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Research Article

Isolation and biochemical characterization of a novel antimicrobial agent produced by *Streptomyces violaceusniger* isolated from Yemeni soil

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ABSTRACT

Background: Infections caused by multidrug-resistant bacteria present daily challenges to infectious disease physicians in hospitals throughout the world and these pathogens are spreading into the community. The development of new antibacterial agents to combat worsening antibiotic resistance is still a priority area in anti-infective research.
Methods: The experiments were carried out to search for new natural antibiotics through isolation of various *Streptomyces* strains from different soil samples from Yemen and studying the antimicrobial effects of metabolites that produced. In the same time, the toxicological and biochemical effects of the extracted antibiotic on animals were studied.
Results: *Streptomyces violaceusniger*, was isolated from Yemeni soil sample produced active metabolite that was designated faqihmycin has substantial antimicrobial potential against different microbial species. Investigations into the possible mode of action of faqihmycin revealed that it affects cell wall synthesis and intracellular macromolecule contents of the Gram-positive bacteria *Bacillus subtilis*. Toxicity studies of faqihmycin confirmed the hepatotoxicity of faqihmycin, there is no strong evidence to suggest that it is nephrotoxic.
Conclusions: Further studies with Faqihmycin are needed in order to elucidate its detailed mechanism of action on bacterial cells, as well as studies with Faqihmycin with different doses in order to determine its potential therapeutic use.

Keywords: Antimicrobial agent, Streptomyces, Antibiotic resistance, Hepatotoxicity, Nephrotoxicity

INTRODUCTION

Drug-resistant bacterial infections are becoming more prevalent and are major health issues facing us today. This rise in resistance has limited our repertoire of effective antimicrobials, creating a problematic situation that has been exacerbated by the small number of new antibiotics introduced in recent years.[1]

The development of new antibacterial agents to combat worsening antibiotic resistance is still a priority area in anti-infective research. However in the post-genomic era it has been more difficult than expected to identify new lead compounds from high-throughput screening, and very challenging to obtain antibacterial activity for lead compounds.[2]

There are several impediments to antibiotic discovery, including the low return on investment (as compared to drugs for chronic diseases) and the rigorous regulatory environment governing the clinical testing of new antibiotics. However, it is likely that technical difficulties

in identifying and developing truly novel and clinically useful antibiotics is the most significant factor.[3] The success of previous screening efforts has created a situation in which conventional screening technologies tend to result in the repeated identification of known molecules. Estimates vary, but it is believed by some that if conventional technology were used it would be necessary to test millions of new isolates of environmental microbes to find a single new antimicrobial compound.[4] The paucity of new antibacterial drugs has led the Infectious Disease Society of America (IDSA) and others to call for action in rebuilding infrastructure and efforts to develop next generation drugs.[5]

It is clear that novel strategies and technologies are required to avoid the rediscovery of known antibiotics and to increase the probability of finding new ones. One encouraging example is the recent discovery of platensimycin, produced by Streptomyces platensis, which represents a novel class of antibiotic that inhibits FabF, an enzyme involved in fatty acid biosynthesis. Platensimycin was identified by screening 83,000 strains grown under different growth conditions against a Staphylococcus aureus strain in which antisense RNA against FabF was used to lower its gene expression, thereby making the bacteria more sensitive to inhibitors of the targeted protein, and increasing the probability of finding a hit.[6] Such occasional successes using novel approaches kept hopes alive to identify natural products from bacterial sources.[7]

During the golden era of antibiotic discovery in the 1950s and 1960s, the then-untapped diversity of soil streptomyces provided most of the antibiotics known today. Over several decades, tens of millions of soil microorganisms were screened for anti-infective activity.[8] Thus, most low-hanging fruits have probably been picked and the discovery of a new antibiotic is nowadays a rare event. Furthermore, newly discovered compounds must possess advantages over the many antibiotics in clinical use, which implies the early recognition of potentially valuable compounds.[9]

Therefore, the present study intended to search for new natural antibiotics from various strains of Streptomyces isolated from Yemeni soil samples. In the meantime we investigated the antimicrobial activities of the selected antibiotic against bacterial cells to elucidate its probable mode of action. The study also investigated the biochemical and toxicological effects of the selected antibiotic on experimental animals in order to evaluate its future potential therapeutic use.

METHODS

Chemicals

Chemicals used in this study were of high analytical grade and were purchased from Merck (Germany), Oxoid (U.K.), Aldrich - Sigma (U.S.A.), Prolabo (France), winlab (UK), El-Nasr pharmaceutical chemical Co. (Egypt), ADWIC (Egypt). Clinical Diagnostic Kits were purchased from Diamond diagnostic Co. (Egypt), Biodiagnostic Co. (Egypt), Biosystem (Spain), Spainreact (Spain), and Biomed (Egypt).

Bacterial strains

The different bacterial strains used were kindly provided from staff members of the Department of Microbiology, Faculty of Science. Ain shams university.

Collection of soil samples

The soil samples were collected from various regions in Bayt al-Faqih area.

Al Hudaydah governorate (west of Yemen; latitude, 14°57'31"N; longitude, 43°32'60"E). Soil samples were collected using clean, dry and sterile polythene bags along with sterile spatula. The samples were immediately taken to the Laboratory, where they were stored at 4 °C in the refrigerator.

Sample processing and isolation of Streptomyces

The soil samples were subjected to dry-heat treatment at 50 °C for 1hr to depress the number of other bacteria for preferential isolation of Streptomyces. One gram of soil was suspended in 100 ml sterile distilled water and incubated in an orbital shaker at 30 °C with shaking at 200 rpm for 1hr. Suspensions were allowed to settle, and then serial dilutions of the clear phase; covering the range of 10-1 to 10-6; were prepared. Isolation of Streptomyces strains was carried out according to the method described by Shirling and Gottlieb on sterilized starch nitrate agar plates supplemented with nystatin 50μg/ml and nalidixidic acid 20μg/ml to inhibit microbial contamination.[10] The plates were incubated at 30 °C for 7 days and the pure colonies were selected, isolated and maintained in starch casein agar slants at 4 °C for subsequent studies.

Assessment of the antimicrobial activities of the different Streptomyces strains

The antimicrobial activity of the Streptomyces broth was assayed after 3, 5 and 7 days of incubation by using the classical diffusion method.[11] After preliminary testing of the isolates for their antimicrobial activities; the most active isolate was selected for further study.

Identification of the selected isolate

Numerous characterizations of the selected strain were carried out according to the method of Waksman that included morphological, cultural and biochemical characterization.[12]

Extraction and purification of the produced antibiotic

Extraction of active metabolites took place on the day of optimal production rate. The culture broth was filtered to remove the biomass. The cell-free filtrate was extracted with 50 % volume of chloroform: ethyl acetate (1:1). The organic phase containing the active substance was then evaporated (using vacuum rotatory evaporator at 60 ºC) till dryness. Purification of the antibiotic was carried out as described by Boudjella et al by passing the crude extract through a Sephadex LH-20 packed column (20x450 mm) and eluted in isocratic mode with 75% methanol in water with a flow rate of 1 ml min-1.[13] The fractions of the eluent were collected sequentially in labeled tubes (each of 5 ml), concentrated in vacuum and examined for their antibacterial activity by paper disk method.

Physicochemical analysis of the antibiotic

Ultraviolet (UV) spectroscopy, Infrared (IR) spectroscopy, Mass spectroscopy, elemental analyses and Nuclear Magnetic Resonance (NMR) were performed to determine the suggested molecular structure of the tested compound.

The mode of action of the antibiotic on Bacillus subtilis

The effects exerted by different concentrations (MIC, 2MIC and 4MIC) of the antibiotic on the growth rate and some biochemical activities of Bacillus subtilis cells were studied. Flasks containing 100 ml nutrient broth medium with different concentrations of the antibiotic were inoculated with Bacillus subtilis cells, and then the flasks were moved to a rotatory shaker of 220 rpm at 37 °C. Another set of flasks lacking the antibiotic was run alongside the experiment as control.

Bacterial samples (10ml) were withdrawn aseptically at regular time interval. The bacterial aliquots were fractionated according to method of Schmidt and Thannhauser to obtain the intracellular components (acid soluble phosphorous, total proteins, total lipids, DNA and RNA) to be assessed.[14] The acid soluble phosphorus and total lipid fractions were estimated according to method of Toribarn et al and Knight et al respectively.[15,16] Total protein concentration was estimated by the dye binding method of Bradford.[17] DNA and RNA contents were determined colorimetrical by the diphenylamine and orcinol reagents respectively.

The negative impact of the antibiotic on bacterial cell wall synthesis was examined using electron microscope and confirmed by assessment of the accumulated cytoplasmic fraction of N-acetylglucosamine (NAG) using method described by Lewy & McAllan.[18] Also, the effect of the antibiotic on the melting temperature (Tm) of salmon tests DNA was assessed according to the method described by Nagai et al.[19]

Developing Bacillus subtilis resistant strain against the antibiotic

The resistance to antibiotic was experimentally induced by the serial passage method described by Sakuri.[20] *Bacillus subtilis* was grown in separate nutrient broth media containing sub-lethal concentration (1/2 MIC) of antibiotic for 48 hours. The first tube in which full growth of the organism was observed in presence of certain concentration of the antibiotic served as inoculums for the next passage. The same procedure was repeated using higher concentrations of the antibiotic. After each passage, the sensitivity was tested by determining the minimum inhibitory concentration (MIC) until reaching bacterial cells resistant to 100 mg/ml of the antibiotic.

SDS-PAGE of proteins extracted from Bacillus subtilis cells resistant to antibiotic was performed according to Laemmli to show differences in the protein pattern when compared with untreated sensitive cells.[21]

Toxicity study

Animals: Eighty six adult male rats were obtained from the Egyptian Company for vaccines & medicines (center to produce natural toxin, Helwan farm). Rats were acclimated to the experimental facility for 1 week and housed in stainless steel cages (12 h dark/light cycle) an ambient temperature of 25º C and relative humidity of 55%. Animal care was followed as recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Determination of acute lethal dose (LD_{100}) and median lethal dose (LD_{50}): The procedure for the determination of LD_{100} and LD_{50} of the tested compound was carried out according to Baß et al.[22] A total of 56 adult male albino rats were divided equally into 7 groups representing doses from 60 mg to 683.4 mg of the antibiotic/ kg body weight with an increasing factor of 1.5. The mice were injected intraperitoneally (i.p.) with the antibiotic. Mortality was recorded after 24 hours, and the LD_{50} was calculated as follows:

Log LD_{50} = log LD next below 50% + (log increasing factor x proportionate distance).

Proportionate distance = (50% - % mortality next below 50%)/ (% mortality above 50% - % mortality next below 50%).

Study design

Thirty rats were randomly assorted into three groups: G1) rats were injected with normal saline and served as normal controls; G2) rats were injected with 1/8 LD50 dose; G3) rats received 1/4 LD50 dose.

Samples collection

At the end of the experiment (7 days), blood samples were taken by heart puncture under light ether anesthesia after a fasting period of 12 h. Serum was separated from the clotted blood samples by centrifugation at 5000 rpm for 5 min and then aliquoted and analyzed. The liver was dissected out, rinsed in isotonic sterile saline, blotted dry on a filter paper and subjected to macromolecule content analysis.

Preparation of liver homogenate

20% whole liver homogenate was prepared in phosphate buffered saline (pH 7.4). Total proteins, DNA and RNA concentrations were estimated in the prepared homogenate.

Biochemical assays

Serum alanine and aspartate aminotransferase activities were determined by the colorimetric method of Reitman and Frankel.[23] Alkaline phosphatase (ALP) activity was determined by the colorimetric method of Kind and King.[24] Serum glucose was estimated according to the method of Cooper.[25] Creatinine, urea and uric acid contents were estimated in the serum samples by the methods of Henry et al, Kaplan, and Fossati et al respectively.[26-28] Total protein concentration was estimated using the method of Bradford.[17] Serum albumin was colorimetrically determined using a commercial assay kit by the method of Doumas et al.[29] Also, total lipids, cholesterol and triacylglycerol contents were estimated according to the method of Kaplan, Richmond, and Bucolo and David respectively.[30-32]

RESULTS

Results from the screening protocol indicate that, forty Streptomyces strains were isolated from Yamini soil samples. Among the isolated strains; twenty two isolates were shown to have the ability to prevent microbial growth (Table 1). With regard to their antagonistic potentialities against different microbial species, the Streptomyces strain designed S37 was selected, as it has the strongest inhibitory action against different test organisms.

Identification and characterization assay suggested that the selected isolate has close relation to Streptomyces violaceusniger where there is close match of the isolate with standard one in Waksman's identification scheme.

Concerning the Physicochemical properties of the purified antibiotic (named faqihmycin), elemental analysis indicated that it consists of 62.96% Carbon, 9.25% Hydrogen, and 11.01% Nitrogen. In the meantime the UV–visible spectra in methanol exhibited maxima at 245 nm and 212 nm. In addition, the infrared spectra of the antibiotic showed the presence of bands at 3347, 3095, and 1762 cm^{-1}, which suggested the presence of NH, OH, and C=O groups respectively. Mass spectrum of the antibiotic showed fragment ions formed by eliminating methyl (m/e, 366), methoxyl (m/e, 350) and carboxyl (m/e, 336) groups

From the ^{1}H NMR spectrum of faqihmycin, a complex profile was obtained and the signals assigned were those of (δH 6.26, 6.62 and 7.5), (δH 3.93), (δH 8.03), (δH 5.11), and (δH 11.0) that revealed alkene protons, a methoxy group, amide group, amine group, and a carboxylic acid OH group respectively (Figure 1). From the ^{13}C NMR data, it was possible to discern an amido-carbonyl group (δC 165.8), carboxylic carbonyl group (δC 180.8), alkene carbons (δC 150.3 and 122.3), and a methoxy group (δC 61.6) (Figure 2).

On the basis of the previous data the suggested molecular formula of faqihmycin was established as being $C_{20}H_{35}O_4N_3$ and the suggested molecular structure in Figure 3.

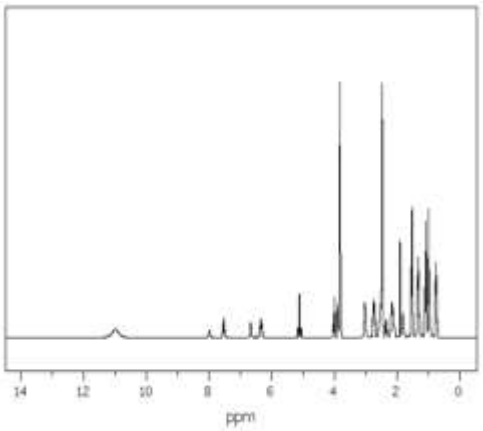

Figure 1: ^{1}H NMR spectral signals of the purified antibiotic faqihmycin

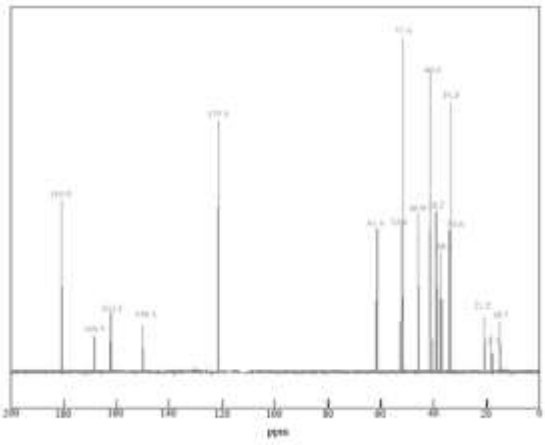

Figure 2: ^{13}C NMR spectral signals of the purified antibiotic faqihmycin.

Table 1: Antagonistic properties of the bioactive Streptomyces isolates against different microbial species.

Test organisms					
Sr. No	Bacteria			Fungi	
	Gram +ve		Gram -ve		
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
01	–	–	+	–	–
02	++	+	–	–	–
04	–	–	++	–	–
06	+	++	+	–	–
09	–	–	–	+	–
10	+	+	+	–	–
11	+	+	–	–	–
13	–	–	++	–	–
14	++	+	–	–	–
16	++	+	+	–	–
17	–	–	+	–	–
19	++	++	–	–	–
20	+	+	–	–	+
21	+	+	+	–	–
23	++	+	–	–	–
26	++	–	–	+	–
27	–	–	++	–	–
28	++	+	–	–	–
30	++	+	+	–	–
35	+	+	–	–	–
36	–	–	–	+	+
37	+++	+	+++	–	–

+++: Inhibition zone diameter ≥ 3 cm
++: Inhibition zone diameter ≥ 2 and < 3 cm
+: Inhibition zone diameter < 2 cm; '-': No inhibition

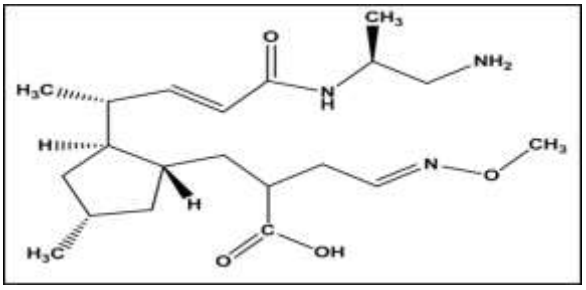

Figure 3: The Suggested molecular structure of the purified antibiotic faqihmycin.

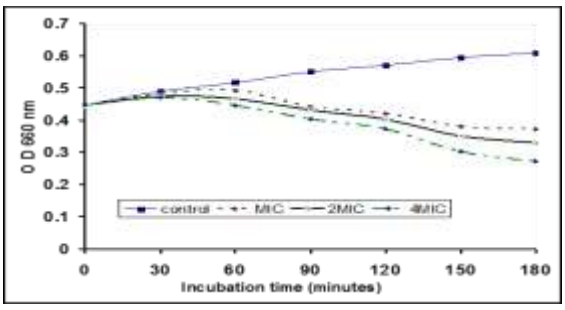

Figure 4: Effect of the antibiotic faqihmycin on the growth rate of *Bacillus subtilis* cells.

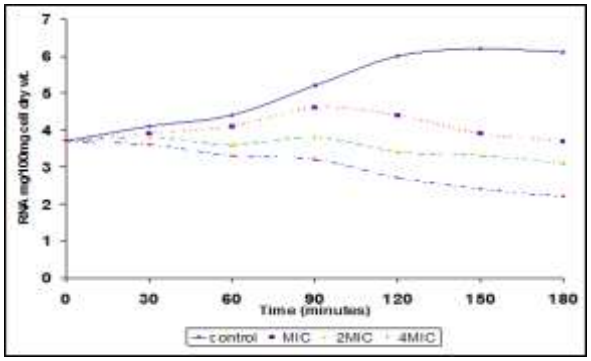

Figure 5: Effect of the antibiotic faqihmycin on the RNA content of *Bacillus subtilis* cells.

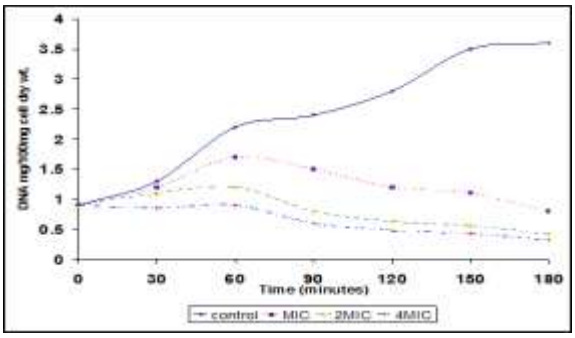

Figure 6: Effect of the antibiotic faqihmycin on the DNA content of *Bacillus subtilis* cells.

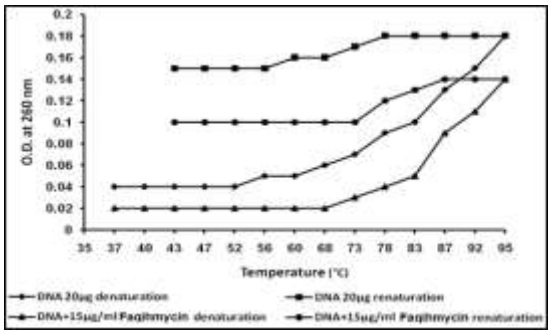

Figure 7: Effect of the antibiotic Faqihmycin (15µg/ml) on thermal denaturation and renaturation of Salmon testis DNA.

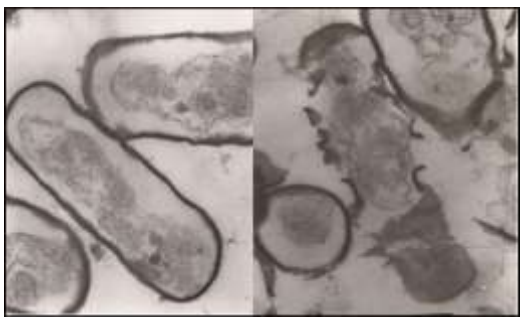

Figure 8: Transmission electron-micrograph of Bacillus subtilis cells. (a) untreated cells (b) faqihmycin treated cells.

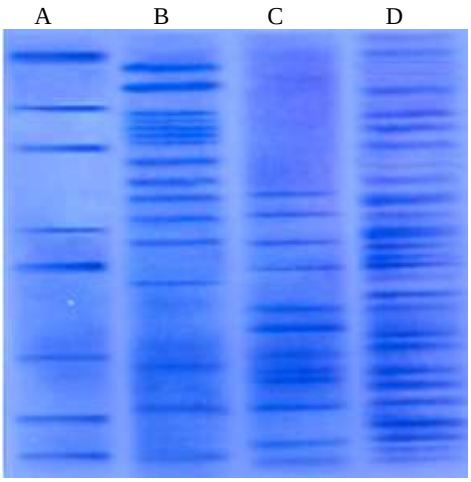

Figure 9: SDS PAGE analysis of the intracellular proteins of untreated, treated and resistant Bacillus subtilis cells. A: SDS-PAGE protein molecular weight marker. B: Protein extracts from untreated Bacillus subtilis cells. C: Protein extracts from faqihmycin treated Bacillus subtilis cells. D: Protein extracts from faqihmycin resistant Bacillus subtilis cells.

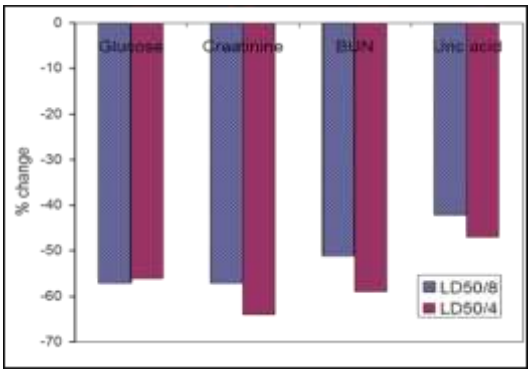

Figure 10: Percent change of serum glucose, creatinine, BUN, and uric acid in faqihmycin- treated rat when compared to the control group.

The Gram-positive bacterium *Bacillus subtilis* was found to be the most sensitive strain to faqihmycin (MIC= 0.75 µg/ml) so that this bacterial strain was used as test organism to elucidate the possible mode of action of the antibiotic.

The results presented in Figure 4 revealed that the tested antibiotic caused a pronounced inhibition in the growth rate of *Bacillus subtilis* cells. The inhibitory effect of the tested compound on bacterial cells was found to be time and concentration dependent, where the maximum inhibitory effect was observed at the 4 MIC level after 180 minutes.

The biochemical profile of the antibiotic-treated cells clearly indicated that, the total lipid, and protein contents were significantly decreased by the effect of faqihmycin at the various concentrations used. Also, the antibiotic exerted an inhibitory effect that reduced the intracellular RNA content of Bacillus subtilis cells. The reduction was found to be 39%, 49% and 64% after 180 minutes at MIC, 2MIC and 4MIC, respectively (Figure 5). In the meantime, the antibiotic reduced the DNA contents in the growing bacterial cells and the effect increased by increasing the concentration of the antibiotic. This reduction reached its maximum level after 180 minutes. The percent reduction was 78%, 88% and 91% for MIC, 2MIC and 4MIC, respectively (Figure 6). In contrast, less significant changes were detected on acid soluble phosphorous content

Results given in Figure 7 clearly indicate that 15 µg/ml of faqihmycin increased the Tm of salmon testis DNA from 54ºC to 70ºC. In the meantime, microscopical examination of faqihmycin untreated and treated bacterial cells is presented in Figure 8, where Figure 8a shows normal rod shape cells for untreated Bacillus subtilis cells while Figure 8b shows some damaged regions of the cell wall of bacterial cells that were treated with faqihmycin.

SDS-PAGE analysis of proteins extracted from *Bacillus subtilis* cells resistant to faqihmycin and faqihmycin-sensitive cells show both qualitative and quantitative differences when compared with untreated cells (Figure 9). Results in Table 2 show that the acute lethal dose (LD_{100}) was 683.4 mg/kg body weight, while the median lethal dose (LD_{50}) was 154.4 mg/kg body weight after intra-peritoneal (i.p.) injection of faqihmycin in albino rats.

Results presented in Table 3 showed that, administration of faqihmycin by different doses caused a dramatic increase in serum levels of AST, ALT and ALP when compared with the control group. Concurrently, the serum levels of total proteins, albumin and globulins were significantly decreased ($p<0.05$) in animals treated with both doses of faqihmycin, when compared to animals of the control group.

In comparison to the normal control group, Implemented doses of faqihmycin significantly decreased the serum levels of glucose, creatinine, blood urea nitrogen (BUN) and uric acid ($p< 0.05$) as shown in Figure 10.

Table 2: Lethal dose (LD_{100}) and median lethal dose (LD_{50}) of the antibiotic faqihmycin.

Dose (mg/kg)	Number of Rats	Survival (S)	Death (D)	Mortality (%)
60	8	8	0	0
90	8	6	2	25
135	8	5	3	37.5
202.5	8	4	4	50
303.8	8	2	6	75
455.6	8	1	7	87.5
683.4	8	0	8	100

Table 3: Serum levels of the selected liver function parameters in control, $LD_{50}/8$- and $LD_{50}/4$- treated rat.

Parameter Groups		ALT	AST	ALP	T. P.	Albumin	Globulin
		(IU/L)			(g %)		
Control	Mean	73.3	36.7	115.8	6.35	4.15	2.35
	±SD	±18.53	±10.2	±23.4	±1.41	±1.02	±0.43
$LD_{50}/8$	Mean	166.7	82	194.2	3.92	2.6	1.42
	±SD	±32.8	±26.3	±29.4	±1.22	±0.84	± 0.35
	p value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
$LD_{50}/4$	Mean	181.8	100.3	207.2	4.11	2.52	1.37
	±SD	± 39.8	±38.1	±16.6	±1.14	± 0.69	± 0.33
	p value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

$LD_{50}/8$ = 38.6 mg/kg body weight; LD50 /4 =77.2 mg/kg body weight; p is significant at ≤ 0.05.

Table 4: Serum levels of the selected lipid profile parameters in control, LD50/8- and LD50/4¬-treated rat.

Parameter Groups		TAG	T. cholesterol	T.lipid
		(mg %)		
Control	Mean	297	147.8	72.2
	±SD	±83.3	±40.9	±20
$LD_{50}/8$	Mean	456.2	229.2	152.7
	±SD	±107.3	±53.8	± 55.5
	p value	N.S.	N.S.	< 0.05
$LD_{50}/4$	Mean	486	242	162
	±SD	± 79.8	±37.4	± 34.9
	p value	<0.05	< 0.05	< 0.05

N.S.: Non- significant
LD_{50} /8 = 38.6 mg/kg body weight.
LD_{50} /4 =77.2 mg/kg body weight.
p is significant at ≤ 0.05.

Table 5: Liver tissue contents of total proteins, DNA, and RNA in case of control, LD50/8- and LD50/4-treated rat.

Parameter Groups		Total prot-eins	DNA	RNA
		(mg %)		
Control	Mean	297	147.8	72.2
	±SD	±83.3	±40.9	±20
$LD_{50}/8$	Mean	456.2	229.2	152.7
	±SD	±107.3	±53.8	± 55.5
	p value	N.S.	N.S.	< 0.05
$LD_{50}/4$	Mean	486	242	162
	±SD	± 79.8	±37.4	± 34.9
	p value	<0.05	< 0.05	< 0.05

N.S.: Non- significant
LD_{50} /8 = 38.6 mg/kg body weight.
$LD_{50}/4$ =77.2 mg/kg body weight.
p is significant at ≤ 0.05.

Results presented in Table 4 clearly indicate that both doses of faqihmycin have a significant increasing effect on serum triacylglycerol ($p<0.05$) by 111% for $LD_{50}/8$ and 124% for $LD_{50}/4$. Moreover, the $LD_{50}/4$ dose caused a significant increase in total lipids and total cholesterol ($p<0.05$) by 64% each. No statistically significant differences in serum total lipids and total cholesterol in the $LD_{50}/8$-treated group when compared with animals of the control group. Faqihmycin administration by different doses induced significant decreases in hepatic protein, DNA and RNA contents compared to their normal control levels (Table 5).

DISCUSSION

There is a growing need for novel and more effective antibiotics to combat multidrug resistant pathogens. Due to aging, immunosuppression and invasive surgical procedures, an ever-larger population is at risk of contracting severe infections, while bacterial pathogens are becoming increasingly resistant to currently available antibiotics.[33] Recently, a large outbreak of diarrhea and the hemolytic–uremic syndrome caused by an unusual serotype of Shiga-toxin–producing Escherichia coli (O104:H4) began in Germany. Cases have subsequently been reported in 15 other countries. One of the many unusual characteristics of strain O104:H4 is that it has resistance genes to multiple classes of antibiotics.[34,35] As a consequence, infections caused by multi-drug resistant bacteria are associated with increased morbidity, mortality, few treatment options, and health care costs. It is now widely accepted that, despite the plethora of novel targets provided by the sequenced genomes of bacterial pathogens, combinatorial chemistry and high throughput screening (HTS) have failed to provide novel drug candidates in the anti-infective field, resulting in a virtually empty pipeline of compounds under development.[36] This has prompted many players in the field to advocate a return to screening natural products, which constitute most of the clinically used antibiotics and the bulk of compounds under development.[37]

The present work deals with the study of antibiotic producing Streptomyces isolated from Yemeni soil samples, where about 40 isolates were included. The isolates were tested for antagonistic potentialities against different strains of Gram-positive, Gram-negative species and fungi. Newly potent antimicrobial agent designated faqihmycin was purified from Streptomyces violaceusniger. The antibiotic was shown to have potential antagonistic potentiality against different microbial species with *Bacillus subtilis* being the most sensitivite strain to faqihmycin.

Faqihmycin was shown to have a negative impact on microbial DNA contents, which might be due to its ability to cause some alterations in the mechanism of DNA polymerase, DNA gyrase or DNA topoisomerase. Alternatively, this could be due to the intercalation effect of Faqihmycin on bacterial DNA as reflected by its ability to raise the Tm of salmon sperm DNA which could interfere with DNA replication and probably transcription. This behavior of the faqihmycin is similar to that of a classical intercalating compound as daunomycin.[38]

The antibiotic faqihmycin caused a decrease in the RNA content compared to control cells. These results are in agreement with Waring and Markeoff, who reported that many antimicrobial agents could partially or even totally suppress the incorporation of uridine into RNA, leading to inhibition of RNA synthesis, which also reflects secondary impairment of protein synthesis.[39]

The extracted faqihmycin caused a significant reduction in the intracellular total lipid contents of treated B. subtilis cells compared to the normal control cells after 180 minutes. This reduction in the total lipid contents is in agreement with the reported data of Novo et al who suggested that the reduction in total lipid contents might be due to the effect exerted by the antimicrobial agent on the permeability of the cell membrane or on the level of the double layer of the bacterial membrane.[40] Also, Gruszecki et al confirmed that, some antibiotics such as amphotericin B exert their action in the lipid environment of liposomes and lipid membrane by forming a pore-like structure which affects the passage of molecules through the membrane.[41] In addition, polypeptide antibiotics were found to induce a structural distortion in the lipid bilayer forming a pore-like structure.[42]

Data obtained from the present study clearly indicated that, faqihmycin arrested the synthesis of bacterial cell wall, and the higher the concentration of the compound, the more pronounced the effect. The prospective reasons for the inhibition or killing of the antibiotic-treated bacterial cells may be due to the effect of faqihmycin on bacterial cell wall synthesis either by inhibition of peptidoglycan synthesis or by preventing the incorporation of cell wall precursors into cell wall. Faqihmycin may exert its effect on bacterial cell wall by interfering with the steps of peptidoglycan synthesis or transglycosylation reaction.

SDS-PAGE analysis of proteins extracted from B. subtilis cells resistant or sensitive to faqihmycin show both qualitative and quantitative differences when compared with untreated cells. The remarkable decrease in total protein content was parallel to the significant decrease in both DNA and RNA contents. The effect of faqihmycin on protein synthesis may be due to its interaction with ribosomes and their subunits, inhibition of one or many steps of protein synthesis, inhibition of microtubular proteins or misreading during protein synthesis. This also, could be explained by loss in RNA synthesis needed for translation. In contrast, faqihmycin resistant B. subtilis produce secondary proteins that may perform the same function as protein of methicillin resistant bacteria. These alternate proteins may be enzymes or anything that damage or cleave the antibiotic; are different enough that antibiotic does not recognize it and so cannot latch onto and inhibit it.

The present study indicated a significant increase in serum ALT, AST, and ALP activity in faqihmycin-treated animals when compared with the control group, which might introduce evidence that the liver can be a target for toxicity. This finding is consistent with previous reports showing that a number of antibiotics induced liver damage in both humans and experimental animals.[43] Other liver function parameters that can detect loss of liver synthetic capacity include albumin, glucose, coagulation factors, ammonia and urea nitrogen. Alterations in these parameters in the blood usually requires significant loss of liver function (>70-80%) since liver has a tremendous reserve capacity for synthesis of these analytes.[44]

In addition to its effect on the aforementioned enzymes, treatment of rats with faqihmycin caused hypoalbuminemia and hypoproteinemia. The reduction in total proteins in treated animals may be due to liver damage by faqihmycin. These results agree with the finding of Ojo et al who stated that the reduction may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein.[45] A similar observation was reported by Farombi et al, who attributed the decrease in total proteins and albumin by antimicrobial agents to liver inability to synthesize proteins at normal rate or decrease in plasma volume because of water loss.[46] The present study also demonstrated a significant increase in serum triglycerides, cholesterol, and total lipids in faqihmycin treated mice when compared with control group. These changes might be consistent with a mechanism involving mobilization of free fatty acids from the peripheral adipose tissue and accumulation of lipids in the plasma and liver.[47] Hypertriglyceridemia can be induced by drugs due to enhancement of hormone-sensitive lipase activity and inhibition of lipoprotein lipase activity.[48]

The observed abnormality in the lipid profile may be due to the hepatic toxicity which was induced by administration of faqihmycin. This conclusion is in agreement with that obtained by Skottova & Krecman who stated that, liver injury of different etiology is often accompanied by disorder of lipid metabolism in the liver and is reflected in altered plasma lipid and lipoprotein as secondary dyslipoproteinaemia.[49]

Kidney functions were also evaluated following antibiotic treatment by measuring serum urea, creatinine, and uric acid. Our results indicate that, blood urea creatinine, and uric acid decreased significantly in both treated groups when compared with their levels of the control group.

Accordingly, although faqihmycin was found to be hepatotoxic, there is no strong evidence to suggest that it is nephrotoxic according to this study.

The results obtained in this study revealed that Faqihmycin caused a significant decrease in serum glucose of faqihmycin-treated groups (hypoglycemic factor). Hypoglycemia can produce a variety of symptoms and effects but the principle problems arise from an inadequate supply of glucose as fuel to the brain, resulting in impairment of function (neuroglycopenia).[50] The decrease in blood glucose concentration may be produced by several mechanisms including increased peripheral glucose utilization, increased release of insulin and/or inhibition of the proximal tubular reabsorption mechanisms for glucose in the kidney.[51] A number of different antibiotics have been associated with hypoglycemia such as levofloxacin, clarithromycin, bacitracin, and tetracycline.[52-55] In addition, certain antimicrobial drugs, such as gatifloxacin have also been associated with a consistent increase in serum insulin levels, with variable effects on glucose homeostasis.[56,57] Rats treated with faqihmycin showed a significant decrease in liver total proteins, DNA and RNA contents compared to untreated control group. A similar observation was reported by Farombi et al who reported a decrease in total liver proteins by antimicrobial agents due to liver inability to synthesize proteins at normal rate or decrease in plasma volume because of water loss.[46]

As for the decrease in hepatic DNA and RNA contents, these results are very consensus with data of Heritage et al who proposed that, lowering the rate of DNA synthesis is a result of decreasing or inhibiting the activity of the enzymes which regulate the synthesis of DNA.[58] It has also been demonstrated that high tendency of the DNA molecule to interact with some drugs forming larger molecules is followed by a gradual increase of degradation and helix instability.[59]

CONCLUSION

In conclusion, a new and potentially potent antibiotic (faqihmycin) was isolated and purified from a Streptomyces violaceusniger. The antibiotic has relatively substantial antimicrobial activities against Gram positive than Gram-negative bacteria while it has no activity against fungi. Regarding its effect on animal cells, faqihmycin caused toxic effects at both acute lower and higher doses. Though Faqihmycin was found to be hepatotoxic, there was no strong evidence to suggest that it is nephrotoxic. Finally, more work is needed to unravel detailed molecular mechanisms of action of the antibiotic.

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