The purpose of crystal engineering is to explore new materials with striking properties that can be used in a variety of implementation areas. Metal complexes with an important place in crystal engineering consist of anions or molecules called ligands, which bind to a metal cation and metal atom at the center of symmetry. Metal complexes have been the subject of interest with the diversity of their structures for many years. The structures of metal complexes were determined to be influenced by the biological and physical properties of metal cations, ligands and intermolecular interactions [1-4]. Medical inorganic chemistry, which has an important place in materials science, is a multidisciplinary field consisting of chemistry, pharmacology, toxicology, and biochemistry. Medical chemists focus on the design and synthesis of new metal-based molecules with greater enhanced biological activity, better selectivity, low toxicity and multiple roles of mechanical effects to overcome the clinical problems of commercially available drugs due to their side effects. Metal complexes are growth inhibitors of bacteria, which is supported by in vitro and in vivo studies [5, 6].

Carboxylic acids have many biological activities such as anti-inflammatory, antibacterial and antifungal. In dermatology, the sodium salt of benzoic acid is used as an antifungal agent. The antibacterial activity of carboxylic acids can be increased by forming complexes with metal ions. The anti-inflammatory and antibacterial activity of metal complexes was determined to be greater than free acid. Therefore, it is crucial to learn about the structure and binding relationships of complexes in the preparation of effective antibacterial species. However, activity is also known to decrease in some similar complexes. Recently, studies on antimicrobial activity of non-steroid anti-inflammatory drugs have shown that the complexes of transition metals...
with various nitrogen-containing ligands show increased antimicrobial activity [7, 8]. Various metal complexes accelerate the effect of the drugs and organic therapeutics. Cobalt (II) mfenamic acid, naproxen, or tolenamic acid [9-11] and mangan (II) tolenamic acid complexes [12], copper (II) mfenamic acid, naproxen, diclofenac, diflunisal, and flu fenamic acid complexes [13-15] exhibit biological activity. Zinc complexes with biologically active ligands have pharmacological effects because they are able to catalyze enzymatic processes in biological systems. Aromatic carboxylates (such as Naproxen, Ibuprofen, Indometacin) and alipic carboxylates (such as Valproic Acid) of zinc complexes with nitrogen-based ligands have been synthesized and their antibacterial and antimarial properties have been studied recently. Most of zinc carboxylates with N-, O-, S- donor ligands have also antibacterial activity [16-19].

In this study, the antibacterial activity of previously synthesized zinc 2-fluorobenzoate complexes with nicotinamide was determined against Bacillus subtilis, Pseudomonas Aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus cereus and Klebsiella pneumoniae by agar well diffusion method. Cytotoxicity of the complex was studied using MTT test method.

MATERIALS AND METHODS

Materials

Chemicals and Instruments

The compound used in the study was a previously synthesized and its structure was determined. The molecular structure of the complex was given in Fig. 1. Mueller Hinton Agar (Oxoid), Phosphate Buffered Saline, Antibiotic Antimycotic Solution, L-Glutamine solution, Histopaque-1077 and Dimethylsulfoxide (Sigma-Aldrich), BIOAMF-1 medium and BIOAMF-1 supplement (Biological Industries) and MTT Cell Proliferation Assay Kit (Cayman Chemical) were purchased commercially. In this study, Nüve Steamart OT 40L autoclave, Nüve BM 101 Water bath, J.P. Selecta Digiheat drying and sterilization oven, ISOLAB vortex mixer, HETTICH EBA 200 centrifuge device, Panasonic MCO-170AICUVH-PE CO2 Incubator and BioTek Epoch Spectrophotometer were used.

Methods

Determination of Antibacterial Activity

Agar well diffusion method was used to evaluate the antibacterial effects of different concentrations of the tested compound. B. subtilis (ATCC 6633), B. cereus (ATCC 8035), S. aureus (ATCC 25923), K. pneumoniae (ATCC 33499), P. aeruginosa (ATCC 27852) and E. coli (ATCC 259222) were used in the study. Bacteria were stored at 4 °C until they were used. For the preparation of the agar to be used, 38 g Mueller Hinton (MH) agar was boiled and mixed in 1 L distilled water until completely dissolved. The prepared agar solution was kept in an autoclave for 15 minutes at 121 °C for sterilization. The agar transferred to Petri dishes and was cooled at the room temperature. Dimethyl sulfoxide (DMSO) was used as a negative control group. The cultivation of microorganisms was done on the prepared agar. Then, 5 mm diameter wells were formed on the agar surface. 50 µL of the tested compound were added to the wells. Petri dishes were incubated for 12 hours at 37 °C, and the diameter of the inhibition zone around each well was measured with the help of a ruler. The experiments were carried out in triplicate.

MTT Analysis

For the preparation of the culture medium, 75 mL of amnion cell culture medium, 15 mL of supplement, 1.5 mL of penicillin+streptomycin+amphotericin B (Antibiotic Antimycotic Solution) and 2 mL of L-glutamine were added in a sterile tube and kept at 37 °C. Lymphocytes were isolated from a human peripheral blood sample and cell counting was performed using a Thoma slide. After that 100 µL culture medium and 100 µL cell suspension (50000 cells/well) were added to the 96-well plates, respectively. The cultivation of microorganisms was done on the prepared agar. Then, 5 mm diameter wells were formed on the agar surface. 50 µL of the tested compound were added to the wells. Petri dishes were incubated for 12 hours at 37 °C, and the diameter of the inhibition zone around each well was measured with the help of a ruler. The experiments were carried out in triplicate.

Figure 1. Molecular structure of the test compound [20]
RESULTS

Antibacterial Activity

Antimicrobial effects of zinc complex against Gram positive (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*) and Gram negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*) bacterial species were evaluated, and the results were given in Table 1 and Fig. 2. The compound at the concentrations of 18.67 and 9.34 mM was found to have antibacterial effects against all bacteria. It was found that the complex had no inhibitory effect on any bacteria at a concentration of 1.17 mM, meaning that the complex was not antibacterial at that concentration. At 2.33 mM concentration, the complex exhibits to have a suppressive effect only against *S. aureus* bacteria. It was determined that the complex showed antibacterial effect in all bacterial species except *B. cereus* and *E. coli* bacteria at 4.67 mM. The inhibition zones formed are seen in Fig. 3.

MTT Test

The results of MTT assay were evaluated. The absorbance values were measured by spectrophotometer. The decrease in cell viability according to the cell control group was calculated and obtained the percent inhibition values (Fig. 4). The % inhibition values were calculated according to the following formula and the obtained values are given in Table 2 and Fig. 4.

\[
\text{Percent inhibition} (\%) = \frac{\text{CVCC} - \text{CVTC}}{\text{CVCC}} \times 100
\]

(CVCC = Cell viability in cell control, CVTC= Cell viability at test concentrations)

When the obtained values are examined, it is seen that cell viability decreases with increasing concentration. At the concentrations of 1.17 mM, 2.33 mM, 4.67 mM and 9.34 mM, cell viability decreased by 13.80%, 13.95%, 27.13% and 35.23%, respectively. The cell death at 18.67 mM, the highest concentration we studied, was 93.58% and the highest cytotoxic effect was occurred at this concentration.

Table 1. Antibacterial activity results

| Derişim       | Gram Positive | Gram Negative |
|----------------|---------------|---------------|
|                | *B. subtilis* | *B. cereus*   | *S. aureus* | *K. pneumoniae* | *P. aeruginosa* | *E. coli* |
| Streptomycin (5.16x10⁻⁴ mM) | 21.33 | 20.33 | 19.33 | 20.67 | 20.67 | 20.00 |
| 18.67 mM       | 18.67        | 16.33        | 20.33 | 18.67 | 14.33 | 16.00 |
| 9.34 mM        | 14.33        | 13.33        | 11.67 | 13.67 | 12.67 | 11.23 |
| 4.67 mM        | 8.67         | -            | 10.00 | 8.67  | 10.00 | -     |
| 2.33 mM        | -            | -            | 6.87  | -     | -     | -     |
| 1.17 mM        | -            | -            | -     | -     | -     | -     |
| DMSO           | -            | -            | -     | -     | -     | -     |

*<6 mm no antimicrobial activity, 6-15 mm weak antimicrobial activity, 15-20 mm strong antimicrobial activity, 20-25 mm very strong antimicrobial activity [21-22]*
The antibacterial effect of zinc 2-fluorobenzoate nicotinamide complex at 1.17, 2.33, 4.67, 9.34 and 18.67 mM concentrations against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* was studied by agar well diffusion method. Cytotoxic effect of the complex on human peripheral blood lymphocyte cultures was determined by MTT method in vitro conditions. In this section, obtained results were evaluated and compared with literature. The synthesis of new compounds has gained importance in the pharmaceutical sector due to the resistance of bacteria to antibiotics. The biological activities of the synthesized compounds are also examined. However, advanced clinical trials are not possible for all compounds for reasons such as time and economy. The antibacterial activities and toxic properties of newly synthesized compounds need to be determined. Therefore, researchers have very big tasks. Because high antibacterial activity and low toxicity make the compound a more important drug material. In this context, metal complexes have an important place in drug chemistry.

Omar and Abu Ali studied the antibacterial effect of zinc ibuprofen's complexes with 4,4'-bipyridine, 1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, 1,2-dimethylimidazole, 1,2-dimimidazole, 2-dimethylimidazole, 2-amino-6-picoline [24] and Ali et al. studied the antibacterial effect of zinc ibuprofen's complexes with 2-aminopyridine, 2-aminomethylpyridine and 2,2'-bipyridine and 2-(Methylaminomethyl)pyridine against *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* at 6 g.L−1 (6000 ppm) concentration [19]. Complexes with 4,4'-bipyridine, 1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline and 2,2'-bipyridine were found to be effective on gram positive bacteria. These two studies show that the antibacterial effect may change depending on the metal and ligand used. The antibacterial effects of these compounds decrease as concentration decreases. There are no findings on toxicity in the studies. Zinc complexes of naproxen, one of the nonsteroidal and anti-inflammatory drugs, have also been synthesized. Antibacterial effects of the complexes obtained using different pyridine derivatives were studied by disc diffusion method against *Escherichia coli* and *Salmonella aureus* and by agar well diffusion method for *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli*. The complexes obtained in these studies were suggested as antibiotics. But the toxicity of the complexes was not investigated [18, 25].

The antibacterial properties of zinc coumarin-3-carboxylate (100-1000 μg/mL) [26], zinc 5-iodo and 5-bromosalicylate (0.001-200 mmol.dm−3) [27], zinc 2-bromobenzoate (0.01-2.0 mmol.dm−3) [28] and zinc salicylates (1 mg/mL) [29] have been investigated. Although the main ligands has antibacterial activity, it has been reported that the antibacterial effect increases in some complexes (especially with pyridine derivatives). However, no additional studies have been carried out the toxicity of these compounds as well. In these studies, the importance of carboxylate complexes was emphasized related to their antibacterial properties. The most prominent feature that distinguishes our study from these studies was the antibacterial activity test as well as studying the cytotoxicity of the complex. In this study, it was determined that the compound has antibacterial properties according to inhibition zone diameters at the concentrations of 0.02 g/mL (18.67 mM) and 0.01 g/mL (9.34 mM). At 0.02 g/mL (18.67 mM) concentration, the complex showed a good antibacterial effect on the *B. cereus*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae*. But the cytotoxicity results showed that the rate of death of lymphocyte cells was 93.58 % at same concentration. Similarly, a moderate antibacterial effect occurred at a concentration of 0.01 g/mL on all studied bacteria, while MTT test results found 35.23% cell death. There is no study on the antibacterial activity of zinc 2-fluorobenzoate and its other pyridine derivatives and any of its complexes in the literature. Antibacterial and cytotoxic effects of zinc 2-fluorobenzoate were investigated for the first time in this study.

There are also some studies in the literature that investigate the cytotoxicity of different zinc complexes. For example, Zhu et al. synthesized zinc 2,4,5-benzetetra-carboxylic acid complex with 1,3,5-Tris (1-imidazolyl) benzene and its cytotoxicity was determined by MTT method on HeLa and KB cancer cell lines at the concentrations of 4, 12, 36, 110 and 330 μg.mL−1 [30]. In a study conducted with the concentration close to the concentrations in our study, it was reported that zinc complex of Schiff bases derived from o-vanillin was cytotoxic on A549, Hela, HL-60 and K562 cancer cell lines at a concentration of 1% [31]. Although the complexes synthesized in these studies were suggested as an
anticarcinogenic agent, their effects on healthy cells at the concentrations studied were not investigated. The results of our study were evaluated as negative, indicating that the tested compound was cytotoxic on the human lymphocyte cell line.

CONCLUSIONS

In this study, antibacterial and cytotoxic properties of pre-synthesized zinc 2-fluorobenzoate nicotinamide complex were evaluated. The complex showed good activity against Gram positive (B. subtilis, B. cereus and S. aureus) and Gram negative (K. pneumonia, P. aeruginosa and E. coli) bacteria at 18.67 mM concentration. At the same concentration, the complex was found to exhibit cytotoxic properties. The antibacterial effect of the concentration was found to decrease as the concentration decreased. The cytotoxicity of the complex increases with increasing concentration. The biological activity of the compound becomes insignificant due to increased death in lymphocyte cells at the concentration at which the antibacterial effect increases. In conclusion, when the findings obtained from this study are evaluated, the zinc 2-fluorobenzoate complex with nicotinamide exhibits antibacterial properties and is not recommended as a drug-specific material because it is cytotoxic at the same concentrations.

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