmbH, Switzerland) [11].

2.4. Drug content study. Griseofulvin concentration in the MEBG formulations was assessed by dissolving known quantity of MEBG in DMSO by sonication for 10 min. Absorbance was measured after centrifugation and suitable dilution at 295 nm using a UV/VIS spectrophotometer (Genesys^{TM} 5, Thermospectronic, USA) and drug content was determined [11].

2.5. Droplet size and size distribution measurement. Droplet size and size distribution of the formulated GF MEBG were carried out using zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) at room temperature. A 1.0 gm sample was dissolved in purified water and agitated to gel homogeneous dispersion [12]. Samples were injected to photocell of zetasizer and mean droplet size and size distribution were obtained.

2.6. Transmission electron microscopy (TEM). Transmission electron microscope (Jeol-JSM 1010, Japan) was used for studying the morphology and size of the MEBG. A drop of the MEBG was placed on a carbon coated copper grid, stained by 1% phosphotungstic acid solution and investigated on TEM [12].

2.7. Rheological studies. The viscosity of the formulated GF MEBG was performed at 25 °C using a cone and plate viscometer with spindle 52 (Brookfield Engineering Laboratories, model HADV-II, Middleboro, MA). Specific amount of the formulation to be inspected was put to the plate and left for equilibrium. The rate of the shear was from 10 to 80 s^{1} with 2 min between each 2 consecutive speeds. The viscosity values were determined from the obtained rheograms and all measurements were made in duplicate [13].

2.8. In vitro release study. In vitro release of GF from different MEBG formulations was performed in 100 ml of the buffered solution of pH 5.5 at 37± 0.5 °C and 50 rpm using the dialysis method [13]. A semipermeable membrane obtained from (Sigma Chem. Co., USA) with molecular cut of 12,000 Daltons, was used. Specific quantity (0.25 gm) of different GF loaded MEBG formulations containing 1.25 mg of GF (to maintain sink condition) were positioned in the dialysis cell. Samples of 5 ml each were withdrawn at different time intervals and measured spectrophotometrically at 295 nm and the withdrawn samples were exchanged by identical quantities of the buffered solution. The release study was carried out for 24 h and each test was repeated three times. The results were expressed as the percentage of cumulative amount of drug released as the function of time.

2.9. Kinetic analysis of the in release data. The drug release data were analyzed to establish the order of drug release. The linear regression was used for analysis of the released data according to zero order, first order and Higuchi diffusion models. Moreover, the equation suggested by Korsmeyer was utilized [13]: $M_t/M_0=kt^n$, where M_t/M_0 is the fractional quantity of the drug released from the MEBG formulation at time t, k is the release rate constant and n is the diffusion exponent that describes the type of the release mechanism. Values of n and k were estimated by the linear regression of log (M_t/ M_0) versus log t.

2.10. Calculation of the permeation parameters of GF across semipermeable membrane. The permeability of GF from different MEBG formulations across semipermeable membrane (0.001 cm thickness) was performed. The membrane samples were placed on glass cylindrical tubes (10 cm in length and 7.07 cm^2 surface area). Weighted amount of MEBG contain 1.25 mg of GF putted on dialysis tubes and the tubes were putted in 100 ml of the buffered solution of pH 5.5 at 37± 0.5 °C and stirred at 50 rpm. The samples were withdrawn at time intervals 0.5, 1, 1.5, 2, 3, 4, 5, 6, 24 h and exchanged with equivalent quantity of fresh buffered solution. The absorbance of the obtained samples was measured spectrophotometrically at 295 nm. The permeation parameters of GF; the steady state fluxes (Jss), permeability coefficients (Kp), and cumulative drug permeated per unit of semipermeable membrane surface area (Q/S) were calculated from the permeation data across semipermeable membrane [26].

2.11. Histopathological examination. Male mice were used for this study weighing from 30-40 g and the hair was eliminated on the dorsal side of mice in the direction of tail to head. As control (no treatment) one mice was reserved. The selected formula F5 was applied evenly on the dorsal area and maintained for 5 h. The mice were killed after that and the exposed dorsal surface were cut. The cutting samples were set in buffered formalin and inserted in paraffin and microtoned. The specimens were stained with hematoxylin and eosin and tested under microscope [8]. Ethical approval to perform the histopathology studies in male mice was obtained from International Animal Ethics Committee of king Saud University and their Guidelines were followed throughout the studies.

2.12. Skin sensitivity test. Ten mice were used for this study (30-40 g). Formulation F5 was uniformly placed to different areas of skin approximately (3x3 cm^2). After 5 h the MEBG was removed and the skin was inspected for any changes as erythema, burning and edema [11].

2.13. Microbiological assay. The clinical strains of *Microsporum audouinii, M. ferrugineum, M. canis, M. gypseum, Trichophyton concentricum and T. interdigitale* used in this study were obtained from Microbiology lab of King Khalid University hospital (Saud Arabia). The isolates were previously identified according to the conventional mycological method. The tested isolates were well-maintained in sterile bi-distilled water at 4 ^{0}C till the experiments

were performed. The standard strains of *Asp. niger* ATCC 16404 and *Candida albicans* STCC 22019 were included. Prior to testing, inoculum of the dermatophytes strains stock suspensions was subcultured on potato dextrose agar (PDA) (Merck, Germany) with gentamicin [14]. Incubation at 28 °C to produce conidia was maintained for seven days under controlled humidity. *Asp. niger* ATCC 16404 and *C. albicans* STCC 22019 were included as reference strains. After growth, the inoculum preparation was accomplished according to Santos et al., 2005 procedures [15]. Briefly, the cultured colonies were mixed with 5 mL of 0.9% sterile normal saline and the conidia were harvested with a sterile loop on the surface, creating a conidial and hyphal fragments mixture. The fragments of conidia and hyphal mixture were filtered. The spores were collected after filtration using a sterile filter with pore diameter 8 mm (Whatman no. 40), which permits passage only of spores and retains hyphal fragments [15]. The conidial suspension was collected in a sterile tube and adjusted to $1.0x10^6$ conidia/mL using a spectrophotometer at 65% transmittance and 520 nm. Quantification of the inoculum was made by plating of 0.01 mL adjusted inoculum on PDA plates. The plates were incubated for 7 days at 28°C and were inspected daily for the existence of fungal growth. The tested inoculated were adjusted to a final dilution of 1:50 in RPMI 1640 buffered with 0.165 M morpholine propane sulfonic acid (MOPS) at a pH of 7.0. The antifungal tests were performed against many strains of dermatophytes using disk diffusion method according to Esteban et al., 2005 [16]. Filter paper disks of 5.0 mm diameter were prepared and sterilized by autoclaving. The cultures were consistently spread on the surface of 10 cm Petri dishes which contained PDA medium and exposed to air dry accordingly. The antifungal disks containing the test agents (the optimized preparation equivalent to 10 µg of griseofulvin) were applied to the plates using an ethanol dipped and flamed forceps, the antifungal disks were aseptically placed on the upper layer of the PDA medium plates sufficiently separated from each other to keep away from overlapping of inhibition zones. The plates were incubated in an upright position at 25°C±2 for 5-10 days. The diameters of the inhibition zones that appear around the disks were measured to the nearest millimeter (mm) and the results were recorded. All tests were performed in triplicate and expressed as average value. Standard plot of griseofulvin against all the dermatophyte strains were prepared in the concentration range 10 µg/100 mL. Griseofulvin (10 µg/disk) was used as a positive control and negative control was prepared using respective media.

2.14. Stability studies. The selected MEBG, F5 formulation was stored away from light in high-density polyethylene bottles at 4°C for one month. After storage, the samples were examined for the physical appearance and rheological performance.

2.15. Statistical analysis. All data were represented as mean ± SD and statistical comparisons were created using Student' s t-test. The differences were considered to be statistically significant when ($p < 0.05$).

3. RESULTS SECTION

3.1. Physical examination. The prepared GF MEBG formulations were white viscous and creamy with a smooth and consistency appearance. They are simply spreadable with suitable bioadhesion properties. The pH of all MEBG formulations ranged from 5.21 to 6.54. The drug content of all formulations was in the range of 85.36 (F9) to 104.3 % (F10) while for F1 and F6 the drug content was 44.70 and 47.70 %, respectively (Table 2).

3.2. Droplet size and size distribution. The mean droplet size of all MEBG was ranged from 122.8 nm ± 4.66 (F1) to 567.75 nm ± 54.09 (F5) as shown in Table 2. It was found that the increase in the oleic acid concentration from 2.5 to 5 % (w/w) resulted in an increase in the droplet size from 122.8 nm ± 4.66 to 273.45 nm ± 50.41 for F1 and F2, respectively. Further increase in the oleic acid concentration to 7.5 % led to non significant decrease in the particle size to 236.45 nm ± 36.13 (F3). For the other formulations it was seen that the increase in the oleic acid concentrations from 2.5 to 7.5 % resulted in increasing in the particle size of the MEBG. The polydispersity index (PDI) of MEBG was from 0.526±0.06 to 1±0 which evidence the consistency of MEBG [12].

3.3. Transmission electron microscopy. TEM examination is applied to approve the droplet size gained by the laser scattering spectroscopy. The MEBG seemed bright and the backgrounds were dark as seen in Fig. 1. Moreover, TEM image of the MEBG was observed which reveals that the droplets size were in the range of microemulsoin and in agreement with the results obtained from zetasizer particle size.

3.4. Rheological studies. Figure 2 shows the rheograms (shear stress vs shear rate) of different GF MEBG formulations. In addition the flow behavior of all GF MEBG formulations is shown in Table 3 and Figs.3 and 4. As evident in the figures, the prepared GF MEBG formulations revealed non Newtonian pseudoplastic flow performance (shear thinning systems) at 25 °C since the viscosity decreased with increasing the shear rate [17]. When the shear rate is enhanced, the usually molecular structure of the gelling matter is caused to disarrange its long axes in the trend of a flow which in turns lessens the internal resistance of the material and hence declines its viscosity. Shear thinning performance is a necessary property of topical semisolid delivery system as it should be thin during usage and thicken otherwise [18]. Figure 3 shows the relationship between the viscosity and concentrations of Carbopol; 0.5 % and 1% (w/w) of MEBG formulations at shear rate of 70 s^{-1}. As seen in the Fig. 3 by increasing the Carbopol concentration, from 0.5 to 1 % (w/w), the viscosity value was significantly ($p<0.05$) increased. The viscosity values were 3.75±0.18, 2.93±0.19, 4.24±0.1 using 0.5 % Carbopol (F1, F2, F3) and 6.44±0.46, 5.26±0.19, 5.85±0.48 using 1 % Carbopol (F6, F7, F8), respectively. The same observations were obtained for formulations F4, and F5 (0.5 % Carbopol) and formulations F9 and F10 containing 1 % Carbopol (Fig. 4). The same remarks were obtained at all shear rates used. This is compatible with the other stated results [11, 13, 19].

Figure 3 and Table 3 also illustrate the effect of applying oleic acid in three different concentrations (2.5, 5 and 7.5 %w/w)

on the viscosity of MEBG formulations. It is noticeable that by rising the concentrations of oleic acid from 2.5 to 5 % (w/w) for F1, F2 respectively, the viscosity is non-significantly decreased from 3.75±0.18 to 2.93±0.19, respectively. Further increasing the oleic acid concentration to 7.5 % (F3), the viscosity value is non-significantly increased (4.24± 0.1) at shear rate of 70 s^{-1}. The same observations obtained using 1 % Carbopol MEBG formulations. The same remarks were obtained at all shear rates used. Moreover, the effect of using DMSO in the MEBG formulations on the viscosity was also studied. It was observed from Fig. 4 and Table 3 that 0.5 % Carbopol MEBG formulations (F1- F3) exhibited greater viscosity than the formulations containing DMSO (F4 and F5). The same notes concluded for 1 % Carbopol formulations.

3.5. Histopathological and skin irritation examinations. The observations of the skin irritation of selected formulation of MEBG (F5) showed no erythema, no redness and also no edema after the application of F5 for 5 h. The microscopic examination of the mice skin for control and F5 as presented in Fig. 5 confirmed no changes with regard to the anatomical and pathological state of mice and hence the safety of the formulated MEBG on mice skin.

3.6. In vitro release study. In vitro release of GF from different MEBG formulations using two different Carbopol concentrations (0.5% and 1% w/w) was studied as presented in Figs. 6 and 7. It is observed from the Figs. that at 0.5% (w/w) Carbopol in F1, F2, F3 and 1 % Carbopol in F6, F7, F8, the % amount of drug released after 24 h is 75.87±0.287, 75.95 ±0.490, 75.50±0.490, 73.44±0.490, 73.20±0.490, 76.10±0.490 %, respectively. The gained results indicated that the release rate of GF from different MEBG non significantly ($p>0.05$) affected by the Carbopol concentrations. While the rate of the release of GF from F4 (81.62 %) and F5 (90.91%) containing 0.5 % Carbopol was higher than F9 (76.1±5.56) and F10 (82.29±0.73) containing 1 % Carbopol after 24 h. The enhance in the viscosity might have influence to the reduced rate of the drug release from F9 and F10 as shown in Table 3. This observation is in agreement with the other recorded results [13, 20].

Moreover, the influence of using (15 % w/w) DMSO as penetration enhancer on the GF release was also assessed. It is clear from the obtained results that the release of GF from the MEBG increases with the addition of DMSO in the formulations. It was observed that the % amount released of GF from F4 (81.63±4.66) and F5 (90.91±9.62) containing DMSO was higher than F1 (75.87± 3.45) and F3 (75.50±0.71) without DMSO. The same notes obtained for F9 (76.10±5.56) and F10 (82.29±0.73) containing DMSO in comparing with F6 (73.44±0.196) and F8 (70.80±7.72) as shown in Figs. 6 and 7. The skin barrier function may be changed by using DMSO as enhancer in the MEBG formulations thus the drug permeation was improved. The obtained results agree with the other reported results [8,9].

The impact of the oleic acid concentration on the GF release was also estimated. Figures 6 and 7 illustrate the release of GF from various MEBG using three different concentrations of oleic acid (2.5, 5 and 7.5 % w/w). It was observed by increasing the oleic acid concentration from 2.5 to 5 and further to 7.5 %, the % amount released of GF from 0.5 % (w/w) Carbopol MEBG is 75.87±0.287, 75.95 ±0.490, 75.50±0.490 for F1, F2 and F3, respectively. The similar achieving was noticed for the MEBG formulations containing 1 % Carbopol in F6, F7 and F8 (Figs. 6 and 7). It is evident from the results that the release of GF from the MEBG was not significantly ($p > 0.05$) affected with an increase of the oleic acid concentrations. F5 and F10 formulations showed the highest drug amount released; 90.91±9.62 and 82.29±0.73, respectively. In formulation F5 and F10, oleic acid is present in its highest level (7.5 %) and also containing DMSO (15 %). This finding evidenced that the effect of oleic acid in enhancing the drug release from the MEBG was more predominant in the presence of DMSO as penetration enhancer. Aggarwal et al., 2013 [9] and Zhu W et al., 2008 [21] reported that oleic acid might disturb the lipid layer in the stratum corneum by making isolated domains which hinder the permanence of the stratum corneum and encourage highly penetrable passage ways in the stratum corneum.

3.7. Kinetic analysis of the release data. The release data of GF from the different MEBG formulations were fitted mathematically according to different kinetic models. The obtained data are elucidated in Table 4. The values of n (release exponent) were between 0.275 and 0.409 for all the formulations revealing a fickian release performance controlled by a diffusion mechanism. The obtained data are compatible with the other informed results [10].

3.8. Calculation of the permeation parameters of GF across semipermeable membrane. The permeation parameters of GF from different MEBG across semipermeable membrane are listed in Table 5. The obtained results showed that Carbopol concentrations had non significantly ($p>0.05$) influence on the cumulative permeated amount of GF from different MEBG. It is noticed from the Table that at 0.5 % (w/w) Carbopol as in F1, F2, F3 and 1 % Carbopol in F6, F7, F8, the cumulative permeated amount after 24 h is 134.24±6.12, 135.36±2.43, 133.53±1.27, 129.94±0.35, 129.94±0.35, and 125.27±13.66, respectively. While the cumulative permeated amount of GF from F4 (144.42±8.26 µg/cm^2) and F5 (160.85±17.04 µg/cm^2) containing 0.5 % Carbopol was higher than F9 (141.44±3.73 µg/cm^2) and F10 (145.6±1.31 µg/cm^2) containing 1 % Carbopol after 24 h. With regard to the flux and permeability coefficient values, F4 and F5 gave the highest flux (2.21±0.0.3 and 2.52± 0.15 µg/cm^2h, respectively) and permeability coefficient (1.76±0.02 and 2.02 ±0.02 cm/h, respectively) compared with the F 9 and F10 (1.69 ±0.07 and 1.81±0.05 µg/cm^2h for flux and 1.36±0.02 and1.45±0.03 cm/h, respectively). It is observed from the obtained results that the cumulative permeated amount, flux and permeability coefficient values increase with the addition of DMSO as shown in F4, F5 compared with F1, F2 and F3. It is concluded also that the cumulative permeated amount, flux and permeability coefficient values were not significantly ($p>0.05$) affected with an increase of the oleic acid concentrations.

3.9. Microbiological assay. Criteria of susceptibility and resistance of two griseofulvin formulations against 6 species of dermatophytes are given in Table 6. There was no growth of fungi in both formulations. It is clear that *T. concentricum M. ferrugineum and T. interdigitale* are most sensitive strains with formulation F5 with mean inhibition zone diameter 57.75, 49.07 and 49.14 mm respectively. Moreover, *T. concentricum, M. canis* and *M. audouinii* are the most sensitive strains with formulation F3 with mean inhibition zone diameter 43.50, 39.10 and 38.30 mm respectively. Antifungal activities of tested formulations were

investigated for their in vitro antifungal activity against many clinical dermatophytes strains. In the present study, agar-based disk diffusion method was used to determine the susceptibility of dermatophytes *M. audouinii* (*n* = 6), *M. ferrugineum* (*n* = 7), *M. canis* (*n* = 9), *M. gypseum* (*n* = 6), *T. concentricum* (*n* = 2), and *T. interdigitale* (*n* = 1). Two isolates *Asp. niger* ATCC 16404 and *Candida albicans* STCC 22019 served as quality control strains. Two formulations showed variable inhibition activities against the tested dermatophytes strains. DMSO is frequently used as a solvent for antifungal drugs in several studies for the determination of antifungal activities with a concentration of 1% or less in most studies [22]. Formulation F5 that additional dissolved in DMSO presented the finest antifungal properties versus all the chosen strains in comparison with formulation F3. These results indicated that the DMSO play an important role in the antifungal potency where the activity was increased when included in the formulation at concentrations ranged between 1.25 to 5% (23). Also, Randhawa reported that DMSO 10% has a good effect with a negligible growth of germ tubes of both the arthrospores and yeast [24]. The distinct effect of the DMSO formulation in a suitable concentration may be due to its bind with the plasma membrane of cells and increases membrane permeability [25]. These results indicated that antifungal formulation containing 15% DMSO concentration could increase griseofulvin effect and can open the door for its application in the pharmaceutical industry.

3.10. Stability studies. The selected formulation F5 was observed to be stable after one month, where no alteration was found in the physical appearance. The viscosity values were 2.837 ±0.10, 2.837 ± 0.10 and 2.048 ±0.22 after 0, two weeks, and one month, respectively upon storage at 4 °C at shear rate of 70 s^{-1}.

Table 1. Composition of griseofulvin microemulsion-based gel (MEBG) formulations, quantity, gm (w/w %).

Formulae Code	Griseofulvin (GF)	Carbopol (934)	Oleic acid	Dimethyl sulfoxide (DMSO)	Tween (20)	Span (20)	Propylene glycol	Methyl paraben	Proplyl paraben	Purified Water to
F1	0.5	0.5	2.5	-	0.5	1	5	0.02	0.01	100
F2	0.5	0.5	5	-	0.5	1	5	0.02	0.01	100
F3	0.5	0.5	7.5	-	0.5	1	5	0.02	0.01	100
F4	0.5	0.5	2.5	15	0.5	1	5	0.02	0.01	100
F5	0.5	0.5	7.5	15	0.5	1	5	0.02	0.01	100
F6	0.5	1	2.5	-	0.5	1	5	0.02	0.01	100
F7	0.5	1	5	-	0.5	1	5	0.02	0.01	100
F8	0.5	1	7.5	-	0.5	1	5	0.02	0.01	100
F9	0.5	1	2.5	15	0.5	1	5	0.02	0.01	100
F10	0.5	1	7.5	15	0.5	1	5	0.02	0.01	100

Table 2. Characterization of griseofulvin MEBG formulations.

Formulae/Code	Droplet diameter (nm)	PDI (polydispersity index)	pH	% Drug content
F1	122.8± 4.66	0.865± 0.03	5.66	44.70
F2	273.15±50.41	0.898±0.14	5.86	92.70
F3	236.45±36.13	1.00±0	6.30	102.0
F4	169.7±38.183	0.526±0.06	6.54	93.0
F5	567.75±54.09	0.694±0.02	6.30	99.70
F6	117.55±6.01	0.773±0.01	5.30	47.70
F7	197.7±14.28	0.906±0.02	5.21	98.3
F8	310.55±37.12	0.948±0.073	5.21	99.25
F9	279.825±27.25	0.898±0.01	5.25	85.36
F10	474.8±24.465	0.791±0.16	5.33	104.30

Table 3. Rheological measurements of MEBG formulations containing GF at 25 °C (n=2, mean ± SD).

Shear rate (s^{-1})	Viscosity (Pas)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
10	16.02 ±1.59	13.01 ±1.31	18.94± 0.72	15.03± 0.91	12.743 ±0.54	28.35± 2.40	24.38± 1.74	20.45± 1.11	22.37± 0.46	24.03± 0.34
20	9.08± 0.61	7.39± 0.67	10.49± 0.58	8.46± 0.46	7.022 ±0.11	16.79± 1.29	13.89± 0.06	11.78± 0.16	13.15± 0.01	12.52± 0.3
30	6.99± 0.71	5.41± 0.38	7.75± 0.31	6.08± 0.41	5.307 ±0.11	12.16± 0.74	10.05± 0.11	8.70± 0.17	9.75± 0.01	9.07± 0.11
40	5.64± 0.48	4.37± 0.33	6.33± 0.19	4.87± 0.35	4.316 ±0.06	9.67± 0.66	8.02± 0.19	7.12± 0.23	7.88± 0.01	7.49± 0.11
50	4.77± 0.43	3.71± 0.25	5.48± 0.02	4.1± 0.29	3.635 ±0.05	8.16± 0.63	6.75± 0.19	6.37± 0.34	6.75± 0.07	6.43± 0.05
60	4.25± 0.28	3.26± 0.21	4.78± 0.05	3.57± 0.26	3.178 ±0.10	7.18± 0.55	5.88± 0.20	5.98± 0.44	5.98± 0.01	5.81± 0.01
70	3.75± 0.18	2.93± 0.19	4.24± 0.1	3.19± 0.21	2.837 ±0.10	6.44± 0.46	5.26± 0.19	5.85± 0.48	5.28± 0.01	5.45± 0.01
80	3.31± 0.04	2.68±	3.88± 0.11	2.9± 0.17	2.553 ±0.07	5.86± 0.41	4.80± 0.14	6.01± 0.58	4.81± 0.07	5.14± 0.03

Table 4. The kinetic parameters of GF MEBG formulations release data according to different kinetic models.

Formulae Code	Linear regression analysis using correlation coefficient (r^2) according to				
	Zero-order	First-order	Higuchi model	Korsmeyer - Peppas	
				r^2	n
F1	0.8487	0.7328	0.8576	0.9922	0.340
F2	0.8238	0.6517	0.7776	0.9577	0.317
F3	0.7832	0.5983	0.7876	0.9379	0.331
F4	0.8276	0.7699	0.8017	0.9904	0.317
F5	0.8619	0.5382	0.8407	0.9987	0.356
F6	0.8718	0.8568	0.8949	0.9785	0.307
F7	0.7951	0.6987	0.7628	0.9865	0.302
F8	0.8018	0.7671	0.7541	0.9678	0.275
F9	0.7506	0.6100	0.6449	0.9903	0.317
F10	0.8299	0.6510	0.8025	0.9950	0.409

Table 5. In vitro permeation parameters of griseofulvin across semi-permeable from different MEBG formulations (mean ± SD, n=3).

Formulae Code	Q/S ($\mu g/cm^2$)	Jss ($\mu g/cm^2 h$)	k_p $x10^{-3}$ (cm/h)
F1	134.24±6.12	1.32±0.01	1.06±0.01
F2	135.36±2.43	1.46±0.12	1.17±0.02
F3	133.53±1.27	1.59±0.65	1.32±0.01
F4	144.42±8.26	2.21±0.0.3	1.76±0.02
F5	160.85±17.04	2.52± 0.15	2.02±0.02
F6	129.94±0.35	1.41±0.21	1.13±0.02
F7	129.94±0.35	1.41±0.21	1.13±0.02
F8	125.27±13.66	1.41± 0.10	1.13± 0.01
F9	141.44±3.73	1.69 ±0.07	1.36±0.02
F10	145.6±1.31	1.81±0.05	1.45±0.03

Jss, the steady state fluxes; k_p, permeability coefficients; Q/S, cumulative drug permeation per unit of semi-permeable membrane surface area.

Table 6. In vitro activities of griseofulvin preparations against 31 strains of dermatophytes.

Species	No. of strains tested (%)	DMSO Formulation F5		Formulation F3	
		Mean IZD* (mm)	IZD range (mm)	Mean IZD* (mm)	IZD range (mm)
M. audouinii	6 (19.35)	41.00	30–55	38.30	30–41
M. ferrugineum	7 (22.58)	49.07	36–65	35.05	33–48
M. canis	9 (29.03)	42.29	35–58	39.10	37–42
M. gypseum	6 (19.35)	48.33	50–56	34.20	41–42
T. concentricum	2 (6.45)	57.75	45–65	43.50	40–48
T. interdigitale	1(3.23)	49.14	33–60	36.00	35–42

IZD* Inhibition zone diameter.

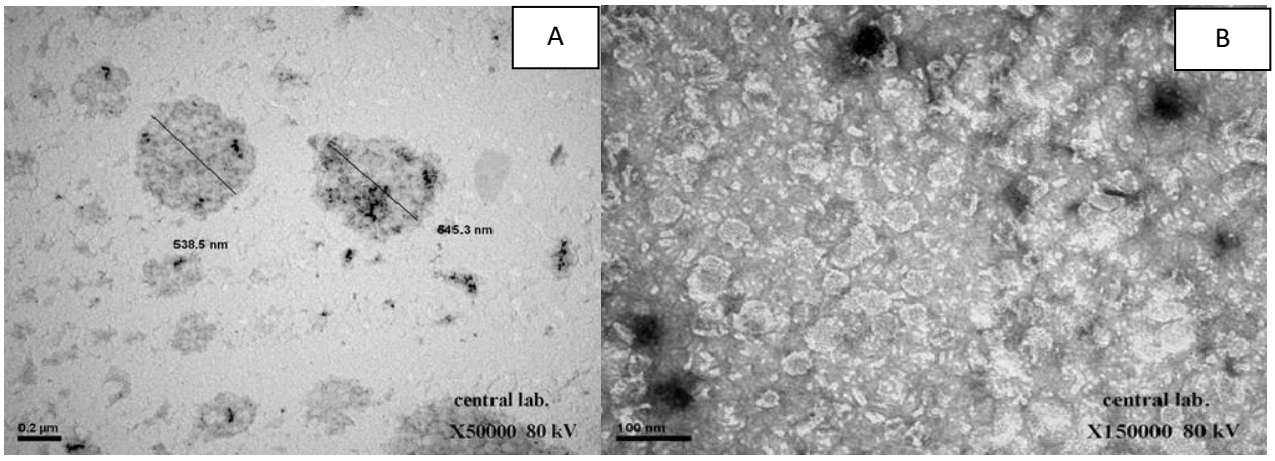

Figure 1. TEM photographs of F5 formulation after magnification of 50000 (A) and 150000 (B).

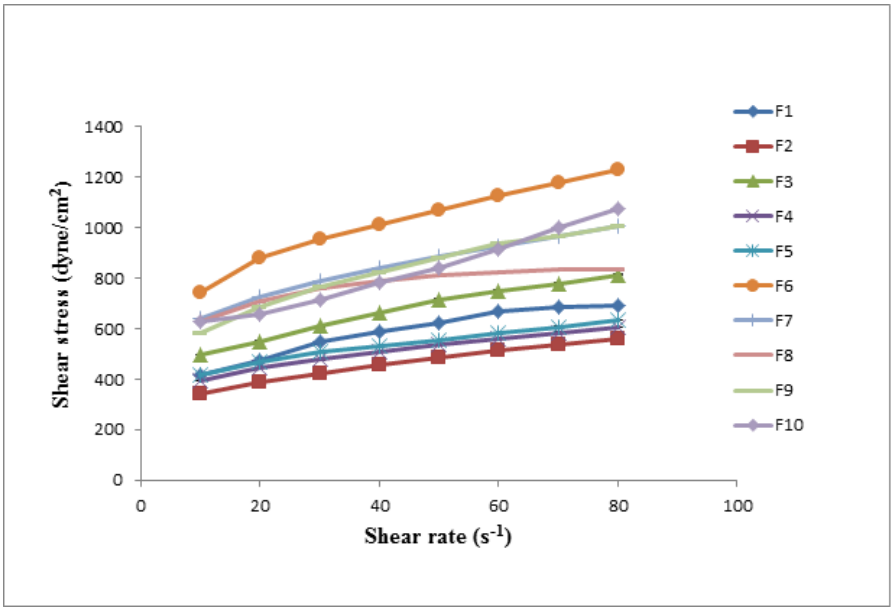

Figure 2. Rheograms of different GF MEBG formulations (n=2, mean ±SD).

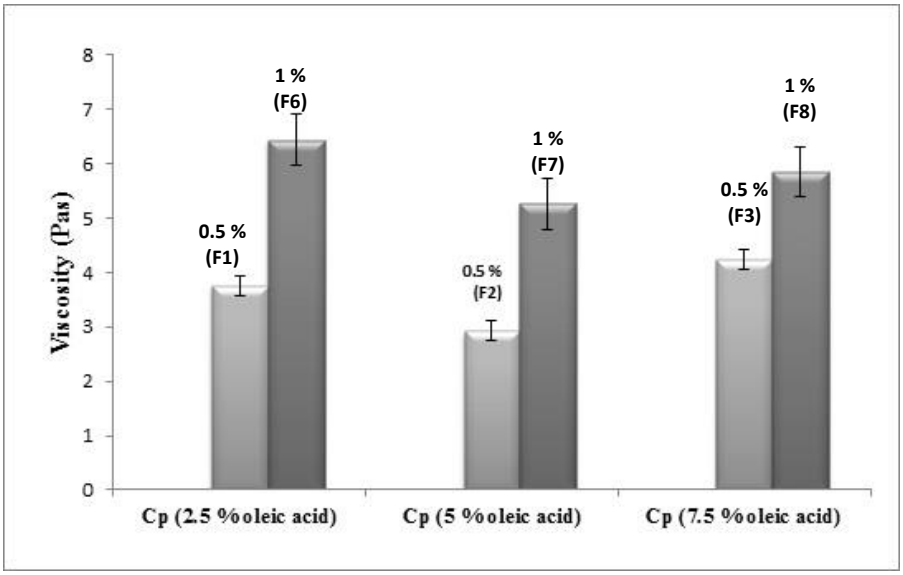

Figure 3. Effect of Carbopol and oleic acid concentrations on the viscosity of GF MEBG at shear rate of 70 s^{-1} (n=2, mean ±SD).

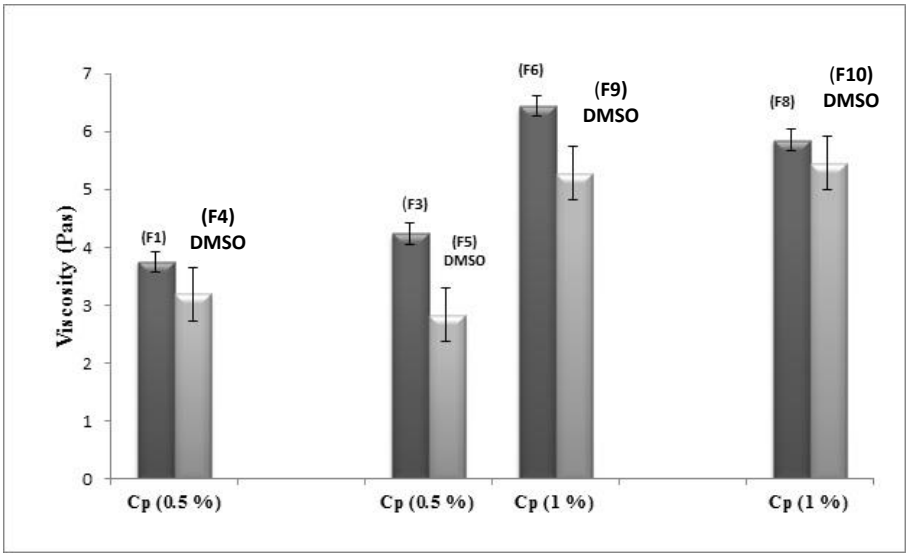

Figure 4. Effect dimethylsulfoxide (DMSO) concentration (15 % w/w) on the viscosity of GF MEBG at shear rate of 70 s^{-1} (n=2, mean ±SD).

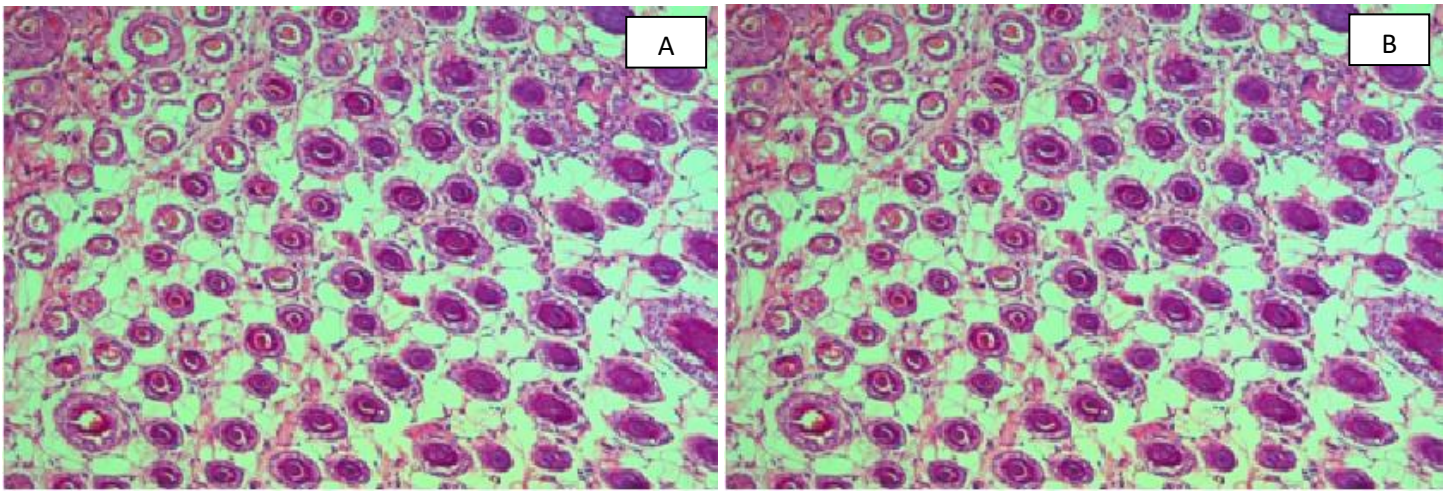

Figure 5. Histopathological photographs of the skin of mice after treatment with control A (no treatment) and B (MEBG, F5).

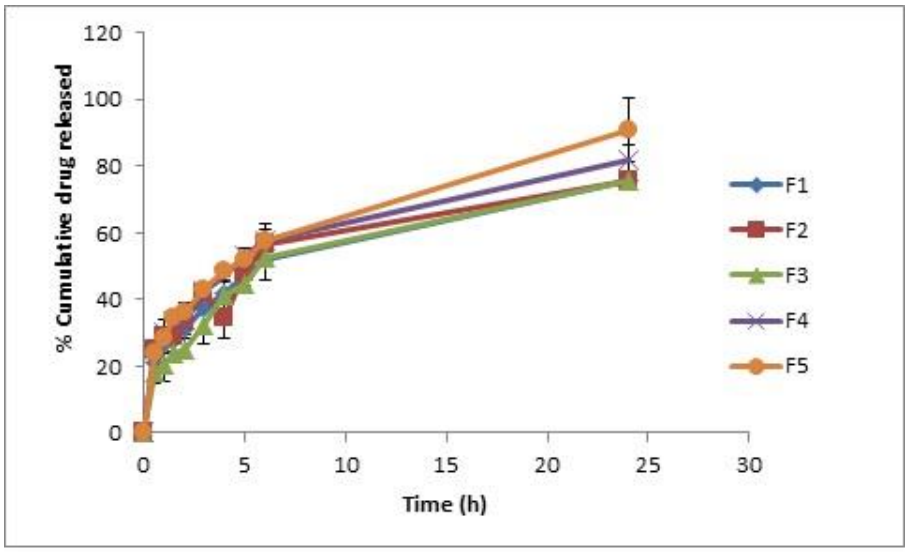

Figure 6. In vitro release profiles of different GF MEBG formulations containing Carbopol (0.5 % w/w) in buffered solution of pH 5.5 (n=3, mean ± SD).

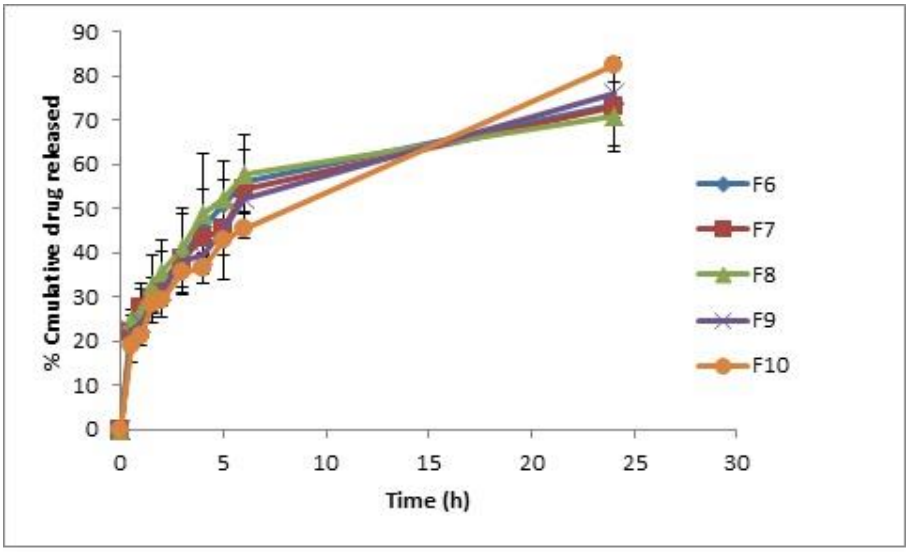

Figure 7. In vitro release profiles of different GF MEBG formulations containing Carbopol (1 % w/w) in buffered solution of pH 5.5 (n=3, mean ± SD).

4. CONCLUSIONS

In this work microemulsion based gel was formulated as delivery system for griseofulvin for topical purpose. The results displayed that they exhibited suitable physical properties and had non-Newtonian pseudoplastic shear thinning behavior. Carbopol concentrations significantly affected on the viscosity while oleic acid concentrations had no effect. Moreover, formulations containing DMSO exhibited lower viscosity than that free from DMSO. The particle size and size distribution were acceptable. The in vitro drug release was enhanced in the presence of DMSO and the rate of the release from formulations containing 0.5 %

Carbopol was higher than that containing 1 % Carbopol. Moreover, the selected F5 formulation had the highest flux and permeability coefficient compared with the other formulations. The histopathological examination of F5 confirmed the safety of the formulated MEBG on mice skin. Also, the selected F5 formulation showed higher antifungal activity in the comparison with F 3 that is free from DMSO and exhibited stability after one month of storage.

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