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Besifloxacin/Mn Novel Metal Complex Ameliorates Hepatic Alterations in Male Rats *via* Modulating NRF-2 Antioxidant Hepatic Signalling Metabolism and NF-κB/ JNK and CHOP Oxidative Stress Pathway

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ABSTRACT

Background: Fluoroquinolone antibacterials are considered as the most beneficial antibacterial agents due to their high activity against various types of bacteria. Besifloxacin (Besi), is a novel fluoroquinolone antibacterial agent with a broad spectrum of activity. Besi demonstrates potent antibacterial activity *in vitro* against a wide range of pathogens; it has also shown high potency against a lot of infectious pathogens and other ocular and systemic infections. Less data are demonstrated in the literature on the tolerability of Besi novel metal drug complexes. The main objective of the current study is the treatment of hepatotoxicity and the oxidative stress damage via using novel metal drug complex in male rats.

Methods: Novel Manganese besifloxacin complex was synthesized. The ability of besifloxacin (Besi) to form chelates with divalent manganese was investigated. Confirmation the structures of the complex has been elucidated by CHN analysis, molar conductance, IR, UV-Vis spectral studies, magnetic moment, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray powder diffraction (XRD). The data suggested that Besi form a 1:2 chelates with Mn (II) ion. The spectral data confirm that Besi act as bidentate *via* C=O and deprotonated COOH group. Eighteen male albino rats (6 Rats/group) were separated randomly into 3 treated groups. Control, Besi (100 mg/kg) and Besi/Mn (100 mg/kg) at the same dosages. At the end of the experiment, liver and blood samples were analyzed for biochemical, molecular and histological variations.

Result: Result revealing that Besi administration significantly elevated the levels of the liver enzymes as a result of Besi administration contrary to Besi/Mn administration. Histopathological examinations revealed inflammatory liver areas. Furthermore, induction of significant increases in NF-κB, JNK and CHOP as compared to the normal control group. Meanwhile, induction of a significant decline in NRF-2 level of Endoplasmic Reticulum oxidative damage, which was detected in the hepatocytes. In contrast to Besi/Mn novel complex treatment, significant hepatic amelioration was observed and a decline in hepatocellular congestion and oxidative stress genes after its administration. Besi/Mn complex was found to be with high antimicrobial potency.

Key words: Besifloxacin, Endoplasmic reticulum, Genotoxicity, Hepatic functions, Metal complex, Oxidative stress.

INTRODUCTION

Quinolone antibiotic compounds are a group of synthetic antibiotics with excellent bioavailability and well oral absorption (Appelbaum and Hunter, 2000; Hooper, 1998). Quinolones are antibacterial agents that inhibit the replication and transcription of bacterial DNA, causing rapid cell death (Cozzarelli,1980 and Mitscher, 2005) by making inhibition for antibacterial key-enzymes, DNA-gyrase (topoisomerase II) and DNA topoisomerase IV. Quinolones interact with the enzyme-DNA complex, forming a drug-enzyme-DNA complex that blocks progression and the replication process (Hooper, 2000 and Maxwell, 1992). Quinolone compounds are zwitterionic due to the presence of COOH carboxylic group at position-3 and a basic N-heterocycle at position-7. Quinolones have good solubility in acidic or basic solutions.

Besifloxacin (Besi, Fig 1) is a 4th-generation fluoroquinolone antibiotic. The marketed compound is besifloxacin hydrochloride that has anti-inflammatory effect in monocytes *in vitro*; this attribute may enhance its efficacy in ocular infections with an inflammatory component and warrants further investigation (Malhotra *et al*., 2013).

Besi is used in the treatment of bacterial conjunctivitis (Besivance, 2009) Besi as 8-chlorofluoroquinolone has R7-aminoazepinyl group with a wide range against gram-positive and gram-negative ocular pathogens and broad spectrum *in vitro* activity, including multidrug-resistant

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strains (Haas *et al.*, 2009; Haas *et al.*, 2010; Haas *et al.*, 2011 and Cambau *et al.*, 2009). The action mechanism of Besi is carried out by inhibition of bacterial DNA enzymes, which are essential for DNA replication (Sanfilippo *et al.*, 2011). Besi has balanced activity against these bacterial enzymes; that decline the resistance and continuous mutations in these bacterial enzymes.

Besi has not available much literature concerning interaction with DNA and metal chelates although the urgent problem of antibiotic resistance that require novel design of antibacterial agents (Lerman, 1961; Waring, 1964; Hollstein, 1974).

Current studies lack a comprehensive understanding of how Mn(II) complexation affects the antibacterial efficacy and toxicity of metal-based compounds. Clarifying whether and how Mn(II) interactions modify biological activity. *Mn* (II) ions can form stable coordination complexes with various ligands, potentially influencing their ability to interact with biological molecules. Such complexation can modify properties like lipophilicity, charge distribution and redox state, which in turn can affect how these compounds penetrate cell membranes, interact with microbial enzymes, or induce oxidative stress. Consequently, Mn (II) complexation might enhance or reduce antibacterial activity or toxicity, depending on the nature of the complex formed.

Ligand-DNA interactions typically involve non-covalent forces such as hydrogen bonding and intercalation, affecting DNA structure and function without actual metal coordination to the bases or backbone. In contrast, metal coordination chemistry involves the formation of covalent or coordinate bonds between metal ions [like Mn (II)] and ligands, which can directly influence the electronic properties and reactivity of the complex. Recognizing these differences is crucial in understanding the mechanisms underlying.

This study aimed to comprehensively assess the Besi ligand drug and its Mn (II) chelate by characterizing their spectroscopic properties (IR, UV, magnetic moments), as well as analyzing their morphological features using SEM and TEM and determining their crystalline structure *via* XRD. Additionally, the study investigated the biological effects *via* a 30-day rat study, gene expression analysis, histopathological examinations and antibacterial testing to assess the pharmacological safety and efficacy of the compounds.

MATERIALS AND METHODS

Experimental

Reagents

Manganese chloride salt ($MgCl_2.2H_2O$), Besifloxacin ($C_{19}H_{21}ClFN_3O_3$, with molar mass 393.84 g·mol^{-1}) drug ligand and chemicals, solvents, were of pure grade and procured from Sigma-Aldrich Chemical Company with purity 99%.

Synthesis besifloxacin/Mn

Besifloxacin/Mn complex was synthesized by adding 0.788 g (2 mmol) of Besifloxacin ligand (with 1 molar mass 393.84 g.mol^{-1}) in 25 ml of ethanol to the aqueous solution of Mn (II) ion (1 mmol) and stirring for about 10 h at 60°C. The solid orange-yellow colour precipitate was formed with a good yield (75%) and analytical purity (99%) and a melting point of 330°C. Then washed and then filtered many times using either hot H_2O or ethanol to remove unreacted manganese salts and ligand, finally washed again and dried in a desiccator.

Chemical characterization methods

Elemental analysis

The perkin elmer CHN 2400 elemental analyser (USA) was used to measure the C and H contents. The Jenway 4010 conductivity meter and a 1.0 10^3 mol/cm^3 dimethyl sulfoxide (DMSO) solution were used to determine the electrolytic or non-electrolytic properties of the produced compounds.

IR measurements

The existence of particular chemical groups in the examined samples was ascertained using FTIR in transmittance mode. A Bruker FT-IR spectrometer was used for the FTIR measurements. Spectra were captured between 4000 and 400 cm^{-1}.

UV- electronic spectra

An analytical method for identifying the presence of substances, such as metal/s ions and strongly conjugated organic molecules, is UV-Vis spectroscopy. It operates by employing UV/visible light to stimulate a metal's d-electron from its ground state configuration to an excited state.

The UV2 Unicam UV-Vis Spectrophotometer was used to measure UV-Vis spectra in DMSO within the 800-200 nm wavelength range.

Magnetic moments

A material's ability to become magnetized in the presence of an applied magnetic field is measured by its magnetic susceptibility. A material's magnetic susceptibility reveals whether it is drawn to or repelled from a magnetic field. Paramagnetic materials are drawn to areas with strong magnetic fields and align with the applied field. Diamagnetic materials, on the other hand, are forced toward areas with

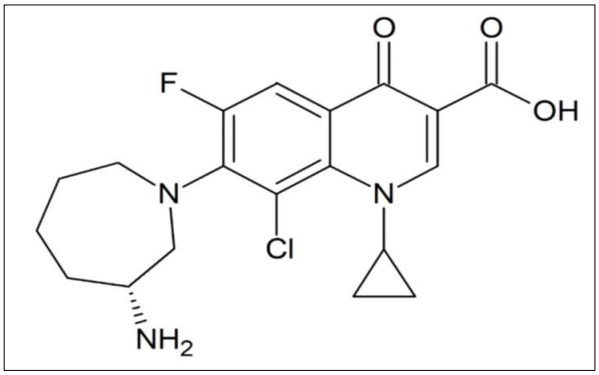


Fig 1: Chemical structure of Besifloxacin.

low magnetic fields and are anti-aligned. The Magnetic Susceptibility Balance from Sherwood Scientific was used to calculate magnetic moments.

Scanning electron microscopy (SEM)

A concentrated beam of high-energy electrons is utilized by a scanning electron microscope (SEM) to produce a variety of signals at the surface of solid objects. The signals include details about the sample, such as its crystalline structure, chemical makeup and exterior shape. A 2-dimensional image showing spatial changes in these attributes is created once data are gathered over a chosen surface area of the sample. SEM examination used a spatial resolution of 50-100 nm and a magnification between 20× and ~30,000×. The Quanta FEG 250 apparatus was used to acquire SEM pictures.

The X-ray diffraction patterns

The most popular method for characterizing materials of all kinds is X-ray spectroscopy. Geometric structure, which provides information on the placements of atoms in a molecule at atomic precision and electronic structure, which focuses on valence and core electrons, were the two types of structural information explored by X-ray spectroscopy. Therefore, when compared to other structural analysis methods, X-ray spectroscopy is the most effective tool. Using copper with secondary monochromate as a target, X-ray diffraction spectra were captured using the X-Pert PRO PAN analytical X-ray powder diffractometer.

Transmission electron microscopy images (TEM)

The material design is made possible by the transmission electron microscope (TEM), a potent tool for structural and chemical characterisation at the nanoscale level that includes imaging, diffraction, microanalytical data and behavior of nanoscale materials. The JEOL 100s microscope was used for TEM examinations.=2.4.

Experimental design and treatment protocol

Ethical approval

This study has been approved and reviewed ZU-IACUC committee in accordance with the U.K. Animals Act.1986 (NIH publications No.8023, revised 1978), This protocol has been reviewed and approved by ZU-IACUC committee in accordance | with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, the National Research Council's Guide for the Care and Use of Laboratory Animals in compliance with the ARRIVE guidelines. (Male albino rats - 18 male albino rats - Age (two months to be mature animals).

- Male rats served as the preferred species for biomedical research due to their physiological and genetic similarity to humans.

As males are not affected by the influence of hormones as in females, this is the pure strain of rats was chosen because it is a suitable strain for less aggressive and anxiety behaviour.

Sample size calculation

Sample size was based on an equal and minimal sample size for both groups to detect a difference at a power of 80% at a 95% confidence level. Thus, I assumed that ALT in the Besi versus Besi/Mn group was 40±2.7 versus 14±1.24 (U/L). At 80% power and a 95% confidence level, the sample size required was 18 and 6 in each group. These calculations used the OPEN EPI software package (Dean *et al.*, 2013). Male albino rats were randomly divided into three groups, with each group consisting of six rats. Group I (Control): Male rats were administered normal physiological saline (1 ml) (Oral gavage)/ day for 30 successive days. Group II (Besiûoxacin) (Besi): The dose for rats (100 mg/Kg), determined based on the previous study of Roy *et al.* (2011), were administered *via* Oral gavage for 30 successive days. Group III (Besi/Mn): the novel complex was administered *via* Oral gavage at the same dosage for 30 successive days based on the previous reference. Both Besi and Besi/Mn were dissolved in normal physiological saline before treatment administration.

Blood samples

Blood samples were collected one day post-experimental procedure *via* the retro-orbital vein, with minimal stress, with light anaesthesia using xylene/ketamine, using heparinized micro-hematocrit capillary tubes to be flowed in another non-heparinized tubes for obtaining the pure serum. The blood samples were clotted at room temperature and serum was obtained through centrifugation at 5000 rpm for 10 minutes. The serum samples were stored at -80°C and then collected for the biochemical parameters, including liver function parameters.

Liver tissue samples were excised, rinsed with ice-cold saline and partitioned for different analyses, including molecular studies and the remaining samples were fixed in neutral buffered formalin for histopathological examination.

Liver function markers

A kit (Spinreact, Spain) was used to measure all of the liver function markers (ALT, AST and LDH). The UV-Vis spectrophotometer (China) is used to measure the following parameters.

Histopathological investigation

Liver specimens were fixed after their collection in buffered formalin, then dehydrated in ethanol in gradual concentration, cleared with xylene, embedded in paraffin and sectioned into semi-thin sections (~10 µm). The sections were dyed and then analyzed or histopathological examination using (Optika) digital microscope (Suvarna *et al.*, 2013).

Gene expression analysis *via* RNA Isolation, cDNA synthesis and qRT PCR

Total RNA was extracted from liver tissues using Qiazol Lysis Reagent, *via* following the approved guidelines.

A NanoDrop spectrophotometer was used to estimate the total RNA concentration, (A260/280) proved it was pure by ratio ~ 1.8. The extracted RNA was then reverse-transcribed into complementary DNA (cDNA) using the cDNA Reverse transcription Kit (Applied BiosystemsTM, Waltham, USA). During the qRT-PCR analysis, the synthesized cDNA was amplified using specific primers, with their sequences listed in Table 1. The reaction setup and thermal cycling conditions were conducted based on (Khamis *et al.*, 2022), with a SYBR Green master mix and a TOPreal™ (Enzynomics, Daejeon, Republic of Korea). Upon Gapdh normalizer gene with other genes were finally calculated and represented as a detailed scientific percentage from the control and then the gene expression levels were normalized and the fold change was estimated as $2^{\Delta\Delta CT}$ (Livak and Schmittgen, 2001). Using Gapdh as a refence gene for hepatic oxidative /Endoplasmic reticulum stress (Zhou *et al.*, 2026 and Hamza *et al.*, 2026).

Antibacterial activity of *E. coli* ATCC 8739

Preparing Inoculum (colony Suspension Method). A disc of *E. Coli ATCC 8739,* was inoculated into 100 ml of broth medium and incubated at 37.0°C for 24.0 hr. A direct sterile saline solution was prepared by inoculating 3-4 colonies, suspension was adjusted to achieve a turbidity. That adjustment results in a suspension containing approximately 1-2 × 10^8 CFU/mL (Alzahrani *et al.*, 2025).

Broth microdilution method

100 µl from the sample was directly inoculated in the first well (without dilution). 50 µl fromMueller-HintonBroth was inoculated in the sterilized wells. All the plates were incubated at 37.0°C for one day. Then, All the plates were placed in the dark to check the bacterial growth. All the wells with recorded growth showed a solution with turbidity, confirming of the bacterial growth (El-Megharbel *et al.*, 2024).

Statistical analysis

The results are presented as (means±S.E.). The data were analysed using a one-way ANOVA and a one-sample t-test plus Wilcoxon test. The SPSS statistical package, version 19.00, was used to perform the analyses. A $P<0.05$ was statistically significant.

RESULTS AND DISCUSSION

Physical data and molar conductance value

Besifloxacin Mn (II) complex is stable, insoluble in water and shows variation degrees of solubility in most organic solvents. Molar conductance value for Besifloxacin Mn (II) compound was measured in 1.0 × 10^{-3} mol/L DMSO to be Λm = 19 (Ω^{-1} mol^{-1} cm^{-1}), which indicates the absence of Cl-1 ions outside the chelation sphere, confirming non-electrolytic behaviour (El-Megharbel *et al.*, 2026; Albogami *et al.*, 2026; Hamza *et al.*, 2026). Carbon, hydrogen and nitrogen analyses (Table 2) and the molar conductance value confirmed the 1:2 (Mn (II): besifloxacin) ratio. The chelation mode of besifloxacin towards Mn (II) metal ion was IR, magnetic moment, SEM, TEM and XRD and the suggested formula for Mn (II) chelation is $[Mn(BFL)_2(H_2O)_2]\cdot 3H_2O$.

Infrared spectra

The interaction of Mn (II) ion with Besifloxacin forms a complex in the form $[Mn(Besi)_2(H_2O)_2]\cdot 3H_2O$. The infrared spectral data and their assignments, comparing mainly the IR frequencies of the Mn (II) complex with Besifloxacin, can be drawn:

1. Strong absorption bands appeared in the Besifloxacin spectrum at 1720 and 1635 cm^{-1} assigned (Maeda and Okawara, 1967) to COOH and the stretching vibrations of C=O .
2. Regarding these IR frequencies, the bands at 1720 cm^{-1} and 1635 cm^{-1} completely vanished in the spectrum of Mn (II) complex as the position of the strong bands at 1585-1575 and 1345-1350 cm^{-1} referring that the carboxylate group appeared due to the two absorption bands $\nu_{asymmet}$ (COO) and ν_{symmet} (COO) and its coordination with Mn (II). Similarly, the band at 1635 cm^{-1} due to the C=O moiety in the spectrum of Besifloxacin ligand disappeared and another new band appeared at 20-35 cm^{-1} lower frequency (1595 cm^{-1}) appeared confirming (Nakamoto, 1986)

Table 1: Primers sequences of targeted genes.

Gene	Forward primer	Reverse primer	Size	Accession no.
Gapdh	ACGGGAAACCCATCACCATC	TCACAAACATGGGGGCATCA	189	NM_017008.4
NF-κβ	CAGGACCAGGAACAGTTCGAA	CCAGGTTCTGGAAGCTATGGAT	150	NM_199267.2
NRF-2	GGTTGCCCACATTCCCAAAC	CAGGGCAAGCGACTGAAATG	124	NM_001399173.1
JNK	AGTGTAGAGTGGATGCATGA	ATGTGCTTCCTGTGGTTTAC	182	NM_053829.2
CHOP	CACAAGCACCTCCCAAAG	CCTGCTCCTTCTCCTTCAT	158	NM_001109986.1

Table 2: Physical and microanalytical values of the Mn-besifloxacin complex.

Compounds	Color	Molecular formula	Λm	Elemental analysis, % Found % (Calcd.)			
				C	H	N	Mn
$[Mn(BFL)_2(H_2O)_2]\cdot 3H_2O$	Yellow orange	$C_{38}H_{50}O_{11}Cl_2N_6F_2Mn$	19	48.96 (49.08)	8.45 (8.61)	9.12 (9.04)	6.01 (5.92)

involvement of C=O carbonyl group in the chelation process. Based on these observations, it can be proposed that Besifloxacin act as a bidentate ligand. Further conclusive evidence (Nakamoto,1986) that was observed was the appearance of a new band at about 550-615 cm^{-1} allocated to (Mn-O) in the spectrum of Mn (II) and not observed in the Besi ligand spectrum (Fig 2).

Electronic spectra and the magnetic measurements

UV-Visible absorption spectra of Besi as a ligand and its Mn (II) complexity can be explained as follows: The electronic transition of Besifloxacin occurred at 290 nm, while on chelation with Mn (II) ion, new bands appeared at 299 nm corresponding to charge transfer from Besifloxacin to Mn (II) ion (Besifloxacin → Mn). The magnetic moment of the present Besifloxacin of Mn (II) complex is 5.93, suggesting octahedral geometry for Mn (II) (Kadyrov *et al.*, 1996; Sonnenburg *et al.*, 1994).

XRD studies of besifloxacin Mn (II) complex

XRD Powder analysis for the Besifloxacin Mn (II) complex explains the degree of crystallinity, where the diffractogram is ranged between 20 and 80° (2θ) values (Fig 3). The XRD patterns confirm the semi-crystalline arrangement for the Mn (II) complex that was investigated at room temperature using the Cu Kα radiation. The crystalline size was determined by using the Scherrer formula (Cullity, 1978).

$$D = \frac{k\lambda}{b\cos\theta}$$

Where,

k = Constant = 0.94.

λ = Wavelength of X-ray (0.154 nm).

The semi-crystalline size for Mn (II) complexes was calculated and has a value of 21 nm.

SEM and TEM investigations

The morphological structure of solid surfaces is described by the SEM technique for Besi and its $[Mn (Besi)_2 (H_2O)_2]\cdot 3H_2O$ complex. A tiny particle agglomerate with apparent projections for the Besi/Mn complex is depicted in (Fig 4). All particles have a high capacity to form agglomerates with a variety of shapes.

TEM pictures for Besi and its $[Mn (Besi)_2(H_2O)_2]\cdot 3H_2O$ Fig 5), are described, where Transmission electron microscopy (TEM) image analysis procedures involve

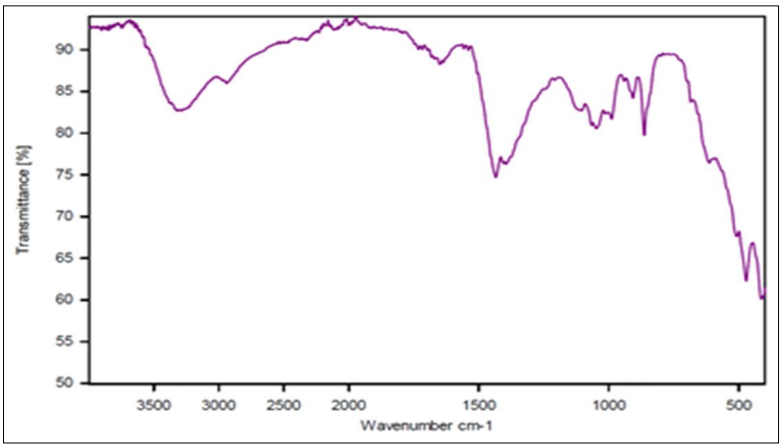


Fig 2: FT-IR of Besi/Mn metal complex.

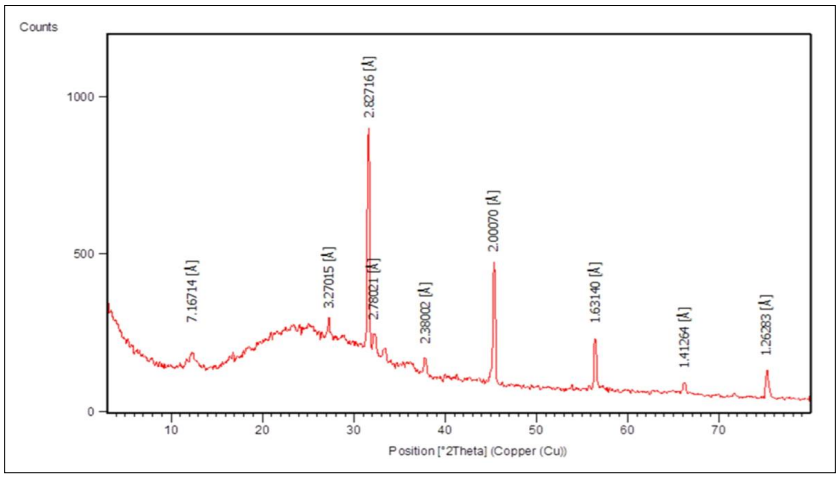


Fig 3: XRD of Besi/Mn metal complex.

calibrating scale bars, enhancing contrast and measuring structural features using software like ImageJ. A homogeneous phase material is confirmed by the micrograph's ordered arrangement of the BFL metal chelate matrix and its $[Mn\ (Besi)_2(H_2O)_2]\cdot 3H_2O$ complex. Determining particle size distribution involves capturing high-resolution micrographs and analyzing the individual particles with image processing software. A spherical black spot shape appears for $[Mn\ (Besi)_2(H_2O)_2]\cdot 3H_2O$ with a particle size of 23 nm.

Effect on liver function parameters

What stands out in the (Table 3) is a clear improvement in hepatic enzymes (ALT and AST) in Besi/Mn treated group, with a significant decrease compared to the control group. Meanwhile, the Besi-treated group afforded a mild significant increment in hepatic enzymes, indicating mild hepatic injury. The same aspect was shown clearly in LDH parameters, with clear marked decline in LDH in Besi/Mn treated group. Meanwhile, the Besi/Mn group showed clear and positive results below those of Besi by declining the hepatic enzyme levels compared to the Besi-treated group only, which clearly indicates the essential role of the novel metal complex in the hepatic repair of hepatic injury.

Histopathology of the hepatic tissues

Fig 6 revealed that (A) control group: A section of the liver tissue showed a patent central vein (C) surrounded by regular rows of rounded hepatocytes (arrow) with central regular vesicular nuclei and deep eosinophilic cytoplasm. No hepatocyte degeneration, inflammation, or congestion could be detected (H and E ×100). (B) Besi group: A section of liver tissue showed a patent central vein (C) surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei and deep eosinophilic cytoplasm (arrow) surrounded by focal minimal periportal inflammatory infiltration (star). This indicates mild liver injury (H and E ×40) (C1) Besi/Mn showing A section in the liver tissue showed a patent central vein (C) surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei and clear cytoplasm filled with fat, indicating mild steatosis (arrow). (H and E × 40). (C2) Besi/Mn showing A

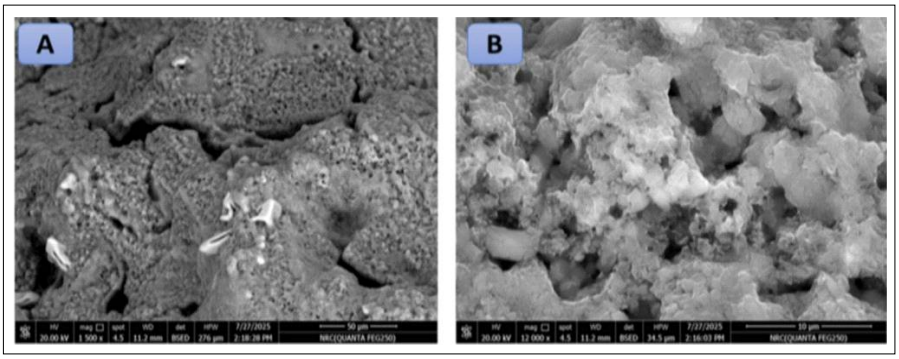


Fig 4: SEM of (A) Besi and (B) Besi/Mn.

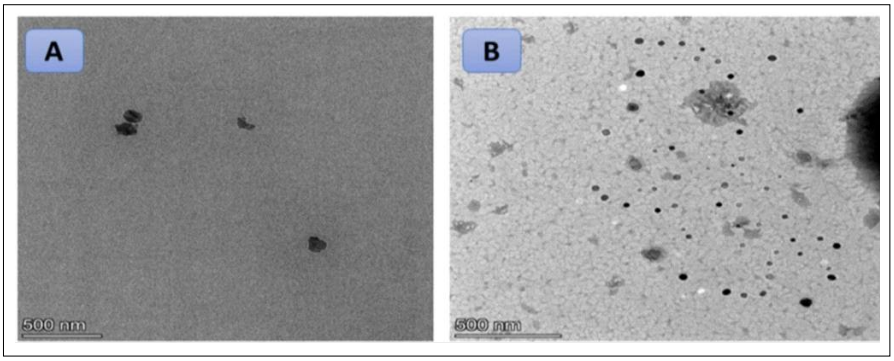


Fig 5: TEM of (A) Besi and (B) Besi/Mn.

Table 3: Effects of Besi or Besi/Mn treatments on liver enzymes in male albino rats.

	ALT (U/L)	AST (U/L)	LDH (U/L)
Control group	47±7.50^b	77±6.51^c	418.00±20.20^c
Besi	55 ±6.21^a	175±6.22^a	1400±24.56^a
Besi/Mn	15±1.48^c	119±8.43^b	715±18.48^b

Values are (Mean ± SE), n=6, Besi: Besifloxacin, Besi/Mn: Besifloxacin/Mn complex, Significance level ($p<0.05$).

section in the liver tissue showed a patent central vein (C) surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei (N) and clear cytoplasm filled with fat, indicating steatosis (arrow), separated by regular patent sinusoids (S). (H and E ×100)

Gene expression

Closer inspection of Fig 7 clarified that the Besi group induced significant increases in NF/-KB, JNK and CHOP levels compared to the control group. Meanwhile, Besi induced a significant decline in NRF-2 levels. On the other hand, Besi/Mn treated group provided strong evidence of antioxidant activity and improvement of the hepatic tissues when compared with the control group.

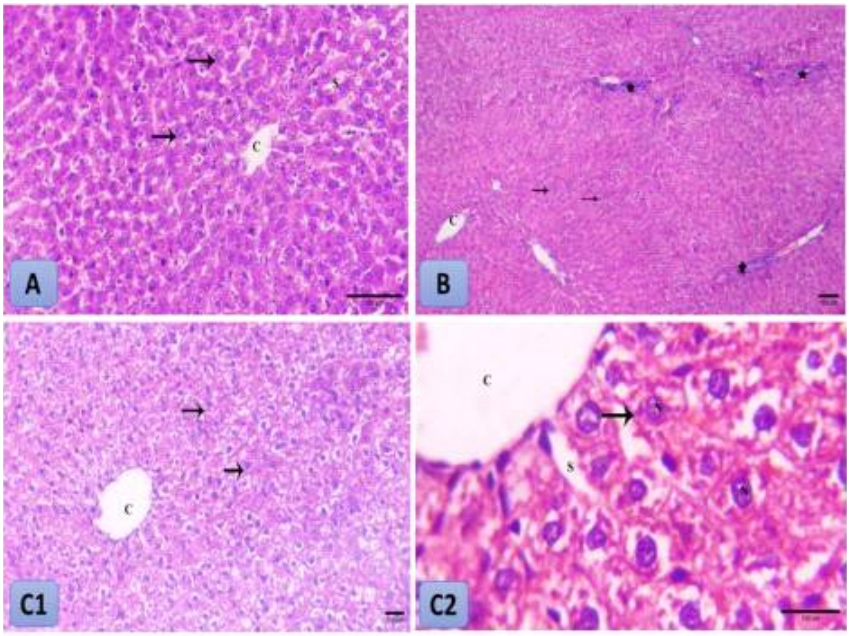
There was a positive and high Confidence level (95% confidence intervals) after performance of one sample T-test and Wilcoxon test, which greatly confirmed the obtained results with highly significant p-values, as shown and clarified in the (Table 4, 5, 6 ,7).

Antibacterial activity

Biological evaluations of the novel complex (Besi/Mn) were performed on the strain of (*Escherichia coli*). The inhibition concentrations of the Besi/Mn metal complex against the tested bacteria were high at approximately the low concentration as compared with Besi alone. Besi/MN novel metal complex was found to possess high antimicrobial activity (Table 8), with MBC activity higher than Besi alone (Fig 8 and Table 9).

Fluoroquinolones are considered as the most useful class that possesses potent antibacterial activities against both Gram-negative and Gram-positive strains (Lu *et al.*, 2008). This category has been used against many infections and targets DNA and some types of bacterial topoisomerases (Khodursky *et al.*, 1995; Hussy *et al.*, 1986). This category is analogous to quinoline, with many pharmacological capacities even with the high modification of its essential structure (Mandell and Tillotson, 2002; Roy *et al.*, 2010).

Besi, is a novel fluoroquinolone agent with a high and broad activity spectrum. Besi possess a high and potent antibacterial activity against a lot of pathogens. Several researchers have examined Besi's *in vitro* and anti-infective properties against a variety of clinical isolates and bacterial conjunctivitis, including (Haas *et al.*, 2010).

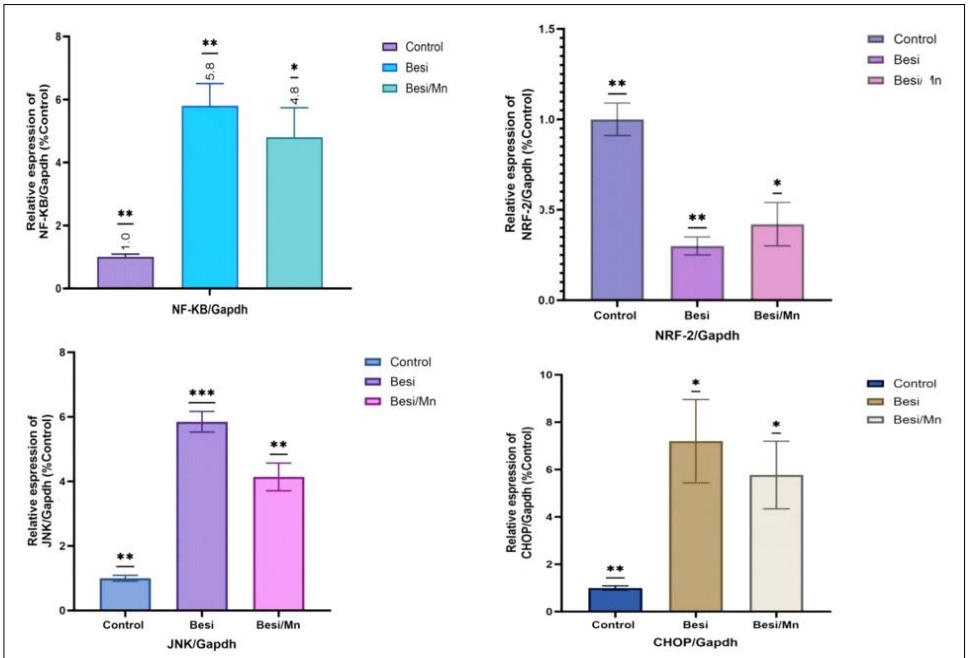


(A) Control group: A cross-section of the hepatic tissue showed normal appearance, with no hepatocyte degeneration, inflammation, or congestion (H and E ×100). (B) Besi: A section of the liver tissue showed a patent central vein surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei and deep eosinophilic cytoplasm, surrounded by focal minimal periportal inflammatory infiltration. This indicates mild liver injury (H and E ×40). (C1) Besi/Mn: A section of liver tissue showed a patent central vein surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei and clear cytoplasm filled with fat, indicating mild steatosis (H and E ×40). (C2) Besi/Mn: A section of liver tissue showed a patent central vein surrounded by regular rows of rounded hepatocytes with central regular vesicular nuclei and clear cytoplasm filled with fat, indicating mild steatosis, separated by regular patent sinusoids (H and E ×100).

Fig 6: Histological sections of different treated groups.

The pharmacodynamics and safety profile of Besi have been studied in animal models and humans (Sanders *et al.*, 2009) and they concluded that Besi showed a favourable genotoxicity and phototoxicity profile.

Limited data are available regarding the adverse effects of Besi. As it is an analogue of quinoline. From the theoretical studies of Besi, Besi has been proved theoretically that it has some phototoxic effect (Roy *et al.*, 2011).



Data are expressed as (mean ± S.E).*: indicate significant at (P<0.05) (Low significant), **: indicate significant at (P<0.01) (Moderate significant), ***: indicate significant at (P<0.001), **** (Highly significant).

Fig 7: Effects of treatments on (A) mRNA expression of Gapdh, (B) mRNA expression of NF-KB, (C) mRNA expression of NRF-2, (D) mRNA expression of JNK, (E) mRNA expression of CHOP.

Table 4: P-values and confidence level of NF-κB/Gapdh expression levels.

CT NF-KB	Control	Besi	Besi/Mn
P- value (two detailed)	0.0027	0.0049	0.0125
P- value summary	**	**	*
Significant (alpha=0.05)	Yes	Yes	Yes
95% confidence interval	0.77 to 1.22	4.04 to 7.55	2.46 to 7.13

Table 5: P-values and confidence level of NRF-2/Gapdh expression levels.

CT NRF-2	Control	Besi	Besi/Mn
P- value (two detailed)	0.0027	0.0091	0.0261
P-value summary	**	**	*
Significant (alpha=0.05)	Yes	Yes	Yes
95% confidence interval	0.77 to 1.22	0.17 to 0.42	0.12 to 0.71

Table 6: P-values and confidence level of JNK/Gapdh expression levels.

CT JNK	Control	Besi	Besi/Mn
P- value (two detailed)	0.0027	0.0010	0.0036
P-value summary	**	***	**
Significant (alpha=0.05)	Yes	Yes	Yes
95% confidence interval	0.77 to 1.22	5.05 to 6.64	3.07 to 5.20

For this reason, I also introduced serum analysis of the male rats in the current study.

Most toxic studies on Besi primarily involved ophthalmic exposures, with no investigations into sub-chronic oral gavage exposure. Additionally, effects from repeated-dose regimens of Besi or its novel complex Besi/Mn remain unstudied, limiting the assessment of Besi due to insufficient animal study data. Furthermore, long-term toxicity studies on Besi and the Besi/Mn complex have not been conducted.

Thus, the current study revealed the high antibacterial efficacy of Besi/Mn compared with Besi alone, beside it's efficacy to protect hepatic tissue from damage or oxidative injury via amelioration of the genes involved in the oxidative stress pathway of the endoplasmic reticulum and thus cellular injury.

The RAT NFE2L2 ENCODING NRF2 antioxidant path way is a crucial me chanism for maint aining cellular ba lance and protecting against oxidative stress. It involves the transcription factor RAT NFE2L2 ENCODING NRF2, which is regulated by associated protein 1 (Keap1). Under normal conditions, Keap1 binds to RAT NFE2L2 ENCODIN GNRF2, tagging it for degradation and keeping its levels low. However, when cells encounter stress, such as reactive oxygen species (ROS) or electrophilic compounds, Keap1 is modified, allowing RAT NFE2L2 ENCODING NRF2 to be released and enter the nucleus (Saha *et al.*, 2020).

Once inside, RAT NFE2L2 ENCODING NRF2 pairs with a small protein partner and binds to specific DNA sequences. This pathway is significant in various diseases, including cancer and Alzheimer's disease and is being explored as a potential therapeutic target and this pathway is greatly proven in the current study via efficacy of Besi/Mn on alleviation of the oxidative stress injury and increasing the expression of NRF-2 as compared with Besi alone and thus enhancing the antioxidant capacities of the cells after treatment with the novel complex of Besi/Mn.

The CHOP antioxidant pathway refers to the role of C/EBP homologous protein (CHOP) in cellular stress responses, particularly in the context of endoplasmic reticulum (ER) stress. CH OP is induced by ER stress and p lays a crucial role in mediating apoptosis, which is the process of

Table 7: P-values and confidence level of CHOP/Gapdh expression levels.

CT CHOP	Control	Besi	Besi/Mn
P- value (two detailed)	0.0027	0.0193	0.0199
P-value summary	**	*	*
Significant (alpha=0.05)	Yes	Yes	Yes
95% confidence interval	0.77 to 1.22	2.82 to 11.57	2.21 to 9.32

Table 8: MIC (Minimum bactericidal concentration) plate reader for Besi and Besi/Mn samples.

Sample	Dilution in (µg/ml)	500	250	125	62.5	31.25	15.62	7.812	3.906	1.953
Besi	*E. Coli* ATCC 8739	C	C	T	T	T	T	T	T	T
Besi/Mn		C	C	C	C	T	T	T	T	T

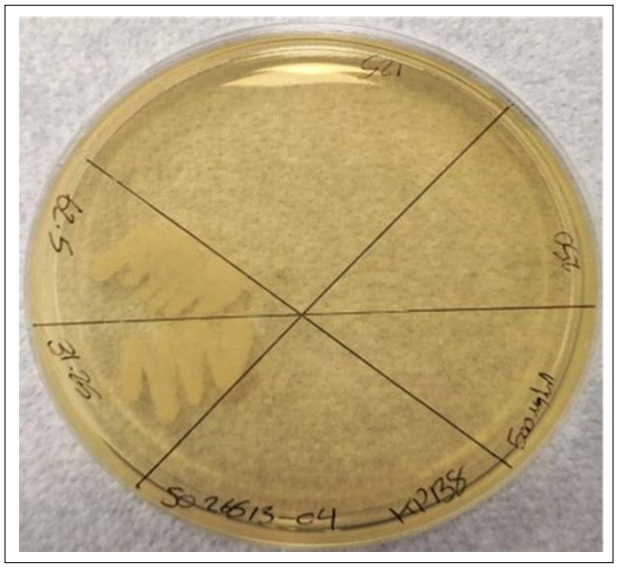


Fig 8: MBC of Besi/Mn against *E. Coli* ATCC 8739.

Table 9: MBC (Minimum bactericidal concentration) results for Besi and Besi/Mn samples.

Sample	Dilution (µg/ml)	500	250	125	62.5	31.25
Besi	*E. Coli* ATCC 8739					
Besi/Mn						

programmed cell death (Wu *et al.*, 2025) and this explains the results of the current study which revealed elevation of gene expression of CHOP, JNK which (c-Jun N-terminal kinase pathway) is a stress-activated branch of the mitogen-activated protein kinase (MAPK) signaling network and It regulates transcriptional responses to environmental stress, inflammatory cytokines, DNA damage and growth and NF-κβ incase of Besi treated groups, meanwhile, it's declining in Besi/Mn treated group which revealed the significant effect of the novel complex in alleviation of the oxidative stress series.

CONCLUSION

Besi/Mn complex demonstrated a significant hepatoprotective effect by attenuating liver enzyme elevations, reducing inflammatory markers and decreasing oxidative stress, with amelioration of hepatic structure and toxicity indicators in treated rats. The structural characterization confirmed successful formation of a 1:2 Mn (II)-Bes chelate, which contributed to its enhanced biological activity. These results suggest that the novel Besi/Mn complex not only retains potent antibacterial properties but also offers promising hepatoprotective and antioxidant benefits, highlighting its potential as a safe and effective therapeutic agent against hepatotoxicity and oxidative stress-related hepatic injury.

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Conflict of interest

The author declares that there is no conflict of interest.

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