Effect of Nanohybrid (Griseovulvin and Magniseum/Alaminium- Bilayers hydroxide) against Dermatophytes spp fungal pathogens

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Abstract

A nanohybrid antifungal Mg/Al-Gri-LDH was prepared by using sol-gel ion exchange method between the antifungal Griseovulvin (Gri) and Bilayers hydroxide (Mg/Al-LDH). The new nanoantifungal were created. FT-IR spectroscopy showed formation a new group in the Mg/Al-Griseovulvin-LDH spectrum that revealed the appearance some new substances. The X-Ray Diffraction (XRD) spectrum showed the formation a new diffraction peaks in the nanohybrid antifungal spectrum when comparison with the spectrum of Bilayers hydroxide. The two- and three-dimensional picture of the Mg / Al-Gri-LDH in atomic force microscope indicated appearance of new antifungal with nanodimensions. The antifungal action of the nanohybrid antifungal was tasted with 12 isolates of Dermatophytes spp. Current results found the higher clear zone was found against Epidermophyton floccosum (15.80 mm) isolated from Tinea pedis and Trichophyton mentagrophytes (15.08 mm) isolated from tinea corporis of body. While Trichophyton mentagrophytes (0.41mm) isolated from Tinea capitis of head showed resistances to antifungal drug.

Key words: Nanohybrid, Bilayers hydroxide, Griseovulvin, biological activity.

1. Introduction

Dermatophytes are most common fungi in its ability to infect millions of humans and animals due to its high tendency to analyze keratin [3]. When Dermatophyt isolated from skin's clinical specimens, the Trichophyton is most frequent genus of these groups, which consists of three fungal genus [1,2]. Trichophyton mentagrophytes are the most common species of Dermatophytes, which also include the Microsporium and Epidermophyton genus, which cause tinea infections. [4] Many reports have suggested the possibility of transmission of this species of fungus by domestic animals, especially dogs (which is considered as part of their normalflora) by direct or indirect contact [2].

The incidence of fungal infections has increased recently reaching to 20-25% of the world's population as well as increased resistance to some traditional antifungal drug, which necessitates many studies to find therapeutic alternatives for these types of difficult fungus infection [5,6].

Griseovulvin antifungal is an effective antifungal drug against many dermatophytes by its ability to prevent cell division due to its direct effect in the formation of achromatic spindle [7, 8]. This antibiotic, which belongs to the Heterocyclic benzofuran group, is a white crystalline, creamy or yellowish white powder that has the molecular formula C17H17ClO6 and has a molecular weight of 352.767 g / mol. It is characterized by its solubility in both ethanol, methanol, and acetone, chloroform and dimethyl form amide, as well as being partially soluble in Water [9,10]. In recent years' nanotechnology has been widely used in the medical and pharmaceutical fields and is assumed to have a serious role in diagnosis, treatment and prevention different infection [11].

The antifungal drugs have gained their share of nonoscale applications that have increased their therapeutics effects for this reason the present study aimed to show apositive effect of Griseovulvin conversion to ananoportic in increasing its inhibition effect against fungus.

2.Materials and Methods

2.1 Preparation of Hybrid Nanoparticle antifungal drugs

The preparation of the Hybrid Nanoparticle antifungal drugs was made according to the method which described by [12] with many modifications. Fifteen ml of the glysofolvinwas added drop after drop to the bilhhydroxide solution (which prepared from dissolve 1 g of the two-hydroxide layers deposition in 50 mL of DDwater. The mixture was stirred magnetically at 25Co for two hours. Then placed in the shaking incubator at 37 Co for 18 hours and transferred to the incubator at 40Co for 24 hours. The precipitate was separated by centrifuge at 5000 rpm for 20 min then washed with D D water

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several times. Finally the precipitate was dried at 40 Co and grind using ceramic motor and stored until use.

2.2. Diagnosis of hybrid nanoparticle compound

The Mg / Al-Gri-LDH hybrid drug was diagnosed with spectral methods and tested with the Atomic Force Microscope (AFM) as follows:

a. Diagnosis by infrared spectrum (FT-IR).

Tablets of A hybrid Nanoparticle with KBr was made after grinding well and measured infrared spectrum in a range of 400-4000 cm

b. Diagnosis by X-ray diffraction XRD).

The Mg / Al-Gri-LDH was diagnosed by using the X-ray diffraction spectroscopy through shows the variation thickness of the Bilayers hydroxide before and after the injection procedure of the Mg / Al-Gri-LDH antifungal using a cod of Brack**nλ = 2dSinθ** after the insertion process.

c. Diagnosis by Atomic Force Microscopy (AFM).

Use the Atomic Force Microscope to examine the Mg / Al-Gri-LDH nanoparticle and measure the diameters, volumes and concentrations of nanoparticles. Then Crystallinity Index was calculated extracted according to formula [13].

Crystallinity Index = Dp / L

d. Determine the antifungal activity of the Mg / Al-Gri-LDH nanoparticle and free Grisofulfin.

Twelve isolates of skin's fungi (were taken from face, hands, legs and nails) were obtained from microbiology lab of biology department of Science / Kerbala University. These isolates included six isolates of *Trichophyton mentagrophytes*, three isolates of *Microsporum canis*, and three isolates *of Epidermophyton flaccosum*. The antifungal activity of studied compounds was tested according to the method described by [14]. The Minimum Inhibition Concentration of the nano-antibiotic was determined by using concentrations ranging of (0.01 -5) mg / ml

2. 3.Results and discussion

Diagnosis of hybrid nanoparticle

3.1. Diagnosis by infrared spectrum

3.1.1 IR spectrum of antifungal glycyofolphin

The results in Fig (1) illustrated that the beam at the frequency of 3392.90 cm $^{-1}$ indicated the low-frequency of OH-H oscillation as it appears to be acute, as well as the C-H at the two frequencies (2941.54 and 2847.03) cm $^{-1}$. While, the two beams at (1708.99 and 1660.77) cm $^{-1}$ was enhanced to the vibration of T = C O.As well as the emergence of the beam at 1460.16 cm $^{-1}$ indicated the bending C-H structural vibration [15].

3.1.2Infrared spectrum for magnesium layers \ aluminum bi-hydroxide.

From the observation of Fig (2) it is clear that the magnesium / aluminum biodiesel layers showed several distinct beams at certain frequencies. The package at 3472 cm -1 is as a results of the shaking OH band, which is a cross-section of the presence of interference from different types of hydroxyl such as hydroxyl, (16,17). While, the characteristic beam at 1380 cm -1 is due to the group of nitrates (NO3) between the layers [18,19] while the beams between (400-600 cm -1) return to vibration association Mg-O and Al-O [20].

3.1.3 Infrared Spectroscopy of Hybrid Nanotubes (Mg / Al-Gri-LDH).

The results in Fig (3) illustrated that the hybrid nanoparticle shows several new characteristic bundles, Which indicated the success of the inserted anti-glycyofolphin between the hydroxide layers. The appearance wide and acute band at 3417.98 cm $^{-1}$ is due to OH, (2995.55 and 2943.98) cm -1 to a constant vibration C = H and has a move to the highi frequency, in addition to disappearance of the band of nitrate group (NO3) of the holder of the hydrohydrate. The band at the frequencies (1708.99 and

1658.84) cm -1 are indicated to a constant vibration C = O [15].

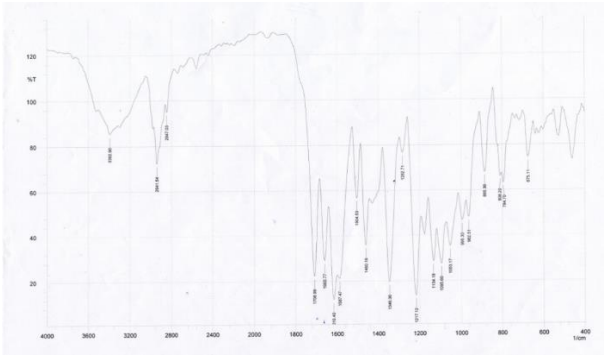

Figure (1) Infrared Spectroscopy of antifungal Griseovulvin

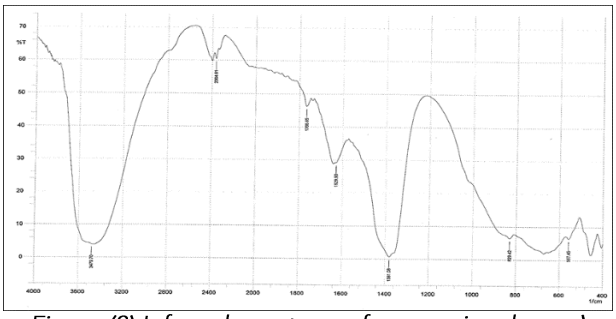

Figure (2) Infrared spectrum of magnesium layers \ aluminum bihydroxide.

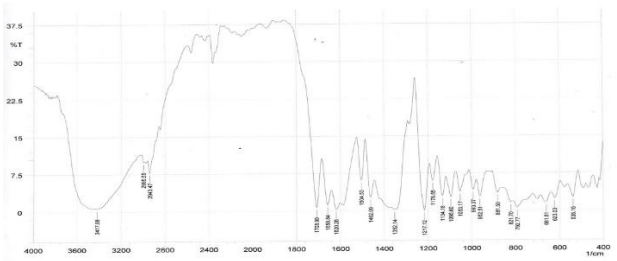

Figure (3) Infrared spectrum of hybrid antifungal Mg/Al-Gri-LDH

3.2. Diagnosis by X-ray diffraction spectra

The X-ray diffraction spectroscopy of the Mg / Al-Gri-LDH hybrid as well as Mg / Al-NO3-LDH layers were studied to determine the difference in thickness of the layer prior to the application of Glyphofoline antifungal between the magnesium / hydroxide. Fig (4) showed that X-ray diffraction spectrum of the Mg / Al-NO3-LDH layer, with crystalline levels (003), (006) and (009).

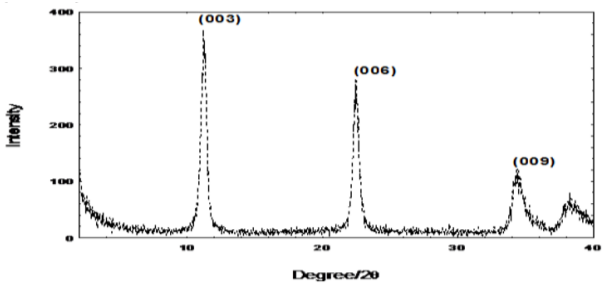

Figure (4) X-ray diffraction spectroscopy for Mg / Al-NO3-LDH

The level (003) is shown at 10.47 ° and with a crystalline distance of 0.84nm. The level (006) is shown at 22.03 ° and a crystalline distance of 0.41nm while the level of 009 is at 34.44 ° and the crystal is 0.26nm. By observing the X-ray diffraction spectroscopy of the Mg / Al-Griseovulvin-LDH hybrid, there is a diffraction of the level (003) with a crystal distance of 2.5 nm, and a diffraction of the level (006) with a crystal distance of 0.94nm and an appearance of (009) 0.7nm as shown in Fig (5).

3.3. Atomic Force Microscopy (AFM).

The outer surface of the hybrid nanoparticle was studied. The results in Fig(a6) shows a two-dimensional image of the Mg / Al-Gri-LDH antifungal drugs and showing molecular clusters of spherical forms. As well as Fig (6b) shows a three-dimensional picture of a sector of the hybrid nanoparticle surface, where the molecular concentrations of n19 (1.19) are observed indicating a hybrid nanoparticle synthesis of the free anti-glyceofolphin and bi-hydroxide layers.

Table1 shows that the mean size of the Mg / Al-Gri-LDH nanoparticle is about 89.44nm. The preparation of this hybrid get particles with diameters of 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 nanometers with ratio of (1.42, 0.95, 3.32, 2.37, 2.84, 3.79, 3.79, 4.27, 3.79, 2.84, 6.64, 5.21, 2.37, 4.27, 4.27, 2.84 and 4.74) respectively.

The results of current study different with result found by [21]. In this study obtained samples of hybrid nanoparticals with spherical shapes at sizes ranging from 50 to 76 nanometers. Based on the data obtained from Table 1 and X-ray diffraction spectra Fig (5).The Crystallinity Index of the antagonist under study was 3.25.

Table (1) Size and diameter of aggregation of the nanohybrid antifungal after tested with Atomic Force Microscopy

	Avg. Diameter:89.44 nm							
Diameter(nm)<	Volume (%)	Cumulation (%)	Diameter(nm)<	Volume (%)	Cumulation (%)	Diameter(nm)<	Volume (%)	Cumulation (%)
20.00	1.42	1.42	75.00	5.21	41.23			
25.00	0.95	2.37	80.00	2.37	43.60	130.00	3.79	82.94
30.00	3.32	5.69	85.00	4.27	47.87	135.00	2.84	85.78
35.00	2.37	8.06	90.00	4.27	52.13	140.00	2.37	88.15
40.00	2.84	10.90	95.00	2.84	54.98	145.00	2.37	90.52
45.00	3.79	14.69	100.00	4.74	59.72	150.00	2.37	92.89
50.00	3.79	18.48	105.00	5.21	64.93	155.00	1.42	94.31
55.00	4.27	22.75	110.00	3.32	68.25	160.00	2.37	96.68
60.00	3.79	26.54	115.00	2.37	70.62	165.00	1.90	98.58
65.00	2.84	29.38	120.00	3.79	74.41	170.00	1.42	100.00
70.00	6.64	36.02	125.00	4.74	79.15			

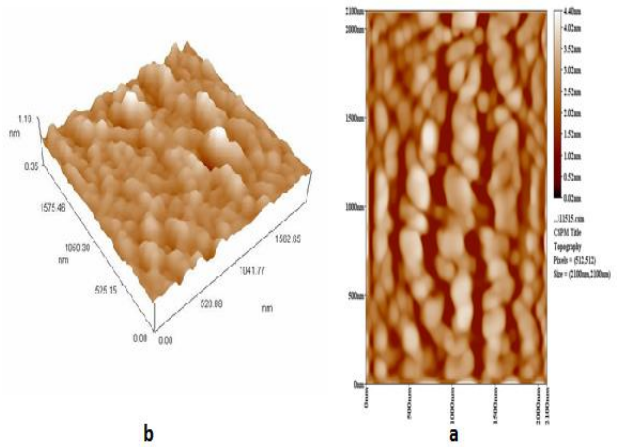

Figure (5 A two-dimensional (a) and 3D (b) image of the Mg / Al-Gri-LDH by atomic force microscope.

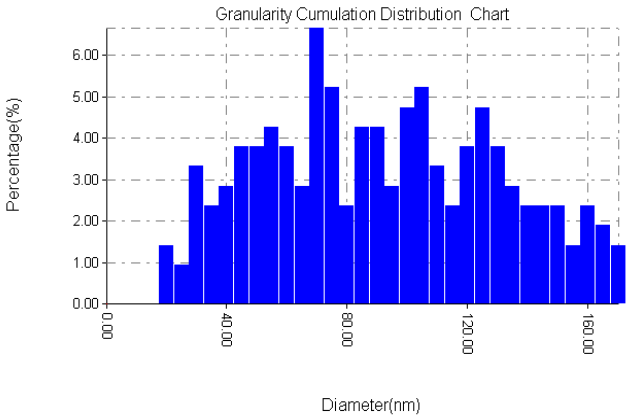

Figure (6) Granulanity accumulation distribution of nanohybrid antifungal Mg/Al-Gri-LDH after tested with Atomic Force Microscopy

3.4. Antifungal activity of Antibody of Mg / Al-Gri-LDH Hybrid Nanoparticle.

The antifungal effect of GRI-LDH was tested with 12 isolates of Dermatophyte including six isolates of Trichophyton mentagrophytes (numbring from 1 to 6), three isolates of Microsporumcanis isolates (numbring from 7 to 9) and three Epidermophyton flaccosum isolates (numbring from 10 to 12).

Table 2 Antibacterial activity of nanohyprid antifungal Gri-LDH and free Gri against 12 Dermatophyte isolates.

Compounds average	12	11	10	9	8	7	6	5	4	3	2	1	Fungal isolates	Compound
	Inhibition zone /mm												Concentration/mg/ml	
6.68B	0	0	0	0	0	0	0	0	0	0	0	0	0.01	Cri -LDH
	0	0	0	0	0	0	0	0	0	0	0	0	0.1	
	0	3	2.6	0	8.6	3.3	11	0	0	0	9.6	0	0.25	
	27	10.3	10	8.6	15.3	10	20.3	0	0	2.6	12.6	6.3	0.5	
	29	12	11.6	9	15.6	13	20.3	9	0	8.3	14	18.6	1	
	31	11.6	17.6	11	16.3	4.6	21.6	11	5	9.6	14	19	5	
16.13A	0	0	0	0	0	0	0	0	0	0	0	0	0.01	Cri- free
	0	0	0	0	0	0	0	0	0	0	0	0	0.1	
	0	0	0	0	0	0	0	0	0	0	0	0	0.25	
	35.3	33.3	42.6	30.6	31.3	29.3	35.3	39.3	0	36.6	30	40.6	0.5	
	38.3	38.6	27.3	31	38.3	36	34.6	36.6	0	34.6	43	33.3	1	
	38.6	36.3	30	41.3	34.3	30	37.6	38	0	35.3	30.6	32.3	5	
	15.80 a	11.83 cd	11.94 cd	10.97 de	13.33 bc	10.52 e	15.08 ab	11.16 cd	0.41 f	10.58 e	12.83 cd	12.47 c	Average of isolates	

* The vertically different capitals indicate significant differences (p <0.05) between the compounds.
* The different small letters horizontally indicate significant differences (p <0.05) between fungal isolates

Concentration	0.01	0.1	0.25	0.5	1	5
average	0D	0D	1.597 C	21.156 B	22.986 A	22.736 A

* The different capitals clearly indicate significant differences (p <0.05) between the concentrations used

Interference	Concentration	Isolates	Compounds	Factor
7.732	1.578	2.232	0.911	LSD0.05

Statistical analyses of the antifungal inhibition activity of the hybrid nanoantifungal of Gri-LDH and the free Gri results as shown at table 2, there was a significant difference at (P <0.05) of the antifungal inhibition activity of free Gri and the Gri-LDHaganst the studied dermatophyte isolates. The inhibition zone of free Gri was 16.13mm, while the inhibition zone of Gri-LDH was 6.63. mm.

From the other hands, the statistical analyses results showed a clear variability in the sensitivity of the studied Dermatophyte isolates toward the free Gri and Gri-LDH with significant differences at (P <0.05). Isolate number 12 (Epidermophyton flaccosum) isolated from Athlete's foot and isolate number 6 (Trichophyton mentagrophytes) isolated from leg were the highest sensitivity toward the nanohybrid antifungal with 15.80mm and 15.08mm inhibition zone respectively. While isolate number 4 (Trichophyton mentagrophytes) isolated from head showed significant lower inhibition zone 0.14mm compared with other isolates.

The Dermatophyte isolates did not affected with 0.01 and 0.1concentrations of antifungal. The rate of inhibition of antifungal was increased significantly (P<0.05) as the concentration of the antifungal increased to reached the highest inhibition zone 22.736 mm of 5 mg/ml of drug.

The mechanism of the inhibitory action of glycophosphon against fungi is shown by its effect on the microtubules, which leads to form achromatic spindle and thus prevents cell division, as it is highly efficient in inhibiting skin fungus [7,8,22].

There have been no previous studies of the GRI-LDH inhibitory action against skin fungal isolates. However, many studies have indicated the inhibitory action of free griseovulvin against these fungus species.

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